A new genus of filamentous epipelic cyanobacteria, *Johansenia*

***Johansenia* – nový rod mezi vláknitými epipelickými sinicemi**

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The cyanobacterial genus *Komvophoron* frequently inhabits sediments in stagnant freshwater among which *K. hindakii* and *K. constrictum* dominate. However, morphological heterogeneity within populations of *K. constrictum* necessitated a closer examination of this species' taxonomic position. Based on Szafer's (1910) concept, *Oscillatoria constricta,* later transferred to *K. constrictum,* is an oscillatorean cyanobacterium and does not form heterocytes. However, Geitler (1925) considers this species to be a member of the genus *Anabaena* due to the presence of heterocytes in some populations. We studied natural and cultured populations using morphological and molecular characters (e.g. the 16S rRNA gene and ITS region) and found that the genus *Komvophoron* is polyphyletic. Thus, we establish a new genus *Johansenia* gen. nov. based on Szafer's original concept of *Oscillatoria constricta*. While *Johansenia* is phylogenetically related to the genera *Spirulina* and *Geitlerinema* (BBD strains), members of the genus *Komvophoron* (*K. hindakii*, *K. kgarii*) are related to members of the family *Gomontiellaceae*.

K e y w o r d s: ecology, *Hormoscilla*, ITS, *Komvophoron*, morphology, phylogeny, 16S rRNA

Introduction

The concept of a species based entirely on morphology (Geitler 1932) has now been replaced by a complex approach to taxonomy, for example in cyanobacteria it takes into consideration all aspects of their biology, genetics, physiology and ecology (Anagnostidis & Komárek 1985, 1988, 1990, Komárek & Anagnostidis 1986, 1989, Komárek 2011). Recent progress in molecular techniques has revealed a high cryptic diversity within cyanobacteria and new genera/species have been described (e.g. Boyer et al. 2002, Casamatta et al. 2003, Řeháková et al. 2007, Siegesmund et al. 2008, Perkerson et al. 2011). The requirement for cultures to generate molecular data can be avoided using single filament/cell PCR techniques (Hayes & Barker 1997, Hayes et al. 2002, Nakayma et al. 2011, Yanagihara et al. 2011).

The order *Oscillatoriales* is a problematic group of globally occurring filamentous, non-heterocytous cyanobacteria. The taxonomy of oscillatorialean cyanobacteria is complicated and needs to be revised using a polyphasic approach sensu Komárek (2011). Thin, motile oscillatorialean cyanobacteria with constrictions at cross-walls have usually been identified as *Pseudanabaena* (e.g. Geitler 1932, Skuja 1948, 1956, Starmach 1966). However, Anagnostidis & Komárek (1988) report differences in morphology within the genus *Pseudanabaena* and transfer several taxa into the genus *Komvophoron*. The generic features of *Komvophoron* include trichome length (brevitrichomy), cell shape (spherical, hemispherical, barrel-like), shape of apical cell (broadly conical, wart-like protrusions),

thylakoid arrangement (fasciculate type known only for *K. bourrellyi*) and autecology – usually benthic in freshwater, epiphytic or epizoic in marine environments (e.g. Turon et al. 1991, Willame et al. 2006, Garbary et al. 2007, Matula et al. 2007, Hašler et al. 2008, Kirkwood et al. 2008, Turicchia et al. 2009, Hašler & Poulíčková 2010). This genus includes two subgenera; *Alyssophoron* (trichomes up to 3.5 μm; type: *K. minutum*) and *Komvophoron* (trichomes above 3.5 μm; type: *K. schmidlei*) and several unclear and unrevised taxa (Komárek & Anagnostidis 2005). In the majority of natural populations the variation in the morphology of life stages (hormogonia) is broadly similar to that in other cyanobacteria (Hašler et al. 2008, Špacková et al. 2009, Hašler & Poulíčková 2010). Detailed knowledge of the biology, ecology and genetic variation is lacking for two main reasons. First, many species inhabit sediments (epipelon), which are less well studied than species that are attached to substrates or occupy planktic niches (Hašler et al. 2008, Špacková et al. 2009, Hašler & Poulíčková 2010). Second, it is difficult to grow some species under laboratory conditions. While epipelic populations of *Geitlerinema splendidum*, *G. carotinosum*, *Microcoleus vaginatus*, *Phormidium autumnale* and *Ph. formosum* can be grown in culture and have been studied in detail across Europe (Hašler et al. 2012), currently many strains of *Komvophoron* cannot be cultured.

In order to circumvent problems with culturing, single filament PCR necessary for molecular work with the genus *Komvophoron* (*K. constrictum* and *K. hindakii*) inhabiting bottom sediments (Špacková et al. 2009, Hašler & Poulíčková 2010) was optimized for use in this study. As epipelic representatives of the genus *Komvophoron* do not grow in cultures, strains are not available in culture collections and there is very little molecular data for comparison.

This study aimed to characterize common epipelic species of *Komvophoron* using a molecular approach (the 16S rRNA gene and the ITS – internal transcribed spacer), using single filament PCR. This resulted in the description of a new genus, *Johansenia*.

Methods

Sampling and study of morphology

Samples of sediments were collected in 2010–2011, using the method introduced by Round (1953), from fishponds in the eastern part of the Czech Republic: Líšnice (A): 49°45'42.5"N, 16°51'37.8"E; Líšnice (B): 49°45'17.3"N, 16°52'35.1"E; Loštice: 49°43'38.8"N, 16°55'43.5"E; Moravičany: 49°44'41.8"N, 16°59'35.5"E; Chropyně: 49°21'21.3"N, 17°22'7.7"E; Bezedník: 49°17'58.7"N, 17°43'27.1"E; Kvasice: 49°14'53.1"N, 17°28'45.3"E (for environmental variables see Hašler et al. 2008). The morphology of epipelic cyanobacteria was studied in semi-natural populations incubated under laboratory conditions: temperature t = 22 °C, photoperiod L/D = 16/8 hrs, irradiance 20 μmol·cm^{-2}·s^{-1}, liquid medium according to Zehnder (Staub 1961). A Zeiss AxioImager light microscope with objectives: EC Plan-Neofluar oil obj. 40×, NA 1.3 DIC; Plan-Apochromat oil obj. 100×, NA 1.4 DIC) was used to observe the cyanobacteria. Images were taken using a Zeiss HRc camera 12MPx, with digital image processing software AxioVision 4.7.

Single filament PCR and sequencing

A previously published protocol for the PCR amplification of the genomic extract from a single filament (Boyer et al. 2002) was modified for use in this study. *Komvophoron* filaments were first examined and characterized using light microscopy. Filaments were harvested in fresh sterile water. A single filament was transferred using a sterile glass capillary to a drop of sterile water. This step was repeated until there were no contaminants then the filament was transferred into 0.2 ml PCR tubes with 9 µl of PCR grade water. To extract the genomic DNA the tubes were frozen 3 times in liquid nitrogen, thawed and vortexed for 15 seconds.

