

A novel genus *Ammassolinea* gen. nov. (Cyanobacteria) isolated from subtropical epipellic habitats

Petr HAŠLER^{1*}, Petr DVOŘÁK¹, Aloisie POULÍČKOVÁ¹ & Dale A. CASAMATTA²

¹ Department of Botany, Faculty of Science, Palacký University Olomouc, Šlechtelů 11, CZ–783 71 Olomouc, Czech Republic; *Corresponding author e-mail: petr.hasler@upol.cz

² University of North Florida, Department of Biology, University of North Florida, Jacksonville, Florida, USA; e-mail: dcasamat@unf.edu

Abstract: A new monospecific genus of an epipellic, filamentous oscillatorean *Phormidium*-like cyanobacterium is established. The genus was first discovered from a pond at Fiddlers Ridge Drive, Jacksonville, Florida, USA and subsequently isolated into uni-algal strains. During the process of cultivation, a combination of morphological, ultrastructural and molecular evidence supports the erection of a novel genus. Based on apomorphies which differ from related taxa such as heap-like colony formation, ultrastructural properties and molecular uniqueness, we propose the generic epithet *Ammassolinea* (sand-dwelling filaments).

Key words: cyanobacteria, Florida, new genus, 16S rDNA, ITS

INTRODUCTION

Cyanobacteria are a group of photo-oxygenic prokaryotes present in nearly all ecosystems (WHITTON & POTTS 2000). Amongst the most ancient lineages of life, cyanobacteria are often studied due to their ubiquity in aquatic habitats and prevalence in freshwater harmful algal blooms. In these cases, cyanobacteria have been shown to produce numerous secondary metabolites which can have negative impacts on associated aquatic flora and fauna (QUIBLIER et al. 2013).

The vast majority of cyanobacteria studied are from planktonic or aerophytic habitats such as soils (POULÍČKOVÁ et al. 2008, 2014). While the biodiversity of cyanobacteria from these habitats is well established, even if the systematic relationships have not been resolved, there are aquatic habitats from which cyanobacterial diversity is poorly known. For example, recent investigations into epipellic habitats (e.g. HAŠLER & POULÍČKOVÁ 2010; HAŠLER et al. 2012; HAŠLER et al. 2014) have revealed a potential wealth of novel taxa. Furthermore, tropical and subtropical regions have been woefully understudied to date.

Cyanobacteria have traditionally been identified using morphological features, although this practice has been shown to be potentially problematic due to phenotypic or culture-induced plasticity, such as commonly seen with sheaths (CASAMATTA et al. 2003; CASAMATTA et al. 2005; PERKERSON et al. 2011; DVOŘÁK et al. 2012; HAŠLER et al. 2012). The systematic relationships among the cyanobacteria are being revised

by the use of molecular markers, most notable the 16S rDNA gene sequence and Internal Transcribed Spacer regions (JOHANSEN & CASAMATTA 2005; PERKERSON et al. 2011; CASAMATTA et al. 2012; HAŠLER et al. 2012, DVOŘÁK et al. 2014; HAŠLER et al. 2014). These molecular approaches have facilitated an explosion of new taxa descriptions by using markers including the secondary folding structures of the ITS regions to provide the much needed phylogenetic signals to character poor or phenotypic plastic taxa. For example, many filamentous cyanobacteria lacking specialized cells (e.g. the Oscillatoriales *sensu stricto*), cannot necessarily be differentiated based solely on morphology. A polyphasic approach employing sequence data, ITS secondary structures, ecology, morphology and chemotaxonomy have allowed a much more robust assessment of phylogenetic relationships over the last decade (CASAMATTA et al. 2012; ENGENE et al. 2013).

The purpose of this paper is to present a novel lineage of cyanobacteria recovered from a seldom sampled epipellic habitat from a seldom sampled subtropical region. This novel lineage superficially resembles the polyphyletic genus *Phormidium*, and yet is genetically quite distinct. Thus, we propose the novel generic epithet *Ammassolinea* to represent this new clade.

MATERIALS AND METHODS

Strain isolation. Strains were isolated from a pond at Fiddlers Ridge Drive, Jacksonville, Florida. Samples were

epipellic on mixed sediment of sand and organic detritus (30°06'31.0"N, 81°42'51.7"W).

The cultures were maintained in 100 ml Erlenmeyer flasks under the following conditions: temperature 22±1 °C, illumination 20 μmol.m⁻².s⁻¹, light regime 12h light/12h dark, and liquid Zehnder medium (STAUB 1961).

