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Barranca variabilis sp. nov.—A New Terrestrial Alga of the Genus *Barranca* (Chaetophorales, Chlorophyta) from the Baikal Region (Russia)

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Abstract: Filamentous green chaetophoralean algae are distributed mainly in aquatic ecosystems, less known from terrestrial habitats. Many of them have a complicated thalli and complex life cycles that make it difficult to determine these organisms by morphology. Description of new representatives of the Chaetophorales continues. In this study, we have explored the filamentous green alga IRK–A 341 from soil of the Baikal Region by light and electron microscopy along with molecular phylogeny. Based on the results of morphological, ecological, and molecular phylogenetic analyses (18S–28S rDNA, *tufA*), we described the studied alga as the new species, *Barranca variabilis* sp. nov. The study complements the data on the diversity of soils green filamentous algae, and their biogeography. For the first time, the data on the structure of the cell walls and the cell ultrastructure of *Barranca* were established. The information on the morphology of the reproductive and resting cells is updated.

Keywords: terrestrial filamentous green microalgae; *Barranca variabilis*; integrative approach; Chaetophorales

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1. Introduction

The order Chaetophorales Wille of the class Chlorophyceae includes filamentous branched, unbranched, and (pseudo)parenchimatous algae, often heterotrichous with a prostrate and erect systems. Uninucleate cells contain a parietal chloroplast with one or several pyrenoids. The characteristics of the cell ultrastructure include the presence of plasmodesmata, and cell division (cytokinesis with the formation of a cell plate in a phycoplast) e.g., [1–11]. Many of these algae have a complex structure of thallus and a life cycle e.g., [10,12–14]. At different stages of the life cycle and/or different conditions, the morphology of thallus can vary significantly. This creates difficulties in species identification based only on the morphological traits. The molecular and ultrastructural data showed that a number of algae previously considered as members of the order Chaetophorales belong to other order or classes of the Chlorophyta e.g., [4,15–17], and some genera such as *Stigeoclonium*, *Chaetophora*, *Aphanochaete* are polyphyletic [18–21]. The taxonomic investigations of chaetophoralean algae at different taxonomical levels and determination of their affinities are presented by the authors in [11,14,18,21–25].

According to the AlgaeBase, the order includes more than 200 species [26], which inhabit mainly aquatic ecosystems. A number of species are found in terrestrial and semi-terrestrial habitats, for example representatives of genera *Uronema*, *Fritschiella*, *Barranca*, *Draparnaldia* [27–29]. Some of the chaetophoralean algae are known to be widespread [26]. Other members are considered as endemic e.g., [30,31]. Aquatic chaetophoralean algae are a large group of primary producers, providing a significant part of the energy to higher trophic levels e.g., [31–38]. The mass development of these organisms can have an impact

on the ecological state of the reservoir or coastal biotopes [35,39–41]. Less is known about the diversity, distribution, and significance of chaetophoralean algae living in terrestrial ecosystems, where their role in the functioning of biogeocenoses can be important [15]. Chaetophoralean representatives can be used for bioindication and biotechnology e.g., [42–45].

In Russia, different terrestrial filamentous green non-conjugate and conjugate algae were registered e.g., [15,46–48]. These are widespread as typical soils inhabitants (non-conjugate) of genera *Klebsormidium*, *Ulothrix*, *Stichococcus*, *Leptosira*. These algae can form a significant biomass and become a part in communities of dominant species in soil algocenoses [46,49,50]. There are a number of records on terrestrial and semi-terrestrial habitats of green algae of genera *Ctenocladus* (*Lochmiopsis*), *Geminella*, *Gloeotila*, *Gongrosira*, *Microspora*, *Microthamnion*, *Oedogonium*, *Pseudoschizomeris*, *Stigeoclonium*, *Rhizoclonium*, and some others. As a rule, the species identity of these algae was established by light microscopy. In many cases, their descriptions are absent, and they are not presented in the culture collections or herbariums.

The genus *Barranca* Caisová et al. (*Barrancaceae* Caisová et al.) was established in 2015 [14]. At present, it includes two species, which were characterized based on morphological traits and life cycle along with molecular data. The type species of the genus *B. multiflagellata* was isolated from an enrichment culture of a soil sample of the volcanic island La Palma (Spain, Canary Islands). Semi-terrestrial *B. yajiagengensis* was described by Gao et al. [42] based on the sample from the stream rock in the highlands of China. In this study, we explore a new strain of the genus from steppe carbonate soil of the Baikal Region (Russia) using an integrative approach.

2. Materials and Methods

2.1. Survey Area and Alga Origin

The studied alga was isolated from enrichment culture of sodium carbonate soil, samples of which were collected in June 2014 in the southern part of the Irkutsk Region, in the vicinity of the Bayandai village. The altitude is about 680 m above sea level; 53°01′ N, 105°29′ E. The research was a part of the study of soil algae of plant communities in which the cyanobacteria *Nostoc commune* develops in mass on the soil surface.

The study area was located within the Central Siberian Plateau, at the junction of the Siberian Platform and the mountains surrounding Lake Baikal. The relief is ridge-hilly. The bedrocks are terrigenous-coal-bearing Jurassic sediments. A limestones and marl formations are common. The vast majority of sediments contain a lot of lime. The climate is sharply continental. The average annual air temperatures are near −4.3 °C. The amount of precipitation per year is up to 300–500 mm, up to 50% falls in the second half of summer. Soils are medium- and relatively well developed, mainly loamy, slightly acidic and neutral, insufficiently and temporarily excessively moist, moderately cold and moderately long-freezing with vegetation of medium and increased productivity. In the survey area, the steppes prevail [51,52].

In an area of anthropogenically disturbed steppe community, three surface soil samples were taken per 1 m^2. Each sample was 4–5 cm^2 in size and 2–3 cm in thickness. In compliance with the conditions of sterility, the materials were collected in a paper bag, and transported to the laboratory. Additionally, soil samples were taken to determine the physicochemical parameters.

The following procedures were performed in the laboratory. For algological analysis, the soil specimens were dried at room temperature, crushed, and mixed well. To obtain an enrichment culture, 1 g of the combined soil sample was placed in a 100 mL flask with a liquid nutrient medium N BBM [53], and incubated in the cultivation (growth) room at 19–23 °C under 12 h photoperiod with cool white lamps ~100 µmol photons m^{-2} s^{-1}. After germination of algae in a flask, 50 µL of the medium with algae was placed on a plate with agar-solidified (1.4–1.6%) medium (N BBM). The individual colonies of filamentous algae were derived to get unialgal cultures. As a result, the studied strain was isolated,

and stored under number 341 in the IRK–A culture collection of algae (Siberian Institute of Plant Physiology and Biochemistry (SIPPB) of the Siberian Branch of the Russian Academy of Sciences, Irkutsk).

Analysis of some physicochemical parameters (pH, the exchangeable cations potassium, sodium, calcium, magnesium, etc.,) was carried out in accordance with certified methods and using equipment of the Shared Research Facilities 'Bioanalitica' (SIPPB of the Siberian Branch of Russian Academy of Sciences) [54]. It was established that soils in the studied area are sodium carbonate with a slightly acidic and neutral pH of organ-accumulative horizons, alkaline in the mineral horizons. They contain increased amounts of calcium and magnesium [55].

