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New species of *Oculatella* (*Synechococcales*, *Cyanobacteria*) from terrestrial habitats of Ukraine

Oxana VINOGRADOVA[1], Tatiana MIKHAILYUK[1], Karin GLASER[2], Andreas HOLZINGER[3], Ulf KARSTEN[2]

[1] M.G. Kholodny Institute of Botany, NAS of Ukraine
2, Tereshchenkivska St., Kyiv 01004, Ukraine
[2]University of Rostock, Institute of Biol. Sci., Department of Appl. Ecology and Phycology
3, Albert-Einstein-Strasse, Rostock D-18057, Germany
[3]University of Innsbruck, Department of Botany, Functional Plant Biology
Sternwartestrasse 15, Innsbruck A-6020, Austria

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Abstract. Here we describe two new species of *Oculatella* Zammit, Billi & Albertano from terrestrial habitats of Ukraine: *O. ucrainica* sp. nov. and *O. kazantipica* sp. nov. The strains were isolated from biological crusts collected at the Sea of Azov conqina beach, and both clay slopes and chalk outcrops in the Kharkiv Region. Five strains evaluated in this study phenotypically and phylogenetically differed both among each other and from other species of this genus. On the phylogenetic tree based on 16S *r*RNA gene sequence comparison, original strains joined already known species of *Oculatella* forming isolated lineages, one of which joined the group of drought-resistant terrestrial species (*O. ucrainica*), while another (*O. kazantipica*) grouped together with terrestrial *O. neakameniensis* Kováčik et Johansen and aquatic *O. hafneriensis* Kováčik et Johansen. The phylogeny based on the 16S *r*RNA gene concatenated with the 16S-23S ITS region, as well as secondary structures of the most informative helices of the 16S-23S ITS confirmed new species designation. Filaments of *O. ucrainica* are narrower (1.5–3.0 μm), and trichomes are wider (1.3–2.7 μm) comparing to *O. kazantipica* (its filaments are 1.3–7.5 μm wide, trichomes 1.1–1.7 μm wide). The new species also differ from one another in sheath morphogenesis, appearence of trichomes, and cell length. *Oculatella ucrainica* morphologically and phylogenetically is close to desert species *O. coburnii* Pietrasiak et Johansen, differing in the higher degree of sheath formation, wider trichomes, apical cells without irregular outgrowth, and by composition and secondary structure of 16S-23S ITS region. *O. kazantipica* is similar to *O. hafneriensis* and *O. neakameniensis*, from which it differs in more abundant sheath, false branching, granulations at cross walls, longer intercalary cells, and by composition and secondary structure of its 16S-23S ITS region.

Keywords: *Synechococcales*, *Oculatella ucrainica*, *Oculatella kazantipica*, new species, biological crusts, Ukraine, molecular sequencing, 16S *r*RNA, 16S-23S ITS, secondary structure
Supplementary Material. Electronic Supplement (Table E1, p. e1) is available in the online version of this article at: https://ukrbotj.co.ua/archive/74/6/509

Introduction

The genus *Oculatella* Zammit, Billi & Albertano, which is morphologically similar to the genus *Leptolyngbya* Anagnostidis & Komárek, was separated from the latter on the basis of differences in sequence of the 16S *r*RNA gene and the secondary structure of the 16S-23S ITS region (Zammit et al., 2012; Osorio-Santos et al., 2014). The name of the genus is due to the photosensitive reddish eyespot (oculus) at the tip of mature apical cells, clearly visible in a light microscope. Recently, we reported the discovery on the Sea of Azov coast morphotypes of thin filamentous cyanobacteria possessing mentioned autapomorph and with nucleotide sequence of the 16S *r*RNA gene completely corresponding to *Oculatella* (Mikhailyuk et al., 2016). A new detailed analysis of these strains, based on the phylogeny of the 16S *r*RNA gene concatenated with the 16S-23S ITS region, as well as secondary structures of the most informative helices of the 16S-23S ITS, showed that our strains differ from all known *Oculatella* species. Further morphological observations and molecular study of the isolated strains revealed that in the crusts of seaside habitats, in fact, there are two species, each having a number of differences from known representatives of this genus. In parallel, studying samples of cyanobacterial crusts from several areas of the cretaceous outcrops in Kharkiv Region, we also found morphotypes of *Oculatella*, which we managed to isolate into culture. It turned out that one of these strains morphologically, as well as by a number of molecular markers, completely coincides with the three strains from the seacoast.

The paper reports on two new species of *Oculatella* from the terrestrial environments of Ukraine described using combined molecular and morphological data.

Materials and methods

Isolation of the strains, culture conditions, light and transmission electron microscopy

The strains in this study were isolated from the samples of biological soil crusts collected on the coast of the Sea of Azov in Kazantip Nature Reserve (Leninsky District, the Crimea) and at the chalk outcrops in Dvorichansky District of Kharkiv Region (Table 1). Sampling and processing of collected material were described in details in our previous paper (Mikhailyuk et al., 2016).

All strains were maintained on 1N BBM (Bischoff and Bold, 1963) and BG-11 (Stanier et al., 1971) agarized media at 12 : 12 light : dark photoperiod at +20 ± 5ºC. Morphological examinations of cultures of cyanobacteria starting from 2 weeks and up to 6 months of cultivation were performed using Olympus BX51 light microscope with Nomarski DIC optics. Photomicrographs were taken from live material with digital camera Olympus UC30 attached to the microscope and processed by software cellSens Entry.

Reference cultures of newly described species (KZ-5-4-1 and KZ-19-s-2) were deposited in the culture collection of University of Göttingen, Germany (*SAG* 2563, 2567). All other *Oculatella* strains are maintained in the algal culture collection at University of Rostock, Germany. For each newly described *Oculatella* species, a herbarium accession was prepared. Young (3–4 weeks) cultures of reference strains were preserved in 4% formaldehyde in a 15 mL glass bottle. The preserved material was then deposited in the Herbarium of the M.G. Kholodny Institute of Botany, NAS of Ukraine (*KW-A* 32375-32376).

Samples were fixed for transmission electron microscopy (TEM) using a standard chemical fixation protocol (2.5% glutaraldehyde, 1% OsO_4 in 10 mM caccodylate buffer, pH 6.8) according to Holzinger et al. (2009). Samples were dehydrated in increasing ethanol concentrations, transferred to modified Spurr´s resin and heat polymerized. For TEM, ultrathin sections were prepared, counterstained with uranyl acetate and Reynold's lead citrate, and investigated by a Zeiss LIBRA 120 transmission electron microscopes at 80 kV. Images were captured with a TRS 2k SSCCD camera and further processed using Adobe Photoshop software (Adobe Systems Inc., San José, California, USA).

DNA isolation, PCR, sequencing and phylogenetic analysis

DNA of the cyanobacterial strains was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Nucleotide sequences of the 16S *r*RNA gene together with 16S-23S ITS region were amplified using Taq PCR Mastermix Kit (Qiagen GmbH) and primers SSU-4-forw and ptLSU C-D-rev (Marin et al., 2005) in a thermocycler Tgradient Thermoblock (Biometra, Germany) under the conditions described in our previous paper (Mikhailyuk et al., 2016). PCR products were cleaned using a Qiagen PCR purification kit (Qiagen GmbH) according to the manufacturer's instructions. Cleaned PCR products were sequenced commercially by Qiagen Company using primers SSU-4-forw, Wil 6, Wil 12, Wil 14, Wil 5, Wil 9, Wil 16 and ptLSU C-D-rev (Wilmotte et al., 1993; Marin et al., 2005). The resulting sequences were assembled and edited using Geneious software (version 8.1.8; Biomatters). They were deposited in GenBank under the accession numbers MG652616-MG652620.