Morphological assessment. Morphology of the strain and natural samples were analyzed using a light microscope Zeiss AxioImager (objectives EC Plan–Neofluar 40×/1.3 N.A., oil immersion, DIC; Plan–Apochromat 100×/1.4 N.A., oil immersion, DIC). Images were taken with a high resolution camera (AxioCam HRc 13MPx). During morphological evaluation of natural samples and strains, the following characters were assessed: cell shape, terminal cell shape, cell dimensions, reproduction, sheaths, and granulation of cells. Measurements were performed on 100 cells of both natural and culture materials.

TEM sample preparation. The sample for TEM was prepared as follows: heap-like colonies of filamentous cyanobacterium were fixed with 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). Sample was washed in 0.05 M phosphate buffer (pH 7.2). Afterwards, the sample was post-fixed in 1% osmium tetroxide diluted in the same phosphate buffer for 2 hours at room temperature. Subsequently, filaments were dehydrated in a gradient of isopropanol (25–100%), and embedded in Spurr's resin. Ultrathin cross-sections (90 nm) were stained with 1% uranyl acetate and Pb-citrate and observed in Jeol JEN 1010 transmission electron microscope (electron emitter: Lanthanum Hexaboride Cathode LaB6, emission intensity: 100 kV).

PCR amplification and sequencing. Genomic DNA was extracted using an UltraClean Microbial DNA Isolation Kit (MOBIO, Carlsbad, CA, USA) from approximately 50 mg of fresh biomass and following the manufacturer's manual. DNA quality and consistence was checked on ethidium bromide stained 1.5% agarose gel. DNA was quantified using the NanoDrop 1000 (Thermo Fisher Scientific, Wilmington, DE, USA).

PCR amplification of the partial 16S rDNA and the whole 16S–23S ITS was performed using primers forward P2 (5'-GGGGAATTTTCGCAATGGG-3'), and reverse P1 (5'-CTCTGTGTGCCTAGGTATCC-3') previously described in Boyer et al. (2002). The PCR reaction, with a total volume of 40 μl, contained: 17 μl of sterile water, 1 μl of each primer (0.01 mM concentration), 20 μl FastStart PCR master (Roche Diagnostics GmbH, Mannheim, Germany), and 1 μl of template DNA (50 ng.μl⁻¹). PCR amplification was performed with the conditions used before in DVOŘÁK et al. (2012). The PCR products were cloned using a StrataClone PCR Cloning Kit (Agilent Technologies, Stratagene Product Division, La Jolla, CA, USA) following the manufacturer's instructions. After the white–blue selection on ampicillin 1.5% agarose plates with Luria Bertani medium, at least 4 positive colonies were transferred into fresh liquid LB medium, and cultured overnight at 37 °C. The plasmid was isolated using a GenElute™ Five-Minute Plasmid Miniprep Kit (Sigma–Aldrich, Co., Saint Louis, MO, USA).

Plasmids were commercially sequenced with primers M13F and M13R. Moreover, two additional internal sequencing primers were added P5 (5'-TGTACACACC-GCCCGTC-3'), and P8 (5'-AAGGAGGTGATCCAGC-CACA-3') (BOYER et al. 2001, 2002). Sequences were as-

sembled and proofread in Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>), accession numbers: KM491920–KM491928.

Phylogenetic analyses. The most similar sequences of 16S rDNA were identified using nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple sequence alignment was performed in MEGA 6 (TAMURA et al. 2013) using Muscle algorithm (EDGAR 2004). All other non-identical reference sequences of *Phormidium* were added. The tree was rooted to a bacterial outgroup. Representative sequences of the heterocyst forming cyanobacteria Nostocales were also added. The most appropriate model for maximum likelihood analyses was determined in jModelTest 0.1.1 (POSADA 2008) based on both the Bayesian and the Akaike Information Criterion as follows: General Time Reversible model with gamma distributed variation across sites. Fifty percent majority consensus tree was constructed in MrBayes 3.2.1 (RONQUIST & HUELSENBECK 2003). Two separate runs were performed, each with 3 heated and 1 cold chains for 15000000 generations. The sampling frequency was each 5000th generation. Twenty five percent of trees were discarded as burn-in. Maximum likelihood analysis was performed in RaxML 8.0.2 (STAMATAKIS 2014) with a model predicted by model test. Rapid bootstrapping analysis was used to test the tree topology. Maximum parsimony analyses were performed in PAUP* 4.0b10 (SWOFFORD 2001), gaps were treated as missing data. All analyses were tested using bootstrapping with 1000 replicates. Similarity matrix was computed using ClustalW 2.0 (LARKIN et al. 2007). The secondary structures of D1–D1' helix and Box–B helix ITS regions were predicted with the Mfold web server version 3.5 (ZUCKER 2003) with temperature set to default (37 °C).