PCR amplification of partial 16S rRNA and complete 16S-23S ITS sequences was performed using cyanobacteria specific primers described in Boyer et al. (2002) forward P2 (5'-GGGGAATTTTCCGCAATGGG-3') and reverse P1 (5'-CTCTGTGTGCCTAGGTA TCC-3').

Premix composed of 0.5 µl of each primer (0.01 mM) and 10 µl FastStart PCR Master (Roche Diagnostics GmbH, Mannheim, Germany) was added to the mixture. The PCR amplification was carried out under the following conditions: initial denaturation for 4 min at 95 °C, followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 57 °C, extension for 1 min 50 s at 72 °C and finally the reaction was finished with an extension for 7 min at 72 °C. The PCR product was checked on a 1.5% agarose gel with 0.5× TBE buffer, stained with Ethidium Bromide. Expected PCR product length was ~1600 bp. Subsequently, all positive bands were isolated using GenElute™ Gel Extraction Kit (Sigma-Aldrich, Co., Saint Louis, MO, USA). Extracted PCR products were cloned using pGEM-T Easy Vector System (Promega Corporation, Madison, WI, USA) following the manufacturer's manual. Transformed competent *Escherichia coli* JM109 cells were spread on ampicilin 1.5% agarose plates with Luria Bertani medium. After white-blue selection, at least four colonies were isolated and placed into 4 ml of fresh Luria Bertani medium and cultured overnight at 37 °C. Plasmid DNA from all clones was isolated using High-Speed Plasmid Mini Kit (Geneaid, Sijhih City, Taiwan) and sent for commercial sequencing.

The plasmids were sequenced using the following primers: M13f and M13r, with the additional internal primers P5 (5'-TGTACACACCGCCCGTC-3') and P8 (5'-AAGGAGGTGATCCAGCCACA-3') after Boyer et al. (2001) and Boyer et al. (2002). Rough sequences were processed (assembled, proof read and trimmed plasmid sequences) in Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA) and deposited in GenBank (http://www.ncbi.nlm.nih.gov; access numbers: KJ140087–KJ140105). Chimeras and other anomalies were checked using program Mallard 1.02 (Ashelford et al. 2005).

Phylogenetic analysis

The most closely related sequences to those of the strains studied were identified using BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Of these, only sufficiently long sequences (at least 1000 bp) were chosen for analysis avoiding uncultured strains. For a broader taxonomical context, additional sequences from taxa of the *Oscillatoriales*, *Nostocales* and *Stigonematales* were added (96 sequences in total). Multiple sequence alignment was performed using the Muscle algorithm (Edgar 2004), implemented in

MEGA 5.05 (Tamura et al. 2011), manually corrected using the text editor implemented in the MEGA software and exported in different formats for further analyses.

An evolutionary model for the maximum likelihood analysis was selected based on both the Akaike Information Criterion and Bayesian Information Criterion. The analysis was performed in jModelTest 0.1.1 (Posada 2008) and both criteria selected the General Time Reversible model with gamma distributed rate variation across sites (GTR+G) as the most suitable model. The phylogenetic tree was inferred in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) via CIPRES Science Gateway web server (Miller et al. 2010). Two parallel Markov chain Monte Carlo (MCMC) simulations were simultaneously run for 10 000 000 generations, each one with one cold and three heated chains. MCMC chains were sampled every 1000th generation. The first 2500 trees were discarded as burn-in. The GARLi (Zwickl 2006) web server (Bazinet & Cummings 2011) was used for bootstrap analysis under the maximum likelihood optimality criterion. Neighbour joining bootstrap analysis was performed in MEGA 5 using the Kimura 2 parameter model (Kimura 1980). Maximum parsimony analysis was performed under the following conditions. Gaps were used as fifth base with Min-Mini heuristic search method (maximum number of trees = 100) in MEGA. All bootstrap analyses were carried out with 1000 replications.

Secondary structures of the D1D1' and Box-B helices were predicted using the Mfold Web server (Zucker 2003) with temperature set to default (37 °C).

Results

Johansenia constricta (Szafer) Hašler, Dvořák et Poulíčková, **gen. nov. et comb. nova** (Fig. 1)

[Basionym: *Oscillatoria constricta* Szafer 1910, Bull. Int. Acad. Sci. Cracovie, Mat-Nat Sci, ser. B: 161–167; synonyms: *Pseudanabaena constricta* (Szafer) Lauterborn 1915, Die sapropelische Lebewelt: ein Beitrag zur Biologie des Faulschlammes natürlicher Gewässer. – Verh. Natur. Med. Ver. Heidelberg 13: 395–481; *Komvophoron constrictum* (Szafer) Anagnostidis et Komárek 1988, Algological Studies 50–53: 327–472; nomen nudum: *Anabaena constricta* (Szafer) Geitler 1925, Cyanophyceae. – In Pascher's Süsswasserflora 12. – 450, G. Fischer-Verl., Jena]

D e s c r i p t i o n: Trichomes are solitairy, 4.6±0.2 μm wide, short to long (more than 50 cells), straight or bent, deeply constricted at cross-walls (mucilaginous bridges, thick cross-walls), usually very motile (gliding). Distinct gelatinous envelopes or sheaths are not present. Trichomes disintegrate into short parts without necridic cells. Vegetative cells are barrel-shaped, isodiametric, rectangular or cylindrical with bright and dark granules in cross-walls, cell contents can be divided into a visible peripheral chromatoplasma and central nucleoplasma. Apical cells are usually broadly rounded.

E t y m o l o g y: The genus is named in honour of Jeffrey R. Johansen, Prof. of John Carroll University in Cleveland, USA and an internationally renowned cyanobacterial researcher.

O c c u r r e n c e: Epipelic species that occurs in muddy sediments, often with organic detritus, in freshwater.

