Kamptonema (*Microcoleaceae*, *Cyanobacteria*), a new genus derived from the polyphyletic *Phormidium* on the basis of combined molecular and cytomorphological markers

***Kamptonema* (*Microcoleaceae*, *Cyanobacteria*), nový rod odvozený z polyfyletického rodu *Phormidium* podle kombinovaných molekulárních a cytomorfologických znaků**

Otakar S t r u n e c k ý[1,2], Jiří K o m á r e k[1,2] & Jan Š m a r d a[3]

[1]*Institute of Botany, Academy of Science of the Czech Republic, Třeboň, Dukelská 135, CZ-37982 Třeboň, Czech Republic, e-mail: otakar.strunecky@gmail.com, jiri.komarek@ibot.cas.cz;* [2]*Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05, České Budějovice, Czech Republic;* [3]*Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, CZ-625 00 Brno-Bohunice, Czech Republic, e-mail: jsmarda@med.muni.cz*

Strunecký O., Komárek J. & Šmarda J. (2014): *Kamptonema* (*Microcoleaceae*, *Cyanobacteria*), a new genus derived from the polyphyletic *Phormidium* on the basis of combined molecular and cytomorphological markers. – Preslia 86: 193–207.

Based on strains obtained from various regions and selected from the CCALA collection (Institute of Botany AS CR) in Třeboň, a special clade of the order *Oscillatoriales* (*Cyanobacteria*), was identified and defined during a taxonomic revision of filamentous cyanobacteria. This study involved combined molecular, cytomorphological and ecological analysis. The new cluster, evaluated as a new genus, *Kamptonema*, is based on a clonal population from thermal waters in Dax, France, and corresponds to the type species, originally described as "*Oscillatoria animalis*" Agardh 1927 from thermal springs in Karlovy Vary, Czech Republic. Members of this new generic unit occur in various freshwater habitats throughout the world and play an important role (production of biomass) in central European aquatic ecosystems, e.g. in many periodical puddles and pools. The genus *Kamptonema*, with type species *K. animale* (Agardh 1927) comb. nova, is characterized by its separate position in a phylogenetic tree, its relatively thin unbranched filaments, characteristic cellular ultrastructure and lack of sheaths, heterocytes and akinetes. The type species is ecologically distinct, occurring in thermal springs. The necessary taxonomic and nomenclatoric transfers of related taxa are included in this article.

K e y w o r d s: cyanobacteria, cyanophytes, ecology, molecular sequencing, morphology, *Oscillatoriales*, taxonomy

Introduction

Our view of cyanobacterial diversity changed after the 16S rDNA based molecular approach to cyanobacterial taxonomy was introduced particularly after the 1980. Numerous newly described taxonomic units were determined by various taxonomists leading to reevaluations and taxonomic revisions of many species and genera. The most recent revision of the *Oscillatoriales* using the polyphasic approach was compiled by Komárek & Anagnostidis (2005), however, the amount of genetic data available, when this comprehensive work was produced, was not good enough for a complete resolution of genera and species of simple filamentous cyanobacteria.

As the main morphological and taxonomic markers of species belonging to the genus *Phormidium* were used the shapes of cells and filaments, types of end cells and facultative presence of sheaths. Initially, 29 species were described for the genus *Phormidium* (Gomont 1892, starting point for this genus), whereas there are 85 species in Geitler's monograph (1932) and more than 260 taxonomic names in the list of *Phormidium*-species by Drouet (1968). Komárek & Anagnostidis (2005) present 163 validly described species, of which many, based on modern criteria, are reclassified and relocated to *Phormidium* from the old traditional genera *Oscillatoria* and *Lyngbya*, mostly because of the structure of their cells and trichomes. However, the genus *Phormidium* Kützing ex Gomont 1892 is confirmed by recent molecular analyses to be polyphyletic (Taton et al. 2006, Palinska & Marquardt 2008, Strunecký et al. 2010, 2011, and others). Geitler's (1932, 1942) description of the type species of *Phormidium*, *Ph. lucidum*, which follows the original descriptions of Agardh (1827, sub *Oscillatoria lucida*), Kützing (1843) and Gomont (1892), is based on an atmophytic mat collected on the walls of thermal springs in Carlsbad (Karlovy Vary, Czech Republic). Komárek & Anagnostidis (2005) emphasize the thermal biotope as one of diacritical features of *Ph. lucidum*, which belongs to group VIII in their classification. The characterization of this cluster, which is considered as typical *Phormidium*, was confirmed by Moro et al. (2010) and Sciuto et al. (2012).

Several other *Phormidium* clusters have been separated and identified using the detailed polyphasic approach, e.g. *Phormidesmis* (Komárek et al. 2009), *Wilmottia* (Strunecký et al. 2011), *Oxynema* (Chatchawan et al. 2012) and *Roseofilum* (Casamatta et al. 2012), or transferred to other genera (the *Phormidium autumnale*-group to the genus *Microcoleus;* Strunecký et al. 2013). There is another cyanobacterial type, traditionally classified in the genus *Phormidium*, *Ph. animale* (*Oscillatoria animalis* Agardh 1827), originally described also from the thermal springs at Carlsbad (Karlovy Vary), Czech Republic, but very different from typical *Phormidium lucidum* according to morphological and molecular criteria. *Phormidium animale* and several related species morphologically resemble "*Ph. autumnale*" type, but contain morphotypes with narrower filaments, mostly lacking sheaths and with reduced or no calyptra; the ends are usually bent, the terminal cells are slightly narrowed and rounded. They form fine and delicate sheaths facultatively, only under specific conditions.

We studied five morphologically similar strains of this group (supported by molecular sequencing) and compared them with descriptions in the literature. Based on polyphasic evaluation, which combines genotypic (16S rRNA gene sequencing) and phenotypic (morphological analysis, ultrastructural sections) methods, a special cluster was defined, which must be separated from the traditional genus *Phormidium* (cf. Geitler 1932, 1942, Anagnostidis & Komárek 1988, Komárek & Anagnostidis 2005) and from populations representing the typical *Phormidium*-species, *Ph. lucidum* (Geitler 1942) and *Ph. irriguum* (cf. Sciuto et al. 2012). This cluster is classified here as a new genus, *Kamptonema* gen. novum. This taxonomic transfer is supported by molecular analyses of many other authors (Boyer et al. 2002, Casamatta et al. 2005, Taton et al. 2006, Comte et al. 2007, Lokmer 2007, Heath et al. 2010, Strunecký et al. 2010).

Material and methods

We carried out a detailed examination of five strains (Table 1) mostly obtained from the Culture Collection of Autotrophic Microorganisms (CCALA), Třeboň, Czech Republic and one strain INDIA 92, isolated and cultivated by the authors. The geographic origin of the strains varied; three were from European localities, one from South America and one from the Indian subcontinent. The corresponding typical strain was isolated from thermal springs, others from shallow pools and wet soil habitats, sometimes at localities where conductivity is high.