RESULTS

Morphology and new genus establishment

Amassolinea gen. nov.

Description: trichomes solitary or aggregated into macroscopic heap-like colonies, straight or slightly bent, more or less attenuated at the apical cells; trichomes without mucilaginous sheath, not or slightly constricted at the cross-walls, motile (bending, gliding), cells shorter than wide to longer than wide and grow into original size before next division, apical cells rounded, broadly conical to conical, trichomes divide into hormogonia via the formation of necridic cells. Cells possess radial arrangement of thylakoids on transversal plane. Thylakoids on longitudinal plane form skew-like structures

Type species: *Amassolinea attenuata*

Etymology: The generic epithet is based on *ammos*, Greek for sand, and *linea*, Latin for line

Amassolinea attenuata sp. nov. (Figs 1–2)

Description: trichomes solitary, later aggregated into macroscopic heap-like colonies, straight or slightly bent, more or less attenuated at the apical cells, tri-

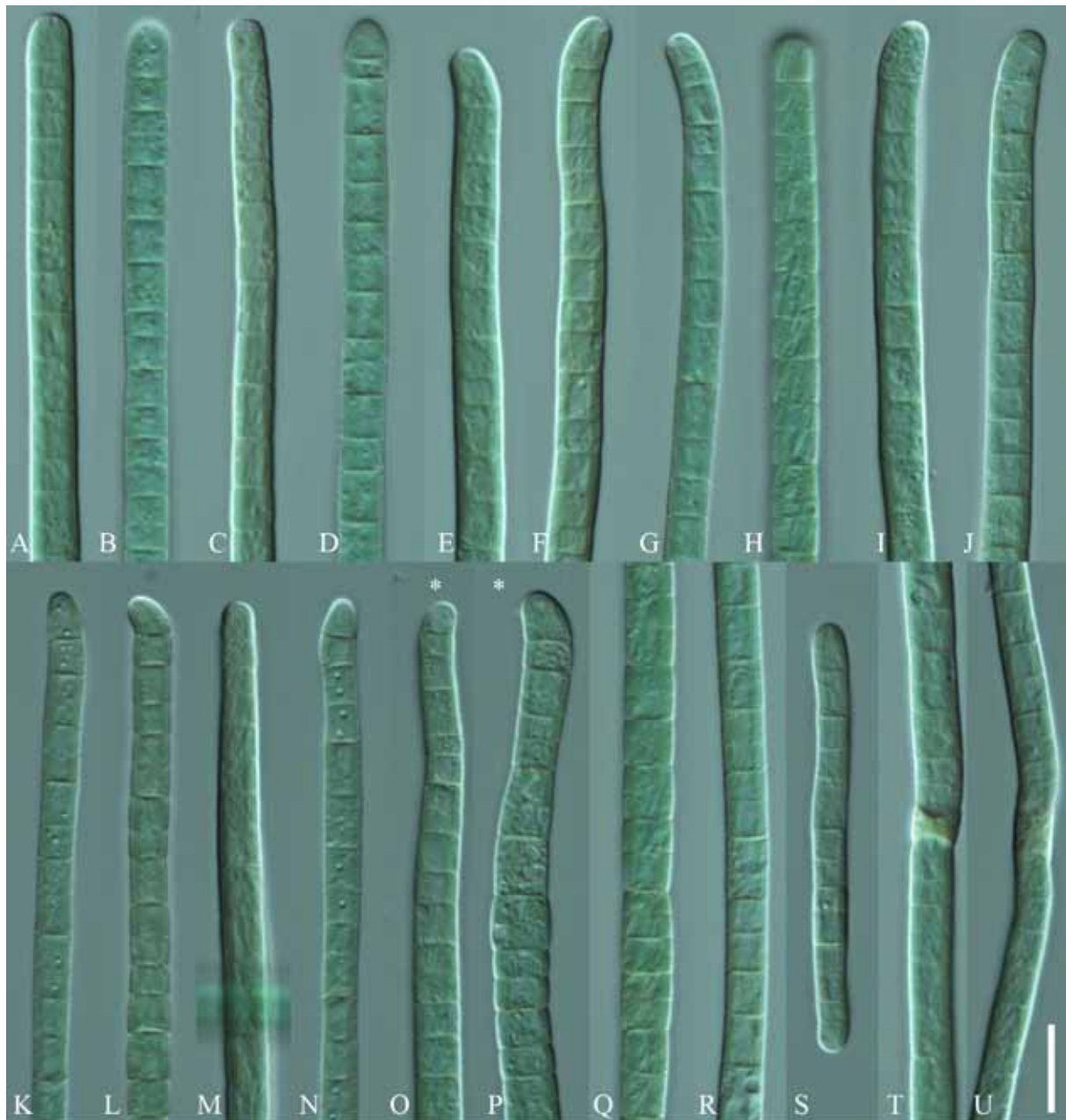


Fig. 1. *Ammasolinea attenuata*, holotype, illustrating the variability of trichomes (A–R); (S) hormogonium; (T–U) formation of necridic cells; * different shapes of trichome ends of the same trichome. Type strain UPOC 61/2013 originating from a Pond at Fiddlers Ridge Drive. Scale bar 10 μ m.

chome without mucilaginous sheath, motile, not or slightly constricted at the cross-walls, cells rectangular or barrel-like, 5.25 ± 0.79 μ m wide, 4.32 ± 1.04 μ m long, cell content green, blue-green or pale blue-green with visible chromatoplasm (especially under laboratory conditions), without granules at the cross-walls, trichomes divide into hormogonia via the formation of necridic cells. Species possesses radial arrangement of thylakoids on transversal plane. Thylakoids on longitudinal plane form skew-like structures.

Etymology: the species epithet is derived from attenuated ends of trichomes.

Type locality: Pond at Fiddlers Ridge Drive, Jackson-

ville, Florida; $30^{\circ}06'31.0''$ N, $81^{\circ}42'51.7''$ W.

Habitat: epipellic on mixed sediment of sand and organic detritus.

Iconotype: Fig. 1.

Holotype: OLM Botany 24: Lichenes and others no. 9218, dried sample is deposited in Regional Museum in Olomouc, Czech Republic. Type strain: UPOC 61/2013, deposited at the culture collection of Department of Botany, Palacký University in Olomouc, Czech Republic.

Isotype: UPOC 64/2013, deposited at the culture collection of Department of Botany, Palacký University in Olomouc, Czech Republic, Fig. 2.

