Botanica Pacifica. A journal of plant science and conservation. 2023. 12(1): 107–120
DOI: 10.17581/bp.2023.12105

BIODIVERSITY STUDIES

Amendments to the description of *Chloromonas actinochloris* (Chlorophyta) inferred from the study of the South Siberian finding

Irina N. Egorova[1], Nina V. Kulakova[1]* & Olga N. Boldina[2]

Irina N. Egorova[1]
e-mail: egorova@sifibr.irk.ru

Nina V. Kulakova[1]*
e-mail: kulakova@sifibr.irk.ru

Olga N. Boldina[2]
e-mail: boldina@binran.ru

[1] Siberian Institute of Plant Physiology and Biochemistry SB RAS, Irkutsk, Russia

[2] Komarov Botanical Institute RAS, St. Petersburg, Russia

* corresponding author

Manuscript received: 06.09.2022
Review completed: 26.12.2022
Accepted for publication: 28.12.2022
Published online: 31.12.2022

ABSTRACT

Chlamydomonas-like green microalgae are unicellular biflagellate organisms from the order Volvocales, which are widely distributed in terrestrial ecosystems. Recent studies have shown that they belong to different phylogenetic lineages. The exact establishment of the taxonomic affiliation of these representatives is based on integrative approach, including multigene phylogeny along with light and electron microscopy, which also allows us to get the most accurate information about their biogeography. In this study, we examined the terrestrial green *Chlamydomonas*-like microalga, isolated from soil of the South-Eastern Altai Mountains in Russia. Based on molecular data of the nuclear 18S and plastid *rbc*L gene, we refer this alga to *Chloromonas actinochloris* within a robust clade of the genus *Chloromonas*, the phylogroup Chloromonadinia. This species was described from North America (USA). It is the first discovery of *C. actinochloris* in the mountains of Southern Siberia, as well as the first record of the species in Russia confirmed by molecular phylogeny and microscopy methods. The description of *C. actinochloris*, information on its biology and geography was supplemented. It is generally accepted that the phylogenetic clade including *C. actinochloris* should be revised and reassigned to a new genus soon. Our study shows that vegetative cells of Chloromonadinia algae can have ornamented cell wall that was mainly known for resting cells (cysts/zygotes) in a number of chlamydomonads, more rarely for vegetative cells. The structures on the wall of vegetative cells of *Chlamydomonas*-like microalgae can serve as an additional diagnostic criterion.

Keywords: terrestrial green microalgae, Chloromonadinia, *Chloromonas actinochloris*, Russian Altai Mountains, taxonomy, integrative approach

РЕЗЮМЕ

Егорова И.Н., Кулакова Н.В., Болдина О.В. Дополнения к описанию *Chloromonas actinochloris* (Chlorophyta) на основе находки в Южной Сибири. *Chlamydomonas*-подобные зеленые микроводоросли – широко распространенные в наземных экосистемах одноклеточные двужгутиковые организмы из порядка Volvocales. Современные исследования показали, что они принадлежат разным филогенетическим линиям. Установление таксономической принадлежности *Chlamydomonas*-подобных водорослей требует применения методов молекулярной филогении наряду со световой и электронной микроскопией, что также позволяет получить наиболее точную информацию об их географии. В настоящей работе нами была изучена зеленая монадная микроводоросль, найденная в почве юго-восточного Алтая (Россия, Республика Алтай, Кош-Агачский район). На основе молекулярных данных она отнесена к самостоятельной кладе в составе филогруппы Chloromonadinia, роду *Chloromonas*, виду *C. actinochloris*. Вид известен из Северной Америки (США). Это первая находка *C. actinochloris* в горах Южной Сибири, а также первая подтвержденная при помощи методов молекулярной филогении и микроскопии находка вида в России. На основе полученных нами данных дополнено описание *C. actinochloris*, сведения о его биологии и географии. Ранее показано, что виды в составе клады, к которой принадлежит *C. actinochloris*, должны быть отнесены к новому роду. Однако, таксономическая ревизия Chloromonadinia еще не проведена. Наши исследования также подтверждают, что вегетативные клетки представителей Chloromonadinia могут иметь оболочку с орнаментом. Скульптурированные оболочки известны преимущественно для покоящихся клеток (цист/зигот) ряда хламидомонад, реже для вегетативных клеток. Структуры на оболочке вегетативных клеток *Chlamydomonas*-подобных микроводорослей могут служить дополнительным диагностическим критерием.

Ключевые слова: почвенные зеленые микроводоросли, Chloromonadinia, *Chloromonas actinochloris*, горный Алтай, таксономия, интегративные исследования

Unicellular green algae which have two equal flagella, chloroplast with or without pyrenoid(s) and cell wall, belong to chlamydomonad-type algae (Ettl 1976, 1983, Pröschold et al. 2001). This type comprises a significant number of species within the class Chlorophyceae order Volvocales (or Chlamydomanadales). Traditionally they were placed in the family Chlamydomonadaceae. According to modern knowledge, they are members of different phylogenetic lineages (Nakada et al. 2008), which can be found in various terrestrial and aquatic ecosystems. These algae are free living organisms,

epibionts and endobionts (Ettl 1983, Boldina 2016, Nema et al. 2019, Boldina & Chunaev 2020, Barcyté et al. 2022). In terrestrial ecosystems, they occupy a variety of ecological niches, such as soils, and different substrates: rocks, plants, snow, ice, etc. Based on the results obtained using the culture-depended methods and light microscopy, it was shown that the diversity and abundance of these algae (morphospecies) were significant in some soils of boreal forests especially after the rain (Gollerbakh & Shtina 1969, Aleksakhina & Shtina 1984). Their representation was less in arid habitats (Gollerbakh & Shtina 1969, Novichkova-Ivanova 1980, Aleksakhina & Shtina 1984). To a certain extent, data obtained by isolation of nucleic acids from natural arid soil samples are consistent with those revealed by culture-depended methods and light microscopy (Lin & Wu 2014). A significant number of species of green volvocalean monads have been found in cryospheric biomes, where they play crucial role in their functioning (Hoham 1975, Broady 1979, Buchheim et al. 1996, Hoham et al. 2002, 2006, Liu et al. 2006, Novis et al. 200, Eddie et al. 2008, Demchenko et al. 2012, Remias et al. 2013, Muramoto et al. 2010, Matsuzaki et al. 2018, 2019, Barcyté et al. 2018a, Procházková et al. 2018, 2019a, b, Gálvez et al. 2021).

About 50 morphospecies of *Chlamydomonas*-like algae are known in the terrestrial habitats of the mountains of Southern Siberia (Egorova et al. 2020a). Most of them were found in soils of the forests, meadows, swamps and others natural and anthropogenic-disturbed plant communities (Sudakova 1975, 1986, Shushuyeva 1977, 1980, Artamonova 1982, Perminova 1989, Faktorovich 2001, Chatta & Lopatovskaya 2001, Lopatovskaya et al. 2017). A number of representatives collected from the bark of trees, rocky substrates, in associations with mosses, or in snow (Middendorf 1867, Komarov 1905, Egorova & Sudakova 2005, Takeushi et al. 2006, Lopatovskaya & Maksimova 2010, Egorova 2012, 2016, Egorova et al. 2020b). The species identity of these algae was established using light microscopy. At the same time, an extremely small number of *Chlamydomonas*-like algae are isolated from natural habitats of Southern Siberia, maintained in culture collections, and studied by light microscopy along with electron microscopy and molecular phylogeny methods. This hinders the understanding of the actual diversity of this group and their biogeography.

The goal of this work is to characterize morphology, ultrastructure and the phylogenetic position of the *Chlamydomonas*-like strain isolated from a dry soil in the stony steppe in the Russian Altai highlands.

MATERIAL AND METHODS

Territory, sampling, strain isolation, cultivation and microscopy

Studied soil specimen was collected in the dry stony steppe located on a mountain slope in the vicinity of the Lake Bol'shoe Boguty, the South-Eastern Altai, the Kosh-Agach District, the Republic of Altai, Russian Federation, in August 2015 (Fig. 1). The territory is inaccessible and remote from populate areas. Altitude 2463 m above sea level; 49.707583°N 89.517283°E. The climate of the territory is extreme continental. Mean annual temperature is -6.7ºC, precipitation 110 mm/year. The greatest amount of precipitation falls in the summer months (up to 70 %). The least of them falls in winter, so there is no permanent snow cover, and the soil freezes hard. The soil is skeletic, sandy, poorly designed. The territory is characterized by a high duration of sunshine (up to 2500 hours/year). The dominant vegetation types are mountain steppe along with mountain tundra and meadows.

The studied soil specimen was consisted of 10 separate soil plots 5×5 cm with thickness of 2–3 cm deep, taken from a site 10×10 m. Sample was collected under sterile conditions in paper bag, air dried and delivered to the laboratory. In laboratory conditions, to obtain enrichment cultures, one gram of the crushed combined sample was placed in a 100 ml flask with a liquid nutrient medium N BBM (Starr & Zeikuss 1973). Individual colonies were derived by methods of Andreeva (1998). The isolated strain is stored under number 167 in the IRK-A collection (Siberian Institute of Plant Physiology and Biochemistry SB RAS, Irkutsk).

For the study, cultures of IRK-A 167 were grown on liquid and agar-solidified (1.4–1.6 %) media 0N, N, 3N BBM with or without vitamins (B_1 and B_{12}), and BG 11 (Stanier et al. 1971). We used different modes of illumination and temperature. The cultures were maintained in a BINDER climatic camera (Germany) at 12–16ºC under ~40 µmol photons m^{-2} s^{-1} irradiance and a 16:8 h light:dark cycle using cool-white fluorescent lamps. Additionally, the strain grew in the growth room at 19–23ºC under 12 h photoperiod with cool-white lamps ~100 µmol photons m^{-2} s^{-1}. Also, IRK-A 167 was cultivated at room temperature and natural light on the window. The cultures of IRK–A 167 were monitored for 2–3(6) months, maximum up to one years.