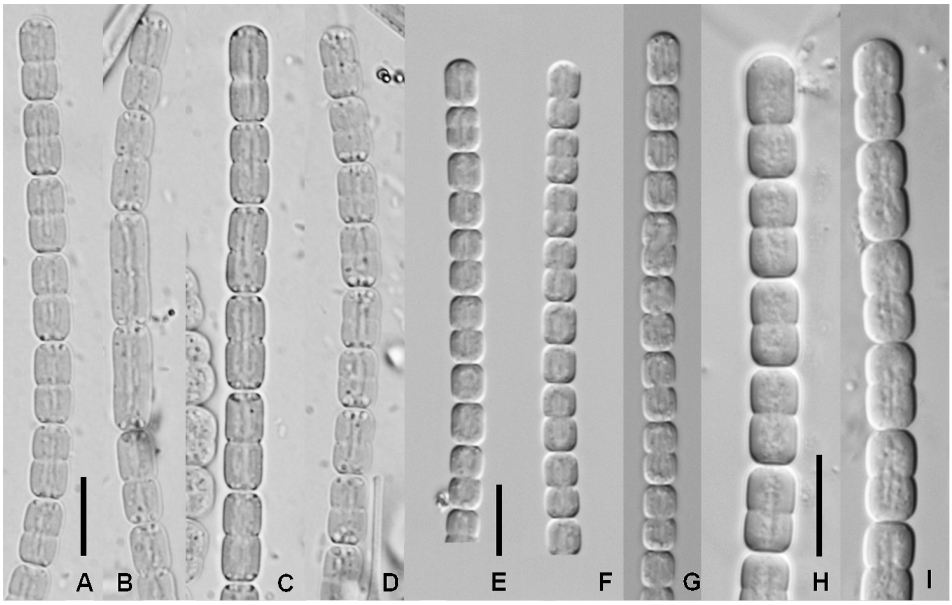

Fig. 1. – Morphological variability of *Johansenia constricta*. Individuals (A–D) from Chropyně pond and (E–I) from Líšnice pond. Scale bars = 10 μm.

Taxonomic note

Our species concept is based on the original description of Szafer (1910), who discusses the similarity of the life stages of *Oscillatoria constricta* and *Anabaena*. However, the author did not observe any heterocytes and akinetes after several months of investigation. Szafer considers this species to be an oscillatorean, non-heterocytous cyanobacterium. The iconotype does not have any trichomes with heterocytes or akinetes (Fig. 2A). Koppe (1924) and some subsequent investigators record *O. constricta* producing heterocytes and thus identify this species as *Anabaena constricta* or *Pseudanabaena constricta* with heterocytes (Koppe 1924, Geitler 1932, Buell 1938, Louis & Peeters 1967, D'Hollander & Caljon 1979, D'Hollander 1980). These authors in their descriptions and drawings apparently show two different species (see Fig. 2B, C). We did not observe any heterocytes or akinetes during our 7 year long study of *Johansenia constricta* from across Europe. Thus, we are confident that the original Szafer's concept and description are accurate and subsequent investigators may have been looking at similar, cryptic strains.

Molecular characterization of Johansenia and Komvophoron

Using single filament PCR, unique sequences of *Komvophoron* were obtained. The similarity of the 16S rRNA sequences with those available in GenBank was low (91–97% similar in BLAST search). The most closely related are members of the family *Pseudanabaenaceae* (*Spirulinoideae*, *Pseudanabaenoideae*) and *Gomontiellaceae*. The analysis of 16S rRNA sequences based on Bayesian inference, maximum likelihood, neighbour joining and maximum parsimony revealed that the genus *Komvophoron* is not

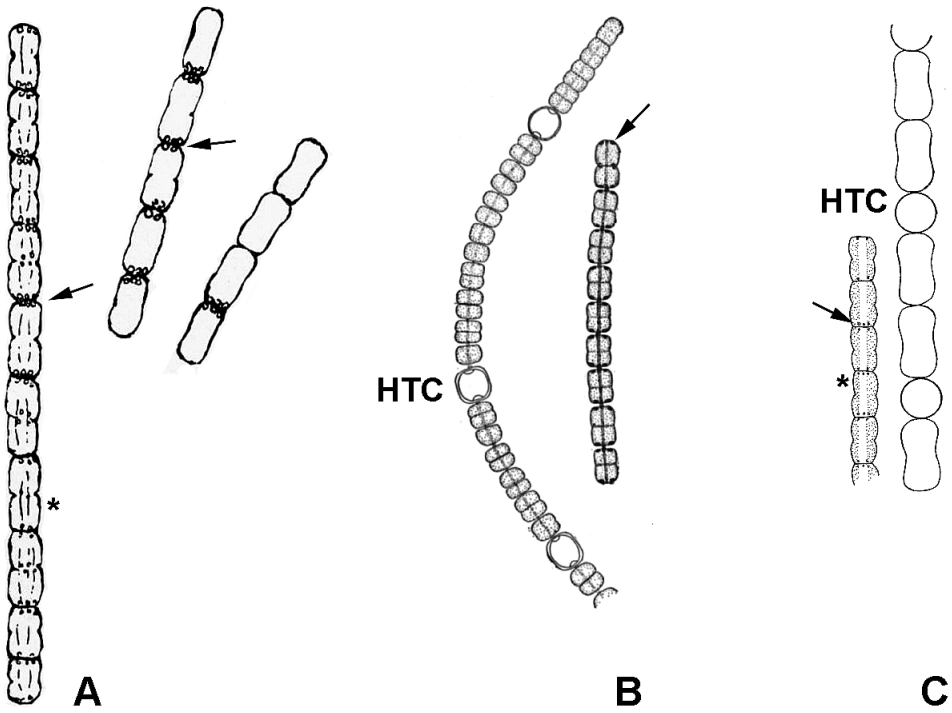

Fig. 2. – Examples of trichomes representing a historical view of *Johansenia constricta*. (A) *Oscillatoria constricta*, iconotype after Szafer (1910), (B) *Anabaena constricta*, after Buell (1938), (C) *Anabaena constricta* after Louis & Peeters (1967). Arrows show characteristic position of dark granules in cross-walls, HTC = heterocytes. In trichomes without heterocytes there is a differentiation into chromatoplasma and nucleoplasma (asterisk). Trichomes containing heterocytes obviously belong to other species.

monophyletic (Fig. 3). The tree topology indicates that two distinct genera exist within the epipelic populations sampled (*K. hindakii* and *K. constrictum*). Moreover the dissimilarity of *K. constrictum* and *K. hindakii* (sequence similarity 88%) allows the separation and description of a new genus, *Johansenia* gen nov. The first clade (Fig. 3, clade A) includes species described here as *Johansenia constricta* sp. nov., which is related to the *Geitlerinema* BBD strains (P2Sb-1, HS223 isolated from Black Band Diseases of Corals) and *Spirulina* (strains isolated from freshwater in Italy, India and brackish water in California). The clade containing *J. constricta* is different from the second clade of pseudanabaenacean cyanobacteria represented by *Pseudanabaena*, *Leptolyngbya* and *Nodosilinea* (posterior probability 0.98). *Komvophoron hindakii* belongs to the second clade (Fig. 3, clade B) together with *K. kgarii* (described as benthic in Australia 2013) and are related to members of the family *Gomontiellaceae*.