2.2. Culture Conditions and Microscopic Observations

For morphological observations, the strain IRK–A 341 was grown in a liquid and agar-solidified media 0N, N, 3N BBM, and BG11 [56] with or without vitamins (B1 and B12), and on the agar-solidified soil extract (pH~6.6) without the addition of salts [57]. The soil extract was obtained from the soil of the survey area. The studied alga was grown in the cultivation room (see above), and in a Binder climatic chamber (Germany) with cool white fluorescent lamps at 12–16 °C under ~40 µmol photons m^{-2} s^{-1} irradiance and a 16:8 h light:dark cycle, and in natural light on the window, where the temperature fluctuated from 10 to 30 °C.

The motile reproductive cells were obtained by transferring IRK–A 341 growing on the agar-solidified medium in the Eppendorf flask with distilled water. Then, the flask was kept overnight in the dark at 12–16 °C. In the next morning, samples were kept in the light, and examined for the presence of motile cells.

Light microscopy was performed using a microscope AxioScope.A1 equipped with a color digital camera ICc5 (Carl Zeiss, Germany). In vivo staining of cells with 0.1% gentian violet and China ink was used to examine the mucilage, structure of cell walls, and a number of flagella of reproductive cells. All available published information was used to identify the species [14,15,17,42]. We followed the terminology as in the works [11,13–15] for descriptions of morphology of the studied alga.

For transmission electron microscopy (TEM), the protocol of sample preparation was applied according to the earlier studies [58]. The cells of the strain IRK–A 341 were observed and photographed with a transmission electron microscope Libra 120+ (Carl Zeiss, Germany) of the Core Facility Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute RAS (St.-Petersburg, Russia).

2.3. DNA Isolation, PCR Amplification, and Sequencing

Total genomic DNA was isolated from living algal culture of the strain IRK–A 341 grown on agar media by the method of Doyle, Doyle [59] with modifications. The 18S–28S rDNA region was amplified and sequenced with primers and conditions as described by authors in [58], with additional primer ITS055R [60]. Partial sequence of the gene tufA was obtained with primers published by Fama et al. [61]. Comparison search for nearest homologues was performed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi accessed on 10 February 2023). Nucleotide sequences were assembled and aligned using BioEdit 7.0.5.3 program [62]. The nucleotide sequences obtained were submitted to the GenBank database with accession numbers OQ589853 (*tufA*), OQ595204 (18S–28S rDNA).

2.4. Phylogenetic Reconstruction and Secondary Structure Analysis of ITS2

Nuclear phylogeny of the studied strain was achieved using the 18S–28S rDNA region. Variable positions of ITS were deleted, and only unambiguously aligned sites were used. Final alignment included 23 sequences with 2627 nucleotide sites (264 parsimony informative sites). Plastid phylogeny was based on 46 sequences of the gene *tufA* with 780 nucleotide sites (262 parsimony informative).

The evolutionary history was inferred using the Bayesian reconstruction, maximum likelihood (ML), and neighbor-joining (NJ) methods. ML trees were reconstructed in IQTREE v.1.6.12 [63] with the ultrafast bootstrap support [64] and model selection using ModelFinder [65] implemented in IQTREE. The model TIM2 + F + I + G4 was chosen as optimal for 18S–28S rDNA and *tufA*. Bayesian trees were obtained in MrBayes 3.2.6 [66] with GTR + I + G model. Effective sample size (ESS > 400) was used as the stopping criterion in Bayesian analysis. NJ trees were reconstructed using Mega 11.0.8 [67]. Bootstrap tests were run for 1000 replicates.

Nucleotide sequences of ITS2 were annotated with the ITS2 Database [68] and secondary structures were predicted in Mfold [69] with default conditions at 37 °C for comparison reasons. The ITS2 secondary structures of strains IRK-A 341 and BEA 0607 folded in Mfold at minimum energies were compared with each other and with the consensus structure of ITS2 proposed by Caisová et al. [70] for Chlorophyta.

3. Results

3.1. Phylogenetic Analysis

Phylogenetic reconstructions show that the studied strain belongs to the genus *Barranca* (Figure 1). This genus is a member of the clade *Barrancaceae*, which is one of the six currently recognized clades (Aphanochaetaceae, Barrancaceae, Chaetophoraceae, Fritschiellaceae, Schizomeridaceae, Uronemataceae) of the monophyletic lineage of the order Chaetophorales. In the nuclear phylogenetic tree, the *Barrancaceae* clade includes (with high support 1/99/93) two branches, one of which unites two strains of the type species of the genus *Barranca*, *B. multiflagellata* BEA 0607 and BEA 0608, and the branch that combines IRK–A 341 and JNU39 (*B. yajiagengensis*) from China (MN891730) (Figure 1). Strains IRK–A 341 and JNU39 are placed together on the same branch with weaker supported node (0.87/97/88). Currently available data do not allow us to attribute the studied strain to the known species of the *Barranca*. The sequence length of JNU39 (1665 nt) was sufficiently shorter than IRK–A 341 (2913 nt) or *B. multiflagellata* strain BEA 0607 (3671 nt) so only partial 18S rDNA was available to compare all these *Barranca* strains (Figure 1). For the partial 18S rDNA, nucleotide similarity of the studied strain with the strain BEA 0607 (*B. multiflagellata*) was 99.1% and with JNU39 (*B. yajiagengensis*) 99.8%. The strain JNU39 differed from IRK–A 341 by two single nucleotide deletions at the 5′ end of 18S rDNA.

The placement of the *Barranca* phylogenetic lineage was similar in 18S–28S rDNA and *tufA* Chaetophorales phylogenetic trees (Figures 1 and 2).

3.2. The Secondary Structure of ITS2

We compared features in ITS2 secondary structure of *Barranca multiflagellata* strain BEA 0607 (similar to BEA 0608) and the strain IRK–A 341 (Figure 3). ITS2 of IRK–A 341 was shorter (216 bp) than in BEA 0607 (226 bp) with different lengths of Helix II and variable Helix 4. The three major helices (Helix I–Helix III according to [71]) were present in secondary structure models of the ITS2 molecule of both strains. Additional helices in BEA 0607 and IRK–A 341 were present between Helices II and III (Helix termed IIA by [71] and at the usual placement of "canonical" Helix IV which was not recognizable (Figure 3). It is noted that not all eukaryotes have the same number of helices, and only Helices II and III are well recognizable and common to essentially all [71]. Helices I and IV are known as most variable in the "four-helix model" of ITS2 structure of different organisms, for example, [72]. The Helix I in the *Barranca* strains was unbranched that is considered a specific feature in all taxa of the Chaetophorales, Chaetopeltidales, and Oedogoniales [70]. Universal conservative sites determined in Helices I–III in the Sphaeropleales, Oedogoniales, Chaetopeltidales, Chaetophorales by [70] were unchanged in both species of *Barranca*. Helix III has its most highly conserved sequence region on the 5′ end [71] while the basal pairings varied between the two compared strains. No compensatory base changes (CBC) were found between these species. Though CBC approach was not able to distinguish BEA 0607 and IRK–A 341, other differences in ITS2 secondary structures

(variable part of the top structure of Helices I, II, basal part of III[d] and fourth helices [to be distinguish with common Helix IV] complete helix 5) support two distinct species.