The strain microphotographs were taken under Axio Scope A1 (Carl Zeiss, Germany) light microscope with a color camera ICc5. The cells were stained with

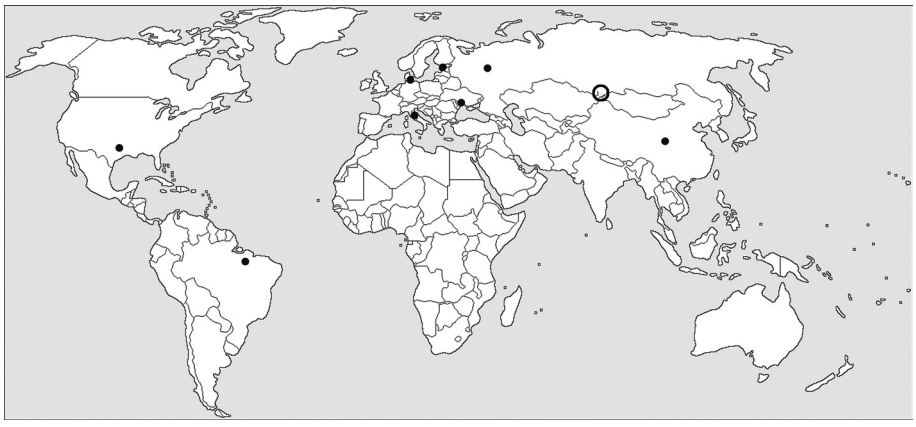

Figure 1 Geographical distribution of *Chloromonas actinochloris* and similar algae (dots). Empty circle shows the sampling site under this study. Black dots indicate the regions of the world where algae of *Chloromonas actinochloris*-like morphology were found

0.1 % gentian violet in vivo to establish the structure of cell wall. For transmission electron microscopy, the cells of week and four-week old agar cultures of IRK–A 167 were included in small agar blocks and fixed in 2.5 % cold glutaraldehyde preliminary dissolved in the liquid culture medium. The specimens were kept on ice for 4 hours. After washing in culture medium, cells were post-fixed in 1 % OsO_4 on ice for 1 h. The procedure was followed by dehydration series in acetone and embedding series in Epon–Araldite resin mixture with DMP–30. The resin samples were stayed for two days at room temperature and two more days at 60°C. Ultrathin sections of polymerized blocks were prepared by LKB III ultratome and glass knives. The sections were placed on copper grids and stained with lead citrate for 10 min. Cells were viewed and photographed using the transmission electron microscope Libra 120+ (Carl Zeiss, Germany).

DNA extraction, PCR and sequencing

The isolation of DNA from the living strain culture grown on an agar media was carried out according to the method of Doyle & Doyle (1990) with modification. Algae were collected from about 1 cm^2 of an agar plate surface in 200 µl of lysis buffer solution and pipetted. The sample was left for cell lysis at 60°C for 60 min, then the standard protocol procedure was followed. The precipitated DNA was dissolved in 25 µl of mQ water and 50–80 ng of DNA used in PCR.

PCR was carried out in 20 µl of the reaction mixture containing 1x PCR buffer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 10 pmol of forward and reverse primer, 5 u. HS-Taq DNA polymerase, 50 ng template DNA, and sterile deionized water.

The 18S rRNA gene and ITS were amplified using the primers published by Katana et al. (2001) and White et al. (1990), respectively. The fragment of the *rbc*L gene 1200 nucleotides in length was amplified using primers published by Levin et al. (2003) and modified for amplification of green microalgae *rbc*LGrF 5'-ATGGYTCCACAAACWGAAAC-3', *rbc*LGrR 5'-GCWCGTAAYGAAGGTCG-3'.

PCR products were purified from 1 % agarose gel and sequenced (Synthol, Moscow) with forward and reverse primers using the Sanger method. Nucleotide sequences were analyzed and assembled using BioEdit 7.0.5.3 program (Hall 1999). Comparison and search for nearest homologues was performed in the BLAST program of the NCBI global service. The 18S-ITS and rbcL sequences were submitted to GenBank under accession numbers OP303232 and OP320792, respectively.

Phylogenetic analyses

Phylogenetic trees were reconstructed based on the 18S *r*RNA, *rbc*L genes, and the combined alignment of these genes (*rbc*L 1062 nt, 18S 1633 nt). For reconstruction of phylogenetic trees, 47 nuclear 18S rDNA sequences and 22 chloroplast rbcL sequences were acquired. The optimal model of nucleotide substitutions for each data set was selected using the jModeltest 2.1.7 program (Darriba et al. 2012). Phylogenetic trees were reconstructed using Maximum Likelihood (ML), Neighbor Joining (NJ) methods in the program Mega 11.0.8 (Tamura et al. 2021) and the Bayesian tree was calculated in the MrBayes 3.2.7 program (Ronquist et al. 2012). The GTR + I + G model (ML and Bayesian reconstruction) and TN93 + I + G nucleotide model (NJ) were used for the concatenated nucleotide alignment. For the 18S phylogeny, TN93 + I + G model was applied. The number of bootstrap replicas was 1000 in the ML and NJ calculations. The Bayesian analysis was run to achieve the SD of split frequencies < 0.01.

RESULTS

LM of cultivated strain IRK–A 167

The solitary cells of IRK–A 167, including monad and hemimonad (without flagella) cells, have ellipsoidal, cylindrical, broadly ellipsoidal or broadly cylindrical, globose, subglobose, ovoid, pyriform or wrong-polygonal shape. This form depends on the mutual pressure of cells in the sporangia (Fig. 2, 3).

The cells are 10–20 µm in length and 5–15.4 µm in width. The cell wall is thin in young cells. It thickens with age, without mucus. In a light microscope, the cell wall often looks smooth. However, we found that it is ornamented (Fig. 4). The bumpes (spikes?) are located on its surface in regular uneven rows. They are especially noticeable in cells growing in liquid cultures. Staining with gentian violet and TEM (see next) confirmed that the cell wall of IRK–A 167 is not smooth. A papilla is almost invisible or expressed, blunt conical. The monad cells with two flagella of equal length, shorter or longer than the cell, or equal to it (Fig. 3). After the movement stops, the cells sometimes drop the flagella. Chloroplast is massive, with radial lobes, sometimes it does not fit tightly to the cell wall, and the ends of the cell appeared empty. The lobes of the chloroplast are not always clearly visible. Chloroplast contains a central pyrenoid, which can be slightly shifted to the cell posterior end. Pyrenoid is surrounded by several, up to 8–10, or numerous starch grains. The stigma is anterior, small, ellipsoidal, elongated-ellipsoid, up to rod-shaped, sometimes curved. It is located in the chloroplast, shifted to the middle of the cells. The stigma is often hidden. With different monad or hemimonad cell positions, the stigma looks dark red or orange in a light microscope. Two contractile vacuoles are present below the papilla. Sometimes, the cells contain a large number of vacuoles in cytoplasm. The chloroplast of such cells is like spongiomorph (Fig. 3K). The nucleus is anterior, placed between the lobes of the chloroplast.

The strain was reproduced asexually, by 2–16 zoo-, hemizoo- and aplanospores, 4 or 8 being the usual number (Fig. 3). Sexual reproduction has not been observed. The daughter cells are released by rupture of the mother cell wall (Fig. 4A). After the liberation of spores, the rest sporangial walls remain in the culture for a long time. Often the spores linger inside the mother cell and are able to start dividing again. As a result, complex sporangia can form. The sporangia of the strain IRK–A 167 in some cases resemble representatives of genus *Tetracystis* and so on. Cultures that have been maintained on agar for a long time, after transferring them to a liquid medium, do not give or form a very small number of zoospores.

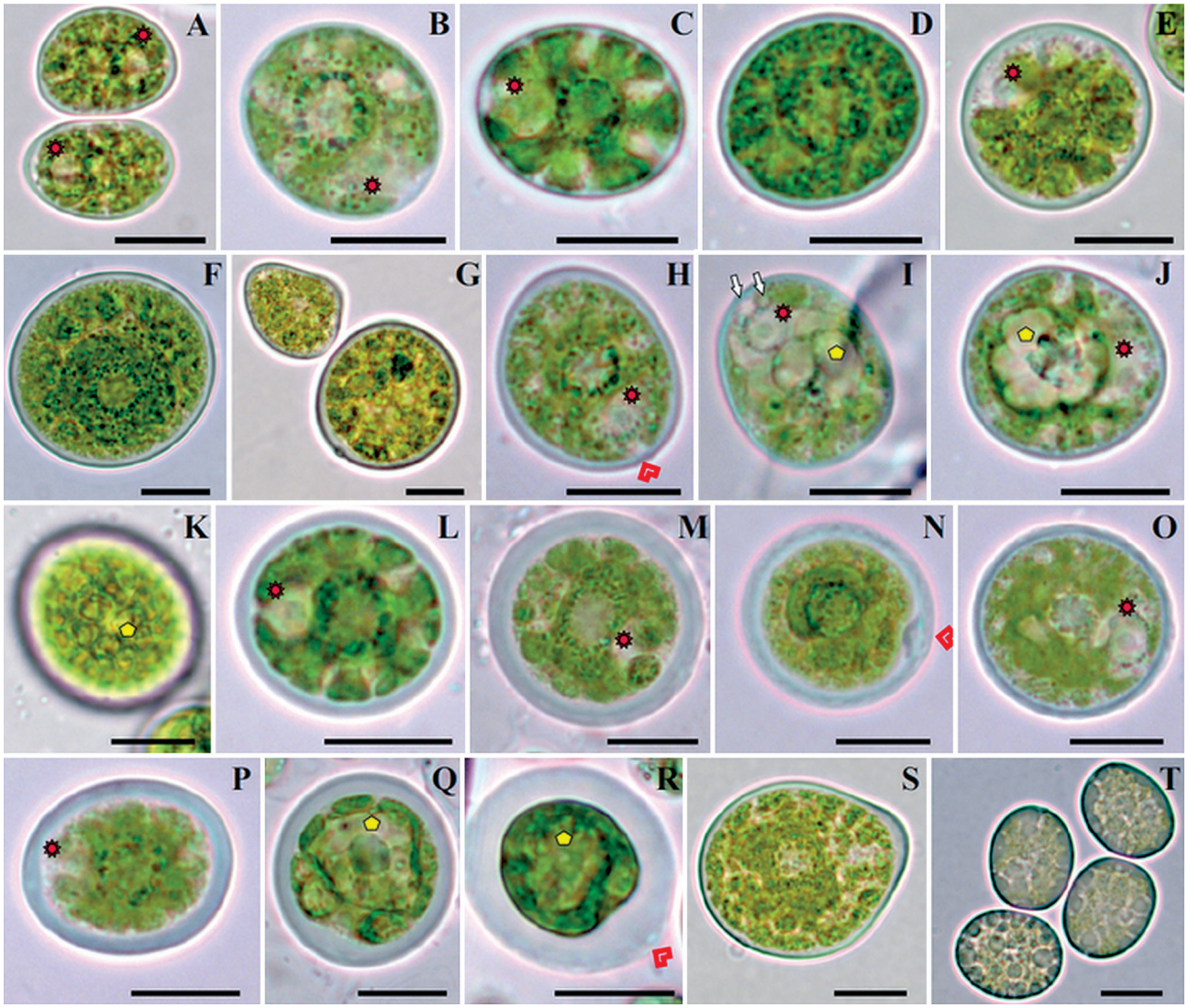

Figure 2 The morphology of the solitary immotile cells of IRK-A 167. A–C, E–J, S, T – the cells of different shape; A–S – shape of the chloroplast, and pyrenoid in the cells of different ages; L–S – the cells with a thickened cell wall; T – old cells with lipid droplets. Red arrowheads point papilla or thickening cell wall in place of the papilla, white arrows – contractile vacuoles, red asterisk – nucleus, yellow polygon – vacuoles in cytoplasm. Scale bars: 10 μm

In agar cultures, cells form colonies without mucilaginous. The solitary cells of IRK–A 167, monad cells after stopping movement and loss of flagella or immotile hemimonad cells, can acquire a spherical, subspherical or ellipsoidal shape over time, up to 25–26 μm in diameter. They are surrounded by a thick cell wall, up to 6 μm in thickness. The thickened wall can be concentrically stratified. Sometimes there is a pronounced thickening of the cell wall at the site of the papilla or in other sites of the cell (Fig. 2). The chloroplast in such cells is asteroid like in *Actinochloris* or *Radiosphaera*, sometimes like in *Chlorococcum*. As a rule, the nucleus is clearly visible. There are especially many such cells in old agar cultures growing at low temperatures (less of 20ºC). These cells are able to divide to form daughter cells, which can remain in the sporangium and be surrounded by a thickened cell wall. The cells of a spherical, subspherical, sub- or ellipsoidal shape, often with a thickened wall, homogeneous pale orange, orange, yellow- or green-orange contents with many drops oil or not can be attributed to akinets (Fig. 2T). Two-month-old or older cultures on agar slants remain green or become yellow-orange.