Secondary structures in the ITS region, both D1-D1' helices and B-box helices, are very different in *Johansenia* and *Komvophoron* (Fig. 4). D1-D1' helices contain numerous loops and bulges. The D1-D1' helix of *J. constricta* is formed by a large hairpin and lower internal loops and by an upper small internal loop and bulge. D1-D1' helices of *K. hindakii* and *H. pringsheimii* are formed by small hairpin loops and large lower internal loops. In the middle part there is a bulge or bulge-like structure. B-box helices are obviously

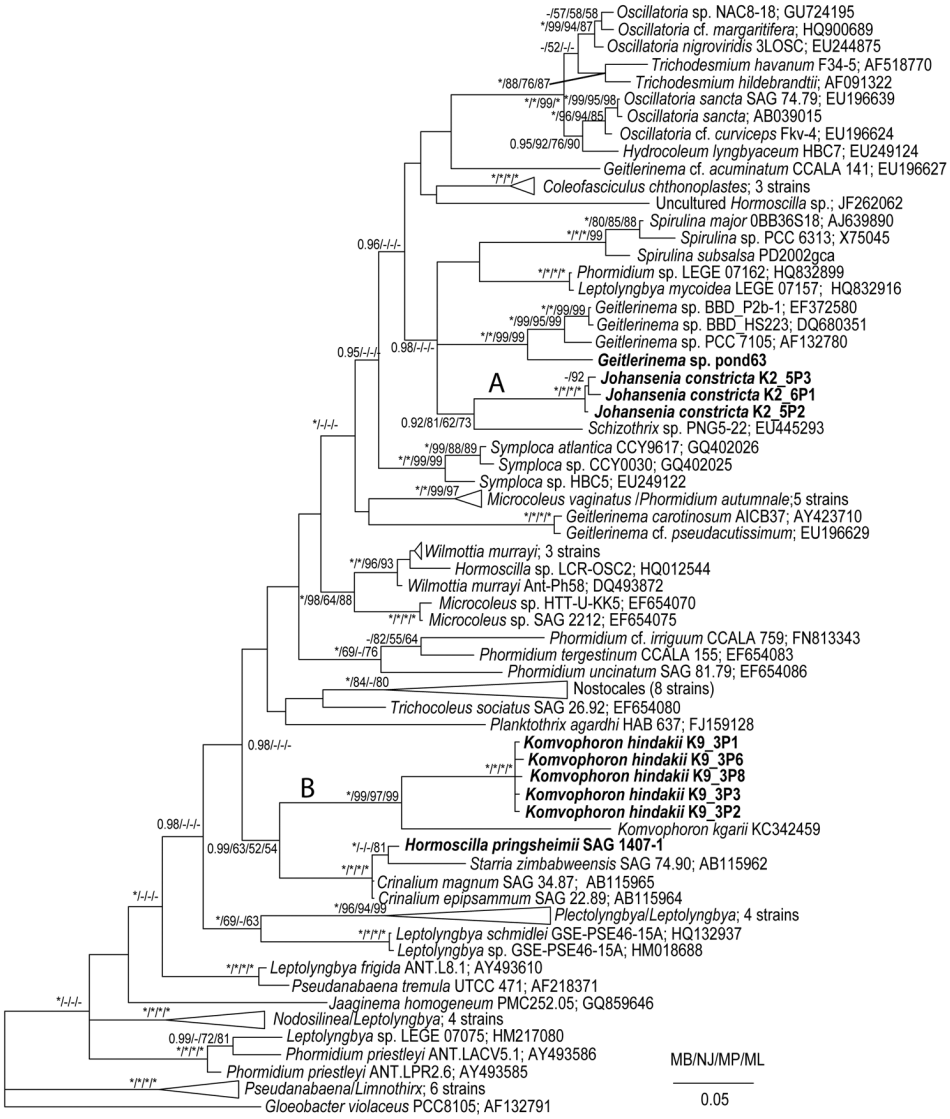

Fig. 3. – Consensus Bayesian tree inferred from 16S rRNA (size 1000 bp), 96 species of filamentous cyanobacteria were added to the analysis, original sequences from 9 isolates of epipelic *Komvophoron*, *Johansenia* and *Hormoscilla pringsheimii* SAG 1407.1 (in bold). Node supports are shown in the following order: Bayesian posterior probabilities (MB), bootstrap values of neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML). (A) clade of *Johansenia constricta* (1.0/100/100/100), (B) *Komvophoron hindakii* and *K. kgarii* (1.0/99/97/99). * absolute bootstrap support, - no bootstrap support.

different in *Johansenia* and *Komvophoron*. B-box in *Johansenia* contains a small hairpin loop and one small bulge and internal loop. On the other hand, the B-box in *Komvophoron* contains a large hairpin loop and a large internal loop underneath. A small bulge occurs at the basis of the stem. B-box in *Hormoscilla* is similar to that in *Komvophoron*. It does not contain a large internal loop.