For comparison with five original strains, we used 63 nucleotide sequences of representatives of the order *Synechococcales* available in GenBank (NCBI*). Sequence of *Oculatella hafneriensis* Kováčik et Johansen used in the study was provided by Jeffrey R. Johansen (John Carroll University, University Heights, USA) during personal communication and deposited by us in GenBank under the accession number and authorship of mentioned person. Sequence of *O. hafneriensis* previously deposited in GenBank (DQ085093) has some doubtful parts and lacking the 16S-23S ITS region.

Multiple alignment of the nucleotide sequences for phylogeny based on the 16S *r*RNA gene was made using Mafft web server (version 7, Katoh and Standley, 2013) followed by manual editing in the program BioEdit (version 7.2). Alignment for the phylogeny of the 16S-23S ITS region was performed manually in BioEdit, taking into account the secondary structure of the RNA in the region. The evolutionary model that is best suited to the used database was selected on the basis of the lowest AIC value (Akaike, 1974) calculated in MEGA (version 6, Tamura et al., 2013). Phylogenetic

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Table 1. **Sampling sites for the *Oculatella* strains reported in this study**

Strain ID	Location	GPS-coordinates	Sampling date	Site description
KZ-5-4-1	The coast of the Sea of Azov, vicinities of Kazantip Nature Reserve, spit of Aqtash Lake	45°43′85″ N 35°85′25″ E	08.08.2012	Coquina beach, 10 m from the water's edge, cyanobacterial-algal crusts with dominance of *Nostoc edaphicum* Kondrat.
KZ-7-1-4	The coast of the Sea of Azov, vicinities of Kazantip Nature Reserve, spit of Aqtash Lake	45°43′85″ N 35°85′25″ E	08.08.2012	Coquina beach, 10 m from the water's edge, cyanobacterial-algal crusts with dominance of *Nostoc edaphicum* and *Hassalia* sp.
KZ-12-1	The coast of the Sea of Azov, Kazantip Nature Reserve, Sharabay Bay	45°46′76″ N 35°84′04″ E	10.08.2012	Clay slope with sparse steppe vegetation, hypolithic under quartz fragments together with other cyanobacteria and algae
KZ-19-s-2	The coast of the Sea of Azov, Kazantip Nature Reserve, Shyrokaya Bay	45°47′04″ N 35°85′47″ E	07.08.2012	Coquina beach, cyanobacterial-algal crusts with dominance of *Microcoleus vaginatus* Gomont ex Gomont
Vin-4-4-1	Vicinities of village Petro-Ivanivka, the Verkhnia Dvorichna River right bank, steep chalk slopes, 121 m above sea level	49°55′32″ N 37°40′45″ E	28.05.2012	The middle part of the slope, growths on the soil with mosses, cyanobacterial-algal crusts with dominance of *Hassalia* sp. and *Nostoc* sp.

trees were constructed in the program MrBayes 3.2.2 (Ronquist, Huelsenbeck, 2003), using an evolutionary model GTR + G + I, with 5,000,000 generations. Two of the four runs of Markov chain Monte Carlo were made simultaneously, with the trees, taken every 500 generations. Split frequencies between runs at the end of calculations were below 0.01. The trees selected before the likelihood rate reached saturation were subsequently rejected. The reliability of tree topology verified by the maximum likelihood analysis (ML) were made using the program GARLI 2.1. Models of the secondary structure of 16S-23S ITS region of the original strains were built according to published data (Osorio-Santos et al., 2014). Helices were folded with the online software mfold (Zuker, 2003) and visualized in the online tool Pseudoviewer (Byun, Han, 2009).

Results and discussion

The study of enrichment cultures of the samples of biological crusts from the coast of the Sea of Azov and chalk outcrops in Kharkiv Region revealed that thin filaments with reddish eyespot in mature apical cells occur quite common: we found them in 57% of the samples from the sea coast and 69% from chalks. Morphological evaluation of selected original strains (Table 2) confirmed taxonomical designation into the genus *Oculatella*. Phylogenetic analysis based on 16S *r*RNA gene sequence comparison supported this matching. Our strains, morphologically attributed to genus *Oculatella*, on the phylogenetic tree joined already known species of this genus, forming an isolated clade (Fig. 1). More detailed phylogenetic analysis on the base of 16S *r*RNA gene sequence concatenated with the 16S-23S ITS region reflected differences in gene identities of the original strains. One of the seaside strains (KZ-19-s-2) grouped in subclade with *O. hafneriensis* Kováčik et Johansen and *O. neakameniensis* Kováčik et Johansen, while the other three together with "chalky" strain joined the group of drought-resistant terrestrial species of *Oculatella* inhabiting arid to semi-arid desert soils: *O. atacamensis* Osorio-Santos et Johansen, *O. mojaviensis* Pietrasiak et Johansen and *O. coburnii* Pietrasiak et Johansen (Osorio-Santos et al., 2014). The four strains (KZ-5-4-1, KZ-7-1-4, KZ-12-1 and Vin-4-4-1) represent a new species *O. ucrainica* sp. nov. (see below); the fifth strain (KZ-19-s-2) despite closeness to *O. neakameniensis* and *O. hafneriensis* formed a highly supported separate lineage which corresponded to another new species described here as *O. kazantipica* sp. nov. (see below).

Comparison of the main helices of 16S-23S ITS secondary structure of our isolates and phylogenetically close species (Fig. 3) showed general similarity of all *Oculatella* strains especially in structure of D1-D1' and Box-B helices. Our newly described species differ from close known taxa by one unique base in D1-D1' and Box-B helices (*O. ucrainica*) and by two unique bases in D1-D1' helix and one unique base in Box-B helix (*O. kazantipica*). V-3 helix is quite similar in all compared taxa, but has the most unique base composition in *O. hafneriensis* (15 unique bases). *O. neakameniensis* differs as well by unique structure