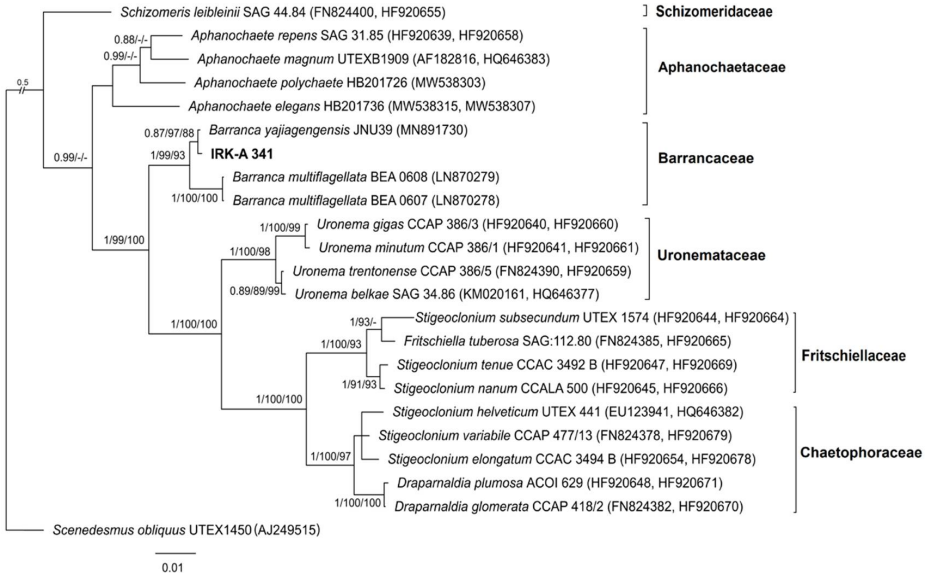

Figure 1. The phylogenetic position of the studied strain IRK–A 341 in the *Barranca* clade of Chaetophorales. Bayesian phylogenetic tree is based on the 18S-ITS2-28S alignment, 2627 nt in length. Bayesian probability support and bootstrap values (PP/ML/NJ) are given next to branch nodes. Values less than 85 (0.85) are not shown. The studied strain is marked with bold font. GenBank accession numbers are given in parentheses. The scale bar indicates the number of substitutions per site. *Scenedesmus obliquus* is shown as outgroup.

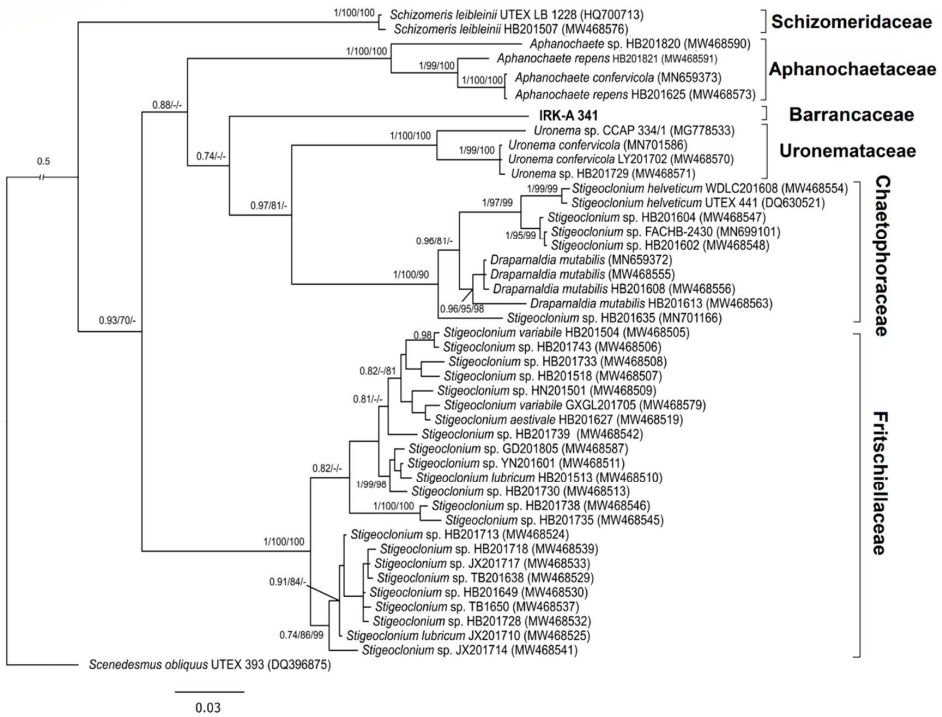

Figure 2. Plastid phylogenetic tree of the strain IRK–A 341 is based on the gene *tufA*. Bayesian tree is reconstructed from aligned 780 nucleotide sites. Bayesian probability support and bootstrap values (PP/ML/NJ) are given next to branch nodes. Values less than 70 (0.70) are not shown. The studied strain is marked with bold font. GenBank accession numbers are given in parentheses. The scale bar indicates the number of substitutions per site. *Scenedesmus obliquus* is shown as outgroup.