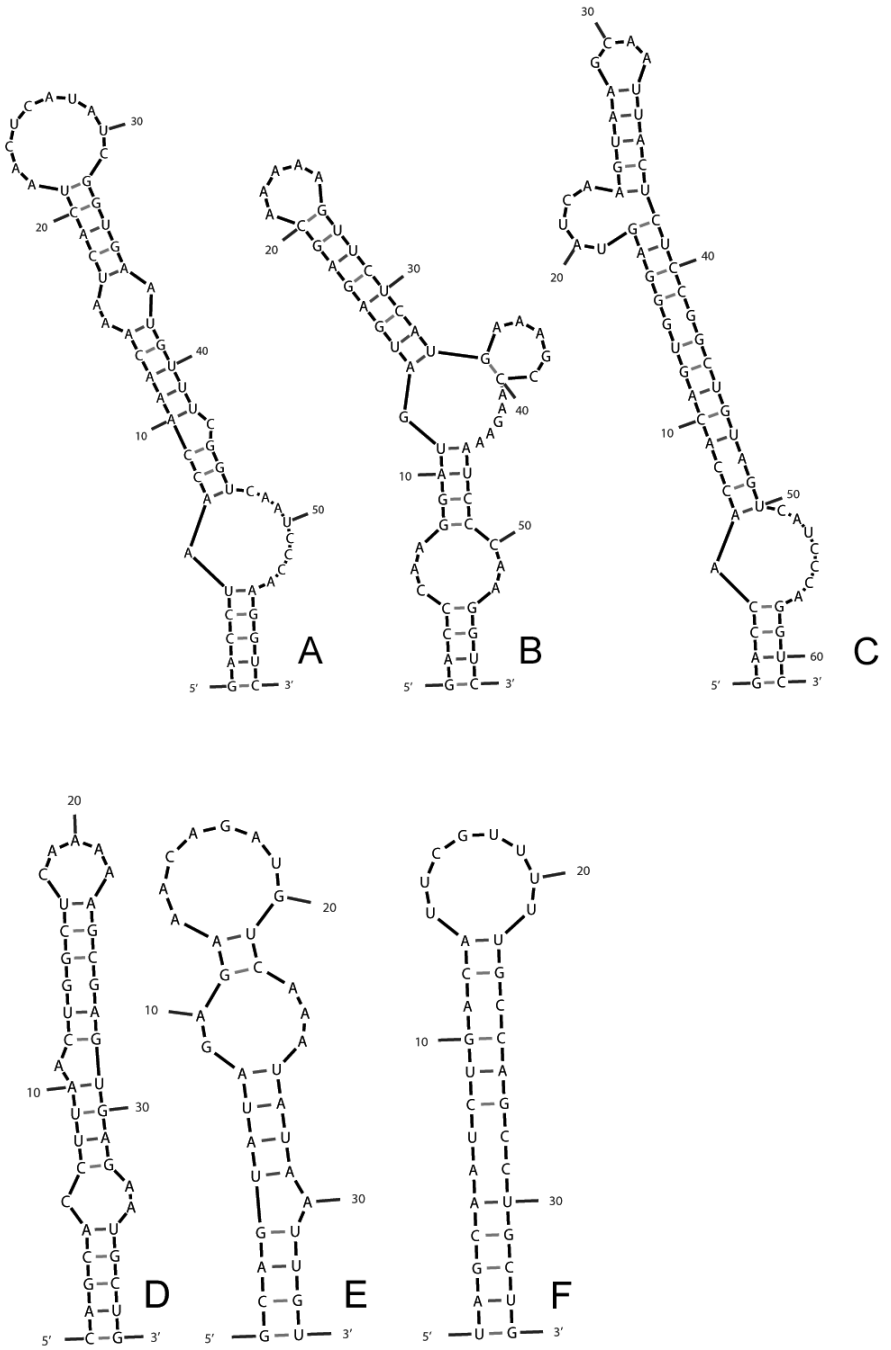

Fig. 4. – 16S-23S rRNA secondary structures. D1-D1'helices: (A) *J. constricta*, population from Líšnice pond (B); B *K. hindakii*, population from Kvasice pond; (C) *H. pringsheimii*, strain SAG 1407.1. B-box helices: (D) *J. constricta*, population from Líšnice pond; (E) *K. hindakii*, population from Kvasice pond; (F) *H. pringsheimii*, strain SAG 1407.1.

Morphological and ecological remarks on Johansenia and Komvophoron

The morphology of *K. hindakii* (population from pond Kvasice) is congruent with the original description by Hašler & Poulíčková (2010). Molecular data suggesting separation of a new genus *Johansenia* from the genus *Komvophoron* are in congruence with the morphology. The morphology of the trichomes of *Johansenia* and *Komvophoron* differ. Trichomes of *K. hindakii* are short (up to 50 cells) and consist of spherical to hemispherical cells. Apical cells are spherical to broadly conical. Conversely, trichomes of *J. constricta* look more like those of *Pseudanabaena*. They are short to long and consist of isodiametric to cylindrical cells. Apical cells are rounded. While we were not able to study the ultrastructure of the cells due to an inability to culture this species, closely related sister taxa to *K. hindakii* have irregular thylakoids while sister taxa to *J. constricta* have a radial arrangement. The details are discussed below.

Both species are motile (gliding) and inhabit fine bottom sediments, usually blackish organic sediments. Epipelic mode of life is typical. Neither species were recorded as either planktonic or epiphytic in this study.

Discussion

The genus *Komvophoron* (*Borziaceae*) is an often overlooked group of cryptically diverse oscillatorialean cyanobacteria. The first study on the genus *Komvophoron* was carried out by Anagnostidis & Komárek (1988), who combined pseudanabaenacean cyanobacteria based on their morphology. Later, a few new species were described using a polyphasic approach and published under the auspices of the International Code of Botanical Nomenclature (Turon et al. 1991, Turicchia et al. 2009, Hašler & Poulíčková 2010, McGregor & Sendall 2013). Although there are few species in the genus *Komvophoron* compared with "wide genera" such as *Phormidium*, the taxonomy and position of this cryptically diverse genus remained unclear.

The phylogenetic relationships of the species within *Komvophoron* have not been previously discussed in detail, partially due to an insufficient number of sequences, especially of the thin members (subgenus *Alyssophoron*). Generally, cyanobacterial phylogenies should include all members of a genus, including the type. However, the type species of the genus *Komvophoron* (*K. schmidlei*) is extremely rare in Europe (Hašler & Poulíčková 2010), not available in culture and no sequence for it exists in GenBank. Molecular studies of other members of *Komvophoron* based on the 16S rRNA gene include either sequences that are too short (*Komvophoron* sp., 520 bp, Willame et al. 2006) or entities whose sequences are not available in GenBank (*K. apiculatum* and *K. rostratum*, Turicchia et al. 2009). McGregor & Sendall (2013) describe *Komvophoron kgarii* as a new epipsamic species from Australia and record the first 16S rRNA sequence of *Komvophoron* longer than 1000bp. In contrast to previous authors, we analyzed a larger number of *Komvophoron* sequences and our analysis strongly supports morphological incongruence in this genus sensu Komárek & Anagnostidis (2005). Our data show that the genus *Komvophoron* is not monophyletic because it appears in two distinct clusters.

Johansenia gen. nov. seems to be a sister to *Spirulina* and marine strains of *Geitlerinema* spp. (Black band disease, strains BBD P2b-1and HS223). The mentioned strains of *Geitlerinema* probably do not represent the genus *Geitlerinema* at all and must

be reclassified (Perkerson et al. 2010). A different position of *Johansenia* group within the phylogenetic tree indicates that these cyanobacteria are not related to similar genera (e.g. *Pseudanabaena*, *Leptolyngbya*, *Nodosilinea* and *Plectolyngbya*). Thus, the family *Pseudanabaenaceae* sensu Komárek & Anagnostidis (2005) consists of two phylogenetic units. *Johansenia* does not cluster with the *Leptolyngbya* group as reported earlier. According to Willame et al. (2006), *Komvophoron* sp. (strain 0RO36S1) shares 90.1% similarity with the *Leptolyngbya* strain 0ES31S2, however this strain is not comprehensively characterized. In our view, *Komvophoron* sp. 0RO36S1 is probably misidentified and does not represent the genus *Komvophoron*. The *K. hindakii* group and *K. kgarii* form a separate clade with the genera *Hormoscilla*, *Crinalium* and *Starria*. Known ultra-structures of *Hormoscilla* and *Crinalium* (Winder et al. 1990, Rosowski & Lee 1991) are identical to those of *K. kgarii* (McGregor & Sendall 2013). Both phylogeny and ultra-structure support an affinity of the genus *Komvophoron* with the family *Gomontiellaceae*. There are other analogies between members of the *Gomontiellaceae* and *Borziaceae*. The similarity between *Borzia* (*Borziaceae*) and *Hormoscilla* (*Gomontiellaceae*) is discussed by Anagnostidis & Komárek (1988) who point out the presence of necridic cells in several *Borzia* species and include them in the genus *Hormoscilla*. Two strains of *Hormoscilla* are not included in this clade in our phylogenetic tree. The strain *Hormoscilla* sp. (described as *Hormoscilla* sp. nov., figs 3, S17, Pereira et al. 2011, access no JF262062) is not a validly described taxon either under the International Code of Botanical Nomenclature or the International Code of Nomenclature of Bacteria and in terms of its morphology does not belong to the genus *Hormoscilla*. On the other hand, the strain *Hormoscilla* sp. LCR-OSC2 (access no HQ012544), which is correctly described as *H. irregularis* Novis & Visnovsky (2011), has all features of the genus *Hormoscilla* and should be studied in detail in order to account for its phylogenetic position. This strain is still available, but it cannot leave New Zealand (P. M. Novis, personal communication).