Strain morphology was studied at up to a 1000× magnification using an Olympus BC51 light microscope. Photomicrographs were taken using an Olympus DP71 Camera, equipped with Quick Photo Micro software. The length and width of at least 50 cells of each strain were measured for morphological evaluation. Our strains correspond morphologically exactly with the descriptions of the traditional species *Phormidium animale* ([Agardh 1827] ex Gomont 1892) Anagnostidis et Komárek 1988 (see Komárek & Anagnostidis 2005).

Table 1. – List of strains studied named according to determinations in original collections (see Fig. 2).

Strain	Name	Collection location	
CCALA761	*Phormidium* cf. *animale*	Czech Republic, Třeboň, Institute of Botany, basin with *Elodea*, periphyton	Kašpárková 1999/4
CCALA771	*Phormidium* sp.	Brasilia, São Paulo, Botanical garden, greenhouse	Komárek 2005/1
CCALA138	*Geitlerinema* sp.	United Kingdom, London, University College	Growther/1459-6
CCALA139	*Phormidium animale*	France, Dax, thermal water	Lefèvre 1956/M97-2b
INDIA92	*Phormidium animale*	India, village Samsing, road pool (N27 01 E 088 47)	Bernardová 2009

Molecular and phylogenetic analysis

DNA from unialgal strains was extracted using the modified method of Yilmaz et al. (2009). The available cultivated cells were suspended in 50 µl of TE buffer at pH 7.4, and 750 µl of XS buffer (1% potassium ethyl xanthogenate; 100 mM Tris-HCl, pH 7.4; 20 mM EDTA, pH 8; 800 mM amonium acetate and 1% SDS) in an Eppendorf tube with glass beads. The trichomes were manually crushed using a micro-pestle. Tubes were incubated for 2 h at 70 °C. After incubation, the tubes were vortexed for 30 s and frozen at –70 °C for 30 min. The sample was thawed and shaken for 15 min, centrifuged for 20 min at 15 000g and the supernatant transferred to a clean micro-centrifuge tube. The DNA was precipitated over night in a 2:3 volume of 100% ethanol with the addition of a 1:20 volume of sodium acetate (3 M, pH 5.2) followed by centrifugation for 30 min at 15,000g. The supernatant was discarded and the pellet was washed with 100 µl of 70% ethanol followed by centrifugation for 15 min. After discarding the supernatant, the pellet was dried and dissolved in 100 µl of miliQ water.

The 16S rRNA gene with the 16S-23S intergenetic segment was amplified using the primers 359F (GGGGAATYTTCCGCAATGGG) (Nübel et al. 1997) and 23S30R (CTTCGCCTCTGTGTGCCTAGGT) (Wilmotte et al. 1993) with the following settings: a starting denaturalization step (94 °C, 5 min); 40 cycles of 30 s at 94 °C, 30 s at 53 °C and 3 minutes at 72 °C; final extension for 7 minutes at 72 °C and cooling to 4 °C. A successful PCR was confirmed by running a subsample on a 1.5% agarose gel stained with ethidium bromide. PCR products were purified using a QIAquick PCR Purification Kit. Sequencing of the 16S rRNA gene fragment was performed on an ABI 3100 sequencer, using BD3.1 (Applied Biosystems, Foster City, USA) chemistry, with six primers (27F, 23S30R, CYA_810R- GTTATGGTCCAGCAAAGCGCCTTCGCCA, CYA783F-TGGGATTAG-ATACCCCAGTAGTC (Strunecký et al. 2010), S17*-GGCTACCTTGTTACGAC and ILE23F- ATTAGCTCAGGTGGTTAG (Wilmotte & Herdman 2001) to obtain complementary sequences.

Sequences for phylogenetic comparison were chosen after extensive evaluation of more than 1300 sequences of *Oscillatoriales* cyanobacteria available in GenBank (www.ncbi.nlm.nih.gov) and the Ribosomal database project (rdp.cme.msu.edu) (unpublished). Two outgroups were used in the construction of the 16S rDNA phylogenetical tree: *Escherichia coli* ATCC 11775 and *Nostocales* from various genera. These sequences were aligned in MAFFT (mafft.cbrc.jp) (Katoh & Toh 2010). Minor changes were done manually using BioEdit 7.0.1 (Hall 1999). A fragment of 1089 nt was used for the phylogenetic analysis of 16S rDNA (starting at *E. coli* ATCC 11775 16S rRNA residues 302).

To allocate the *Kamptonema* cluster to *Oscillatoriales*, the Maximum Likelihood method based on the Tamura 3-parameter model in MEGA 5 (Tamura et al. 2007) was employed, topology was validated using the Bayesian analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) and unweighted maximum parsimony (MP) was implemented in PAUP* (Swofford 2003). For the Bayesian analysis, two runs of four Markov chains of over 15 000 000 generations, sampling every 1000 generations, were employed. The initial 25% of the generations were discarded as burn-in.

Ultrastructure

Filaments of strains CCALA 761 and CCALA 771 were prepared for electron microscopy in two ways: (a) fixed by placing them for 3 hours at 4°C in an osmium solution (standard bacteriological fixative sec. Kellenberger, slightly modified), which consisted of 1% (w/v) osmium tetroxide in 0.7% or 1.4% (w/v) veronal-acetate buffer, pH 6.5, with traces of sodium chloride and calcium chloride, or (b) fixed for 3 hours at 4 °C in glutaraldehyde: 3% (w/v) glutaraldehyde in 100 mM cacodylate buffer, pH 7.3, followed by post fixation in 1% (w/v) osmium tetroxide in the same buffer for 2 hours, again at 4 °C.

Following fixation, the material was washed with the respective buffer overnight at 4°C. Thereafter, it was instilled in 2% (w/v) agar and, within it, dehydrated by a series of ethanol at gradually increasing concentrations from 50% to 80% (w/v) for 20 min each, then at 96% (w/v) for 40 min and, finally, at 100% (w/v) for 45 min. The material was then infiltrated with metacrylate LR White, first in 1:1 ethanol for 30 min, then in 2:1 ethanol for 60 min, 3:1 ethanol for 2 hours and, finally, in pure LR White at 4°C for 3 days. Thereafter, it was encapsulated and polymerized using UV light at 4°C, again for 3 days. Then ultrathin sections of the material were cut using a Reichert-Jung ultramicrotome Ultracut E.

All the sections were placed on supporting grids and contrasted using 2.5% (w/v) uranyl acetate and alkaline Reynolds solution (3%, w/v, lead nitrate with 3%, w/v, sodium citrate).

The sections were photographed at various magnifications using a digital transmission electron microscope (FEI Morgagni 268D).