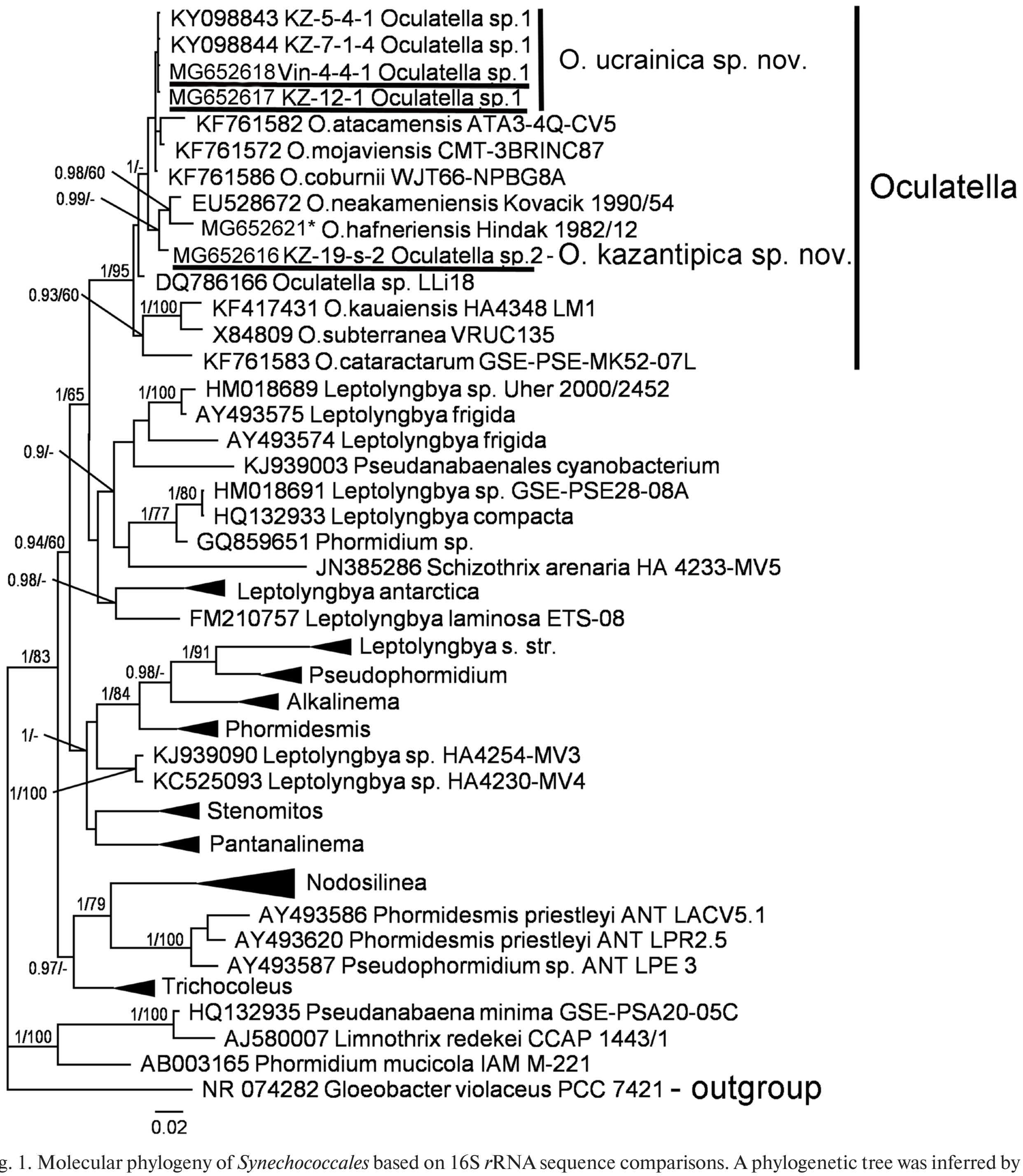

Fig. 1. Molecular phylogeny of *Synechococcales* based on 16S *r*RNA sequence comparisons. A phylogenetic tree was inferred by the Bayesian method with Bayesian Posterior Probabilities (PP) and Maximum Likelihood bootstrap support (BP) indicated at nodes. From left to right, support values correspond to Bayesian PP and Maximum Likelihood BP; BP values lower than 50% and PP lower than 0.8 not shown. Strains marked with underline are newly sequenced cyanobacteria. Clade designations follow Osorio-Santos et al., 2014 and Miscoe et al., 2016

* Sequence of *Oculatella hafneriensis* used in the study was provided by Jeffrey R. Johansen (John Carroll University, University Heights, USA) via personal communication.

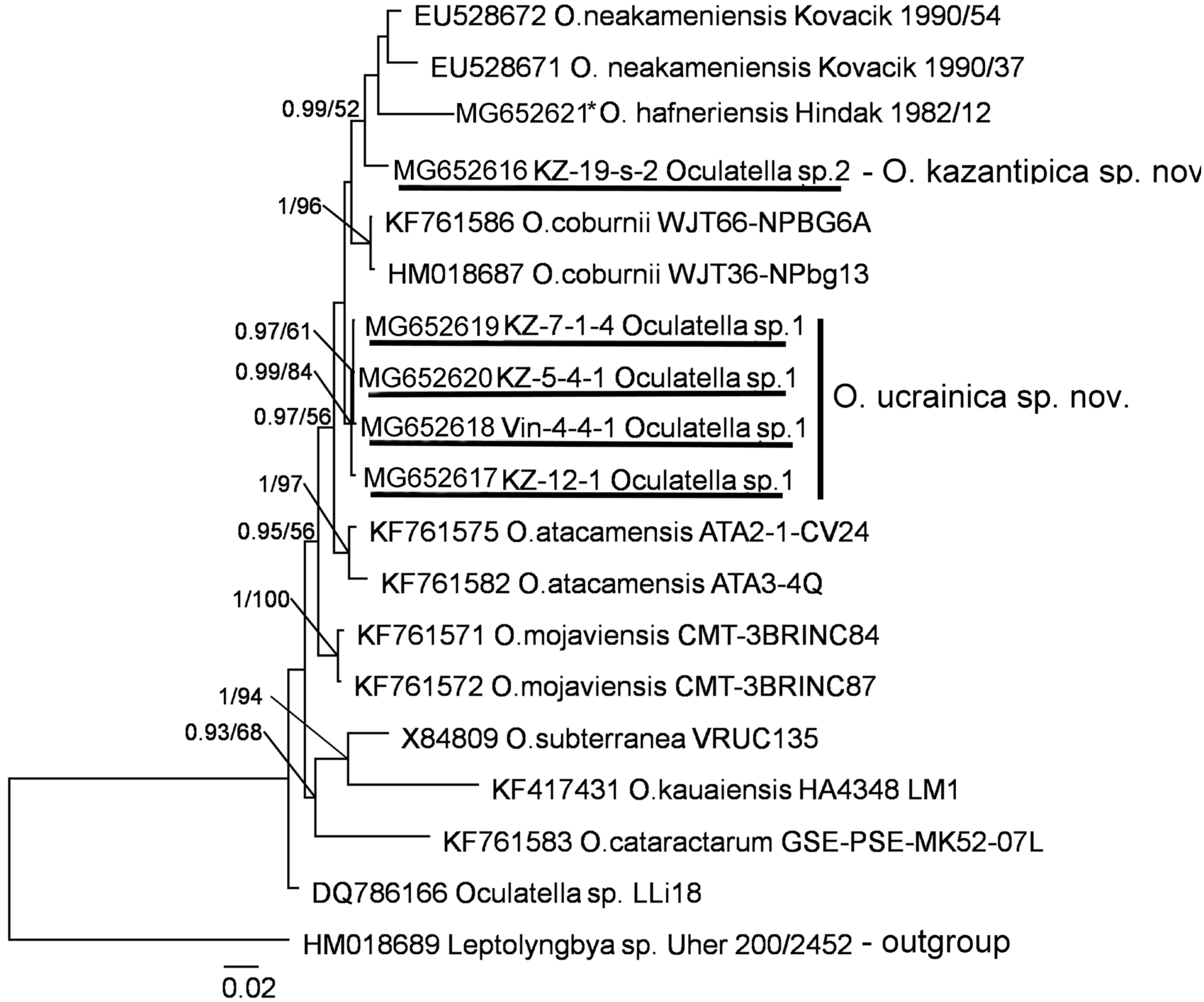

Fig. 2. Molecular phylogeny of genus *Oculatella* based on 16S-23S ITS sequence comparisons. A phylogenetic tree was inferred by the Bayesian method with Bayesian Posterior Probabilities (PP) and Maximum Likelihood bootstrap support (BP) indicated at nodes. From left to right, support values correspond to Bayesian PP and Maximum Likelihood BP; BP values lower than 50% and PP lower than 0.8 not shown. Strains marked with underline are newly sequenced cyanobacteria