Within the genus *Komvophoron* sensu Anagnostidis & Komárek (1988) we can distinguish two separate morphological groups: (i) species with spherical or hemispherical cells, usually without obvious separation of vegetative cells (hyaline bridges, thick cross-walls) such as *K. minutum*, *K. groenlandicum*, *K. breve*, *K. jovis*, *K. schmidlei*, *K. hindakii* and *K. kgarii* (currently described by McGregor & Sendall 2013); (ii) species with barrel-shaped or angular-like cells often obviously separated such as *J. constricta* (previously *K. constrictum*), *K. bourrellyi*, *K. crassum*, *K. pallidum* and *K. skujae*. We consider the species mentioned in the first group to be typical members of the genus *Komvophoron* s. str., which correspond to the description of the type species *K. schmidlei*. On the other hand, the second group (barrel shaped to cylindrical cells) is represented by *Johansenia constricta*, which is one of the most frequently recorded epipelic morphospecies in Europe (e.g. Hašler et al. 2008, Hašler & Poulíčková 2010). This taxon is discussed in detail by Anagnostidis & Komárek (1988), who combine it with similar types, separates them from the genus *Pseudanabaena* and places them in *Komvophoron constrictum*. Pairs of large black granules are reported as an important diagnostic feature (Szafer 1910, Komárek & Anagnostidis 2005). We observed that these granules are not always present or poorly visible and the presence of granules in cross-walls probably depends on the physiological state of cells. We found that under unfavourable conditions in the laboratory trichomes lost these granules and then fragment and disappear. Although there is no molecular data for other species in this group they share some morphological features with our new genus. For instance, the

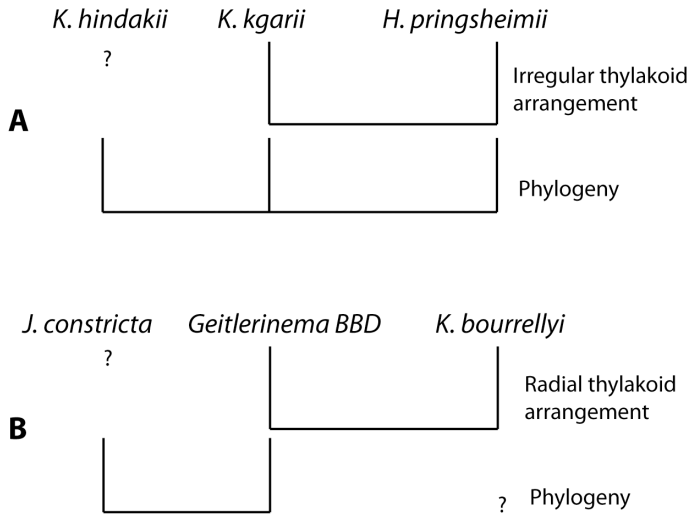

Fig. 5. – Relationships between *Komvophoron*-like taxa. (A) first group *Komvophoron* s. str. + family *Gomonteliaceae*; (B) *Johansenia constricta* and morphologically similar taxa.

description of *K. crassum* is very similar to that of *Johansenia* and this species is very similar to *J. constricta*. However, it lacks the typical granules in the cross-walls. It occurs in the mountain area in Tadzhikistan and La Nga River in Vietnam (Nhan & Tung 2008). On the other hand, *J. constricta* usually lives on the fine organic sediments in stagnant freshwater. In the same way, *K. skujae* (formerly *Pseudanabaena minuta* sensu Skuja 1948) is morphologically very similar to *J. constricta*. Skuja (1948) discusses the morphological similarity of his *P. minuta* (*K. skujae*) with *P. constricta* (*J. constricta*) and indicates that the absence of dark granules in the cross-walls is a distinquishing feature of *P. minuta* (*K. skujae*). Moreover, *K. skujae* is planktonic or tychoplanktonic not benthic as is *J. constricta*. The *Komvophoron* species mentioned above are rare and are not available in culture collections.

A large biomass of cyanobacteria is required for ultrastructural studies and this can only be obtained from either collecting many samples or cultured strains. The majority of the members of the genus *Komvophoron* do not grow under laboratory conditions and do not occur in sufficiently large aggregations in the field. Currently, only two different patterns of thylakoid arrangement within the genus *Komvophoron* are described (Fig. 5). A radial structure of thylakoids is reported in the epizoic species *K. bourrellyi* (Turon et al. 1991) and irregular (*Hormoscilla*) type in *K. kgarii* (McGregor & Sendall 2013, biomass collected in the field). Incongruity in thylakoid arrangement supports the idea that the *Komvophoron*-like taxa are polyphyletic and must be split into better defined units. For the first group (*Komvophoron* s. str. + *Gomonteliaceae*, Fig. 5A) the only missing information is the type of thylakoid arrangement in *K. hindakii*. The second clade (Fig. 5B) is still missing a detailed analysis of the phylogenetic position of *K. bourrellyi* and thylakoid structure of *J. constricta*.

In summary, epipelic cyanobacteria comprise species well adapted to life on bottom sediments, dominated by specialized filamentous, motile species such as *Johansenia constricta* and *K. hindakii*. This study gathered critical evidence that the genus *Komvophoron* is not a monophyletic lineage and has to be divided into two genera: *Johansenia* and *Komvophoron*. This study (based on molecular, morphological and eco-

logical data) confirmed the validity of *K. hindakii* and its phylogenetic relation to the family *Gomontiellaceae*. A new genus *Johansenia* is described and its phylogenetic affinity to *Spirulina* and *Geitlerinema* confirmed.