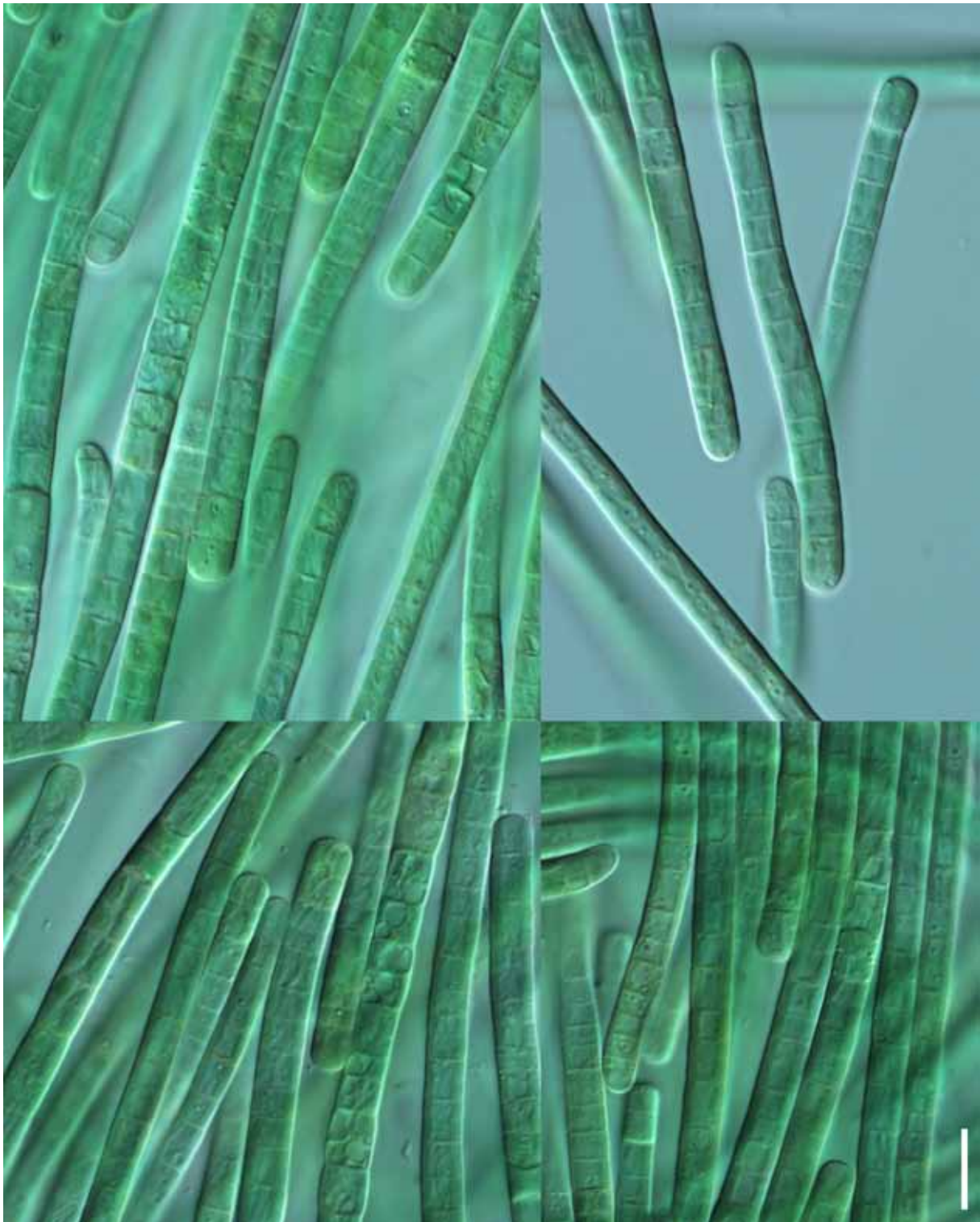


Fig. 2. *Ammassolinea attenuata*, isotype, illustrating the variability of trichomes. Strain UPOC 64/2013 originating from a pond at Fiddlers Ridge Drive. Scale bar 10 μ m.

Phylogenetics

Phylogenetic trees constructed using 16S rDNA gene sequence data yielded a tree typology with an uncertain placement of *Ammassolinea* (Fig. 4). The clade containing the isolates and their clones was highly supported (100%), but the placement of this clade was