TEM description of studied strain

The vegetative cells of IRK–A 167 are covered by three-layered cell wall (Fig. 5A–F). The outer layer is composed of elongated outward electron dense formations (Fig. 5A–F). At the base, such structures have a different shape, but they have the regular orientation (Fig. 5C). The middle layer is hardly visible, sometimes in the form of repeating crystalline structures, less often lamellar (Fig. 5B). The inner one is formed by densely packed fibrils with parallel orientation to the cell surface, and it is tightly attached to the middle layer. Near to the plasmalemma, the fibrils of the inner layer are loose. At the apical end the inner layer is thicker, forming papilla (Fig. 5E).

The plasmalemma is wavy. The chloroplast is surrounded by a double membrane and contains few small starch grains and multiple bands of long thylakoids packed to 2–3 units. Some of thylakoids form short bands of 5–7 units. Thylakoid bands are usually strait or slightly curved (Fig. 5A, D, E). Pyrenoid is embedded in the chloroplast body. It is delineated from the rest of chloroplast by the envelope formed by 8 rather small starch grains, which lay

apart from each other (Fig. 5E). The pyrenoid stroma is darker than the stroma of chloroplast. It is dissected by single thylakoid bands entering from chloroplast. The reduction in the number of thylakoids to one is found quite deep in the pyrenoid stroma (Fig. 5H). The thylakoid shape in bands stays flattened when entering the pyrenoid stroma. The cytoplasm contains nucleus (not shown), some small profiles of mitochondria located more or less centrally (Fig. 5E, F), Golgi body (Fig. 5H) and vacuoles (Fig. 5F). During cell aging, large light heterogeneous structures are found (Fig. 5G)

Molecular analyses

The phylogenetic analyses presented in the Figs 6 and 7 showed that IRK-A 167 was placed within the Chloromonadinia phylogroup (sensu Nakada et al. 2008), clade 3 (by Hoham et al. 2002). The phylogeny based on 18S rDNA sequences allowed us to include greater number of *Chloromonas* sequences in comparison with that based on the *rbc*L gene.

On the 18S-based phylogenetic tree the clustering of the studied strain with the closest relatives *C. actinochloris* SAG 1.72 (autenthic strain) and UTEX 578 (previously registered as *C. mutabilis*) was supported with high probability and bootstrap values (≥0.99/99) (Fig. 6). In addition, strains IRK-A 167 and UTEX 578 clustered together on the tree based on the concatenated alignment of 18S and *rbc*L while there is no *rbc*L sequence of SAG 1.72 in GenBank (Fig. 6).

Comparison of IRK-A 167 nuclear marker (18S rDNA) with that of SAG 1.72 and UTEX 578 revealed their high nucleotide similarity, 99.4 % and 99.2 %, respectively. Two other species of the clade 3 *C. asteroidea* SAG 11.47 and *C. radiata* UTEX 966 showed nucleotide similarity 98.3 % and 98.2 %, respectively. Analysis of *rbc*L gene sequence revealed, that IRK-A 167 differed by 0.6 % nucleotides from *Chloromonas actinochloris* UTEX 578, by 3.1 % nucleotides from *C. asteroidea* SAG 11.47, and by 4 % from *C. radiata* UTEX 966.

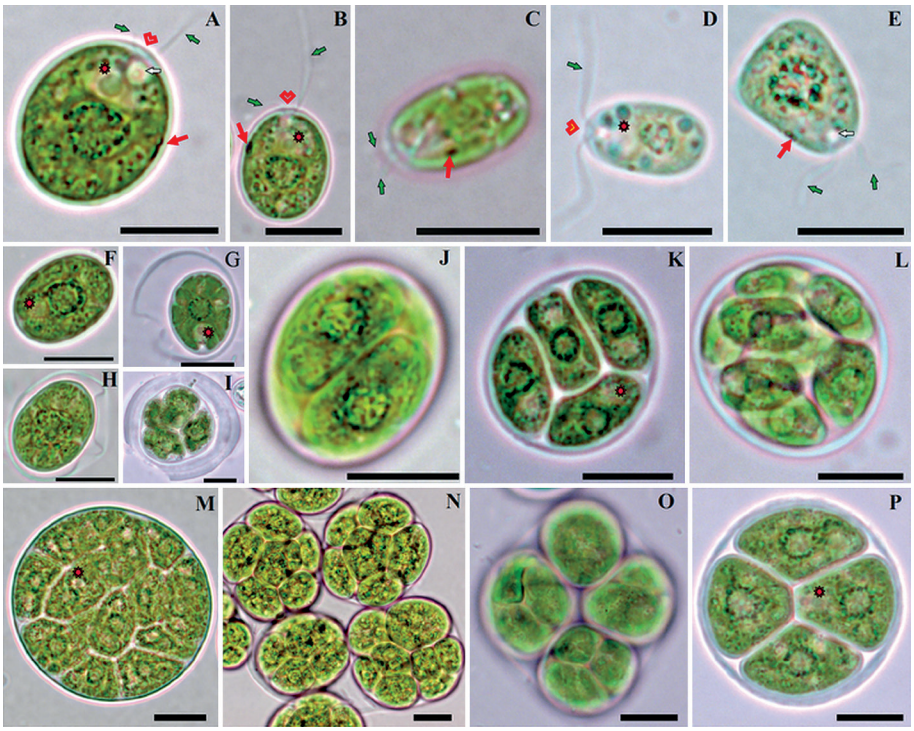

Figure 3 The monadic cells and sporangia of IRK-A 167. A–E – monad cells (including zoospores); F – hemimonad cell; G–P – cells in the sporangia; G, H – the rest of mature cell wall with single spore; I, P – sporangia with thickened cell wall; J–M – four, eight, sixteen spores in the sporangia; N, O – complex sporangia. Red arrowheads point papilla, red arrows – stigma, green arrows – flagella, white arrows – contractile vacuoles, red asterisk – nucleus. Scale bars: 10 μm

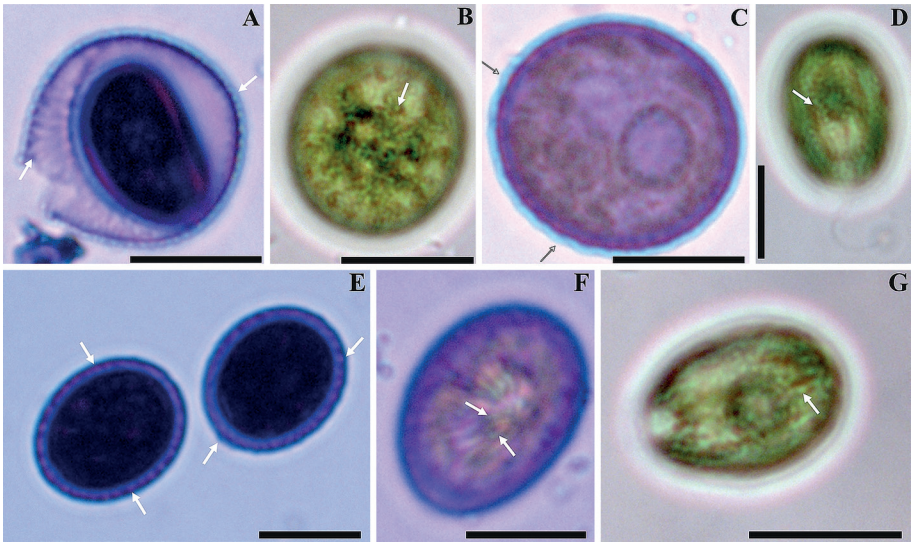

Figure 4 The cell wall of the sporangia (A) and vegetative cells (B–G) of IRK-A 167. Cells stained with gentian violet (A, C, E, F). The arrows show the structures of the cell walls. Scale bars: 10 μm

DISCUSSION

A large number of pyrenoid-bearing biflagellate green microalgae with flagella of equal length and cell wall are being considered as representatives of the genus *Chlamydomonas* Ehrenberg. This genus includes up to several hundred species, many of which were described by light microscopy (Ettl 1976, 1983, Guiry & Guiry 2022). Based on the molecular data, it was demonstrated that the traditional genus *Chlamydomonas* is polyphyletic and *Chlamydomonas*-like algae are members of several phylogroup within Volvocales