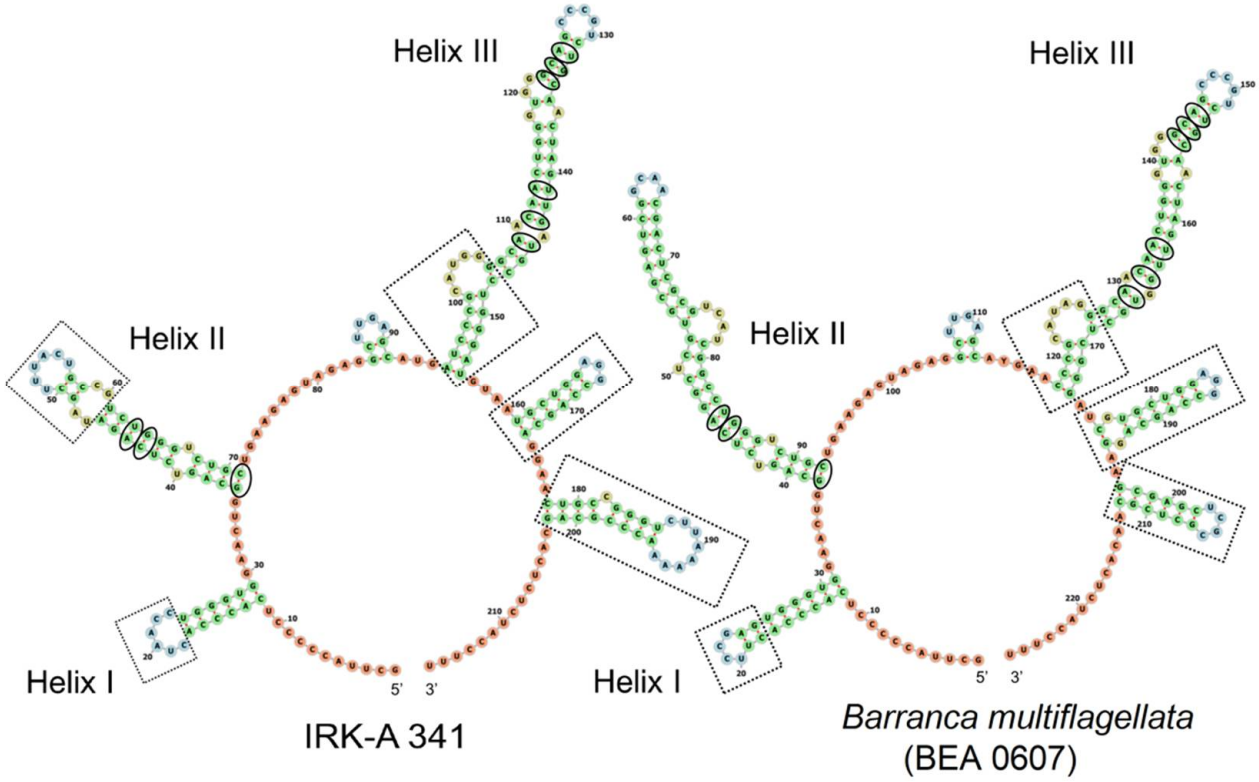

Figure 3. ITS2 secondary structures of *Barranca* strains. Regions (tips of Helices I and II, basal part of Helix III and two additional helical structures) which are variable between the studied strain IRK–A 341 and *Barranca multiflagellata* BEA 0607 are highlighted with dotted lines. Count of nucleotide positions starts from 5′ end of ITS2. Common for Chlorophyta sites of CBC in conservative parts of Helixes II and III are ringed (no specific CBCs in *Barranca*). Structures with minimum free energies (dG) −37.05 for IRK–A 341 and −58.27 for BEA 0607 were compared.

3.3. Morphology

The studied strain IRK–A 341 forms filaments of uncertain length, branched and unbranched, straight or slightly curved, waved, especially when they grow on the agar (Table 1; Figure 4). Filaments are mainly uniseriate or less frequent biseriate. Young filaments developed from zoospores or some resting cells have apical-basal polarity (Figure 5).

Table 1. Comparison of morphological traits of the *Barranca* species.

Species/ Trait	***B. multiflagellata* BEA 0607 [a]**	***B. yajiagengensis* JNU39 [b]**	***B. variabilis* IRK–A 341 [c]**
1. Filaments:			
uniseriate	+	+	+
biseriate	–	–	+
branching	no or rarely simply	no or rarely simply	no or expressed, simply
polarity: a holdfast and a ring-like structure	+	+	+
2. Vegetative cells:			
shape of apical cells	straight, mostly rounded, slightly acute or acuminate	–//–	–//–, slightly expanded
shape of intercalary cells	cylindrical	–//–	–//–, frequently with a bulge on the middle, globose, dumbbell-, pear-, barrel-shaped, ovoid, square, wrong polygonal
length × width, μm	4 − 2 × 4 − 13	4 − 30 × 4 − 11	2.3 − 32 × 4.5 − 13(15)

Table 1. *Cont.*

Species/ Trait	*B. multiflagellata* BEA 0607 [a]	*B. yajiagengensis* JNU39 [b]	*B. variabilis* IRK–A 341 [c]
nucleus	One	–//–	–//–
chloroplast	parietal, single, lobed	–//–	–//–, or with a smooth unlobed edges
pyrenoid (P)	1–4	–//–	1-2(4)
P starch envelope	+	+	3 and more, sometimes absent
3. Reproduction: vegetative asexual	fragmentation of filaments	–//–	–//–
by zoospores (Z)	+	+	+
shape of Z	oval or nearly spherical #	–//–	fusiform, rod-shaped, oval, spherical
sizes of Z, μm	10–20 of length #	$7-13\times4-9$	$6-20\times4.5-8$
number of flagella per Z	4, 8, 12–24	2, 4	4, 8 and more
by aplanospores (A)	+	+	+
shape of A	spherical, cylindrical, occasionally constricted	spherical	spherical, cylindrical, ellipsoid, occasionally constricted
sizes of A, μm	14–15 in diameter, or $10-21\times8-16$	8–11 in diameter	$12-17\times6-12$

Notes: [a]—according by Caisová et al. [14], [b]—by Gao et al. [42], [c]—present study. "+"—present; "–"—absent; "–//–"—the same; "#"—based on chemically fixed cells.

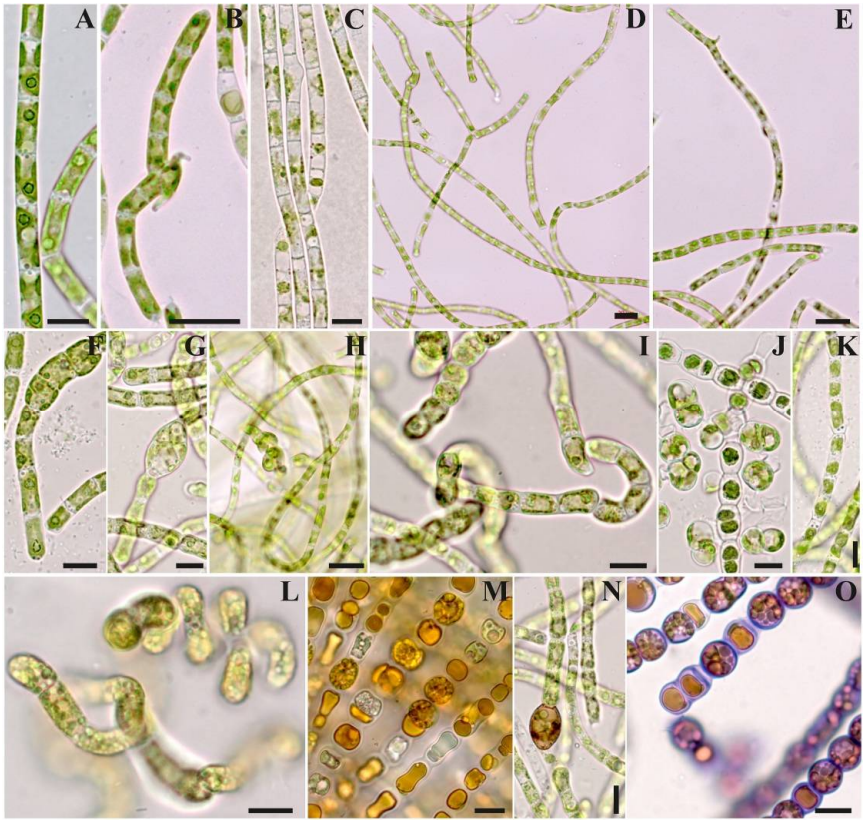

Figure 4. Morphology of filaments of the strain IRK–A 341 on the different stages of development. (**A**–**O**). Filaments of different shape and age; cells of different shape and sizes. (**B**,**D**,**E**). Branching. (**F**–**H**,**J**). Filaments with dividing cells, and forming two or more rows of cells. (**K**). Fragmented filaments with the formation H-pieces. (**M**–**O**). Resting cells of different shape. Scale bars: (**A**–**C**,**F**–**O**)—10 μm, (**C**–**E**)—20 μm.