not well supported. All sequenced clones formed a coherent cluster with minimal differences among them. A similarity matrix constructed from all 16S rDNA clones showed 99.59–99.9% similarity. Our new isolates shared only 93.15% similarity with the closest related Blast hit (*Phormidium* DVL 1003c, JQ771628),

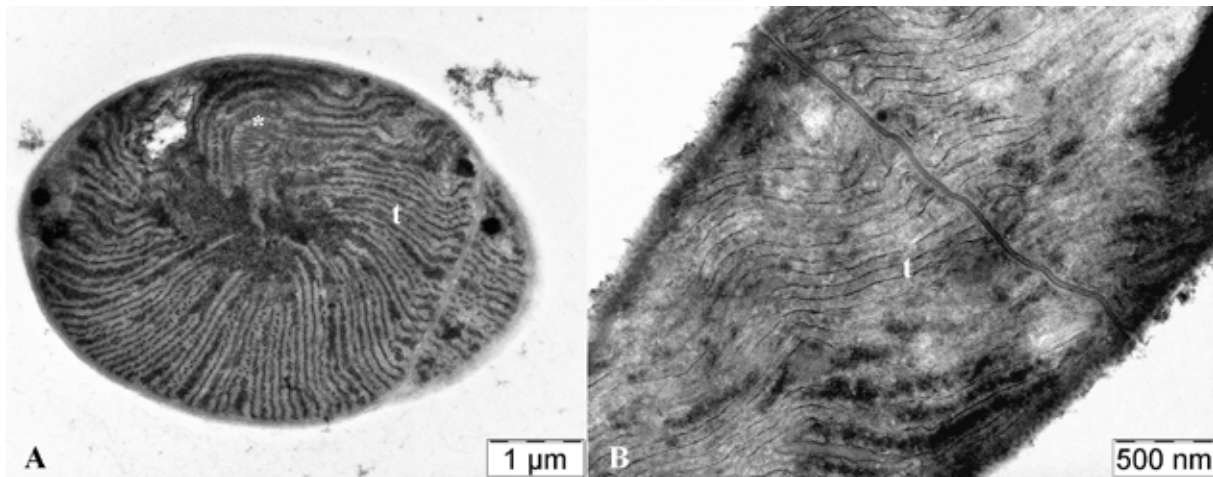


Fig. 3. *Ammassolinea attenuata* cross-sections: (A) transversal section, (t) radial arrangement of thylakoids, (*) membranous stack; (B) longitudinal section, (t) skew-like oriented thylakoids.