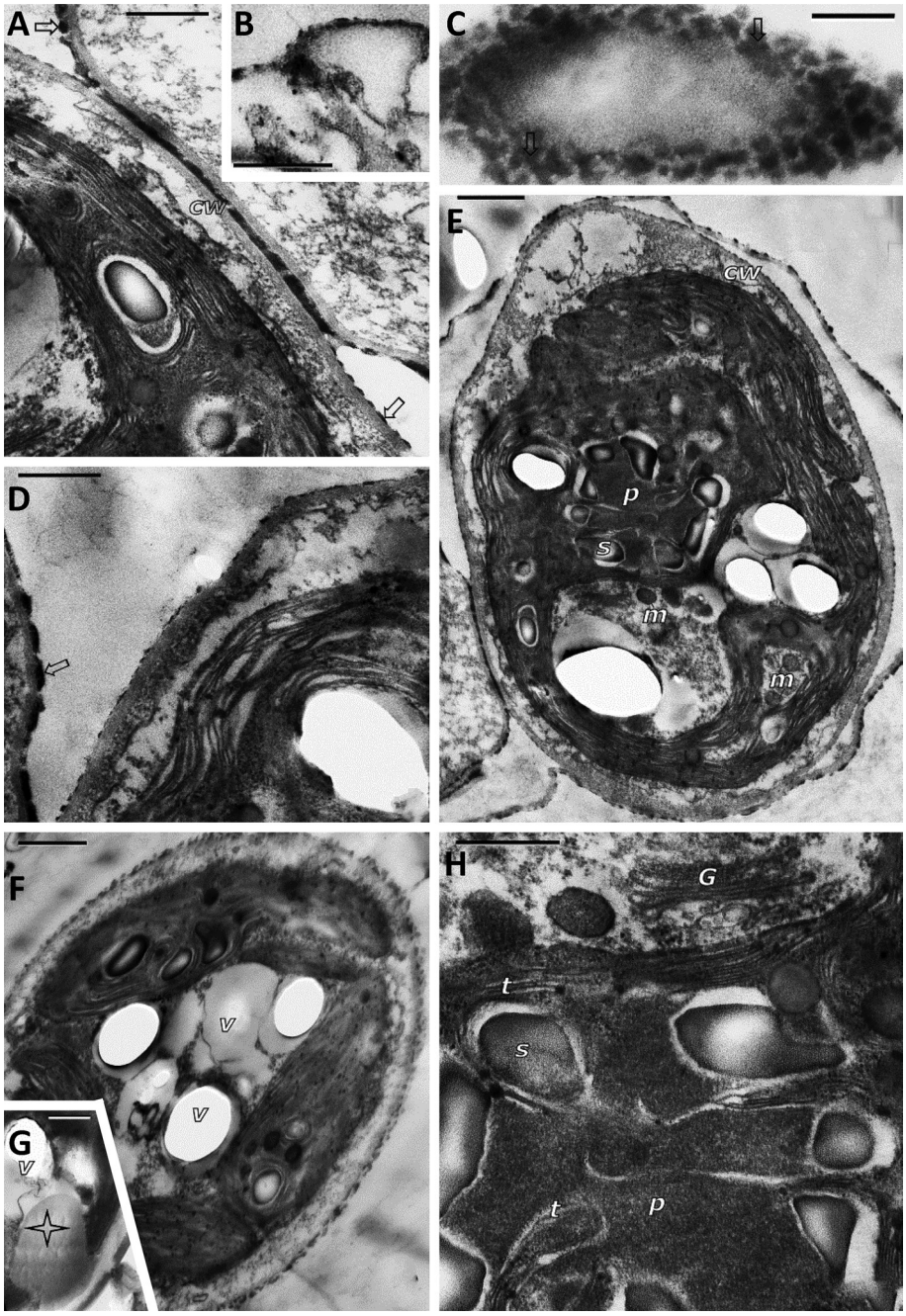

Figure 5 Transmission electron micrographs of studied strain IRK-A 167. A–H – ultrastructure of the IRK-A 167 cells. CW – cell wall, p – pyrenoid body, t – thylakoids, s – starch grains, v – vacuole, *G* – Golgi body, m – mitochondria. Arrows indicate outer layer elements, asterisk – heterogeneous structure. Scale bars: 1–4, 6, 7 – 0.5 μm; 5, 8 – 1.0 μm

(Buchheim et al. 1990, 1996, Pröschold et al. 2001, Pröschold & Leliaert 2007, Nakada et al. 2008, 2016, Procházková et al. 2019a, Gálvez et al. 2021). One of them is the phylogroup of Chloromonadinia (Nakada et al. 2008) that initially unites species of the genus *Chloromonas* Gobi. This genus was described by light microscopy just like *Chlamydomonas*, but without pyrenoid in its chloroplast. It consisted of more than 100 species (Ettl 1970, 1983). Later it was established by the molecular data that the traditional genus *Chloromonas* is also polyphyletic (Buchheim et al. 1997). *Chloromonas* was considered as a monophyletic clade containing the type species of genus, *Chloromonas reticulata* (Goroschankin) Gobi. It corresponds to the *Chloromonas reticulata* clade according to Pröschold et al. (2001) or the *Chloromonas* lineage defined by Buchheim et al. (1997), or *Chloromonadinia* by Nakada et al. (2008; Fig. 6). All species within this clade were classified as *Chloromonas*, and they are both pyrenoid-bearing and -lacking algae, including originally described *Chlamydomonas* (Pröschold et al. 2001). Now, as a result of numerous integrative studies, it is shown that the phylogroup *Chloromonadinia* comprises morphologically, physiologically, genetically diverse organisms. Along with representatives of *Chloromonas*, it also includes other genera: *Gloeomonas* G.A. Klebs (Nozaki et al. 2010), *Chlainomonas* Christen (Novis et al. 2008), *Ixipapillifera* Nakada (Nakada et al. 2016), *Ostravamonas* Barcyté et Hodač (Barcyté et al. 2020). In addition, phylogenetic studies focused on Chloromonadinia representatives revealed the existence of three main Chloromonadinia subclades (1, 2 and 3), which are not always statistically supported (Barcyté et al. 2018b, 2020). The sister relationships of the subclades and some smaller clades are not robust, and may vary in different analyses. The same results also obtained in our study (Figs 6, 7). Nevertheless, the question is raised that a number of species currently considered as *Chloromonas* in Chloromonadinia should be assigned to other new genera (Matsuzaki et al. 2012, Barcyté et al. 2018b, 2020).

Now, the Chloromonadinia contains only a few sequences of aero-terrestrial strains of different genera, and taxa with terrestrial lifestyle are understudied (Barcyté et al. 2020). In this work, we studied terrestrial strain IRK-A 167 by light, electron microscopy and molecular phylogeny methods. Based on light microscopy, this strain was previously published as cf. *Chlamydomonas* (Egorova et al. 2019). Our integrative research has shown that IRK-A 167 is a member of clade 3 of the Chloromonadinia and belongs to the species of the genus *Chloromonas*: *C. actinochloris* (Deason et H.C. Bold) Pröschold et al. *Chloromonas actinochloris* was originally described by Deason et Bold (1960) as pyrenoid-bearing flagellate *Chlamydomonas actinochloris*. This alga was

Table 1. Morphological characteristics of *Chloromonas actinochloris* and *Chlamydomonas mutabilis*.

Morphological and ecological features	Taxon		
	***Chloromonas actinochloris* strains**		***Chlamydomonas mutabilis*[c, e]**
	SAG 1.72 (=UTEX 965)	**IRK-A 167[a]**	
Cells shape	globose, subglobose[b], ellipsoidal, ellipsoid-ovoid, ellipsoid-cylindrical[c,d]	globose, subglobose, ellipsoidal, ellipsoid-cylindrical, ovoid, pyriform, wrong-polygonal	ellipsoidal, elongated-ellipsoidal, ellipsoid-cylindrical
Flagella	two, equal to or slightly shorter or longer than the length of the cell[b]	two, equal to or slightly shorter than the length of the cell[3]	two, approximately equal to the length of the cell
Cell wall	thin, without mucus, in old cultures it is thickened with uni- and bipolar shape[b]	thin or thick, without mucus, ornamented, it is thickened up to 6 μm, with or without polar fashion	moderately thick
Chloroplast	asteroid[b,c,d]	central, asteroid	parietal, cup-shaped, of varied form
Papilla	absent[b], present, low, wide, blunt[d]	present, wide, blunt conical	present, blunt cone-shaped up to low, semi-round
Stigma	anterior[b], in the first third of the cell, small[c,d]	anterior, in the first half of the cell, small, elongated-ellipsoid, ellipsoidal, rod-shaped, dark red or orange	in the first third or half of the cell, narrow elliptical
Pyrenoid	large, spherical, axial, central[b,c,d]	large, spherical, axial, central or slightly eccentric, surrounded by several or numerous starch grains	large, spherical, central or slightly eccentric
Nucleus	above pyrenoid	above pyrenoid	above pyrenoid
Vacuoles	two, contractile, below papilla[b,c]	two, contractile, below papilla; also numerous in cytoplasm of the individual cells	two, contractile, below papilla
Length×Width (μm)	10–20×4–9[b,d]	10–20×5–15.4	17–25×7.5–15[c,e]
Reproduction, a number of zoospores	asexual, up to 8[b], 4–8[c]	asexual, 2–16	asexual, 2–4
Akinete	unknown	known	known
Gloeocysts	unknown	unknown	known
Habitat	soils, including under the oak trees (Texas, USA)[b,c,d], in a slimy mass of algae in a small pond (Denmark)[c]	steppe soil (Russain's Altai highlands)	sample of algae from iron irrigation gutters in Dahlem (Germany)[c]

Notes: a – data obtained in frame of this work; b – data taken from Deason & Bold (1960); c – by Ettl (1976, 1983); d – by Ettl & Gärtner (2014), e – by Gerloff (1940).

isolated from soils of North America (USA), and deposited in culture collections UTEX (USA), SAG (Germany), NIES (Japan) and other under number UTEX 965/SAG 1.72/NIES 2201, respectively. Based on the 18S phylogeny Pröschold et al. (2001) classified the strain SAG 1.72 as a member of the genus *Chloromonas* (see above) and designated this species as *Chloromonas actinochloris*. Additionally, the strain SAG 34.72/ UTEX 578/NIES 2224 registered by U.G. Schlösser as *Chlamydomonas mutabilis* Gerloff was attributed to this species (Pröschold et al. 2001). The origin of the strain SAG 34.72 is not known (EPSAG, 2022). The results obtained in this study are consistent with the data of Pröschold et al. (2001). The 18S nucleotide sequences of SAG 1.72 (=UTEX 965/ NIES 2201) and SAG 34.72 (=UTEX 578/ NIES 2224) are combined together into one clade. *Chlamydomonas mutabilis* was described by Gerloff (1940) based on light microscopy observations. This species was found in a sample of algae (mainly *Haematococcus pluvialis*) from iron irrigation gutters of buildings at the biological department of the Botanical Garden in Dahlem (Germany). The authentic strain of this species is not represented in culture collections, and no of its sequences available in Genbank. The morphological features both of *Chlamydomonas mutabilis* and *Chloromonas actinochloris* are given for comparison in Table 1.

Unlike *Chloromonas actinochloris*, *Chlamydomonas mutabilis* has variable cup-shaped chloroplast, narrow-elliptical stigma, and gloeocysts (Table 1). At present time, it is not possible to conclude that *Chloromonas actinochloris* and *Chlamydomonas mutabilis* Gerloff are the same species.

Boldina had previously studied the ultrastructure of the strains SAG 1.72 and SAG 34.72 (Konstantinova & Boldina 2003, Boldina 2008). Although the strain SAG 34.72 is still less studied. Data obtained by TEM indicate that ultrastructure of the strains SAG 1.72, SAG 34.72 and IRK–A 167 are similar. All mentioned strains have the same pyrenoid type and cell wall structure. According by TEM, the IRK-A 167 ultrastructure of cellular components is more similar to that of SAG 1.72 than to SAG 34.72. Perhaps this is a phenomenon of intraspecific variability.