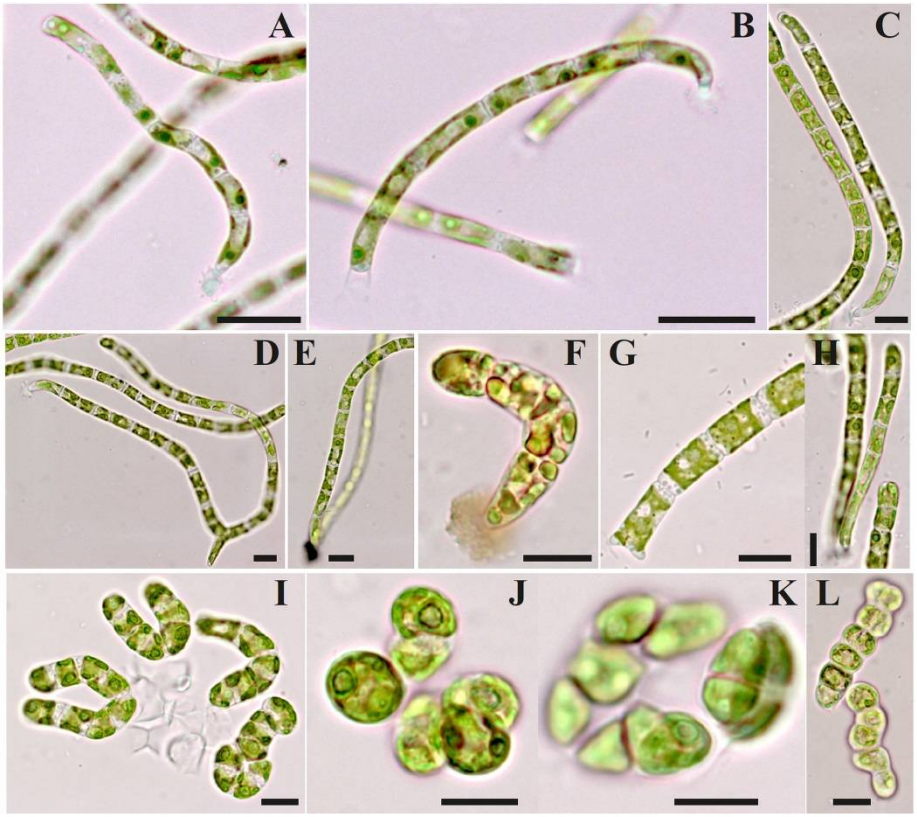

Figure 5. Growing filaments and cell aggregates of the strain IRK–A 341. (**A**–**H**). Filaments with the apical-basal polarity. (**A**–**D**,**F**–**H**). Filaments with a holdfast and/or ring-like structure. (**I**–**K**). Germinating cells from resting or vegetative cells. (**L**). Filaments similar with protococcalean algae. (**A**–**E**,**G**–**L**). The culture from mineral medium. (**F**). The culture from soil medium. Scale bars: (**A**–**E**,**H**)—20 µm, (**F**,**G**,**I**–**L**)—10 µm.

The polarity is due to the fact that the basal end of the filament forms a holdfast and a ring-like structure by which the filament is attached to the substrate (Figure 5). From 1 to 3(5) of such structures per one filament were observed. Usually apical ends of these filaments are acute, more rarely expanded and/or rounded, sometimes bisected. The polarity is well visible in filaments consisting of a small number or several dozen cells.

As a rule, cells in filaments are cylindrical, uninucleate, with parietal chloroplast. The chloroplast is girdle-shaped, band-shaped, it is lobed or with a smooth edge, sometimes it can be cut in half or one-third along the long axis. It stretches the entire length of cell or occupies its middle part (Figure 4). The well-visible 1–2 (up to 4) pyrenoids are located in the chloroplast. The pyrenoid is surrounded by starch grains (3 or more) or naked (for example, in oldest cells). Starch grains can also be present in the chloroplast.

The uniseriate active growing filaments consist of cylindrical cells with similar width, not constricted at the transverse walls. The length of cells is up to five times more the width, equal to it, or slightly less (Table 1). These filaments are unbranched or rarely branched (Figures 4 and 5). The branch can be short or relatively long. The branching is usually single per one filament. More rarely 2–3 branches are formed. Uniseriate filaments can produce the cylindrical cells that are wider than cells formed initially (Figure 4F). Moreover, swollen cells of various shape (near spherical, barrel-, dumbbell-, pear-shaped, or irregular shape) are produced (Figure 4B,C,E,G,N). The filaments consisting of such cells can be constricted at the cross walls. Swollen cells often contain one or more big and small vacuoles, and able to divide. The division can occur in one or more planes. As a result, a barrel-shaped or cells of different shape are formed, or a section of filaments consisting of two or more rows, or a dyads, tetrads of cells, or cell packages like some in protococcalean or other green algae (Figure 4H,J, Figures 5L and 6C). Sometimes, the alga produced thalli consisting of

swollen cells of different shape and shortly branched. These thalli resemble those in species of the genus *Leptosira* (not shown) or some growth stages of *Stigeoclonium* representatives, or other chlorophyte algae.

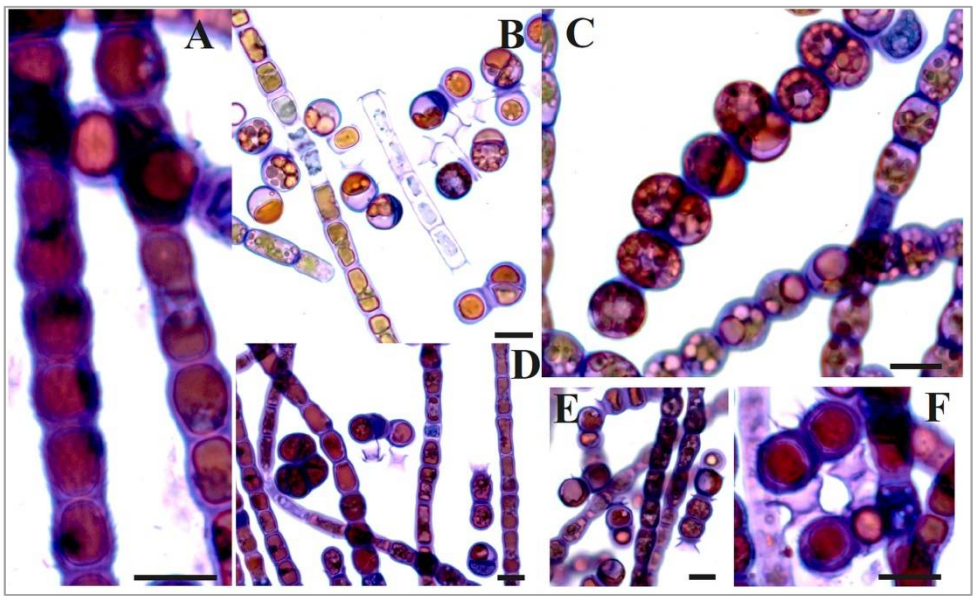

Figure 6. The cell wall of aging cells of IRK-A 341 (stained with gentian violet). (**A**–**C**). Filaments cells with striated (**A**) and smooth (**B**–**E**) thickening cell wall. (**B**,**D**–**F**) H-pieces at the ends of filaments, and caps remaining after the release of resting cells from filaments. Scale bars: 10 μm.