which belongs to a different clade. There are another five clades that contain taxa putatively identified as “*Phormidium*”, further illustrating the polyphyletic nature of this genus and revealing additional unrevised, polyphyletic lineages.

ITS analysis

Secondary structures of the ITS region revealed that both strains have two different operons. One operon contains both tRNAs for isoleucine and alanine and the other lacks both tRNAs. The D1–D1’ helices are identical, but significant differences were found in Box B helices (Fig. 5). The basal sequence was identical (5’–CAGCA–UGCUG–3’). Clones lacking tRNAs had only small terminal loop and now basal loop in Box B. On the other hand, clones having both tRNAs had larger terminal loop than clones lacking tRNAs. Moreover, they contained large basal loop (Fig. 5).

DISCUSSION

Cyanobacteria are exceedingly common components of freshwater ecosystems, yet certain habitats, such as epipelon or epiphyton, are rarely sampled for their cyanobacterial components. This may be due to the difficulties in collecting, identifying, and culturing these organisms, but recent investigations into epipellic samples have revealed numerous new taxa (POULÍČKOVÁ et al. 2008, 2014; HAŠLER et al. 2012, 2014). Further, most aquatic ecologists have been slow to appreciate the potential role that microbes in these habitats may play in the larger aquatic ecology. While we did not examine the putative ecological role that *Ammassolinea* may have in epipellic habitats, we do note that the morphologically similar genus *Phormidium* is commonly grazed upon, and thus may be of ecological importance for the invertebrate community. Moreover,

these epipellic assemblages may also play a role in substrate stabilisation and create microhabitats for bacterial communities involved in nutrient transformation. Morphologically, trichomes of our new genus resemble those of *Phormidium*. *Phormidium* is the most commonly encountered stream macroalgae in North America (SHEATH & COLE 1992), but is highly polyphyletic and character poor (CASAMATTA et al. 2003). Further, there are numerous cryptic taxa within this genus (CASAMATTA et al. 2003). Thus, it is not surprising that *Ammassolinea* is nearly indistinguishable morphologically from *Phormidium*. However, based on a range of evidence we conclude that *Ammassolinea* is a novel genus. The ecology (epipellic habitats from a subtropical region) is very different from other similarly described *Phormidium* taxa. The colony morphology of *Ammassolinea* (heap-like) is also different than other currently described taxa of *Phormidium sensu lato*. The ultrastructures of members of *Phormidium* are heterogeneous and do not exhibit a uniform pattern (MARQUARDT & PALINSKA 2007) among which both parietal and radial arrangements of thylakoids prevail. Thylakoids of *Ammassolinea attenuata* exhibit radial orientation (Fig. 3) but form wavy rather than straight strips as found in *Phormidium* or *Microcoleus* (e.g. in *Phormidium cf. irriguum*, *Microcoleus autumnalis*; LOKMER 2007; STRUNECKÝ et al. 2013). Longitudinal sections of *Ammassolinea* do not show a fasciculate thylakoid arrangement, rather they are arranged slightly diagonally throughout the whole cell. So in general, thylakoids form skew-like structures along the longitudinal cell axis. Other members of *Phormidium* (*Ph. inundatum* or *Ph. tergestinum*) form parietal thylakoids which are different from the orientation observed in *Ammassolinea*. We identified membranous stacks in the structure of *Ammassolinea* but we do not have any information whether this structure also occurs in *Phormidium*. These patterns were previously reported in *Cylindrospermum* sp., several chroococcalean endo-