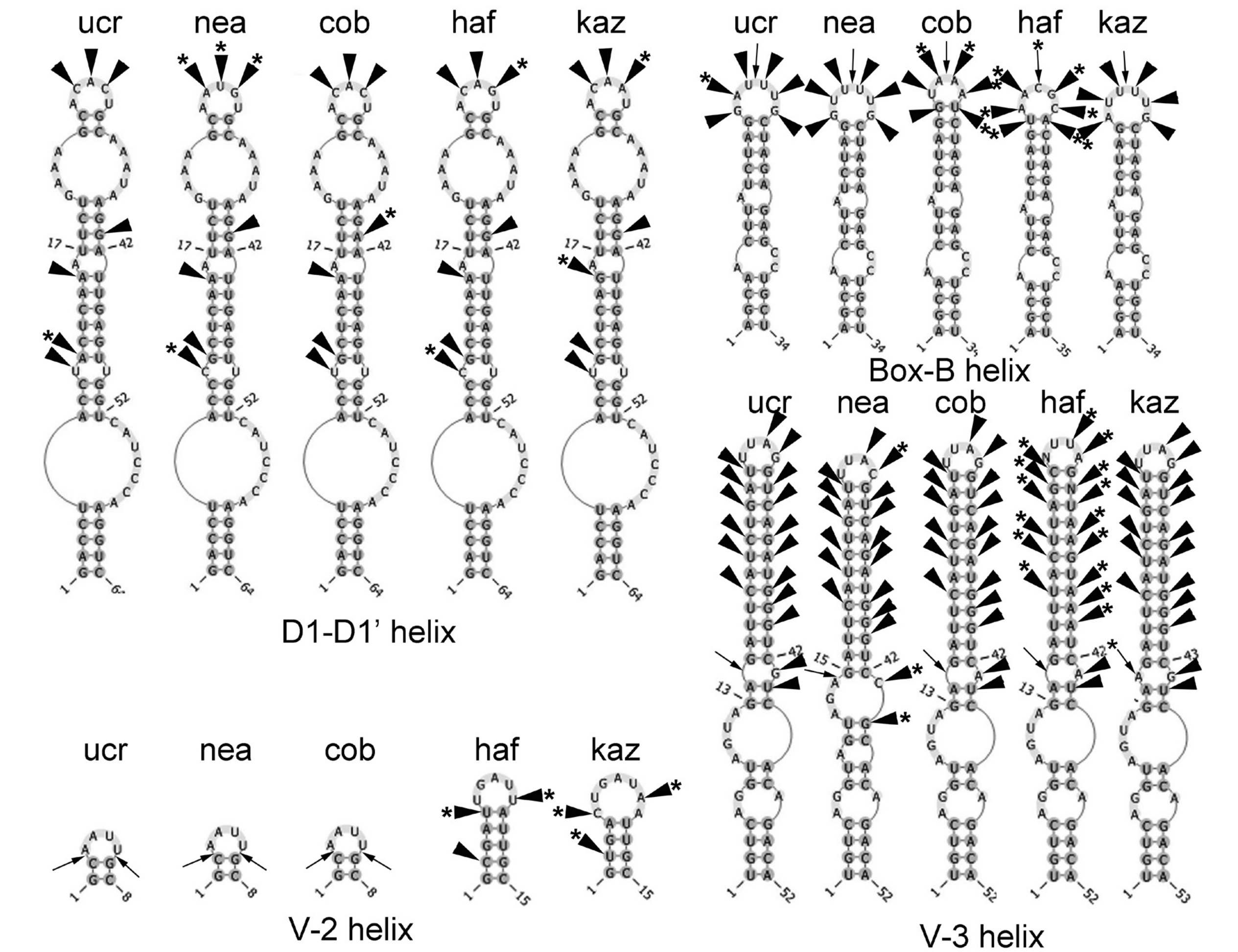

Fig. 3. Secondary structure of the main informative helices of region 16S-23S ITS of newly described species (*Oculatella ucrainica* (ucr) and *O. kazantipica* (kaz)) and comparison with the most close known species of *Oculatella* (*O. neakameniensis* (nea), *O. coburnii* (cob) and *O. hafneriensis* (haf)). Variable bases are shown with arrowheads, places of insertions/deletions of base pairs are marked with arrows, unique bases are indicated with asterisk

of V-3 helix in the base part due to one unique base difference. Our new species have V-3 helices similar to *O. coburnii*, but differed by one base in the basal loop (*O. ucrainica* and O. *kazantipica*) and by one unique insertion (O. *kazantipica*). V-2 helix of *O. ucrainica* was identical with the helices of all terrestrial species of *Oculatella*. *O. kazantipica* had V-2 helix similar to *O. hafneriensis* but differed by 3 unique bases.

Revealed morphotypes differ both among the two new species and from other species of *Oculatella* (Table 2, Figs 4, 5). As can be seen from the table and figures, the width range of the filaments in *O. kazantipica* (1.3–7.5 μm) is significantly higher than that of *O. ucrainica* (1.5–3.0 μm); it relates to the different nature of the sheath formation in these species. In old cultures of both species, the sheath became broader and stronger, but in *O. ucrainica* the extension of the filament is because the sheath somewhat retreated from the trichome, whereas in *O. kazantipica* the sheath gradually expanded, sometimes becoming lamellar. The new species also differ one another in appearance and trichome width. Trichomes of *O. ucrainica* are broader (1.3–2.7 μm), clearly constricted and rarely with granulations at cross walls. In contrast, *O. kazantipica* trichomes unconstricted or weakly constricted but usually with granulations at crosswalls; the width of the trichome (1.1–1.7 μm) is smaller, but the length of the cells (4.7–7.5 μm) exceeds that of the first species (Table 2).

From the type species *O. subterranea* Zammit, Billi et Albertano both new species differ by the blue-green

Table 2. **Morphological comparison of *Oculatella ucrainica* sp. nov. and *O. kazantipica* sp. nov. with known* *Oculatella* species****

Species	Filament width, µm	Sheath	False branching	Trichome width, µm	Constrictions	Granulations at crosswalls	Necridia	Cell length, µm	Apical cells width / length, µm	Habitat
O. atacamensis	1.8–4.1	common	rare	1.5–2.3	weak	sometimes	–	1.5–7.4	1.4–2.1 / 2.5–9.9	Soils and under quartz rocks in desert
O. coburnii	1.7–2.8	common	rare	1.4–1.8	clear	absent	–	1.8–4.8	1.4–1.8 / 2.4–5.4	Granitic soil in hot desert
O. mojaviensis	2.0–2.6	common	rare	1.6–2.2	absent/ weak	sometimes	+	1.5–5.0	1.4-2.0 / 2.4-6.8	Dolomitic soil in hot desert
O. neakameniensis	1.2–4.1	common	absent	1.2–1.7	absent/ weak	sometimes	–	1.5–5.4	1.1–1.7 / 2.3–7.7	Semi-arid volcanic soil
O. *kazantipica*	(1.3)1.5 – 5-7.5	common	rare	1.1–1.3–1.7	absent/ weak	frequently	–	(2)–2.3–4.7(7.5)	1.3–1.5 / (4)5–7(8.7)	Conquina beach
O. ucrainica	(1.5)2.5 – (3.0)	common	rare	(1.3)–1.7–2.5 (3.0)	clear	sometimes	–	(1.3)1.7–3.7(4.7)	1.3–1.7 (2.3)/(2.3) 3.3–6.7 (7.7)	Conquina beach, chalk outcrops
O. cataractarum	1.3–1.7	rare	rare	0.8–1.3–(1.7)	absent/ weak	frequently	–	(1.4)–1.6–6.8–(8.7)	/2.1–7.7–(12.8)	Dripping sandstone rocks
O. hafneriensis	1.4–2.4	common	absent	1.1–1.9	absent/ weak	sometimes	–	1.0–4.4	1.0–1.7 / 2.0–5.8	Lake benthos
O. kauaiensis	1.2–1.7	common	absent	0.9–1.4	absent/ weak	absent	–	1.0–4.4	0.9–1.5 / 1.3–7.8	Sea cave

* We did not include *O. subterranea* in the comparative table because of obvious morphological and ecological differences with our strains. ** After Osorio-Santos et al., 2014.

color, much longer intercalary cells, and the shape and dimensions of apical cells. Ukrainian species also differ from already known representatives of the genus by the appearance and width of trichomes, the length of intercalary and apical cells.