The data we obtained supplemented the information about the biology and biogeography of *Chloromonas actinochloris*. The strain IRK-A 167 isolated from steppe soil of highland have both common morphological traits with previously reported for this species (Deason & Bold 1960, Ettl 1976, 1983, Ettl & Gärtner 2014). The features of life cycle of IRK–A 167 are similar to .those of *Chloromonas* (*Chlamydomonas*) *augustae* (Skuja) Pröschold et al. (Hoffmann et al. 2007). As a result of our study, the traits of *Chloromonas*

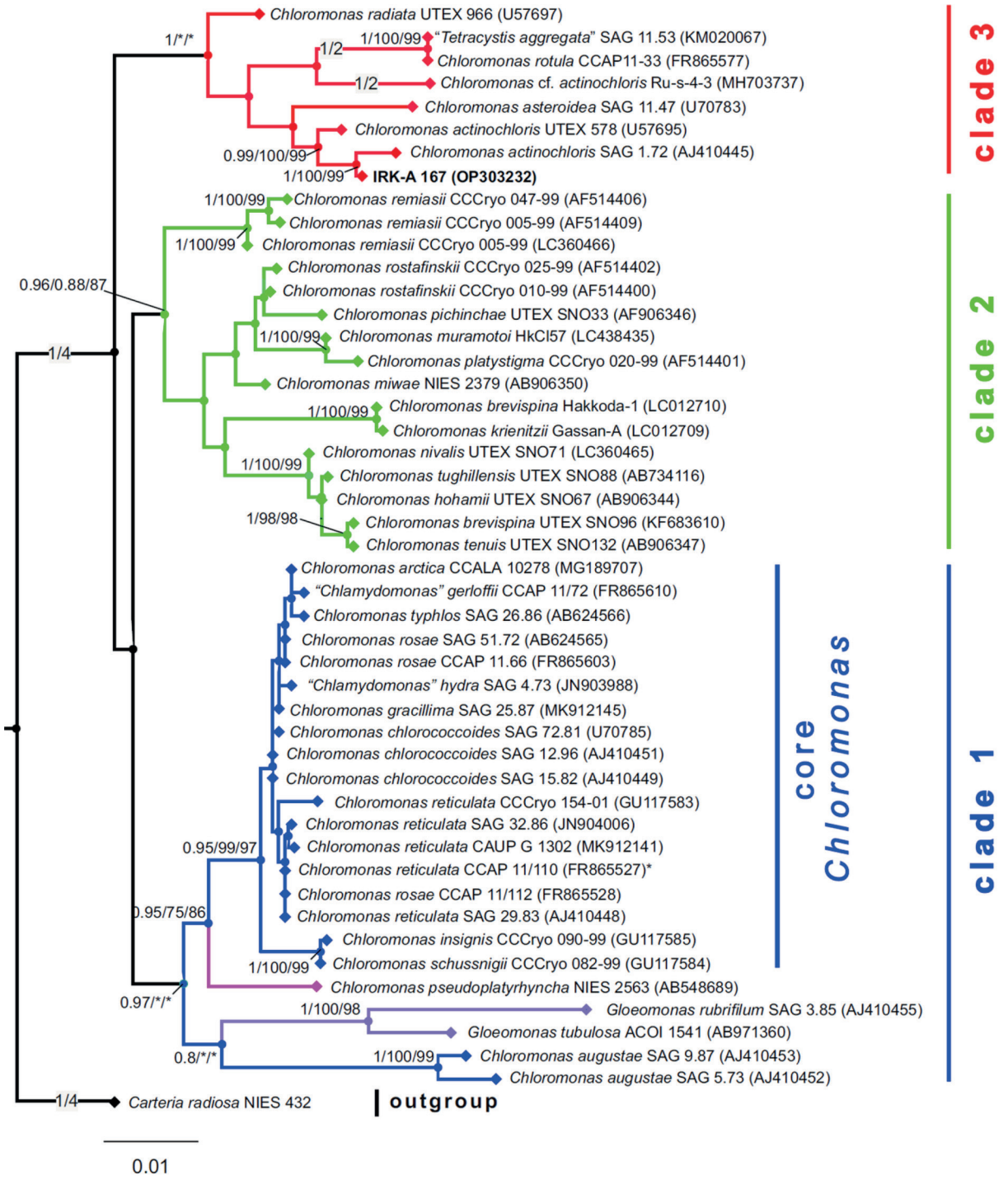

Figure 6 The ML phylogenetic tree of Chloromonadinia and relationship of the studied strain IRK-A 167 were reconstructed based on the 18S rDNA data. Bayesian posterior probability, Maximum Likelihood and Neighbor Joining bootstrap supports are shown at the nodes (PP/ML/NJ). Values less than 0.75/75 % are omitted. The studied strain is in bold. The 18S rDNA sequence accession numbers are given in parentheses. The authentic strain *Chloromonas reticulata*, the type species of the genus *Chloromonas*, is marked with an asterisk. The clades 1, 2 and 3 are delimited according to Hoham et al. (2002)/Barcyté et al. (2020)

actinochloris have been significantly supplemented: the stigma and the cell wall are characterized, together with features of life cycle, and a description of the ultrastructure of the species. We have established that the vegetative cells of the Chloromonadinia clade representatives may have ornamented cell walls. Among chlamydomonad-like algae, ornamented cell walls are known mainly for resting cells (cysts/zygotes). It is known that closely related species may have resting cells with different wall ornaments. The ornaments of the cell wall may be considered a diagnostic trait (Matsuzaki et al. 2019). The walls of vegetative cells of chlamydomonads are generally smooth (Ettl 1983). There is little information that the shells of a number of representatives are not smooth. For example, Boldina found by TEM that *Chlamydomonas parallelistriata* Korshikov (the strain SAG 2.73) has cell wall with spikes (Konstantinova & Boldina 2003). The vegetative cells of *Microglena antarctica* Trentin et al. have cell walls with non-regular big bulges (Trentin et al. 2022). Probably, such a trait

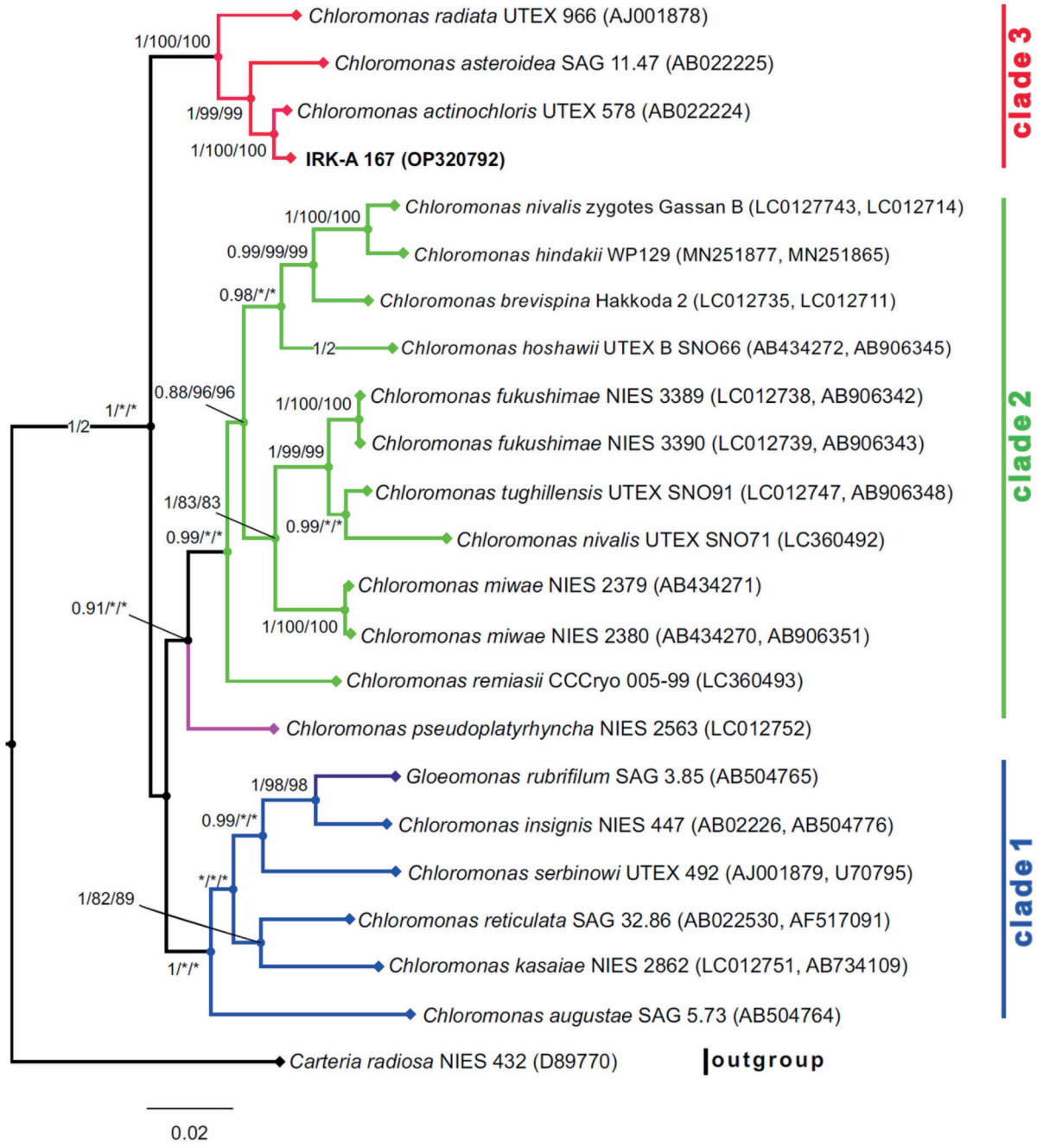

Figure 7 The Bayesian phylogenetic reconstruction and position of the studied strain IRK-A 167 within clade 3 of the Chloromonadinia lineage are based on the nucleotide alignment of the 18S *r*DNA and rbcL concatenated sequences. Bayesian posterior probability, Maximum Likelihood and Neighbor Joining bootstrap supports are shown at the nodes (PP/ML/NJ). Values less than 0.75/75 % are omitted. The studied strain is marked in bold font. The *rbc*L and 18S rDNA sequence accession numbers are given in parentheses. The 18S *r*DNA sequence numbers are partially indicated in Fig. 6. The clades 1, 2 and 3 are highlighted according to Hoham et al. (2002)/Barcyté et al. (2020)

as ornamented walls of vegetative cells is more common among *Chlamydomonas*-like algae than it is commonly thought.

Chloromonas actinochloris IRK-A 167 did not form monad cells quickly and in large numbers, which is characteristic of some others chlamydomonad algae. In the conditions of a prolonged absence of drip-liquid water, it reproduces only by immotile non-monadic cells, which may be the predominant type of cells during the life cycle. Probably, this is an adaptation to adverse environmental conditions. Deason & Bold (1960) found that the strains of *Chloromonas actinochloris* they studied can grow mixotrophically (on media with glucose, maltose, xylose, arabinose, acetate in light) and possibly heterotrophically (trace in glucose in darkness). We have not studied the growth of the strain IRK-A 167 on media with the addition of sugars or acetate. IRK-A 167 grew equally well on media containing vitamins or without them. It is able to grow on nutrient media with different concentrations of salts. The research results show that *Chloromonas actinochloris* can inhabit a wide range of environmental conditions. Further studies of chlamydomonad algae from terrestrial habitats will allow us to better understand the features of their adaptation.