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Souhrn

Epipelické populace vláknitých sinic rodu *Komvophoron* představují velmi komplikovanou skupinu, ve které není zcela jasné vymezení některých druhů. Široká morfologická variabilita uvnitř rodu a druhů naznačuje nesoulad v taxonomii s nutností revize. Při studiu jsme se opřeli o molekulární analýzu 16S rRNA genu a ITS oblasti u *K. constrictum*, *K. hindakii* a *H. pringsheimii* a získaná data jsme využili pro srovnání s morfologickými znaky jmenovaných druhů s následnou taxonomickou diskuzí. *Komvophoron constrictum* z dlouhodobého hlediska představoval taxonomický problém s ohledem na morfologickou variabilitu druhu a podobných druhů, zejména nostokálních sinic rodu *Anabaena.* Na zmíněný druh bylo nahlíženo podle dvou protichůdných konceptů. Szafer (1910) popsal tento druh jako sinici netvořící heterocyty s jasnou příbuzností k rodu *Oscillatoria*. Údaje o možné tvorbě heterocytů a příbuznost k rodu *Anabaena* s ohledem na svoje dlouhodobá pozorování nepovažuje za správné. Lauternborn (1915) s ohledem na odlišný typ struktury vláken a buněk přesunul tento druh do nového rodu *Pseudanabaena.* Koppe (1924) své předchůdce mylně interpretoval a pravděpodobně ovlivnil řadu autorů v průběhu první poloviny 20. století. Geitler (1925) následně provedl kombinaci a původní druh *Oscillatoria*/*Pseudanabaena constricta* označil jako *Anabaena constricta*. Zmiňovanou přítomnost heterocytů uvnitř populací diskutovali např. Buell (1938) a zejména Komárek & Anagnostidis (2005). S ohledem na dosavadní znalosti o druhu a výsledky našeho studia považujeme koncepci Geitlera jako nostokální sinice za chybnou. Navíc výsledky molekulární analýzy ukazují, že rod *Komvophoron* je polyfyletický. Z tohoto důvodu navrhujeme popis nového rodu *Johansenia*, kde typový druh *J. constricta* je založen na původním konceptu Szafera (1910). Naše dlouhodobé studie napříč Evropou potvrzují původní Szaferovu domněnku o nepřítomnosti heterocytů a akinet u *Oscillatoria constricta*. *Komvophoron hindakii* vykazuje zcela odlišnou morfologii vláken a podle 16S rRNA a ITS oblasti je zcela odlišný od rodu *Johansenia*. S ohledem na morfologii jej považujeme za typického zástupce rodu *Komvophoron* s. str. Mimo jiné je morfologicky a molekulárně podobný nedávno popsanému druhu *K. kgarii* z Austrálie. Jak *K. hindakiii,* tak *K. kgarii* vykazují vysokou fylogenetickou příbuznost k sinicím čeledi *Gomontiellaceae*.

References

Anagnostidis K. & Komárek J. (1985): Modern approach to the classification system of cyanophytes 1. Introduction. – Algol. Stud. 38–39: 291–302.

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

Anagnostidis K. & Komárek J. (1990): Modern approach to the classification system of cyanophytes 5. *Stigonematales*. – Algol. Stud. 59: 1–73.

Ashelford K. E., Chuzhanova N. A., Fry J. C., Jones A. J. & Weightman A. (2005): At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. – Appl. Environ. Microbiol. 71: 7724–7736.

Bazinet A. L. & Cummings M. P. (2011): Computing the Tree of Life: leveraging the power of desktop and service grids. – In: Mueller F., Beaumont O., Oliker L., Kandemir M. T. & Nikolopoulos D. (eds), Proceedings of the Fifth Workshop on Desktop Grids and Volunteer Computing Systems (PCGrid 2011), IEEE International Symposium 16–20 May 2011, p. 1896–1902, IEEE, Anchorage, AK.

Boyer S. L., Flechtner V. R. & Johansen J. R. (2001): Is the 16S-23S rRNA Internal Transcribed Spacer Region a good tool for use in molecular systematics and population genetics? A case study in *Cyanobacteria*. – Mol. Biol. Evol. 18: 1057–1069.

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.

Buell H. F. (1938): The taxonomy of a community of blue-green algae in a Minnesota. – Bull. Torrey Bot. Club 65: 377–396.

Casamatta D. A., Vis M. L. & Sheath R. G. (2003): Cryptic species in cyanobacterial systematics: a case study of *Phormidium retzii* (*Oscillatoriales*) using RAPD molecular markers and 16S rDNA sequence data. – Aquat. Bot. 74: 295–309.

D'Hollander E. (1980): Le phytoplancton du massif du tagant (Mauritanie) à l'exclusion des diatomées. – Bull. Soc. R. Bot. Belg./Bull. KoninklijkeBelg. Bot. Ver. 113: 75–90.

D'Hollander E. & Caljon A. (1979): Le phytoplancton de l'adrar mauritanien à l'exclusion des diatomées. – Bull. Soc. R. Bot. Belg./Bull. KoninklijkeBelg. Bot. Ver. 112: 201–215.

de Winder B., Stal L. J. & Mur L. R. (1990): *Crinalium epipsammum* sp. nov.: a filamentous cyanobacterium with trichomes composed of elliptical cells and containing poly-β-(1,4) glucar (cellulose). – J. Gen. Microbiol. 136: 1645–1653.

Edgar R. C. (2004): MUSCLE: multiple sequence alignment with high accuracy and high throughput. – Nucl. Acids Res. 32: 1792–97.

Garbary D. J., Bourque G., Herman T. B. & McNeil J. B. (2007): Epizoic algae from freshwater turtles in Nova Scotia. – J. Freshw. Ecol. 22: 677–685.

Geitler L. (1925): *Cyanophyceae*. – In: Pascher's Süsswasserflora, Vol. 12, G. Fischer-Verl., Jena.

Geitler L. (1932): *Cyanophyceae*. – In: Rabenhorst L. (ed.), Kryptogamen Flora von Deutschland, Österreich und der Schweiz, Vol. 14, Akademische Verlagsgesellschaft, Leipzig.

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.

Hašler P. & Poulíčková A. (2010): Diversity, taxonomy and autecology of autochtonous epipelic cyanobacteria of the genus *Komvophoron* (*Borziaceae*, *Oscillatoriales*): a study on populations from the Czech Republic and British Isles. – Biologia 65: 7–16.

Hašler P., Štěpánková J., Špačková J., Neustupa J., Kitner M., Hekera P., Veselá J., Burian J. & Poulíčková A. (2008): Epipelic cyanobacteria and algae: a case study from Czech ponds. – Fottea 8: 133–146.

Hayes P. K. & Barker G. L. A. (1997): Genetic diversity within Baltic Sea populations of *Nodularia* (*Cyanobacteria*). – J. Phycol. 33: 919–923.

Hayes P. K., Barker G. L. A., Batley J., Beard S. J., Handley B. A., Vacharapiyasophon P. & Walsby A. E. (2002): Genetic diversity within populations of *Cyanobacteria* assessed by analysis of single filaments. – Antonie van Leeuwenhoek 81: 197–202.

Kimura M. (1980): A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. – J. Mol. Evol. 16: 111–120.