In their ecology, both new species are terrestrial xerophytes. On the marine beach, where they were initially revealed, the biota is exposed to high solar radiation and salinity of the environment; water stress is mitigated by the proximity of the sea. For chalk outcrops, where *O. ucrainica* is common, the main limiting factor is the lack of moisture. It is logical to assume that morphologically and in molecular features Ukrainian species would be closer to the "desert" group of *Oculatella* (*O. atacamensis*, *O. coburnii*, *O. mojaviensis*, *O. neakameniensis*) than to species from aquatic habitats (*O. cataractarum* Bohunická et Johansen, *O. hafneriensis*, *O. kauaiensis* Miscoe et Johansen). A comparative analysis of the main morphological features of *Oculatella* species (Table 2) shows that the width of the filaments of *O. ucrainica* is indeed similar to the "desert" and differs from "aquatic" species, but in the trichome width it exceeds all known species of genus *Oculatella*. Morphologically *O. ucrainica* is the most similar to *O. coburnii*: common features are constrictions at cross walls and length of intercalary cells. It is interesting that *O. ucrainica* represents the sister lineage to *O. coburnii* in 16S-23S ITS phylogeny (Fig. 2) and shows most similarties in the secondary structure of 16S-23S ITS region (Fig. 3). TEM investigation of *O. ucrainica* strains showed 4–6 parietal thylakoids in vegetative cells and dark granules near cross walls (Fig. 6). The ultrastructure with parietal arrangement of thylakoids are typical for both other species of *Oculatella* (Zammit et al., 2012; Osorio-Santos et al., 2014) and representatives of *Synechococcales*.

The appearance and dimensional features of *O. kazantipica* differ from other species of this genus to a lesser degree: the distinctive characters are the upper limit of the filament width, and the clearly visible granulations at cell walls; they are also present in *O. cataractarum*. Morphologically *O. kazantipica* is most similar to *O. hafneriensis* and *O. neakameniensis*:

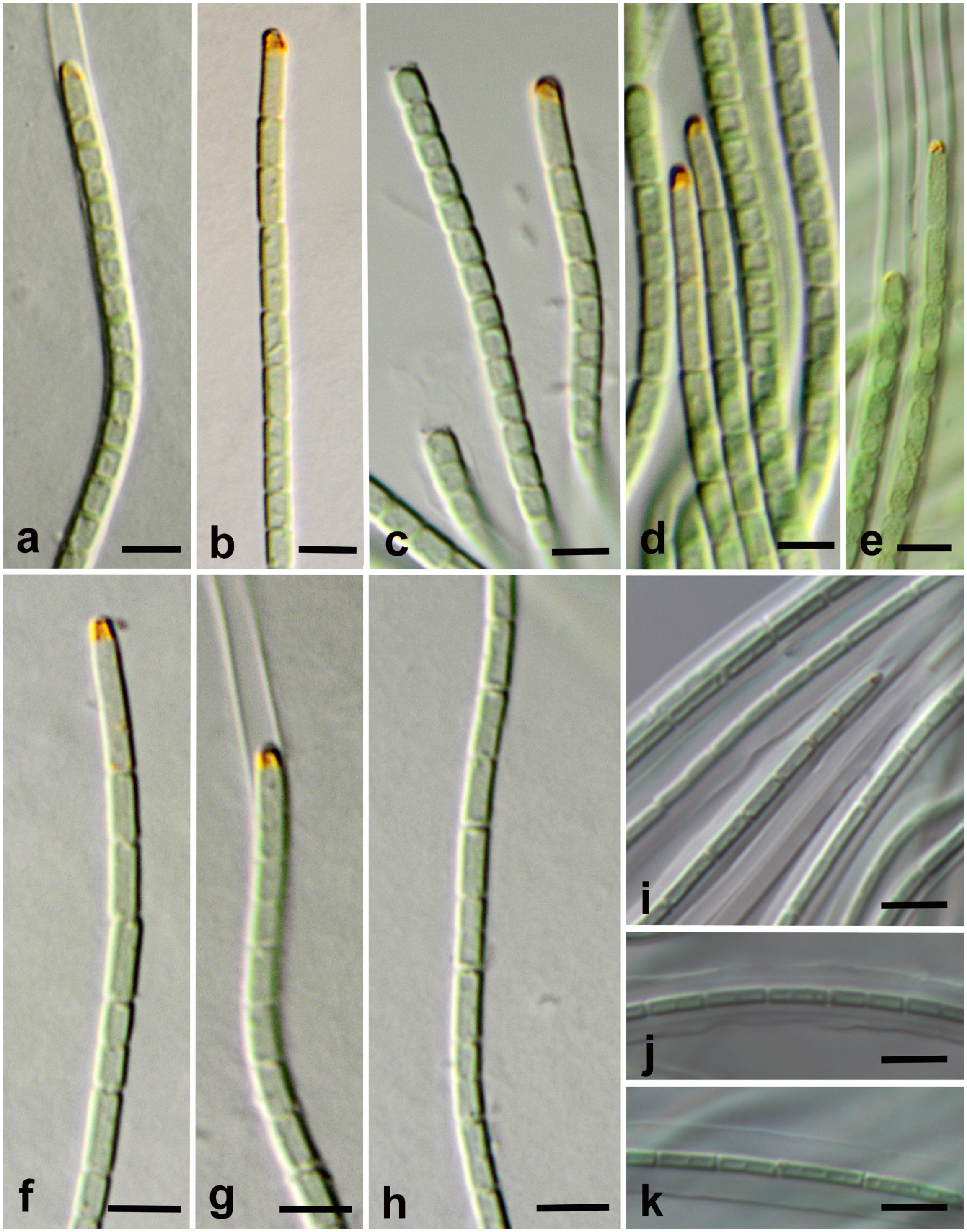

Fig. 4. Micrographs of new species of *Oculatella*; *O. ucrainica* sp. nov.: *a–d* – young filaments of strains KZ-5-4-1 (*a–c*) and KZ-12-1 (*d*) with clear photosensitive granules in terminal cells, *e* – old filament with narrow sheath (KZ-5-4-1); *O. kazantipica* sp. nov. (KZ-19-s-2): *f–h* – young filaments with clear photosensitive granules in terminal cells and narrow sheath (g), *i–k* – old filaments with wide and slightly lamellar sheaths. Scale 5 μm