Chloromonas actinochloris was isolated from soils at two sites of Texas (USA), Caldwell County and Williamson

County (Deason & Bold 1960). Ettl (1983) reported that this species was found also in the pond of Danmark (as *Chlamydomonas actinochloris*). Ettl & Gärtner (2014) mentioned only locations in Texas. There are known records of this morphospecies on the territory of Ukraine (Kostikov et al. 2001, Darienko 2012), Brazil (Menezes 2010), Italy (Cărăuș 2012), and China (Hu et al. 2015 cit. by Guiry & Guiry 2022) (Fig. 1). In Russia, morphospecies of *Chloromonas actinochloris* (*Chlamydomonas actinochloris*) was detected in soil of spruce forest in vicinity of Syktyvkar (Patova & Novakovskaya 2018). Pivovarova (1974) studied soil algae of the steppe in the vicinity of Kosh-Agach (the Republic of Altai), and found one chlorophycean monad alga *Chlamydomonas* sp. There is no description of this representative. Currently, in the GenBank there are sequences of *Chloromonas actinochloris* from Texas and from unknown habitat (SAG 34.72), complemented by sequences of IRK-A 167 obtained in this work. In framework of a project to study the global distribution of fungi in terrestrial ecosystems, fragments of nucleotide sequences (18S-V9, ITS?) attributed to cf. *Chloromonas actinochloris* were isolated from soils of Estonia (Tedersoo et al. 2021, GBIF 2022). Unfortunately, these data are not presented in the GenBank. Moreover, the nucleotide sequence 18S-V9 is too short to include it in our research, and there are no sequences of ITS region for the strains SAG 1. 72 and 34.72 in the GenBank (NCBI 2022). The question of the distribution and frequency of *Chloromonas actinochloris* records remains open. Mikhailyuk et al. (2019) reported finding *Chloromonas* cf. *actinochloris* Ru-s-4-3 in biological soil crusts from sand dunes on the Baltic Sea coast (Germany). The 18S nucleotide sequence of this strain was not clustered with authentic strain SAG 1.72. It formed a separate lineage within the clade 3 of the Chloromonadinia phylogroup (Mikhailyuk et al. 2019), which is consistent with our data (Fig. 6). It is probably a new species. The finding of a morphologically similar alga with *Chloromonas actinochloris*, but genetically different from this species, indicates a higher diversity of algae with such morphology.

Earlier studies have shown that the representatives of the clade 3 of Chloromonadinia phylogroup should be allocated to an independent genus (Barcyté et al. 2018b, 2020). As a result, *Chloromonas actinochloris* should be considered as a member of a new genus. But, taxonomic revision of Chloromonadinia is only expected.

Based on the obtained data, as well as taking into account the previous studies, we propose to expand the description of the species.

Chloromonas actinochloris (Deason & H.C. Bold) Pröschold, Marin, U.G. Schlösser & Melkonian. 2001, Protist 152: 265–300 emend. I.N. Egorova, Kulakova, O.N. Boldina (≡ *Chlamydomonas actinochloris* Deason et H.C. Bold, 1960, Phycological Studies I, p. 13, fig. 1–3 (descr. et ic. prima, iconotypus; epitype is the cryopreserved strain UTEX 965)

Amended description: The vegetative cells are ellipsoidal, globose, subglobose, ellipsoid-cylindrical, ovoid, rarely pyriform or wrong-polygonal, 10–20 μm in length, 4–15.4 μm in width. The equal flagella approximate cell length or slightly exceed it. The cells usually lack flagella on agar, except in freshly transferred cultures. The cell wall is thin or thick, closely adpressed to the protoplast. It thickens to 6 μm with age. In old cells, it can be stratified, with one or more thickenings. The cell wall is ornamented, with bumps on the surface. The bumps are more noticeable in cells growing in liquid cultures, and during staining with gentian violet or under electron microscope. They are arranged in regular uneven rows. The papilla is low, often poorly visible, wide, blunt conical. The radiating chloroplast is a massive, with a single central, slightly eccentric, axial pyrenoid. The pyrenoid is surrounded by a starch grains, dissected by single thylakoid bands entering from chloroplast. The reduction in the number of thylakoids to one is found quite deep in the pyrenoid stroma. The stigma is anterior in the first third or half of the cell, small, elongated-ellipsoid, ellipsoidal, rod-shaped, dark red or orange. The nucleus is the clearly visible, anterior to the pyrenoid. There are two anterior contractile vacuoles.

The reproduction is asexual, accomplished by repeated endogenous bipartitions to form 2–16 daughter cells which are liberated by a rupturing of the cell wall. The daughter cells can linger in the sporangia and start dividing again. The complex sporangia are formed. The akinetes are globose, subglobose, ellipsoidal, subellipsoidal shape. They have a thickened layered cell wall, homogeneous pale orange, orange, yellow- or green-orange contents with many drops of oil or not. The two-month-old or older cultures may turn yellow-orange.

ACKNOWLEDGEMENTS

The work was supported by the Ministry of Science and Higher Education of the Russian Federation, the grant 075-15-2020-787 for implementation of Major scientific projects on priority areas of scientific and technological development (the project «Fundamentals, methods and technologies for digital monitoring and forecasting of the environmental situation on the Baikal natural territory»). The authors are grateful to T.I. Morozova and to the staff of the Sailyugem National Park (the Republic of Altai, Russian Federation) for their help in expedition research.

LITERATURE CITED

Aleksakhina, T.A. & E.A. Shtina 1984. *Soil algae of forests biogeocenoses*. Nauka, Moscow, 130 pp. (in Russian). [Алексахина Т.А., Штина Э.А. 1984. Почвенные водоросли лесных биогеоценозов. Москва: Наука, 130 с.].

Andreeva, V.M. 1998. *Terrestrial and aerophilic green algae (Chlorophyta: Tetrasporales, Chlorococcales, Chlorosarcinales)*. Nauka, Saint Petersburg, 351 pp. (in Russian). [Андреева В.М. 1998. Почвенные и аэрофильные зеленые водоросли (Chlorophyta: Tetrasporales, Chlorococcales, Chlorosarcinales). Санкт-Петербург: Наука, 351 с.].

Artamonova, V.S. 1982. Soil algae of aspen-fir forest of scientific biological station "Kotorovo". In: *Microbiological processes in the soils of Western Siberia* (I.L. Klevenskaya, ed.), pp. 161–175, Nauka, Novosibirsk (in Russian). [Артамонова В.С. 1982. Почвенные водоросли осиново-пихтового леса стационара «Которово» // Микробиологические процессы в почвах Западной Сибири / отв. ред. И.Л. Клевенская. Новосибирск: Наука, 1982. С. 161–175].

Barcyté, D., M. Pusztai, P. Škaloud & M. Eliáš 2022. When you like other Algae: *Adglutina synurophila* gen. et sp. nov. (*Moewusinia*, Chlorophyceae), a clingy green microalga associated with *Synura* colonies. *Protist* 173:125858.

Barcyté, D., L. Hodač & L. Nedbalová 2020. Overlooked diversity with terrestrial lifestyle in the predominantly fresh-

water and snow phylogroup *Chloromonadinia* (Volvocales, Chlorophyceae). *European Journal of Phycology* 55:207–222.

Barcytė, D., L. Hodač, L. Nedbalová & J. Elster 2018a. *Chloromonas arctica* sp. nov., a psychrotolerant alga from snow in the High Arctic (Chlamydomonadales, Chlorophyta). *International Journal of Systematic and Evolutionary Microbiology* 68: 851–859.

Barcyté, D., L. Hodač, L. Nedbalová & J. Elster 2018b. *Chloromonas svalbardensis* n. sp. with insights into the phylogroup *Chloromonadinia* (Chlorophyceae). *Journal of Eukaryotic Microbiology* 65:882–892.

Boldina, O.N. 2008. Morphological types of pyrenoid organizationin green monads. *Vestnik Tverskogo gosudarstvennogo universiteta, seria "Biologia i ecologia"* 9:21–26 (in Russian). [Болдина О.Н. 2008. Морфологические типы организации пиреноидов у зеленых монад // Вестник ТвГУ, серия «Биология и экология». Вып. 9. С. 21–26].

Boldina, O.N. 2016. Chlamydomonads (Chlamydomonadaceae) from St. Petersburg and the Leningrad Region: current knowledge and new records. *Novosti Sistematiki Nizshikh Rastenii* 50:5–22 (in Russian). [Болдина О.Н. 2016. Хламидомонады (Chlamydomonadaceae) Санкт-Петербурга и Ленинградской области: состояние изученности и новые находки // Новости систематики низших растений Т. 50. С. 5–22].

Boldina, O.N. & A.S. Chunaev 2020. Symbiotic chlamydomonads in algological department collection of BIN RAS. *Voprosy sovremennoi algologii* 2(23):79–84 (in Russian). [Болдина О.Н., Чунаев А.С. 2020. Симбиотические хламидомонады в коллекции лаборатории альгологии БИН РАН // Вопросы современной альгологии. № 2 (23). С. 79–84].

Broady, P.A. 1979. A preliminary survey of the terrestrial algae of the Antarctic Peninsula and South Georgia. *British Antarctic Survey Bulletin* 48:47–70.

Buchheim, M.A., M. Turmel, E.A. Zimmer & R.L. Chapman 1990. Phylogeny of *Chlamydomonas* (Chlorophyta) based on cladistic analysis of nuclear 18S rRNA sequence data. *Journal of Phycology* 26:689–699.

Buchheim, M.A., C. Lemieux, C. Otis, R.R. Gutell, R.L. Chapman & M. Turmel 1996. Phylogeny of the Chlamydomonadales (Chlorophyceae): a comparison of ribosomal RNA gene sequences from the nucleus and the chloroplast. *Molecular Phylogenetics and Evolution* 5:391–402.

Buchheim, M.A., J.A. Buchheim & R.L. Chapman 1997. Phylogeny of Chloromonas (Chlorophyceae): a study of 18S ribosomal RNA gene sequences. *Journal of Phycology* 33:286–293.

Cărăuș, I. 2012. Algae of Romania. A distributional checklist of actual algae. Version 2.3 – third revision. *Studii și cercetări de Biologie* 7:1–809.

Chatta, E.N. & O.G. Lopatovskaya 2001. Soil algoflora of the mineral spring in valley of Rivulet Chelolek (Vitim nature reserve). In: *Modern problems of Baikalogia. Proceeding of young scientists,* pp. 86–94, Izdatel'stvo Irkutskogo gosudarstvennogo universiteta, Irkutsk (in Russian). [Чатта Е.Н., Лопатовская О.Г. 2001. Почвенная альгофлора минерального источника долины ручья Челолек (Витимский заповедник) // Современные проблемы Байкаловедения. Сборник трудов молодых ученых. Иркутск: Из-дво Иркут. гос. ун-та. С. 86–94].