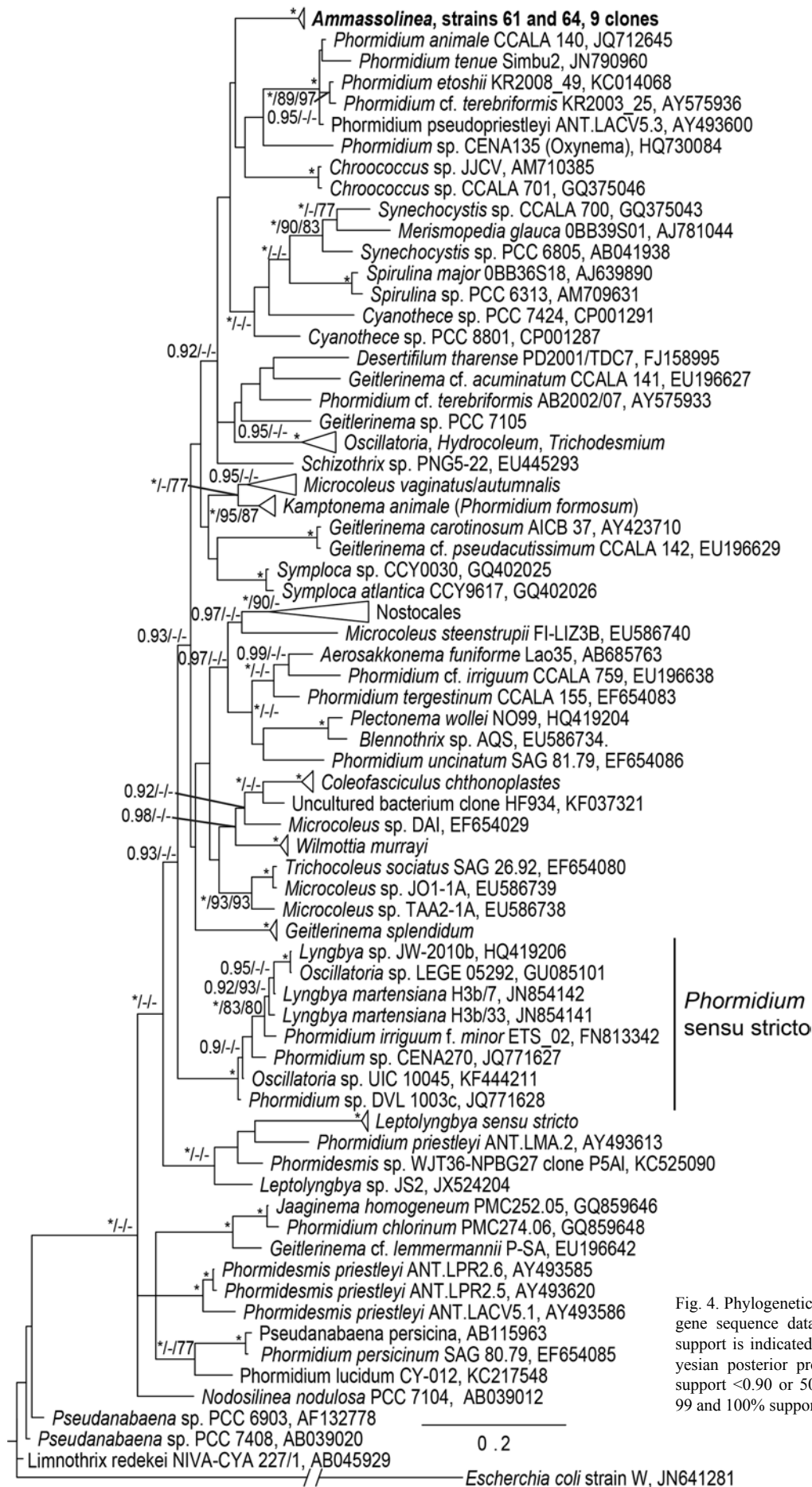


Fig. 4. Phylogenetic analyses of 16S rDNA gene sequence data from 76 taxa. Node support is indicated as bootstraps and Bayesian posterior probabilities; “-“ means support <0.90 or 50% and asterisk means 99 and 100% support.

lithic species and in the genus *Synechococcus* (JENSEN 1993). The importance of membranous stacks for cyanobacteria is not clear. JENSEN (1993) suggests that these structures are important for cellular syntheses, but their role in *Ammassolinea* is unclear at this time.