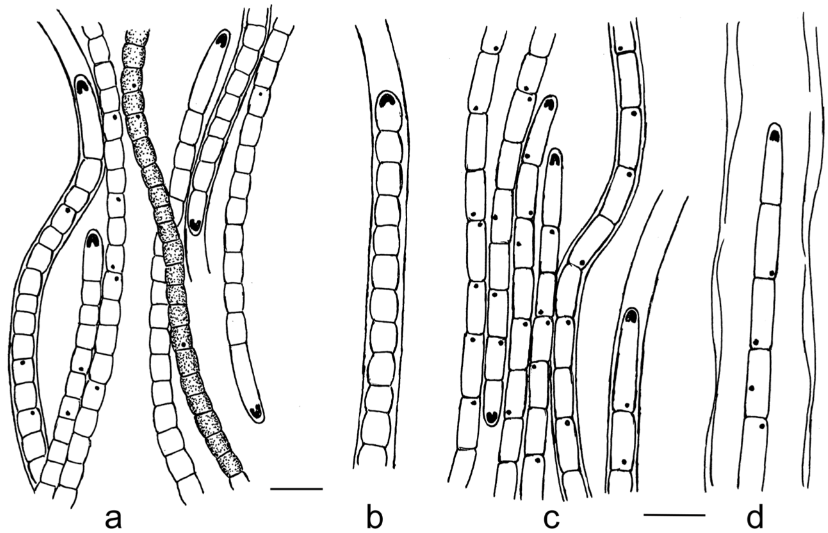

Fig. 5. Drawings of new species of *Oculatella*. *O. ucrainica* sp. nov. (KZ-5-4-1): *a* – young filaments, *b* – old filament; *O. kazantipica* sp. nov. (KZ-19-s-2): *c* – young filaments, *d* – old filament. Scale 5 μm

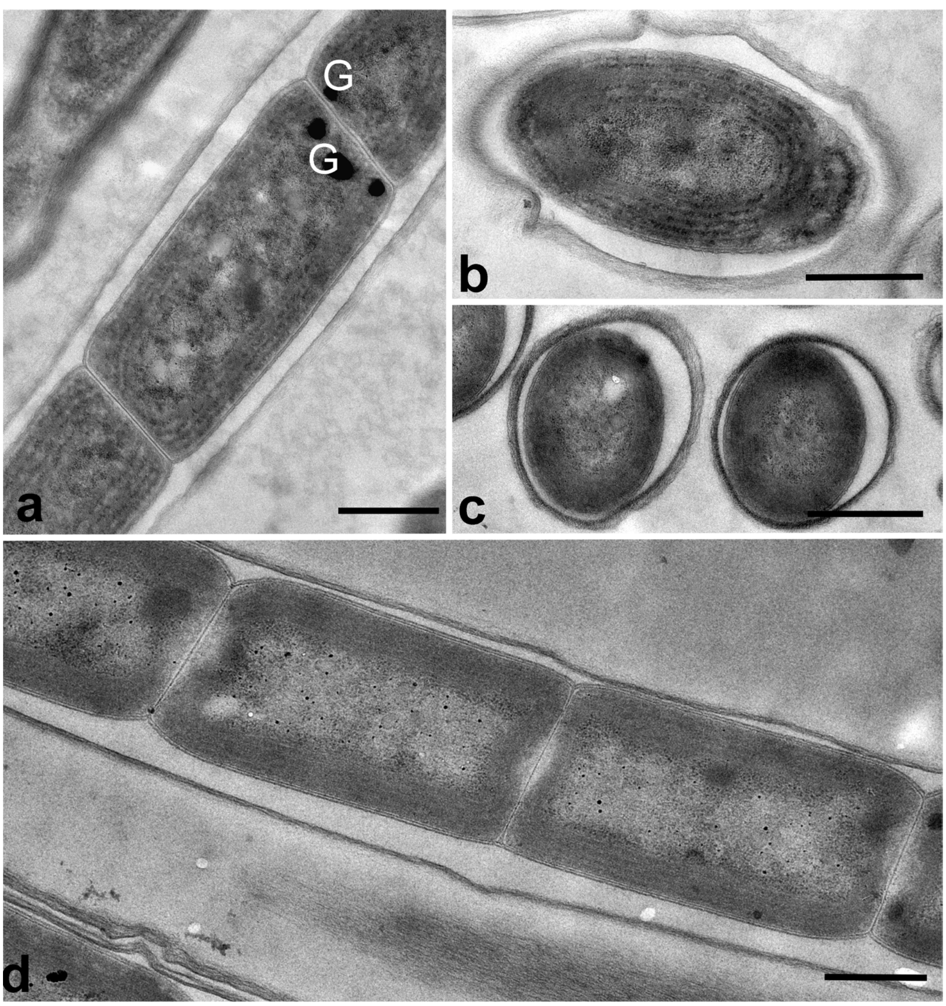

Fig. 6. Transmission electron micrographs of *Oculatella ucrainica*: *a*, *d* – longitudinal section of filaments, *b*, *c* – cross sections of filaments; *a–c* – strain KZ-5-4-1, *d* – strain KZ-12-1. Strains are characterized by parietal thylakoids and the presence of granules (marked G). Scale 1 μm

common features are trichome width, lack of constrictions at cross walls, shape and dimensions of apical cells. *O. kazantipica* phylogenetically is close to both mentioned above species (Fig. 2) and represents similar secondary structure of 16S-23S ITS region, especially close to unique V-2 helix of *O. hafneriensis* (Fig. 3). *O. neakameniensis* is a terrestrial species isolated from semiarid volcanic soil (Greece) and therefore ecologically similar to our isolate. The phylogenic position of *O. hafneriensis* between terrestrial species is surprising because this species is inhabiting fresh waters and occupies basic phylogenetic position among other aquatic representatives in Osorio-Santos et al. (2014). But *O. neakameniensis*, *O. hafneriensis* and our isolate KZ-19-s-2 are close biogeographically because were isolated in Europe and differ from other known species found from tropical islands, North and South America. *O. hafneriensis* were originally described from benthos of a temperate lake in Austria (Osorio-Santos et al., 2014).

Taxonomic descriptions

Oculatella ucrainica O.M. Vynogr. et Mikhailyuk sp. nov.

D i a g n o s i s: Morphologically and phylogenetically is the most similar to *O. coburnii*, from which differs in the higher degree of sheath formation, wider trichomes, apical cells without irregular outgrowth and by composition and secondary structure of 16S-23S ITS region.

Thallus flat, thin, spreading diffusely from the center, penetrating into the agar, blue-green. Filaments flexuous, (1.5)1.9–2.5(3.0) µm wide, mostly unbranched. Sheath very common, nearly obligate in older cultures, thin and tightly attached when young, later distinctly widened, colorless. Trichomes motile only in young state, olive-green, (1.3)1.7–2.3(2.7) µm wide, clearly constricted at cross walls (especially in young cultures) and sometimes with granules, lacking necridia, not attenuated to the end. Cells isodiametric to longer than wide, (1.3)1.7–3.7(4.7) µm, with nongranular cytoplasm and parietal thylakoids clearly visible in the light microscope. Mature apical cells bluntly conical, longer than vegetative cells, 1.3–1.7(2.3) µm wide, (2.3)3.3–6.7 (7.7) µm long, with a reddish-orange spot in the apex of the cell. D1-D1′ helix of the 16S-23S ITS region 64 nucleotides long, with a large subterminal bilateral bulge of 9 nucleotides. V2 helix with only 8 nucleotides. Box-B with 34 nucleotides, with 6 nucleotides in the terminal loop. V3 helix with 52 nucleotides, with a unilateral bulge on the 5′ side of the helix.

H a b i t a t: isolated from biological crusts dominated by *Nostoc edaphicum* and *Hassalia* sp. on the surface of coquina, clay and chalk outcrops, and hypolitically under pieces of quartz.

T y p e l o c a l i t y: The coast of the Sea of Azov, vicinities of Kazantip Nature Reserve, Aqtash Lake spit.

I c o n o t y p e: Figs 4 *a–c*, *e*, 5 *a*, *b*.

H o l o t y p e here designated: *KW-A* 32375, Herbarium of M.G. Kholodny Institute of Botany of NAS of Ukraine.

R e f e r e n c e s t r a i n: KZ-5-4-1 (*SAG* 2563).