Darienko, T.M. 2012. Terrestrial algae of Zmiinyj island (Black Sea, Ukraine): general characteristic and peculiarities of species composition. *Ukrainian Botany Journal* 69(1):110–124.

Darriba, D., G.L. Taboada, R. Doallo & D. Posada 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8):72.

Deason, T.R. & H.C. Bold 1960. Phycological studies. I. Exploratory studies of Texas soil algae. *The University of Texas publication* 6022:1–72.

Demchenko, E., T. Mikhailyuk, A.W. Coleman & T. Pröschold 2012. Generic and species concepts in *Microglena* (previously the *Chlamydomonas monadina* group) revised using an integrative approach. *European Journal of Phycology* 47:264–290.

Doyle, J.J. & J.L. Doyle 1990. Isolation of Plant DNA from Fresh Tissue. *Focus* 12(1):13–15.

Eddie, B., C. Krembs & S. Neuer 2008. Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. *Marine Ecology Progress Series* 354: 107–117.

Egorova, I.N. & E.A. Sudakova 2005. Epiphytic algae of the south Baikal region. *Novosti Sistematiki Nizshikh Rastenii* 38:47–57 (in Russian). [Егорова И.Н., Судакова Е.А. 2005. Эпифитные водоросли Южного Предбайкалья // Новости систематики низших растений. Т. 38. С. 47–57].

Egorova, I.N. 2012. The species diversity of algae in association with *Rhytidium rugosum* (Bryophyta) in Sokhondo reserve (Zabaikalsky territory). *Botanicheskii Zhurnal* 97(8): 1051–1061 (in Russian with English summary). [Егорова И.Н. 2012. Видовой состав водорослей в ассоциациях с *Rhytidium rugosum* (Bryophyta) в Сохондинском заповеднике (Забайкальский край) // Ботанический журнал. Т. 97, № 8. С. 1051–1061].

Egorova, I.N. 2016. About of epilithic algae some mountain areas of Russia and Mongolia. *Izvestiya Samarskogo nauchnogo centra RAN* 5(2):257–263 (in Russian). [Егорова И.Н. 2016. О водорослях скально-каменистых субстратов некоторых горных районов России и Монголии // Известия Самарского научного центра РАН. Т. 5, № 2. С. 257–263].

Egorova, I.N., G.S. Shambuyeva & T.I. Morozova 2019. Additional information about terrestrial algae of the Altai Mountains (Republic of Altai, Russia). *Problemy botaniki Yuzhnoi Sibiri i Mongolii* 254–259 (in Russian with English abstract). [Егорова И.Н., Шамбуева Г.С., Морозова Т.И. 2019. Дополнительные сведения о наземных водорослях горного Алтая (Республика Алтай, Россия) // Проблемы ботаники Южной Сибири и Монголии. С. 254–259].

Egorova, I.N., E.A. Sudakova, E.N. Maksimova & G.S. Tupikova 2020a. Terrestrial Algae of the Mountains of South Siberia and North Mongolia. *Botanicheskii Zhurnal* 105(2): 107–132 (in Russian with English summary). [Егорова И.Н., Судакова Е.А., Максимова Е.Н., Тупикова Г.С. 2020. Наземные водоросли гор Южной Сибири и Северной Монголии // Ботанический журнал. Т. 105, № 2. С. 107–132].

Egorova, I.N., M.S. Konovalov, O.V. Shergina, N.V. Dudareva & G.S. Tupikova 2020b. Algae and epilitic mosses associations of the *Hedwigia* P. Beauv. genus in mountain taiga of the Khentey Uplands (Zabaykalskiy Krai, Russia). *Sibirskii Lesnoi Zhurnal* 6:64–80 (in Russian with English abstract and references). [Егорова И.Н., Коновалов М.С., Шергина О.В., Дударева Н.В., Тупикова Г.С. 2020. Ассоциации водорослей и мохообразных рода *Hedwigia* P. Beauv. в горной тайге Хэнтэя (Забайкальский край, Россия) // Сибирский лесной журнал. № 6. С. 64–80].

EPSAG. 2022. *Experimental Phycology and Culture Collection of Algae at the University of Goettingen*. Available from: http://www.uni-goettingen.de. Last accessed 10.08.2022.

Ettl, H. 1970. Die Gattung *Chloromonas* Gobi emend. Wille (*Chlamydomonas* und Die Nächstverwandten Gattungen I). *Nova Hedwigia, Beiheft* 34:1–283.

Ettl, H. 1976. Die Gattung *Chlamydomonas* Ehrenberg (*Chlamydomonas* und die nächstverwandten Gattungen II). *Nova Hedwigia, Beiheft* 49:1–1122.

Ettl, H. 1983. Chlorophyta I. *Phytomonadina*. In: *Süßwasserflora von Mitteleuropa* (H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer, eds), pp. 1–807, Gustav Fischer Verlag, Yena.

Faktorovich, L.V. 2001. Composition and structure of soil algae association of the wood mountain profile of Aryskanyg Shivilig-Chem in Uvs-Nuur Region of Tuva. *Sibirskii Ekologicheskii Zhurnal* 8(4):423–429 (in Russian). [Факторович Л.В. 2001. Состав и структура почвенных водорослей лесного пояса Арысканыг Шивилиг-Хемского кластера Убсунурской котловины Тувы // Сибирский экологический журнал. Т. 8, № 4. С. 423–429].

Gálvez, F.E., M. Saldarriaga-Córdoba, P. Huovinen, A.X. Silva & I. Gómez 2021. Revealing the characteristics of the Antarctic snow alga *Chlorominima collina* gen. et sp. nov. through taxonomy, physiology, and transcriptomics. *Frontiers in Plant Science* 12:662298.

GBIF Backbone Taxonomy. 2022. *Checklist dataset. SH1178808.09FU (*cf. *Chloromonas actinochloris) in GBIF Secretariat*. Available from: https://doi.org/10.15468/39omei. Last accessed 15.12.2022.

Gerloff, J. 1940. Beiträge zur Kenntnis der Variabilität und Systematik der Gattung *Chlamydomonas*. *Archiv für Protistenkunde* 94:311–502.

Gollerbakh, M.M. & E.A. Shtina 1969. *Soil algae*. Nauka, Leningrad, 228 pp. (in Russian). [Голлербах М.М., Штина Э.А. 1969. Почвенные водоросли. Ленинград: Наука, 228 с.].

Guiry, M.D. & G.M. Guiry 2018. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: https://www.algaebase.org. Last accessed 08.08.2022.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.

Hoffmann, L., L. Ector & I. Kostikov 2007. Algal flora from limed und unlimed forest soils in the Ardenne (Belgium). *Systematics and Geography of Plants* 77: 15–90.

Hoham, R. W. 1975. The life history and ecology of the snow alga *Chloromonas pichinchae* (Chlorophyta, Volvocales). *Phycologia* 14:213–26.

Hoham, R.W., T.A. Bonome, C.W. Martin & J.H. Leebens-Mack 2002. A combined 18S rDNA and rbcL phylogenetic analysis of *Chloromonas* and *Chlamydomonas* (Chlorophyceae, Volvocales) emphasizing snow and other cold-temperature habitats. *Journal of Phycology* 38: 1051–1064.

Hoham, R.W., J.D. Berman, H.S. Rogers, J.H. Felio, J.B. Ryba & P.R. Miller 2006. Two new species of green snow algae from Upstate New York, *Chloromonas chenangoensis* sp. nov. and *Chloromonas tughillensis* sp. nov. (Volvocales, Chlorophyceae) and the effects of light on their life cycle development. *Phycologia* 45:319–330.

Hu, H.J., M. Yang, L.M. Luo & S. Chen 2015. Chlorophyta, Chlorophyceae, Order Volvocales (II), Genus *Chlamydomonas*. In: *Flora algarum sinicarum aquae dulcis. Vol. 20*, pp. [i]-xvii, 1–174, 2 pls. Beijing: Science Press (in Chinese).

Katana, A., J. Kwiatowski, K. Spalik, B. Zakrys, E. Szalacha & H. Szymanska 2001. Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. *Journal of Phycology* 37:443–451.

Komarov, V.L. 1905. A trip to the Tunka region and to the lake of Kosogol in 1902. *Izvestiya Imperatorskogo Russkogo Geograficheskogo Obshchestva* 41(1):23–154 (in Russian). [Комаров В.Л. 1905. Поездка в Тункинский край и на оз. Косогол в 1902 г. // Известия Императорского Русского географического общества. 41(1):23–154].

Konstantinova, I.A. & O.N. Boldina 2003. Comparative analisis on the cell walls of monadoid and coccoid green algae. In: *Botanical explorations in Asia Russia. Materials of XI Congress of Russian Botanical Society*, p. 107, Izdatel'stvo AsBuka, Barnaul (in Russian). [Константинова И.А., Болдина О.Н. 2003. Сравнительный анализ клеточных оболочек монадных и коккоидных водорослей // Ботанические исследования в Азиатской России. Материалы XI сьезда Русского Ботанического общества. Барнаул: АзБука. С. 107].

Kostikov, I.Yu., P.O. Romanenko, E.M. Demchenko, T.M. Darienko, T.I. Mikhailyuk, O.I. Rybchinskii & A.M. Solonenko 2001. *Soil algae of Ukraine (history and research methods, system, conspectus of flora)*. Fitosotsiotsentr, Kiev, 300 pp. (in Ukrainian). [Костіков І.Ю., Романенко П.О., Демченко Е.М., Даріенко Т.М., Михайлюк Т.И., Рыбчинський О.И., Солоненко А.М. 2001. Водорості ґрунтів України (історія та методи дослідження, система, конспект флори). Київ: Фітосоціоцентр. 300 с.].

Lin, Ch.-S. & J.-T. Wu 2014. Environmental factors affecting the diversity and abundance of soil photomicrobes in arid lands of subtropical Taiwan. *Geomicrobiology Journal* 31:350–359.

Liu, C., X. Huang, X. Wang, X. Zhang & G. Li 2006. Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. *Phycologia* 45:190–198.

Levin, R.A., W.L. Wagner, P.C. Hoch, M. Nepokroeff, J.C. Pires, E.A. Zimmer & K.J. Sytsma 2003. Family-level relationships of Onagraceae based on chloroplast *rbc*L and *ndh*F data. *American Journal of Botany* 90:107–115.

Lopatovskaya, O.G. & E.N. Maksimova 2010. Ionic-salt and algological characteristic of the snow cover as one of elements of the estimation of the condition of the environment. *Problemy regional'noi ekologii* 3:35–39 (in Russian). [Лопатовская О.Г., Максимова Е.Н. 2010. Ионно-солевая и альгологическая характеристика снежного покрова как один из элементов оценки состояния окружающей среды // Проблемы региональной экологии. 3:35–39].