In the growing filaments, young cells have a thin and smooth cell wall. The aging cells have a thickening cell wall. It is homogeneous or stratified in the older cells (Figure 6). The staining with gentian violet inferred that the cell wall of older cells is not smooth. The outer layers of the cell wall rupture over time, and the cell wall of such cells looks spiky (Figure 6A). Cells with thickening cell walls germinate after being placed in a fresh medium (Figure 5). Filament cells grow intercalarily. When vegetative cells elongate and/or divide, the outer layer of the mother cell wall breaks. Remnants of the mother cell wall are visible as the cap at the end of filaments or as H-pieces (Figures 4K, 5B,I and 6B,D–F). Sometimes they resemble the caps of the *Oedogonium* species. Remnants of maternal cells are uneven at the edges, where the shell bursts. Edges of these caps can band outwards, and they become noticeable even in the middle or inner parts of the actively growing filaments (Figure 6B,E,F). Some aging vegetative cells of filaments are eventually filled with starch and oil droplets. Their contents become granular or relatively homogeneous, and orange. The cell wall is thickened, sometimes red-brown or brown. These cells can be considered as akinete-like or resting cells (Figures 4M–O, 5F and 6). In a fresh medium or water, they leave filaments through lateral pore or breaking of the cell wall, and germinate. Remnants of maternal cells in the form of quadrangular or octagonal caps are well noticeable in the culture for a long time (Figures 5I and 6B,D).

The reproduction of the studied alga occurs in several ways. The vegetative reproduction is by division of cells into two or more planes (see above), and fragmentation of thalli as described by Caisova et al. [14] for *Barranca multiflagellata* and/or Chaetophoraceae representatives [10]. Asexual reproduction of IRK–A 341 occurs by zoospores and aplanospores. One zoospore or aplanospore is formed in one filament cell (Figure 7). The size of reproductive cells depends on the size of the mature cells. Zoospores actively move inside the mother cell. Their release occurs in the same ways as akinete-like cells (Figure 7A–C). Immediately after leaving the mature cell, zoospores may be irregular in shape (Figure 7D) or as described in the Table 1. They have a papilla, two (probably more?) anterior contractile vacuoles, elongated anterior stigma, chloroplast with visible pyrenoid (Figure 7). The

flagella are isokont up to 10 µm in length. After the stopping, zoospores retract the flagella into the cell body. A part of the monad cells is rounded off by the end of the movement (Figure 7G). Others monad cells do not take a spherical or subspherical shape (Figure 7I–L,O). The shape of some monads cells (Figure 7E,I,O), and the process similar with mating (Figure 7P–U), allow us to make an assumption about the presence of a copulation or about the violation of cell division. The filaments of IRK–341, which was grown by zoospores, are narrower than those germinating from resting cells. This corresponds to previously obtained data for *Barranca* species [14].

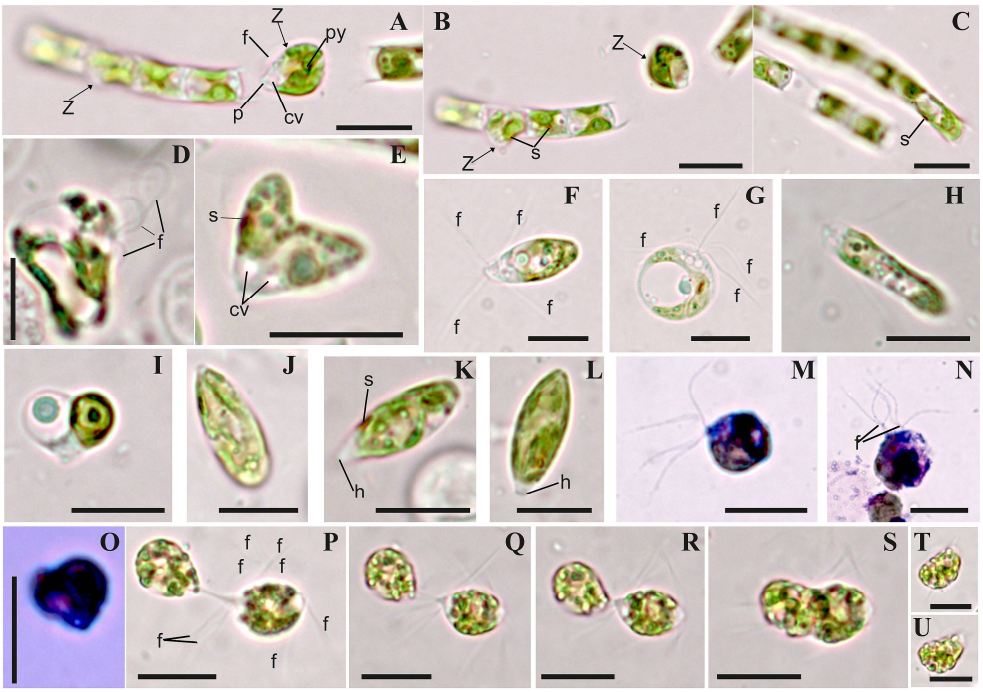

Figure 7. Reproductive and vegetative cells of IRK–A 341. (**A**–**C**) Liberation of zoospores from mature filament cells. (**A**,**B**,**D**–**I**,**M**–**O**) Zoospores of the different shape. (**N**) Zoospore with more than four flagella. (**I**–**L**,**O**) Static zoospores without flagella. (**E**,**P**–**U**) A merging of two halves of one cell or crossing (?). (**M**–**O**) Stained with gentian violet. Designations: cv—contractile vacuole; f—flagella, h—holdfast; p—papilla, py—pyrenoid; s—stigma (eyespot); z—zoospore. Scale bars: 10 µm.

The aplanospore formation is similar to that as reported by Caisova et al. [14]. We have not observed the formation of brown ruptured shell by aplanospores. However, it is possible that origin of the resting (akinete-like) cells of IRK–A 341 was different, and some of them were the stages of development of aplanospores.

The strain IRK–A 341 study showed that this representative of the genus *Barranca* has morphological differences from known species of the genus (Table 1, Figures 4–7).

3.4. Ultrastructure

The cell wall of individual vegetative cell is two-layered: the thin outer lamellar layer and inner fibrillary (Figure 8A,C,F). The outer multilayered fibrillary sheath, covering the filaments is often stratified. Fibrillar caps are visible at the ends of the cells (Figure 8A,B,H,I). Possibly, they are remnants of maternal cell wall after intercalary growth (Figure 8I,M). Cell division occurs at different angles to the filament growth axis, sometimes perpendicular to the long axis of the cell (Figure 8,C,J) or at an angle of 45 grades or more (Figure 8B,E,L,M). Most of the observed cellular profiles are more or less constricted on one side (Figure 8A,F,H). An oval or elongated nucleus with a centrally positioned heterogeneous nucleolus is observed at the cell center (Figure 8A,D,H,J). The nucleus is of a simple chromocentric type (Figure 8A,D). Usually a pair of Golgi bodies (Figure 8D,E,K) and some vacuoles (Figure 8A,K) are seen near the nucleus. Mitochondria are located along

the chloroplast lobes. They have small oval, rarely elongated profiles (Figure 8A,H). A chloroplast occupies just one half of the cell. It is lobed (Figure 8A,B,E,H,I,M). Some of its profiles are located peripherally, along the cell wall, some are more centrally. In young forming cells, it is parietal (Figure 8C,J,L). Chloroplast thylakoids are long, stretched along the long axis of the cell, and organized in packs mostly of two or three units (Figure 8A,F,G,K). Sometimes short profiles of thylakoids could be seen (Figure 8K). The pyrenoid is embedded in the chloroplast. It is formed by a dark intact stroma and surrounded by 3–4 starch plates (Figure 8B,E,G,I,J). In rare cases, the starch envelope is not formed completely, and the pyrenoid looks naked and comes outside (Figure 8A,F,H). Thylakoids do not cut the pyrenoid stroma, they terminate on its periphery (Figure 8F,G).