Additional strains: KZ-7-1-4, KZ-12-1, Vin-4-4-1.

Additional sampling localities: the coast of the Sea of Azov, Kazantip Nature Reserve, Sharabay Bay. Kharkiv Region, Dvorichansky District, vicinities of Petro-Ivanivka village.

E t y m o l o g y: *ucrainica* = from Ukraine.

Oculatella kazantipica O.M. Vynogr. et Mikhailyuk sp. nov.

D i a g n o s i s: Morphologically and phylogenetically it is the most similar to O. *hafneriensis* and *O. neakameniensis*, from which differs in higher degree of sheath formation, false branching, granulations at cross walls and longer intercalary cells, and by composition and secondary structure of 16S-23S ITS region.

Thallus a thin film penetrating into the agar, spreading diffusely from the center of thallus, blue-green. Filaments weakly waved, rarely with false branching or more than one trichome in common sheath. Sheath nearly obligate, first thin and tightly attached, (1.3)1.5–1.9 µm wide, in old cultures firm, gradually expanded, sometimes lamellate, 5–7.5 µm wide, colorless. Trichomes olive-green, (1.1)1.3–1.7 µm wide, unconstricted to slightly constricted at the cross walls (especially in young cultures) often flanking with granules, lacking necridia, not attenuated to the end. Cells consistently longer than wide, with nongranular cytoplasm, with parietal thylakoids clearly visible in the light microscope, (2)2.3–4.7(5) µm long, in old cultures up to 7.5 µm long. Mature apical cells elongated-conical, longer than vegetative cells, 1.3–1.5 µm wide, (4.0)5.0–7.0(8.7) µm long, with a reddish-orange spot in the apex of the cell. D1-D1′ helix of the 16S-23S ITS region 64 nucleotides long, with a large subterminal bilateral bulge of 9 nucleotides. V2 helix 15 nucleotides long, with a terminal loop of 7 nucleotides. Box-B with 34 nucleotides, with 6 nucleotides in the terminal loop. V3 helix region with 53 nucleotides, with a unilateral bulge on the 5′ side of the helix at nucleotides.

H a b i t a t: coquina beach exposed to direct sunlight, in biological crusts with dominance of *Microcoleus vaginatus* at seaside.

T y p e l o c a l i t y: The coast of the Sea of Azov, Kazantip Nature Reserve, Shyrokaya Bay.

I c o n o t y p e: Figs 4 *f–k*, 5 *c*, *d*.

H o l o t y p e here designated: *KW-A* 32376, Herbarium of M.G. Kholodny Institute of Botany of NAS of Ukraine.

R e f e r e n c e s t r a i n: KZ-19-s-2 (*SAG* 2567).

E t y m o l o g y: *kazantipica* = from the Cape Kazantip.

In contrast to previous studies, which reported *Oculatella* as a rare genus (Osorio-Santos et al., 2014), we found this genus in over half of our soil crust samples from the Sea of Azov coast and chalk outcrops in Kharkiv Region. Recent studies suggest that *Oculatella* might be even more widely distributed as it was found in biological soil crust in Iran (Dulić et al., 2017). From the isolated strains, we described two new species after detailed investigations of their morphology, phylogeny, and ultrastructure. Both, the morphological and phylogenetic characterstics, indicate that the new strains represent new species. This is strengthen by the ecology and biogeography of the new strains, both different to the previously describted *Oculatella* species. It is interesting that type populations of these new species were found on small territory of Kazantip Nature Reserve and vicinities similar to the earlier described species *O. coburnii* and *O. mojaviensis* found also on a limited territory, in desert soils of California, USA. Moreover, the terrestrial species *O. kazantipica* is morphologically and genetically close to the freshwater European taxon *O. hafneriensis*.

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Виноградова О.М.[1], Михайлюк Т.І.[1], Глазер К.[2], Хольцингер А.[3], Карстен У.[2] **Нові види роду *Oculatella* (*Synechococcales*, *Cyanobacteria*) з наземних місцезростань України.** Укр. бот. журн., 2017, 74(6): 509–520.

[1] Інститут ботаніки ім. М.Г. Холодного НАН України вул. Терещенківська, 2, Київ 01004, Україна

[2] Університет м. Росток, Інститут біологічних наук Альберт Ейнштейн штрассе, 3, Росток D-18057, Німеччина

[3] Університет м. Інсбрук, кафедра ботаніки Штернвартештрассе, 15, Інсбрук A-6020, Австрія

Описані нові для науки види з роду *Oculatella* Zammit, Billi & Albertano: *O. ucrainica* sp. nov. та *O. kazantipica* sp. nov., виділені з біологічних кірочок, відібраних на ракушняковому пляжі біля Азовського моря (АР Крим), а також на крейдяних та глинистих схилах у Харківській області. Комплексне дослідження п'яти отриманих штамів *Oculatella* показало, що фенотипично та філогенетично вони відрізняються від усіх відомих видів цього роду. На філогенетичному дереві, побудованому на основі порівняння послідовностей генів 16S *p*РНК, штами нових видів приєднались до вже відомих видів *Oculatella*, які утворювали окремі лінії, при цьому один з них (*O. ucrainica*) увійшов до групи засухостійких наземних видів, а інший (*O. kazantipica*) потрапив до однієї клади з терестріальним видом *O. neakameniensis* Kováčik et Johansen та озерним *O. hafneriensis* Kováčik & Johansen. Філогенетичний аналіз за ділянкою 16S *p*РНК, зв'язаною з регіоном 16S-23S ITS, а також вторинні структури найінформативніших хеліксів 16S-23S ITS підтвердили виділення нових видів, які морфологічно також чітко відрізняються. Нитки *O. ucrainica* вужчі (1,5–3,0 мкм), а трихоми ширші (1,3–2,7 мкм), ніж у *O. kazantipica* (нитки 1,3–7,5 мкм шир., трихоми 1,1–1,7 мкм шир.), є відмінності у морфогенезі піхов, перетягнутості трихомів та довжині клітин. *Oculatella ucrainica* найбільш подібний до пустельного виду *O. coburnii* Pietrasiak & Johansen, від якого відрізняється інтенсивністю формування піхов, ширшими трихомами, відсутністю неправильного виросту на кінцевих клітинах, а також конфігурацією вторинної структури регіону 16S-23S ITS. *Oculatella kazantipica* близька до *O. hafneriensis* та *O. neakameniensis*, від яких відрізняється за морфологією піхов, наявністю несправжнього галуження, грануляціями біля поперечних перегородок, більшою довжиною інтеркалярних клітин, та деталями будови вторинної структури регіону 16S-23S ITS.

Ключові слова: *Synechococcales*, *Oculatella ucrainica*, *O. kazantipica*, нові види, біологічні корочки, Україна, молекулярна філогенія, 16S *p*РНК, 16S-23S ITS, вторинна структура

Виноградова О.Н.[1], Михайлюк Т.И.[1], Глазер К.[2], Хольцингер А.[3], Карстен У.[2] **Новые виды рода *Oculatella* (*Synechococcales*, *Cyanobacteria*) из наземных местообитаний Украины**. Укр. бот. журн., 2017, 74(6): 509–520.