Lopatovskaya, O.G., E.N. Maksimova & E.R. Khadeeva 2017. Saline Soils and Diversity of Soil Algae of Ol'khon Island at Baikal Lake. *Izvestiya Irkutskogo gosudarstvennogo universiteta. Seriya "Biologiya. Ekologiya"* 20:73–88. (in Russian). [Лопатовская О.Г., Максимова Е.Н., Хадеева Е.Р. 2017. Засолённые почвы острова Ольхон и видовое разнообразие почвенных водорослей // Известия Иркутского гос. ун-та. Серия «Биология. Экология». Т 20. С. 73–88].

Matsuzaki, R., H. Nozaki, N. Takeuchi, Y. Hara & M. Kawachi 2019. Taxonomic reexamination of "*Chloromonas nivalis* (Volvocales, Chlorophyceae) zygotes" from Japan and description of *C. muramotoi* sp. nov. *PLoS One* 14:e0210986.

Matsuzaki, R., H. Nozaki & M. Kawachi 2018. Taxonomic revision of *Chloromonas nivalis* (Volvocales, Chlorophyceae) strains, with the new description of two snow-inhabiting *Chloromonas* species. *PLoS One* 13:e0193603.

Menezes, M. 2010. Chlorophyceae. In: *Catálogo de plantas e fungos do Brasil. Vol. 1* (R.C. Forzza, ed.), pp. 335–352, Rio de Janeiro: Andrea Jakobsson Estúdio; Instituto de Pesquisas Jardim Botânico do Rio de Janeiro.

Mikhailyuk, T., K. Gläser, P. Tsarenko, E. Demchenko & U. Karsten 2019. Composition of biological soil crusts from sand dunes of the Baltic Sea coast in the context of an integrative approach to the taxonomy of microalgae and cyanobacteria. *European Journal of Phycology* 54(3):263–290.

Middendorf, A.F. 1867. Travel to the North and East of Siberia. In: *Rastitelnost' Sibiri*, pp. 69 (XLIX)–54 (LIV) Saint-Peterburg. Chast. 1. Otdelenie. 4. (in Russian). [Миддендорф А.Ф. 1867. Путешествие на север и восток Сибири // Растительность Сибири. Санкт-Петербург. Ч.1, отд. 4. С. 69 (XLIX)–54 (LIV)].

Muramoto, K., T. Nakada, T. Shitara, Y. Hara & H. Nozaki 2010. Reexamination of the snow algal species *Chloromonas miwae* (Fukushima) Muramoto et al., comb. nov. (Volvocales, Chlorophyceae) from Japan, based on molecular phylogeny and cultured material. *European Journal of Phycology* 45:27–37.

Nakada, T., K. Misawa & H. Nozaki 2008. Molecular systematics of Volvocales (Chlorophyceae, Chlorophyta) based on exhaustive 18S *r*RNA phylogenetic analyses. *Molecular Phylogenetics and Evolution* 48:281–291.

Nakada, T., M. Tomita, J.-T. Wu & H. Nozaki 2016. Taxonomic revision of *Chlamydomonas* subg. *Amphichloris* (Volvocales, Chlorophyceae), with resurrection of the genus *Dangeardinia* and descriptions of *Ixipapillifera* gen. nov. and *Rhysamphichloris* gen. nov. *Journal of Phycology* 52:283–304.

NCBI 2022. *National Center for Biotechnology Information, U.S. National Library of Medicine*. Available from: www.ncbi.nlm.nih.gov. Last accessed 20.12.2022.

Nema M., M.L. Hanson & K.M. Müller 2019. Phylogeny of the egg-loving green alga *Oophila amblystomatis* (Chlamydomonadales) and its response to the herbicides atrazine and 2,4-D. *Symbiosis* 77:23–39.

Novichkova-Ivanova, L.N. 1980. *Soil algae of phytocenoses of the Sahara-Gobi desert region*. Nauka, Leningrad, 256 pp. (in Russian). [Новичкова-Иванова Л.Н. 1980. Почвенные водоросли Сахаро-гобийской пустынной области. Ленинград: Наука, 256 с.].

Novis, P.M., R.W. Hoham, T. Beer & M. Dawson 2008. Two snow species of the quadriflagellate green alga *Chlainomonas* (Chlorophyta, Volvocales): ultrastructure and phylogenetic position within the *Chloromonas* clade. *Journal of Phycology* 44:1001–1012.

Nozaki H., T. Nakada & S. Watanabe 2010. Evolutionary origin of *Gloeomonas* (Volvocales, Chlorophyceae), based on ultrastructure of chloroplasts and molecular phylogeny. *Journal of Phycology* 46:195–201.

Patova, E.N. & I.V. Novakovskaya 2018. Soil algae of the northeastern European Russia. *Novosti sistematiki nizshikh rastenii* 52(2): 311–353 (in Russian with English abstract). [Патова Е.Н., Новаковская И.В. 2018. Почвенные водоросли северо-востока европейской части России // Новости систематики низших растений. 52(2):311–353].

Perminova, G.N., I.S. Gutishvili, & E.V. Kitaev 1989. Soil algae of the plant communities of the Baikal nature reserve. In: *Algae, lichens, fungi, and mosses in the reserves of the RSFSR. Sbornik nauchnykh trudov TsNIL RSFSR*, pp. 17–26, Moscow (in Russian). [Перминова Г.Н., Гутишвили И.С., Китаев Е.В. 1989. Почвенные водоросли фитоценозов Байкальского заповедника // Водоросли, лишайники, грибы и мохообразные в заповедниках РСФСР. Сб. науч. трудов ЦНИЛ Главохоты РСФСР. Москва. С. 17–26].

Pivovarova, Zh.F. 1974. Comparative characteristic of algosinusiae under some plants of mountainous steppe of the Issyk-Kul hollow of the Tien-Shan and South-East Altai. *Izvestia Sibirskogo otdelenia Akademii nauk SSSR. Seria Biologicheskikh nauk* 1:25–32 (in Russian). [Пивоварова Ж.Ф. 1974. Сравнительная характеристика альгосинузий под некоторыми растениями горных степей Иссык-кульской котловины Тянь-Шаня и юго-восточного Алтая // Известия Сибирского отделения Академии наук СССР. Серия биол. наук. 1:25–32].

Procházková, L., T. Leya, H. Křížková & L. Nedbalová 2019a. *Sanguina nivaloides* and *Sanguina aurantia* gen. et spp. nov. (Chlorophyta): the taxonomy, phylogeny, biogeography and ecology of two newly recognized algae causing red and orange snow. *FEMS Microbiology Ecology* 95:fiz064.

Procházková, L., D. Remias, T. Řezanka & L. Nedbalová 2019b. Ecophysiology of *Chloromonas hindakii* sp. nov. (Chlorophyceae), causing orange snow blooms at different light conditions. *Microorganisms* 7:1–22.

Procházková, L., D. Remias, T. Řezanka & L. Nedbalová 2018. *Chloromonas nivalis* subsp. tatrae, subsp. nov. (Chlamydomonadales, Chlorophyta): reexamination of a snow alga from the High Tatra Mountains (Slovakia). *Fottea* 18:1–18.

Pröschold, T., B. Marina, U.G. Schlösser & M. Melkonian 2001. Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). I. Emendation of *Chlamydomonas* Ehrenberg and *Chloromonas* Gobi, and description of *Oogamochlamys* gen. nov. and *Lobochlamys* gen. nov. *Protist* 152:265–300.

Pröschold, T. & Leliaert 2007. Systematics of the green algae: conflict of classic and modern approaches. In: *Unravelling the algae: The past, present, and future of algal systematics* (J. Brodie & J. Lewis, eds), pp. 123–153, Taylor & Francis, London.

Remias, D., H. Wastian, C. Lütz & T. Leya, 2013. Insights into the biology and phylogeny of *Chloromonas polyptera* (Chlorophyta), an alga causing orange snow in Maritime Antarctica. *Antarctic Science* 25:648–656.

Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard & J.P. Huelsenbeck 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3):539–542.

Shushueva, M.G. 1977. Formation of algae groups on the dumps of coal mines in Kuzbass. In: *Natural complexes of lower plants of Western Siberia*, pp. 57–85, Novosibirsk (in Russian). [Шушуева М.Г. 1977. Формирование водорослевых группировок на отвалах угольных разработок в Кузбассе // Природные комплексы низших растений Западной Сибири. Новосибирск. С. 57–85].

Shushueva, M.G. 1980. Algae of gray forest soils of the South of Western Siberia. In: *Algae, fungi, and lichens of the South of Siberia*, pp. 128–137, Moscow (in Russian). [Шушуева М.Г. 1980. Водоросли серых лесных почв юга Западной Сибири // Водоросли, грибы и лишайники юга Сибири. Москва. С. 128–137].

Stanier, R.Y., R. Kunisawa, M. Mandel & G. Cohen-Bazire 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriological Reviews* 35:171–205.

Starr R.C. & Zeikus J.A. 1993. UTEX: The culture collection of algae at the University of Texas at Austin. *Journal of Phycology* 29:1–106.

Sudakova, E.A. 1975. Soil algae of some plant communities of the valley of the Ushakovka River (Angara basin). In: *Flora, Vegetation, and Plant Resources of Transbaikalia and Adjacent Territories. Theses of the Scientific Conf. Issue 5*, pp. 62–63, Chita (in Russian). [Судакова Е.А. 1975. Почвенные водоросли некоторых фитоценозов долины р. Ушаковки (бассейн Ангары) // Тезисы к науч. конф. «Флора, растительность и растительные ресурсы Забайкалья и сопредельных территорий». Чита. Вып. 5. С. 62–63].

Sudakova, E.A. 1986. Soil algae of meadow biogeocenoses. In: *Ecology of meadows in the western section of the BAM zone*, pp. 35–44, Novosibirsk (in Russian). [Судакова Е.А. 1986. Почвенные водоросли луговых биогеоценозов // Экология лугов западного участка зоны БАМ. Новосибирск. С. 35–44].

Takeuchi, N., J. Uetake, K. Fujita, V.B. Aizen & S.D. Nikitin 2006. A snow algal community on Akkem glacier in the Russian Altai mountains. *Annals of Glaciology* 43:378–384.

Tamura, K., G. Stecher & S. Kumar 2021. MEGA11: Molecular evolutionary genetics analysis Version 11. *Molecular Biology and Evolution* 38(7): 3022–3027.

Tedersoo, L., V. Mikryukov, S. Anslan, M. Bahram, A.N. Khalid, A. Corrales, A. Agan, et al. 2021. The Global soil mycobiome consortium dataset for boosting fungal diversity research. *Fungal Diversity* 111:573–588.

Trentin, R., E. Negrisolo, E. Moschin, D. Veronese, M. Cecchetto & I. Moro 2022. *Microglena antarctica* sp. nov. a new Antarctic green alga from Inexpressible Island (Terra Nova Bay, Ross Sea) Revealed through an integrative approach. *Diversity* 14:337.

White, T.J., T. Bruns, S. Lee & J. Taylor 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and amplifications* (M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White, eds), pp. 315–322, Academic, New York.