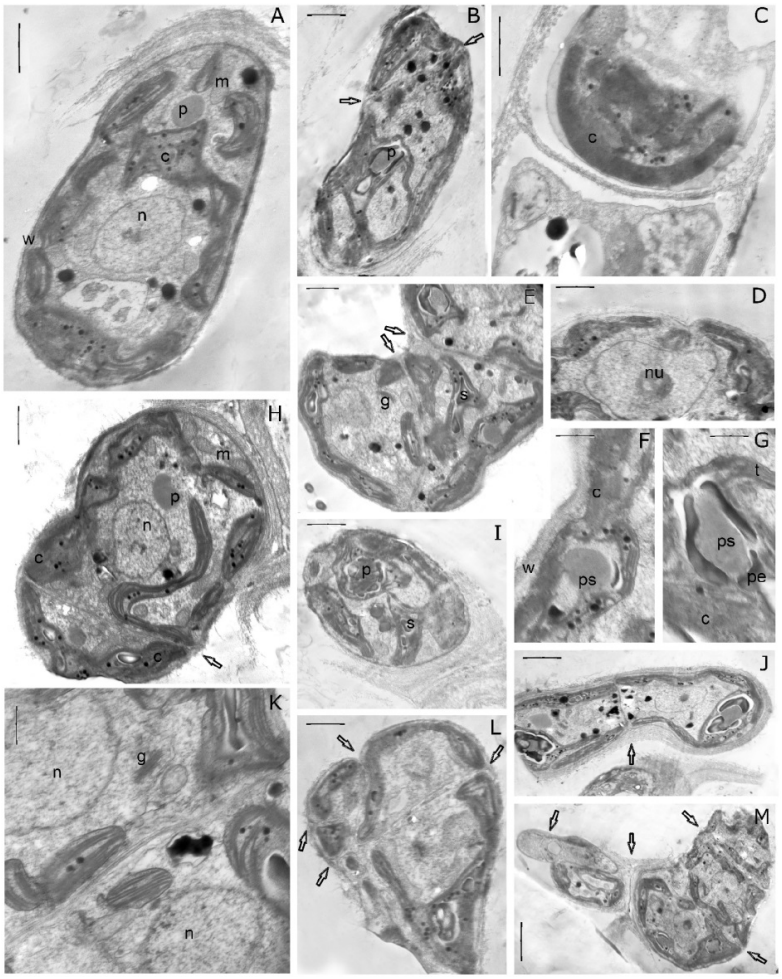

Figure 8. Ultrastructure of the studied strain IRK–A 341. (**A**) An individual cell with its cell wall and remnants of a filamentous cell wall on top of the cell; (**B**) a young cell ready to form a new filament, arrows pointed the cleavage furrow; (**C**) a fragment of the filament with a completely formed wall between cells, note the intact parietal chloroplast in forming cell; (**D**) a nucleus with heterogeneous nucleolus; (**E**) small filament with three cells, note different angles of cell divisions; (**F**,**G**) pyrenoids with intact stroma and almost absent (**F**) and developed (**G**) starch envelope; (**H**) just divided cell with a constriction on the left side; (**I**) possibly forming young cell in the filament; (**J**) a two-celled filament, formed by the division perpendicular to its long axis; (**K**) fragments of two young cells, forming new cell walls, note closely located nuclei, Golgi body and mitochondrion; (**L**,**M**) filaments with more than tree cells, note different plans of division. Designations: c—chloroplast, g—Golgi body, m—mitochondrion, n—nucleus, nu—nucleolus, p—pyrenoid, pe—pyrenoid starch envelope, ps—pyrenoid stroma, t—thylakoids, w—cell wall. Arrows showed plans of the cell division. Scale bars: (**A**–**E**,**H**,**I**,**L**)—1 μm, (**F**,**G**,**K**)—0.5 μm, (**J**,**M**)—2 μm.

3.5. Taxonomic Conclusion

Barranca variabilis I.N. Egorova, N.V. Kulakova, O.N. Boldina and G.S. Tupikova sp. nov.

Etymology: The species epithet refers to the diversity of the morphology of the thallus during the life cycle.

Description: Thalli are uniseriate unbranched or simply branched filaments or they have irregular shape. Thalli of irregular shape are with biseriate and more filaments or a part of filaments, can be often short branched, resemble some protococcalean or ulvophycean algae. Sometimes cell aggregates from 2–4 and more cells are formed. The young filaments are heteropolar or not. Heteropolar filaments have a holdfast and a ring-like structure at the basal end by which they are attached to the substrate. The apical end of the filament is slightly acute or acuminate, rounded, sometimes expanded and/or bisected. H-pieces can be presented at the ends of young and aging filaments. The cells' actively growing filaments are usually cylindrical, and with increasing age they are often swollen, globose, dumbbell-, pear-, barrel-shaped, ovoid, square, wrong polygonal ($2.3 - 32 \times 4.5 - 13(15)$ μm). The filaments constricted or not at the cross cell walls. The chloroplast is parietal, band- or girdle-shaped, often lobed or with a smooth edge, stretching along the length of the cell or located only at some part of the cell. From 1 to 2(4) pyrenoids usually with starch envelope are located in the chloroplast. Thylakoids do not cut the pyrenoid stroma, they terminate on its periphery. One nucleus is well visible in the cell. The cell wall is thin, with age thickening up to 2–3 μm and more. The cell wall of older cells is striated, rupture, and it may look like spikes. The maternal cell wall often is noticeable as cups at the polar ends of cells.

The reproduction occurs by vegetative cell division and fragmentations of filaments. There is asexual reproduction by zoospores and aplanospores. The one reproductive cell is formed in the one filament cell. The zoospores are of various shapes, often fusiform, and can be rounded. The zoospore sizes are $6 - 20 \times 4.5 - 9$ μm. They have a papilla, two contractile vacuole below papilla, parietal chloroplast with pyrenoid, anterior or shifted to the middle of the cell-elongated stigma. Four, eight, and possible more flagella are isokont. After zoospores stop moving and retract flagella, they attach to the substrate by the apical end. A holdfast is formed at the papilla.

There are resting cells (akinete-like, other?), filled with starch and oil drops. The contents of such cells are granular or homogeneous, orange or red. They have thickened cell walls. The cell wall can be red-brown or brown, ruptured. The old cultures on the agar are colored red or orange.

Diagnosis: *Barranca variabilis* IRK–A 341 is phenotypically different from type species of the genus *B. multiflagellata* by the ability to form thalli not only in the form of uniseriate filaments, but also more complexly organized (biseriate filaments, thalli similar to protococcalean algae). Additionally, *Barranca variabilis* has shorter ITS2 (216 bp) than *B. multiflagellata* (226 bp), and differences in the secondary structure of ITS2. Studied alga IRK–A 341 differs from species *B. yajiagengensis* by the presence of four and more flagella in motile cells (zoospores) and larger sizes of these cells. The new species is isolated from steppe carbonate soil of the Baikal Region. *B. multiflagellata* was registered from soil of the oceanic island La Palma (Spain). *B. yajiagengensis* was found in semi-terrestrial habitat (the stream rock) in the highland of China.