Results

Genotypic characters

The 16S rDNA gene sequences of our strains were BLASTed against GenBank, which revealed a more than 97% match with the uncultured bacterium clone GBII (Severin & Stal 2010) from the Netherlands, uncultured cyanobacterium clone B107211D (Schmidt et al. 2010) from Alaska, 5 strains of *Phormidium formosum* isolated by Hašler et al. (2012) and *Phormidium animale* SAG 1459-6, which is identical to "*Geitlerinema*" sp. CCALA 138. The "whole genome sequencing consortium GEBA-Cyano (http://genome.jgi.doe.gov /programs/bacteria-archaea/GEBA-Cyano.jsf)" sequenced as "*Oscillatoria*" sp. PCC 6506 and assigned to *Oscillatoria* Cluster IV (according to Rippka & Herdman 1992), was also identical with the *Kamptonema* cluster.

The strains identified using 16S rRNA gene sequences form a compact and separate cluster (Fig. 1). The similarity of 16S rDNA within this cluster was always higher than 97% (Table 2). Because it differs from other cyanobacterial clades by more than 5% (Table 3) and is definable cytomorphologically, it is designated as a separate generic taxon *Kamptonema* (strains in Table 2). The exact P distance is 5.01% from *Microcoleus*, represented by *Microcoleus vaginatus* FGP2, and 6.6% from *Oscillatoria*, represented by *O. sancta* SAG 7479 (CCALA 135). The nucleotide distance from other genera, such as *Phormidium*, *Wilmottia*, *Coleofasciculus*, *Oxynema*, *Oscillatoria* and *Planktothrix*, is always greater than 7%. The phylogenetic analysis indicates that *Kamptonema* is well separated from the nearest clusters represented by strains labelled as belonging to the genera *Microcoleus*, *Trichodesmium*, *Lyngbya*, *Geitlerinema* and *Arthrospira.*

Phenotypic characters

All strains included in the *Kamptonema* cluster have a similar morphology, which differs from that of typical *Phormidium* (based on the type species *P. lucidum* and *Ph. irriguum*; cf. Sciuto et al. 2012) and *Microcoleus* (based on *M. vaginatus*; cf. Strunecký et al. 2013), and this difference can be used to characterize this taxon morphologically at the generic level (Fig. 2).

Filaments are solitary or in colonies and almost always grow without sheaths. Sometimes there are several or many parallel arranged trichomes, which lack common firm sheaths. Disintegrating fascicles are not enveloped in slime. Trichomes are simple, cylindrical along their whole length (up to the end), only usually slightly narrowing towards the ends, uniseriate, pale blue-green, principally slightly constricted at the cross-walls, uniformly 3–5 μm wide, sometimes bent and rounded at the apex, commonly without calyptra. Cells are mostly ± isodiametric or slightly longer or shorter than wide. End cells are rounded or slightly conical; 2–3 end cells show bending or folding around the longitudinal

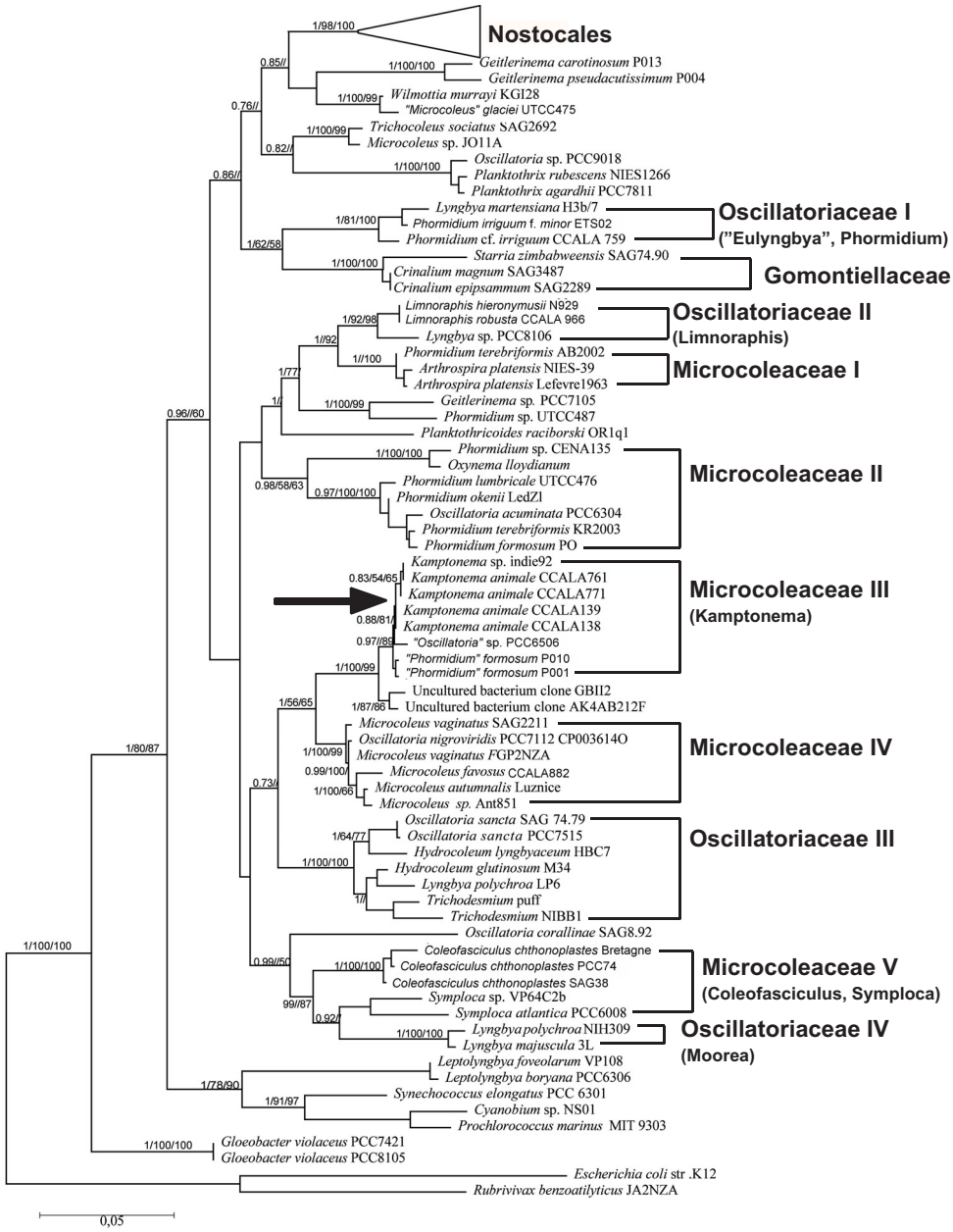

Fig. 1. – Phylogenetic relationships of the 16S rRNA gene within the Oscillatoriales, showing the relationship of the genus *Kamptonema* within the order Oscillatoriales. This was done using the Maximum Likelihood (ML) method based on the Tamura 3-parameter model in MEGA 5. Topology was validated by Bayesian analysis in MrBayes 3.1.2 and unweighted maximum parsimony (MP) was implemented in PAUP*. Values indicate Bayesian posterior probabilities/MP/ML; values lower than 50% are not shown. A fragment of 1089 nt was used for the phylogenetic analysis of the 16S rRNA gene.

axis; this bending is distinct especially during the trichome movement. Trichomes are highly motile with gliding motility. All cells are capable of division (without meristematic zones). There were no typical necridic cells (important difference from *Phormidium autumnale* = *Microcoleus* type), however, fragmentation follows the formation of ± short segments of dying cells. The cell content looks heterogenous, sometimes with distinct peripheral or irregular chromatoplasma (agglomeration of thylakoids). Solitary granules are present in the cells (Fig. 2).