Kirkwood A. E., Buchheim J. A., Buchheim M. A. & Henley W. J. (2008): Cyanobacterial diversity and halotolerance in a variable hypersaline environment. – Microbiol. Ecol. 55: 453–465.

Komárek J. (2011): Introduction to the 18th IAC symposium in České Budějovice 2010, Czech Republic: some current problems of modern cyanobacterial taxonomy. – Fottea 11: 1–7.

Komárek J. & Anagnostidis K. (1986): Modern approach to the classification system of cyanophytes 2. *Chroococcales*. – Algol. Stud. 43: 157–226.

Komárek J. & Anagnostidis K. (1989): Modern approach to the classification system of cyanophytes 4. *Nostocales*. – Algol. Stud. 56: 247–345.

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

Koppe F. (1924): Die Schlammflora der ostholsteinischen Seen und des Bodensees. – Arch. Hydrobiol. Plankt. 14: 619–672.

Louis A. & Peeters F. (1967): Observations écologiques et floristiques sur la florule algale du nord de la Campine belge. – Bull. Jardin Bot. Natl. Belg./Bull. National Plant. België 37: 357–443.

Matula J., Pietryka M., Richter D. & Wojtun B. (2007): *Cyanoprokaryota* and algae of Arctic terrestrial ecosystems in the Hornsund area, Spitsbergen. – Pol. Polar Res. 28: 283–315.

McGregor G. B. & Sendall B. C. (2013): *Komvophoron kgarii* sp. nov. (*Oscillatoriales*), a new epipelic cyanobacterium from subtropical eastern Australia. – Phycologia 52: 472–480.

Miller M. A., Pfeiffer W. & Schwartz T. (2010): Creating the CIPRES science gateway for inference of large phylogenetic trees. – In: Pierce M., Thomas M. & Wilkins-Diehr N. (eds), Proceedings of the Gateway Computing Environments workshop, p. 1–8, IEEE, New Orleans, LA.

Nakayama T., Ikegami Y., Nakayama T., Ishida K-i., Inagaki Y. & Inouye I. (2011): Spheroid bodies in rhopalodiacean diatoms were derived from a single endosymbiotic cyanobacterium. – J. Plant. Res. 124: 93–97.

Nhan L. T. T. & Tung N. T. (2008): Planktonic *Cyanobacteria* species composition and seasonal change in La Nga River. – Science & Technology Development 11.

Novis P. M. & Visnovsky G. (2011): Novel alpine algae from New Zealand: *Cyanobacteria*. – Phytotaxa 22: 1–24.

Pereira A. R., Etzbach L., Engene N., Müller R. & Gerwick W. H. (2011): Molluscicidal metabolites from an assemblage of Palmyra Atoll *Cyanobacteria*. – J. Nat. Prod. 74: 1175–1181.

Perkerson R. B., Johansen J. R., Kovácik L., Brand J., Kaštovský J. & Casamatta D. A. (2011): A unique pseudanabaenalean (*Cyanobacteria*) genus *Nodosilinea* gen. nov. based on morphological and molecular data. – J. Phycol. 47: 1397–1412.

Perkerson R. B., Perkerson E. A. & Casamatta D. A. (2010): Phylogenetic examination of the cyanobacterial genera *Geitlerinema* and *Limnothrix* (*Pseudanabaenaceae*) using 16S rDNA gene sequence data. – Algol. Stud. 134: 1–16.

Posada D. (2008): jModelTest: phylogenetic model averaging. – Mol. Biol. Evol. 25: 1253–1256.

Řeháková K., Johansen J. R., Casamatta D. A., Xuesong L. & Vincent J. (2007): Morphological and molecular characterization of selected desert soil *Cyanobacteria*: three species new to science including *Mojavia pulchra* gen. et sp. nov. – Phycologia 46: 481–502.

Ronquist F. & Huelsenbeck J. P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics 19: 1572–1574.

Rosowski J. R. & Lee K. W. (1991): Junctional pores and cell wall depressions of *Hormoscilla pringsheimii* (Cyanophyceae). – J. Phycol. 27: 257–268.

Round F. E. (1953): An investigation of two benthic algal communities in Malham Tarn, Yorkshire. – J. Ecol. 41: 174–179.

Siegesmund M. A., Johansen J. R., Karsten U. & Friedl T. (2008): *Coleofasciculus* gen. nov. (*Cyanobacteria*): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. – J. Phycol. 44: 1572–1585.

Skuja H. (1948): Taxonomie des phytoplanktons einiger seen in uppland, Schweden. – Symbol. Bot. Upsal. 9: 1–399.

Skuja H. (1956): Taxonomische und biologishe Studien über das phytoplankton schwedisher Binnengewässer. – Nova Acta Reg. Soc. Sci. Upsal., ser. IV, 16: 1–404.

Špačková J., Hašler P., Štěpánková J. & Poulíčková A. (2009): Seasonal succession of epipelic algae: a case study on a mesotrophic pond in a temperate climate. – Fottea 9: 121–133.

Starmach K. (1966): *Cyanophyta*. – Panstwowe Wydawnictwo Naukowe, Warszawa.

Staub R. (1961): Ernährungsphysiologisch-autökologische Untersuchungen an der planktischen Blaualge *Oscillatoria rubescens* DC. – Aquatic Sciences/Research Across Boundaries 23: 82–198.

Szafer W. (1910): Die sapropelische Lebewelt: ein Beitrag zur Biologie des Faulschlammes natürlicher Gewässer. – Verh. Natur. Med. Ver. Heidelberg 13: 395–481

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. (2011): MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – Mol. Biol. Evol. 28: 2731–2739.

Turicchia S., Ventura S., Komárková J. & Komárek J. (2009): Taxonomic evaluation of cyanobacterial microflora from alkaline marshes of northern Belize. 2. Diversity of oscillatorialean genera. – Nova Hedwigia 89: 165–200.

Turon X., Hernandez-Marine M. & Catalan J. (1991): A new species of *Komvophoron* (*Cyanophyta*, *Borziaceae*) epibiote on ascidians from the Mediterranean Sea. – Algol. Stud. 64: 249–259.

Willame R., Boutte C., Grubisic S., Wilmotte A., Komárek J. & Hoffmann L. (2006): Morphological and molecular characterization of planktonic *Cyanobacteria* from Belgium and Luxembourg. – J. Phycol. 42: 1312–1332.

Yanagihara K., Niki H. & Baba T. (2011): Direct PCR amplification of the 16S rRNA gene from single microbial cells isolated from an Antarctic iceberg using laser microdissection microscopy. – Polar Sci. 5: 375–382.

Zucker M. (2003): Mfold web server for nucleic acid folding and hybridization prediction. – Nucl. Acids Res. 31: 3406–3415.

Zwickl D. J. (2006): Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. – PhD. Diss., University of Texas, Austin.

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