Holotype (designated here): dried specimen deposited in the herbarium of the Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences (IRK), Irkutsk, Russia, under the accession number IRK 003116.

Type locality: Russia. Irkutsk Region. The vicinity of Bayandai village. The altitude is about 680 m above sea level; 53°01′ N, 105°29′ E. Sodium carbonate soil in anthropogenic disturbed steppe communities. Collected in 13 June 2014.

Reference strain: IRK–A 341 deposited in the IRK–A culture collection of algae of Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia.

Genbank numbers: OQ595204, OQ589853.

4. Discussion

The morphological and molecular data conclusively show that the studied alga belongs to the genus *Barranca* of the order Chaetophorales. Many representatives of this order have a complexly organized thalli and complicated life cycle that makes it difficult to study these algae [13,15]. The thalli of the *Barranca* species are simpler in comparison with those of *Stigeoclonium, Chaetophora, Fritschiella,* and some others. The similarity of *Barranca* and *Uronema* (one of genera of Chaetophorales) was reported by Caisová et al. [14]. Representatives of these two genera have similarities in the morphology of filaments (uniseriate, polar, holdfast), and reproduction (by zoospores, aplanospores, fragmentation of filaments, reproductive cells are released through lateral pore of the cell wall), that correspond with the data obtained in this study. Additionally, in the frame of this research, it presents that *Barranca* representatives have more in common with other chaetophoralean. The ability to form thalli such as protococcalean algae, the thickening of cell walls in aging cells and their striation, features of the structure of the cell wall (H-pieces, presence of caps at the ends of cells), and ultrastructure of cells, reproduction, presence of resting akinete-like cells are similar to those of some representatives of *Stigeoclonium*, *Chaetophora*, *Draparnaldia*, and others. For example, the study of the ultrastructure of the strain IRK–A 341 revealed that the pyrenoid structure (without the penetrating of the pyrenoid stroma by thilakoids) is similar to *Uronema*, *Stigeoclonium*, *Chaetophora*, *Draparnaldia*, *Fritschiella*, but not *Schizomeris* or *Aphanochaete*. The species of the latter two genera have a pyrenoid with traversing membranes as shown by the authors of [4,5]. In a culture, the *Stigeoclonium*, *Chaetophora* species produce filamentous thalli along with protococcalean packages [13]. Cell walls of the *Stigeoclonium* aging filament cells show thickening, [10,13]. These and others morphological and life cycle traits of chaetophoralean including *Stigeoclonium*, *Chaetophora*, *Uronema*, and some others are significantly similar with such characteristics of *Barranca*. These features along with molecular data confirm the place of the genus *Barranca* in the system of the Chaetophorales (Figures 1 and 2). Nevertheless, the organization of thalli of the considered algae is much simpler than that of Aphanochaetaceae, Chaetophoraceae, Fritschiellaceae representatives. The motile reproductive cells formed by *Barranca* may have two to four and more flagella. It distinguishes the species of the genus from many chaetophoralean, for which mainly two or four flagella are known [13].

The distinct position of the genus in the system of the Chaetophorales is confirmed by molecular phylogeny based on the nucleotide sequences of the nuclear region [14,42] (Figure 1 in this study). The chloroplast (*tufA*) phylogeny also corroborated with the special place of *Barranca* in the order (Figure 2). However, the topology of the nuclear and chloroplast phylogenetic trees does not completely correspond to each other. The position of Fritschiellaceae on phylogenetic trees is different. The data obtained in this study is consistent with the data of Liu et al. [21,25], which showed that it is necessary to use additional chloroplast markers for more deep phylogenetic analysis of the Chaetophorales.

Barranca variabilis IRK–A 341 shares the characteristics of the phenotype with the other two species of the genus (Table 1). It differs from the type species of the genus *B. multiflagellata* by more complex thalli and ecology. The relatively conservative 18S rDNA promoted the application of the ITS2 secondary structure to resolve phylogenetic relationships between these species [70,71]. The secondary structure of ITS2 of two strains of *Barranca* (IRK–A 341 and BEA 0607) was analyzed in this study. Folding ITS2 sequences of the *Barranca* strain IRK–A 341 and the strain BEA 0607 did not result in "universal four-helix model" and six helices were observed for each structure. It was noted that not all eukaryotes have the same number of helices, and only Helices II and III are well recognizable and common while Helices I and IV are known as most variable [70,71]. Helix I in the *Barranca* strains was unbranched that is considered a specific feature in all taxa of the Chaetophorales, Chaetopeltidales, and Oedogoniales [70]. It had six common conservative base pairs without any changes in both strains. Universal nucleotides determined in Helices

I–III in the Sphaeropleales, Oedogoniales, Chaetopeltidales, Chaetophorales by Caisova et al. [70] were also unchanged in both strains of *Barranca*. Basal pairings in Helix III varied between two compared strains but were unchanged in the highly conserved sequence region at the top of this helix. Though CBC approach was not able to distinguish BEA 0607 and IRK–A 341, differences in secondary structures (variable parts in all helices) support two distinct species.

B. yajiagengensis JNU39 is characterized to a lesser extent than *B. multiflagellata*. Nevertheless, the studied strain IRK–A 341 is more similar with *B. yajiagengensis* based on the description of JNU39 and its micrographs as well as 18S molecular phylogeny (42; Figure 1). The differences in the zoospore structure (a number of flagella, the sizes of zoospores), and ecology do not allow us to consider these two alga as one species. In addition, the branch of the nuclear phylogenetic tree that unites these two representatives is sufficiently supported (97) in the ML nuclear phylogenetic tree but not in Bayesian (0.87) or NJ (88) trees. ITS2 and 28S rDNA data are not available in GenBank for JNU39 to completely resolve the phylogenetic relationships between strains IRK–A 341 and JNU39.

The data obtained In this study show that algae of the genus *Barranca* are not fully characterized. For example, there is no data on the features of the structure of cell walls in known species of the genus. It does not allow us to make a conclusion about the use of this feature as a diagnostic criterion. The origin of the resting cells can be different. As mentioned above for *Barranca variabilis*, the filamentous vegetative cells are capable to form the resting (akinete-like) cells. Caisová et al. [14] described resting cells, which were formed by aplanospores of *B. multiflagellata*. In our opinion, the reproductive process of these algae, the formation of the resting cells, as well as other morphological traits still need to be analyzed in greater depth.

The soil sample from which *Barranca variabilis* IRK–A 341 was isolated was collected in June 2014. Later, the alga of similar morphology has been repeatedly found in soils at the studied area about 500 m^2 (in June 2014, July 2018, and August 2021). The same phenotype and similar conditions (soil and vegetation) allow us to suggest the same species and assume that algae of such morphology can be more widespread than is currently known. The studied strain grew on a medium containing only soil extract. Probably, the alga can grow mixotrophically. The variability of morphology of the *Barranca* thalli makes it difficult to determine them under light microscopy. Sudakova repeatedly found green filaments algae registered as *Ulotrichopsis cylindrica* Wichmann in the soils of the Baikal Region [73,74]. Currently, this alga is attributed to the class Ulvophyceae with uncertain taxonomic position [17]. *Ulotrichopsis cylindrica* resembles *Barranca* representatives in some stages of their development. Future investigations on green filaments algae in the soils of the Baikal Region are needed to expand our knowledge on these organisms.

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