Table 2. – Proportional similarity matrix (based on P-distance) of partial 16S rDNA gene sequences within the cluster of *Kamptonema* strains.

		1	2	3	4	5	6	7	8
1	*Kamptonema* sp. CCALA139		1.00	1.00	0.99	1.00	0.97	0.98	0.99
2	*Kamptonema* sp. CCALA771	1.00		1.00	0.99	1.00	0.98	0.98	0.99
3	*Kamptonema* sp. CCALA138	1.00	1.00		0.99	1.00	0.97	0.98	0.99
4	*Kamptonema* sp. PCC6506	0.99	0.99	0.99		0.99	0.97	0.97	0.99
5	*Kamptonema formosum* P010	1.00	1.00	1.00	0.99		0.97	0.98	1.00
6	Uncultured cyanobacterium clone GBII2	0.97	0.98	0.97	0.97	0.97		0.99	0.97
7	Uncultured cyanobacterium clone AK4AB212F	0.98	0.98	0.98	0.97	0.98	0.99		0.97
8	*Kamptonema formosum* P001	0.99	0.99	0.99	0.99	1.00	0.97	0.97	

Table 3. – Similarity of 16S rDNA of *Kamptonema* with that of similar strains of other related clusters (genera).

		1	2	3	4	5	6	7	8	9	10	11
1	*Kamptonema* sp. CCALA139		0.92	0.95	0.92	0.93	0.93	0.93	0.91	0.90	0.92	0.90
2	*Phormidium irriguum* f. *minor* ETS02	0.92		0.91	0.91	0.91	0.92	0.90	0.89	0.88	0.89	0.89
3	*Microcoleus vaginatus* FGP2	0.95	0.91		0.90	0.93	0.93	0.91	0.91	0.91	0.92	0.91
4	*Phormidium tergestinum* CCALA155	0.92	0.91	0.90		0.90	0.92	0.93	0.89	0.88	0.90	0.88
5	*Oscillatoria sancta* CCALA135 (= SAG7479)	0.93	0.91	0.93	0.90		0.91	0.92	0.91	0.89	0.91	0.89
6	*Wilmottia murrayi* CCALA843	0.93	0.92	0.93	0.92	0.91		0.93	0.89	0.91	0.93	0.91
7	*Microcoleus chthonoplastes* SAG38	0.93	0.90	0.91	0.93	0.92	0.93		0.91	0.90	0.93	0.90
8	*Oxynema thaianum* CCALA960	0.91	0.89	0.91	0.89	0.91	0.89	0.91		0.89	0.90	0.89
9	*Oscillatoria* sp. PCC9018	0.90	0.88	0.91	0.88	0.89	0.91	0.90	0.89		0.89	0.99
10	*Symploca* sp. PCC8002	0.92	0.89	0.92	0.90	0.91	0.93	0.93	0.90	0.89		0.89
11	*Planktothrix agardhii* PCC7811	0.90	0.89	0.91	0.88	0.89	0.91	0.90	0.89	0.99	0.89	

Ultrastructure

The thylakoidal system is organized more or less parietally and, in material from well grown cultures, occupies more than 50% of the inner volume of most cells. Thick, wavy bundles are formed by four to 22 ± parallel arranged thylakoids (Fig. 3C, D, Fig. 4B–D). Usually, thylakoids are aggregated along the inner side of cell walls, often forming irregular waves and circles, and also in the central part of cells (Fig. 3C, D, Fig. 4). However, a minority of the cells differ in slightly modified ultrastructure, depending on cultivation conditions. Their thylakoid structure changes (checked also by optical microscopy) especially after being kept in the dark for a few days. Thalykoids keep their parietal location, but the circular structure of their bundles becomes wavier and extends into the

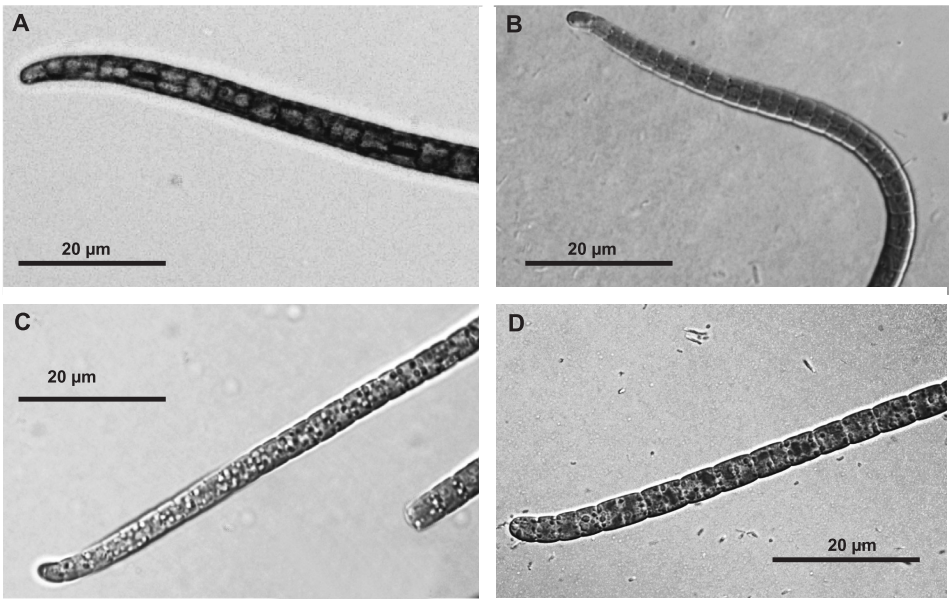

Fig. 2. – Microphotographs of four revised strains of *Kamptonema*: A – CCALA 761, B – CCALA 771, C – CCALA 138, D – CCALA 139.

centroplasm (Fig. 4). Thylakoids show a limited tendency to keritomy (organization of thylakoids) and some thylakoids are transformed into bizarre, thick and "prickly" membranes.

The solitary irregular granular inclusions consist of polyphosphates, cyanophycin and carboxysomes (Fig. 3C–F, Fig. 4A, B). Cyanophycin granules are of various sizes (up to very large) and forms, and are scattered in any cell compartment, just like the mostly smaller, round and rare carboxysomes. Those of cyanophycin show a tendency to be located mainly along the cross walls (Fig. 3E–F). Cross walls growing centripetally first push away and later break through the thylakoid bundles (Fig. 4C); at the same time they become wider and straighter. Flower-bud-like membranous corpuscules appear in the chromatoplasm. In the nucleoplasm there are often membrane-covered vesicles filled with structured contents. Similar, but superficial membrane lacking vesicles are found more or less regularly surrounded by large cyanophycin granules.