[1] Институт ботаники им. Н.Г. Холодного НАН Украины ул. Терещенковская, 2, Киев 01004, Украина

[2] Университет г. Росток, Институт биологических наук Альберт Эйнштейн штрассе, 3, Росток D-18057, Германия

[3] Университет г. Инсбрук, кафедра ботаники Штернвартештрассе, 15, Инсбрук A-6020, Австрия

Описаны новые для науки виды из рода *Oculatella* Zammit, Billi & Albertano: *O. ucrainica* sp. nov. и *O. kazantipica* sp. nov., выделенные из биологических корочек, отобранных на ракушечниковом пляже у Азовского моря (АР Крым), а также на меловых и глинистых склонах в Харьковской обл. Комплексное исследование пяти полученных штаммов *Oculatella* показало, что фенотипически и филогенетически они отличаются от всех известных видов этого рода. На филогенетическом дереве, построенном на основе сравнения последовательностей генов 16S *p*РНК, штаммы новых видов присоединялись к уже известным видам *Oculatella*, образующим отдельные линии, при этом один из них (*O. ucrainica*) вошел в группу засухоустойчивых наземных видов, а другой (*O. kazantipica*) попал в одну кладу с наземным видом *O. neakameniensis* Kováčik et Johansen и водным *O. hafneriensis* Kováčik & Johansen. Филогенетический анализ по участку 16S *p*РНК, связанному с регионом 16S-23S ITS, а также вторичные структуры наиболее информативных хеликсов 16S-23S ITS подтвердили выделение новых видов, которые морфологически также хорошо различимы между собой. Нити *O. ucrainica* ýже (1,5–3,0 мкм), а трихомы шире (1,3–2,7 μm), чем у *O. kazantipica* (нити 1,3–7,5 мкм шир., трихомы 1,1–1,7 мкм шир.), отличия также касаются морфогенеза влагалища, перешнурованности трихомов и длины клеток. *Oculatella ucrainica* наиболее сходен с пустынным видом *O. coburnii* Pietrasiak & Johansen, от которого отличается интенсивностью формирования влагалища, более широкими трихомами, отсутствием неправильного выроста на конечных клетках, а также конфигурацией вторичной структуры региона 16S-23S ITS. *Oculatella kazantipica* близка к *O. hafneriensis* и *O. neakameniensis*, от которых отличается морфологией влагалища, наличием ложного ветвления, грануляциями у поперечных перегородок, большей длиной интеркалярных клеток и деталями строения вторичной структуры региона 16S-23S ITS.

Ключевые слова: *Synechococcales*, *Oculatella ucrainica*, *O. kazantipica*, новые виды, биологические корочки, Украина, молекулярная филогения, 16S *p*РНК, 16S-23S ITS, вторичная структура

Table E1. **List of species and strains used for the 16S rRNA and 16S-23S ITS sequence comparisons**

Species	Strain	Accession number[1]	
		16S rRNA	16S-23S ITS region
Oculatella ucrainica sp. nov.	KZ-5-4-1, SAG 2563	KY098843	**MG652620**
Oculatella ucrainica sp. nov.	KZ-7-1-4	KY098844	**MG652619**
Oculatella ucrainica sp. nov.	Vin-4-4-1	**MG652618**	
*Oculatella ucrainica*sp. nov.	KZ-12-1	**MG652617**	
Oculatella kazantipica sp. nov.	KZ-19-s-2, SAG 2567	**MG652616**	
Oculatella hafneriensis	Hindak 1982/12	MG652621[2]	
Oculatella atacamensis	ATA3-4Q-CV5	KF761582	
Oculatella atacamensis	ATA2-1-CV24	–	KF761575
Oculatella mojaviensis	CMT-3BRINC87	KF761572	
Oculatella mojaviensis	CMT-3BRINC84	–	KF761571
Oculatella coburnii	WJT66-NPBG6A	KF761586	
Oculatella coburnii	WJT36-NPbg13	–	HM018687
Oculatella neakameniensis	Kovacik 1990/54	EU528672	
Oculatella neakameniensis	Kovacik 1990/37	–	EU528671
Oculatella sp.	LLi18	DQ786166	
Oculatella kauaiensis	HA4348 LM1	KF417431	
Oculatella subterranea	VRUC135	X84809	
Oculatella cataractarum	GSE-PSE-MK52-07L	KF761583	
Leptolyngbya sp.	Uher 2000/2452	HM018689	
Leptolyngbya frigida	ANT.L52.2	AY493575	–
Leptolyngbya frigida	ANT.L70.1	AY493574	–
Pseudanabaenales cyanobacterium	WJT40-NPBG3	KJ939003	–
Leptolyngbya sp.	GSE-PSE28-08A	HM018691	–
Leptolyngbya compacta	GSE-PSE28-08A	HQ132933	–
Phormidium sp.	PMC301.07	GQ859651	–
Schizothrix arenaria	HA4233-MV5	JN385286	–
Leptolyngbya antarctica	ANT.LG2.5	AY493603	–
Leptolyngbya antarctica	ANT.L18.1	AY493607	–
Leptolyngbya antarctica	ANT.L67.1	AY493572	–
Leptolyngbya laminosa	ETS-08	FM210757	–
Leptolyngbya tenerrima	AF218368	UTCC 77	–
Leptolyngbya boryana	PCC 6306	EF429289	–
Leptolyngbya boryana	CCAP 1446/2	HF678483	–
Leptolyngbya foveolarum	VP1-08	FR798945	–
Leptolyngbya sp.	HA4236-MV8	KJ939018	–
Pseudophormidium sp.	WJT71-NPBG25	KJ939062	–
Pseudophormidium sp.	ATA5-5-1-KO9	KC311902	–
Alkaline mapantanalense	CENA531	KF246497	–
Alkaline mapantanalense	CENA530	KF246496	–
Phormidesmis priestleyi	ANT.L66.1	AY493581	–
Phormidesmis priestleyi	ANT.LG2.4	AY493580	–
Phormidesmis sp.	WJT36-NPBG15	KJ939033	–
Leptolyngbya sp.	HA4254-MV3	KJ939090	–
Leptolyngbya sp.	HA4230-MV4	KC525093	–
Leptolyngbya frigida	ANT.L52B.3	AY493612	–
Leptolyngbya frigida	ANT.L64B.1	AY493577	–
Leptolyngbya tenuis	PMC304.07	GQ859652	–
Pseudanabaena tremula	UTCC 471	AF218371	–
Pantanaline marosaneae	CENA539	KF246503	–
Pantanaline marosaneae	CENA521	KF246488	–
Nodosilinea epilithica	Kovacik 1998/7	HM018677	–
Nodosilinea epilithica	Kovacik 1990/52	HM018679	–
Leptolyngbya margaretheana	1T12	FR798934	–
Nodosilinea nodulosa	UTEX 2910	KF307598	–
Nodosilinea conica	SEV4-5-c1	EU528667	–
Nodosilinea sp.	Prim-5-5	KY098847	–
Nodosilinea bijugata	Kovacik 1986/5a	EU528669	–
Oscillatoria neglecta	AM M-82	AB003168	–
Leptolyngbya subtilissima	EcFYyyy700	KC463197	–
Phormidesmis priestleyi	ANT.LACV5.1	AY493586	–
Phormidesmis priestleyi	ANT.LPR2.5	AY493620	–
Pseudophormidium sp.	ANT.LPE.3	AY493587	–
Trichocoleus desertorum	ATA4-8-CV2	KF307604	–
Trichocoleus badius	CRS-1	EF429297	–
Pseudanabaena minima	GSE-PSE20-05C	HQ132935	–
Limnothrix redekei	CCAP 1443/1	AJ580007	–
Phormidium mucicola	IAM M-221	AB003165	–
Gloeobacter violaceus	PCC 7421	NR074282	–

[1]Accession numbers marked with Bold are newly sequenced cyanobacteria.
[2] Sequence of *Oculatella hafneriensis* used in the study was provided by Jeffrey R. Johansen (John Carroll University, University Heights, USA) via personal communication and submitted by us to NCBI.