Ecology and distribution

The strains of the *Kamptonema* type studied are generally of freshwater origin. The ecological characters of the strains, where the type of environment before isolation is known, are recorded in Table 1: CCALA 139 – thermal water, CCALA 771 – greenhouse, CCALA 761 – shallow basin with *Elodea canadensis* and used for cultivation for water plants. The strain INDIA92 originated from puddles on a road in a subtropical area of India. The strains cultivated by Hašler et al. (2012) and identified as *Phormidium formosum* PO001–PO010, were collected from eutrophic fishponds with large bird colonies in various parts of the Czech Republic. The other sequences within this cluster are

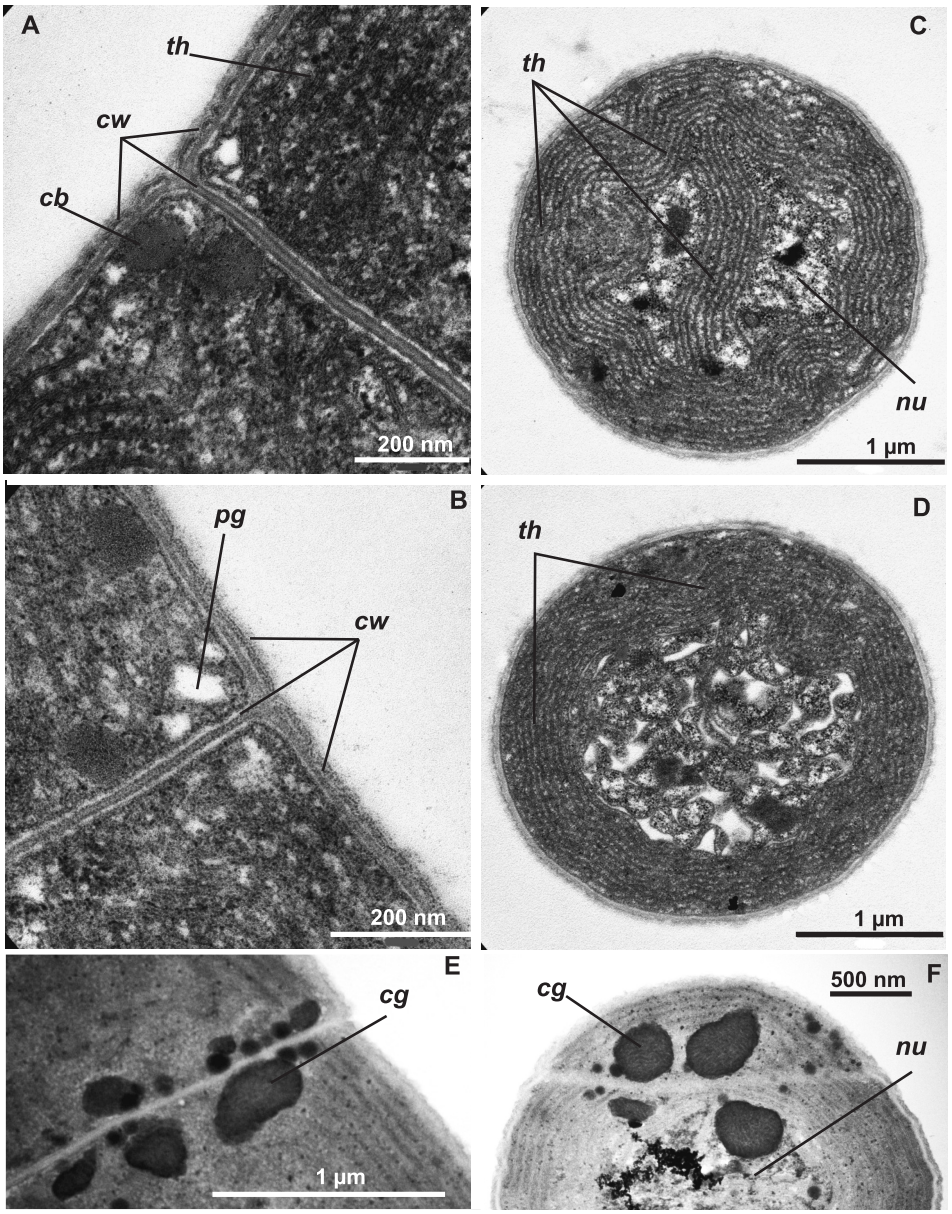

Fig. 3. – Ultrastructure of strains of *Kamptonema*: A, B, E – longitudinal sections with position of thylakoids (th), details of cell walls (cw), carboxysomes (cb), and polyphosphate (pg) and cyanophycin (cg) granules; C, D, F – cross sections with the same parameters, nucleoplasm (nu); A, D, E – strain CCALA 771, B – strain CCALA 761; C, F – strain CCALA 139.

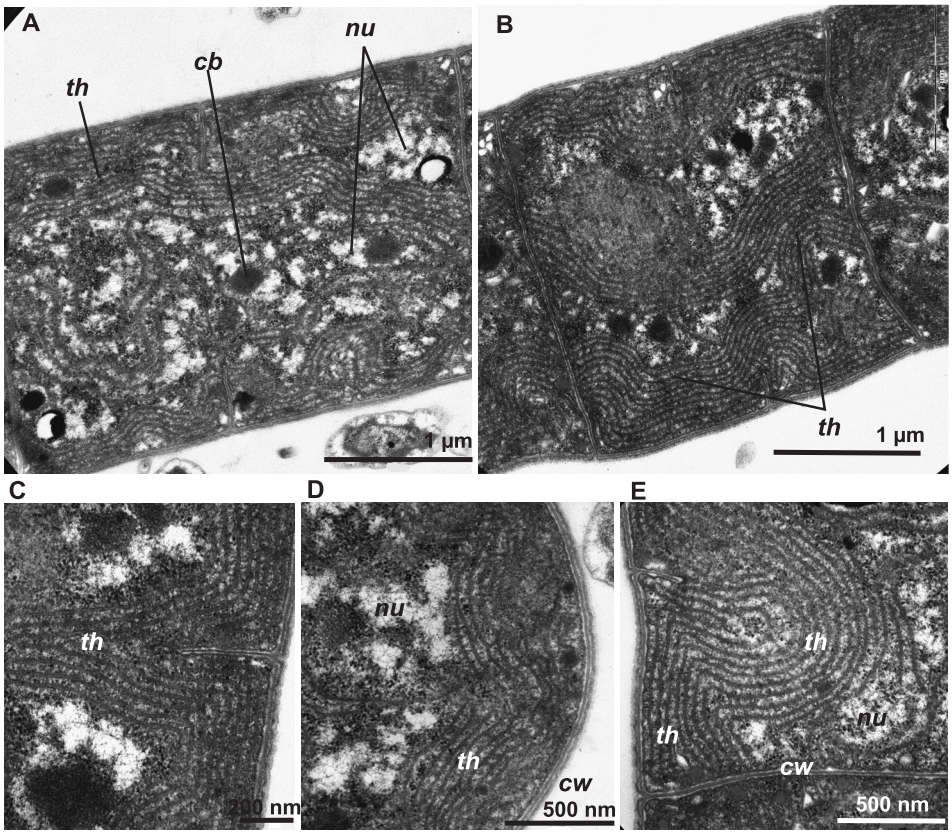

Fig. 4. – Ultrastructure of strains of *Kamptonema*, characteristic position of thylakoids (th), ribosomes are visible in cells as black points: A, D – strain CCALA 761; B, C, E – strain CCALA 771.

represented by "uncultured bacterium clone AK4AB2_12F" and "uncultured bacterium clone GBII-2", which originated from recently deglaciated soil near the Mendenhall Glacier in Alaska (S. Schmidt, pers. comm.) and sand dunes on an island in the North Sea close to the Dutch coastline, respectively. The geographic origin of the typical *Kamptonema* strains therefore varies and, in particular, those in the cluster containing the CCALA strains were collected in Europe, South America and Indian subcontinent. The genus *Kamptonema*, therefore, has a worldwide distribution, but the different strains (possible species) have distinct ecological requirements and can be considered to be different specific taxa. This is confirmed by the results of the genetic analyses.

Kamptonema is widely distributed in the Czech Republic. The type species came from thermal waters at Karlovy Vary, but is also common in shallow pools in field roads, in periodically flooded inundation areas along rivers, on the bottom of shallow pools, ponds, etc. This is particularly the case for the species *K. animale* and *K. formosum.*

Formal description of the genus

From the genetic, morphological and cytological (ultrastructural) analyses, following the criteria of modern cyanobacterial taxonomy, it follows that the strains studied belong to a special genus. The formal taxonomic description is as follows:

Kamptonema gen. novum

D i a g n o s i s: Filaments solitary without sheaths, or with very fine, facultative and diffluent sheaths, sometimes with several to many trichomes associated more or less in parallel without common firm sheaths. The fascicles are not enveloped in slime. Trichomes simple, cylindrical along their whole length (up to the end), uniseriate, pale blue-green, slightly constricted or unconstricted at cross-walls, motile, (2.5) 3–5 (5.3) μm wide. Cells isodiametric or slightly longer or shorter than wide. End cells rounded, bent and hooked without calyptra, 2–3 end cells usually bent or folded from the longitudinal axis, with distinct movement. All cells are capable of division (without meristematic zones). The cell content is heterogenous, sometimes with a slightly more distinct peripheral chromatoplasma. Arrangement of thylakoids in cells is parietal or semiparietal with a circular pattern containing bundles of thylakoids, crossing the interior of cells. Solitary granules of polyphosphate and cyanophycin are present in cells. Comparison of 16S rDNA revealed it is phylogenetically similar (95%) to the *Microcoleus vaginatus* cluster, whereas its similarity when compared with all the other orders of cyanobacteria is under 93% (Table 3).

T y p e s p e c i e s: *Kamptonema animale* (Agardh ex Gomont) comb. nova; type strain CCALA 139, collected by Lefèvre 1956 from Dax; thermal spring, France (locus classicus), deposited in CCALA 139 (= SAG72.79).

The nomenclatoric transfers to the genus *Kamptonema* concern the following taxa (traditional morphospecies with their predominant ecology – E):

Kamptonema animale (Agardh ex Gomont) comb. nova
Basionym: *Oscillatoria animalis* Agardh, Flora 10(1:40): 632, 1827, ex Gomont, Ann. Sci. nat. 7. Bot. 16: 221, 1892
E: warm, thermal and sulphur springs, wet soils, rarely in puddles, in stagnant cold water, walls of greenhouses, widely distributed in temperate and tropical zones, possibly cosmopolitan

Kamptonema pavlovskoense (Elenkin) comb. nova
Basionym: *Phormidium pavlovskoense* Elenkin, Monogr. Alg. Cyanoph. Aquidulc. Terr. URSS, Fasc. 2, 1501–1502, 1949
E: periphytic in stagnant and flowing waters; several localities in E Europe and temperate Asia

Kamptonema cortianum (Meneghini ex Gomont) comb. nova
Basionym: *Oscillatoria cortiana* Meneghini, Consp. Algol. Eugan., p. 326, 1937 ex Gomont Ann. Sci. nat. 7. Bot. 16: 231, 1892
E: in thermal and mineral springs

Kamptonema okenii (Agardh ex Gomont) comb. nova
Basionym: *Oscillatoria okenii* Agardh, Flora 10(1:40): 633, 1827; ex Gomont, Ann. Sci. nat. 7. Bot. 16: 232, 1892
E: mineral and sulphur springs, rocky pools, ditches and rice fields (also in sewage), with high conductivity and usually alkaline water; distributed worldwide, cosmopolitan?

Kamptonema laetevirens (Crouan ex Gomont) comb. nova
Basionym: *Oscillatoria laetevirens* Crouan, Fl. Finistère p. 112, 1867; ex Gomont, Ann. Sci. nat. 7. Bot. 16: 226, 1892
E: salty and sulphur thermal springs, widely distributed in Europe

Kamptonema gebhardtianum (Claus) comb. nova
Basionym: *Oscillatoria gebhardtiana* Claus, Hydrobiologia 19(2): 199, 1962
E: described from a calcareous substrate (Abaliget Caves, Hungary)

Kamptonema chlorinum (Kützing ex Gomont) comb. nova
Basionym: *Oscillatoria chlorina* Kützing, Phycol. Gener., p. 185, 1843; ex Gomont, Ann. Sci. nat. 7. Bot. 16: 223, 1892
E: benthic on organic mud, in sulphuretes, sometimes also in slightly salty water; recorded from many localities throughout the world, probably cosmopolitan

Kamptonema jasorvense (Vouk) comb. nova
Basionym: *Oscillatoria jasorvensis* Vouk, Jugosl. Akad. Prirod. Istr. Hrvatske i Slovenie, Mat. Prir. Razr. 14: 128, 1919
E: thermal springs in Slovenia, recorded from similar localities in Greece, Libya and the Himalayan range (India), rarely recorded from the Czech Republic

Kamptonema formosum (Bory ex Gomont) comb. nova
Basionym: *Oscillatoria formosa* Bory, Dict. Class. d´Hist. Nat. 12: 474, 1827; ex Gomont, Ann. Sci. nat. 7. Bot. 16: 230, 1892
E: wet soils, shallow pools and ponds, probably cosmopolitan

Kamptonema proteus (Skuja) comb. nova
Basionym: *Oscillatoria proteus* Skuja, N. Acta Reg. Soc. Sci. Upsal., ser. 4, 14(5): 48, 1949
E: benthos and free floating in stagnant water, tropical species

Discussion

Recent molecular analyses, primarily of the 16S rRNA gene, have helped us to define various groups of oscillatorialean species, which can also be separated by morphological features. We have gathered the strains originally described as *Ph. animale*, *Ph. formosum*, *Phormidium* sp. (sine typo) and *Geitlerinema* sp. (sine typo) to form a special phylogenetic cluster designated as *Kamptonema* genus novum. Both morphologically and genetically identical strains were recently cultivated and described also by Hašler et al. (2012) as *Ph. formosum*. The genus *Kamptonema* is definable by both morphological and genetic (16S rDNA gene sequencing) characteristics, which are distinctly different from those of other oscillatorialean genera and thus they are separable from the traditional genus *Phormidium.* The whole sequence of the genome of *Oscillatoria* sp. PCC 6506, assigned to *Oscillatoria* Cluster 4 (Rippka & Herdman 1992), has identical genetic (based on 16S rDNA) and morphological characteristics to those of other strains of *Kamptonema*.

The modern taxonomy and reclassification of cyanobacteria is based on a "polyphasic approach". Numerous traditional genera appear to be heterogeneous and need be placed in different taxonomic clusters. This is true also of the genus *Phormidium*, in which several groups are already separated and reorganized, e.g. *Oxynema* (Chatchawan et al. 2012), *Roseofilum* (Casamatta et al. 2012), *Phormidesmis* (Komárek et al. 2009) and the group of *Phormidium autumnale*, which was transferred into the genus *Microcoleus* (Strunecký et

al. 2013), etc. Certain phenotypic features are recognizable in the morphologically relatively simple traditional genus *Phormidium*, but these were usually neglected or considered as variable and not important for characterization of taxa at the supraspecific level. In the case of *Kamptonema*, all the strains and populations of this group are in one phylogenetic cluster and are morphologically unified by certain phenotypic markers, which are described in detail in the taxonomic diagnosis. Other species of *Phormidium* possibly also belong in the genus *Kamptonema*, but this needs to be studied in detail in the future. Only the species that corresponded exactly morphologically with this new unit were transferred to the genus *Kamptonema*. Several other described species belong evidently to this genus, but their position should be confirmed by future studies and molecular analyses. These are e.g. *Phormidium boryanum*, *Ph. chalybaeum*, *Ph. crassivaginatum*, *Ph. deflexoides*, *Ph. dudicsianum*, *Ph. insigne*, *Ph. kolkwitzii*, *Ph. pseudocortianum*, *Ph. pseudopriestleyi*, *Ph. subuliforme* (marine), *Ph. tanganyikae*, *Ph. terebriforme*, *Ph. viscosum* etc.

Up to now, the morphological characters of all the revised taxonomic units (genera) correspond with units, determined by generic position. For example, the related genera *Microcoleus*, *Geitlerinema*, *Phormidesmis*, *Leptolyngbya*, *Wilmottia* and *Trichodesmium* are well characterized by morphological markers. The autapomorphic character of the "cluster *Microcoleaceae* III" in our phylogenetic tree (*Kamptonema*, Fig. 1) is clearly defined.

The genus *Kamptonema* clearly belongs (according to its phylogenetic position and morphology) to the family *Microcoleaceae*, as a related cluster to the genus *Microcoleus* (IV). It was originally classified as *Phormidium* in the family *Phormidiaceae*. However, a recent study indicates that the type of the genus *Phormidium* belongs in the family *Oscillatoriaceae* and therefore the genus *Phormidium* sensu stricto also belongs to this family (related to *Oscillatoria* and *Lyngbya*). The family *Phormidiaceae* in its original concept must be rejected.

The autapomorphic characters of *Kamptonema* are as follows: solitary filaments or filaments aggregated in irregular clusters, monoseriate, simple, usually without sheaths, motile, cylindrical, at the end (last few cells) slightly narrowed and bent, end cells rounded. Heterocytes and akinetes are lacking. The genus *Kamptonema* does not have sheaths, or they are very facultative. The majority of species of *Kamptonema* were described, therefore, as members of the genus *Oscillatoria*. The absence of sheaths is possibly connected with the main biotope of species of *Kamptonema*. Strains of species in this cluster were originally collected from submerged colonies in warm microenvironments; the original *Ph. animale* Agardh 1842 was described from thermal waters in Carlsbad (Karlovy Vary, Czech Republic). The genus *Kamptonema* should be placed in the family *Microcoleaceae*, which consists of both freshwater and soil types (like *M. vaginatus*). Keritomy (net-like structure) of the protoplast of cells, which results from the arrangement of the thylakoids in cells, is very characteristic of many species of *Kamptonema*.

Acknowledgements

The work was funded by grants GACR P506/12/1818 and RVO67985939. The access to computing and storage facilities owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum, which was provided under the programme Projects of Large Infrastructure for Research, Development and Innovations (LM2010005), was greatly appreciated. The authors are indebted to Dana Švehlová and Jana Šnokhousová for

technical help, to Dobromila Klemová (laboratory preparations) and Ladislav Ilkovics (TEM) for their excellent technical assistance with the electron microscopy, Keith Edwards for language correction and Tony Dixon for editing the English of the final manuscript.

Souhrn

V rámci modernizace cyanobakteriálního systému dochází k četným změnám v jednotlivých řádech sinic. V řádu *Oscillatoriales* byl rozdělen zejména rod *Phormidium*, který představuje podle kombinovaných, molekulárních, morfologických, ultrastrukturálních a ekologických kritérií několik fylogenetických linií, hodnocených na úrovni rodů. Na základě těchto analýz byl oddělen a definován rovněž nový rod *Kamptonema*, odpovídající podle posledních taxonomických kritérií skupině II z rodu *Phormidium* (podle Komárek & Anagnostidis 2005). Tento rod má velice charakteristickou morfologii a představuje jednotnou skupinu i z ekologického hlediska. U starých autorů byly tyto druhy původně řazeny do rodu *Oscillatoria* kvůli absenci pochev. Podle nového systému musí být rod *Kamptonema* zařazen do čeledě *Microcoleaceae*, revidované po zrušení čeledě *Phormidiaceae* (typický rod *Phormidium* patří geneticky do okruhu rodů *Oscillatoria* a *Lyngbya*). Do rodu *Kamptonema* byly převedeny všechny nejblíže příbuzné druhy odpovídající revidované rodové diagnóze. Typem rodu *Kamptonema* je *K. animale.*

References

Agardh C. A. (1827): Aufzählung einiger in den österreichischen Ländern gefundenen neuen Gattungen und Arten von Algen, nebst ihrer Diagnostik und beigefügten Bemerkungen. – Flora 10: 625–640.

Anagnostidis K. & Komárek J. (1988): Modern approach to the classification system of cyanophytes 3. *Oscillatoriales*. – Algol. Stud. 50–53: 327–472.

Boyer S. L., Johansen J. R., Flechtner V. R. & Howard G. L.(2002): Phylogeny and genetic variance in terrestrial *Microcoleus* (*Cyanophyceae*) species based on sequence analysis of the 16s rRNA gene and associated 16s-23s ITS region. – J. Phycol. 38:1222–1235.

Casamatta D. A., Johansen J. R., Vis M. L. & Broadwater S. T. (2005): Molecular and morphological characterization of ten polar and near-polar strains within the *Oscillatoriales* (*Cyanobacteria*). – J. Phycol. 41:421–38.

Casamatta D., Stanić D., Gantar M. & Richardson L. L. (2012): Characterization of *Roseofilum reptotaenium* (*Oscillatoriales, Cyanobacteria*) gen. et sp. nov. isolated from Caribbean black band disease. – Phycologia 51: 489–499.

Chatchawan T., Komárek J., Strunecký O., Šmarda J. & Peerapornpisal Y. (2012): *Oxynema,* a new genus separated from the genus *Phormidium* (*Cyanophyta*). – Cryptog. Algol. 33: 41–59.

Comte K., Šabacká M., Carre-Mlouka A., Elster J. & Komárek J. (2007): Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. – FEMS Microbiol. Ecol. 59: 366–376.

Drouet F. (1968): Revision of the classification of the *Oscillatoriaceae.* – Acad. Nat. Sci. Philadelphia, Fulton Press, Pensylvania.

Geitler L. (1932): *Cyanophyceae*. – In: Rabenhorst's Kryptogamenflora von Deutschland, Österreich und der Schweiz, Akad. Verlagsges., Leipzig.

Geitler L. (1942): *Schizophyta* (Klasse *Schizophyceae*). – In: Engler A. & Prantl K. (eds), Natürliche Pflanzenfamilien 1b, Duncker & Humblot, Berlin.

Gomont M. (1892): Monographie des Oscillariées (Nostocacées homocystées). – Ann. Sci. Nat., Sér. VII, Bot., 15: 263–368, 16: 89–264.

Hall T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95//98//NT. – Nucleic Acids Symp. Ser. 41: 95–98.

Hašler P., Dvořák P., Johansen J. R., Kitner M., Ondřej V. & Poulíčková A. (2012): Morphological and molecular study of epipelic filamentous genera *Phormidium*, *Microcoleus* and *Geitlerinema* (*Oscillatoriales, Cyanophyta/Cyanobacteria*). – Fottea 12: 341–356.

Heath M. W., Wood S. A. & Ryan K. G. (2010). Polyphasic assessment of fresh-water benthic mat-forming cyanobacteria isolated from New Zealand. – FEMS Microbiol. Ecol. 73: 95–109.

Huelsenbeck J. P. & Ronquist F. (2001): MRBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics 17: 754–755.

Katoh K. & Toh H. (2010): Parallelization of the MAFFT multiple sequence alignment program. – Bioinformatics 26: 1899–1900.

Komárek J. & Anagnostidis K. (2005): *Cyanoprokaryota* 2. Teil/ 2nd Part: *Oscillatoriales*. – In: Büdel B., Krienitz L., Gärtner G. & Schagerl M. (eds), Süsswasserflora von Mitteleuropa 19/2, Elsevier/Spektrum, Heidelberg,.

Komárek J., Kaštovský J., Ventura S., Turicchia S. & Šmarda J. (2009): The cyanobacterial genus *Phormidesmis*. – Algol. Stud. 129: 41–59.

Kützing F. T. (1843): Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange. – F. A. Brockhaus, Leipzig.

Lokmer A. (2007): Polyphasic approach to the taxonomy of the selected oscillatorian strains (*Cyanobacteria*). – Thesis, University of South Bohemia, České Budějovice, Czech Republic.

Moro I., Rascio N., La Rocca N., Sciuto K., Albertano P., Bruno L. & Andreoli C. (2010): Polyphasic characterization of a thermo-tolerant filamentous cyanobacterium isolated from the Euganean thermal muds (Padua, Italy). – Eur. J. Phycol. 45: 143–154.

Nübel U., Garcia-Pichel F. & Muyzer G. (1997): PCR primers to amplify 16S rRNA genes from cyanobacteria. – Appl. Environ. Microbiol. 63: 3327–3332.

Palinska K. & Marquardt J. (2008): Genotypic and phenotypic analysis of strains assigned to the widespread cyanobacterial morphospecies *Phormidium autumnale* (*Oscillatoriales*). – Arch. Microbiol. 189: 325–335.

Rippka R. & Herdmann M. (1992): Pasteur culture collection of cyanobacterial strains in axenic culture, catalogue and taxonomic handbook. – Institut Pasteur, Paris.

Schmidt S. K., Lynch R. C., King A. J., Karki D., Robeson M. S., Nagy L., Williams M. W., Mitter M. S. & Freeman K. R. (2010): Phylogeography of microbial phototrophs in the dry valleys of the high Himalayas and Antarctica. – Proc. R. Soc. B, Biol. Sci. 278: 702–708.

Sciuto K., Andreoli C., Rascio N., La Rocca N. & Moro I. (2012): Polyphasic approach and typification of selected *Phormidium* strains (*Cyanobacteria*). – Cladistics 28: 357–374.

Severin I. & Stal L. J. (2010): NifH expression by five groups of phototrophs compared with nitrogenase activity in coastal microbial mats. – FEMS Microbiol. Ecol. 73: 55–67.

Strunecký O., Elster J. & Komárek J. (2010): Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? – Polar Biol. 33: 1419–1428.

Strunecký O., Elster J. & Komárek J. (2011): Taxonomic revision of the freshwater cyanobacterium "*Phormidium*" *murrayi* = *Wilmottia murrayi*. – Fottea 11: 57–71.

Strunecký O., Komárek J., Johansen J., Lukešová A. & Elster J. (2013): Molecular and morphological criteria for revision of the genus *Microcoleus* and its relation to *Phormidium autumnale, Cyanobacteria*. – J. Phycol. 49: 1167–1180.

Swofford D. (2003): PAUP* v.4beta Phylogenetic analysis using parsimony (* and other methods) Version 4. – Sinauer Associates, Sunderland, Massachusetts.

Tamura K., Dudley J., Nei M. & Kumar S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. – Mol. Biol. Evol. 24: 1596–1599.

Taton A., Grubisic S., Balthasart P., Hodgson D. A., Laybourn-Parry J. & Wilmotte A. (2006): Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. – FEMS Microbiol. Ecol. 57: 272–289.

Wilmotte A. & Herdman M. (2001): Phylogenetic relationships among cyanobacteria based on 16S rRNA sequences. – In: Boone D. R. & Castenholz R. W. (eds), Bergey's manual of systematic bacteriology, p. 487–493, Springer, New York.

Wilmotte A., Van der Auwera G. & De Wachter R. (1993): Structure of the 16 S ribosomal RNA of the thermophilic cyanobacterium *Chlorogloeopsis* HTF ("*Mastigocladus laminosus* HTF") strain PCC7518, and phylogenetic analysis. – FEBS Lett. 317: 96–100.

Yilmaz M., Philips E. J. & Tillett D. (2009): Improved methods for the isolation of cyanobacterial dna from environmental samples. – J. Phycol. 45: 517–521.

Received 10 June 2013
Revised 20 December 2013
Accepted 20 December 2013