The 16S sequence data clearly distinguishes *Ammassolinea* from sister taxa, sharing only 93.15% sequence similarity with its closest neighbour *Phormidium* DVL 1003c which clusters with *Phormidium sensu stricto* (STRUONEKÝ et al. 2014). Thus, it is clear that *Ammassolinea* is new genus clustering aside the type of *Phormidium*. The secondary folding patterns of the ITS are also very different from closely related taxa. The secondary folding pattern of the D1–D1' helix resembles that of other *Phormidium*-like taxa, such as *Kamptonea* (HAŠLER et al. 2012; STRUONEKÝ et al. 2014), *Microcoleus vaginatus*, and *Microcoleus autumnalis* (HAŠLER et al. 2012). It possesses a larger terminal loop and two rather small loops close to the basis of the helix. This character in particular has increasingly been employed as a stable marker for distinguishing cryptic genera (JOHANSEN et al. 2011; CASAMATTA et al. 2012). Finally, the heap-like colony morphology of *Ammassolinea* is distinct and different from typical *Phormidium* which is characteristically expanded, widely attached to the substrate, gelatinous, and mucilaginous according to KOMÁREK & ANAGNOSTIDIS (2005). Morphologically, *Ammassolinea* trichomes resemble *Phormidium taylorii*, which is also recorded from the United States, but the latter do not taper toward the apices (KOMÁREK & ANAGNOSTIDIS 2005).

Cyanobacterial species concepts are subject to much debate. Recently, a proliferation of new cyanobacterial taxa have been precipitated by the use of molecular markers coupled with an apomorphic species concept more conducive to the erection of new taxa (e.g., JOHANSEN & CASAMATTA 2005). Our ecological, morphological and molecular data indicates that our isolates are sufficiently removed from any other sister taxa to warrant the erection of a new genus. The subtropical habitat of isolation represents a depauperate area in terms of its ecological and information.

While some may be reluctant to erect a new genus without a clear morphological distinction (at the trichome level), there are several reasons that this is acceptable. Firstly, recent works dealing with tropical marine cyanobacteria have revealed numerous new genera which share morphological similarity with "*Lyngbya*", but possess widely divergent chemotaxonomic and genetic (16S rDNA) markers (ENGINE et al. 2012, 2013). Secondly, geno-genera (those units erected based on genetic signatures without a corresponding morphological character) may harbor environmentally or culturally induced morphological differentiation currently not realized. For example, the recently erected genus *Nodosilinea* was originally described as a "*Leptolyngbya*" but with inducible formation of nodules on the trichomes at low light levels (LI & BRAND

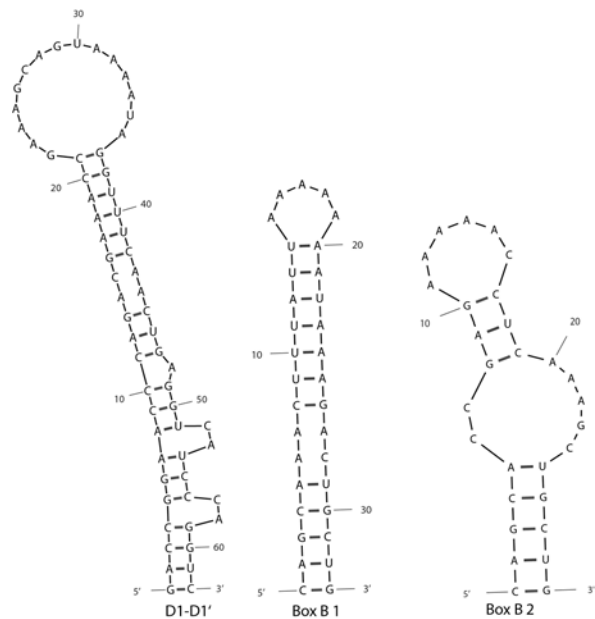


Fig. 5. ITS secondary structures found for the most important helices as follows: D1–D1', Box B1, Box B2.

2007). This genus was at first a geno-genus, with a fortuitous morphological apomorphy subsequently discovered (PERKERSON et al. 2011). Lastly, morphological similarity may mask ecological divergence. As a whole, cyanobacteria are intimately tied to convergent evolution constrained by simple bacterial morphology. For example, the ubiquitous *Synechococcus* has been described in marine and freshwaters throughout the world. However, recent inversions have shown that this genus contains numerous lineages (at least seven new genera) masked by lack of morphological evidence (DVOŘÁK et al. 2014).

Acknowledgements

Minor gratefulness belong to the grants ESF Post-UP II CZ.1.07/2.3.00/30.0041 and IGA PrF 2014001.

REFERENCES

- BOYER, S.L.; FLETCHNER, V. & JOHANSEN, J.R. (2001): Is the 16S–23S rRNA internal transcribed spacer (ITS) region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. – *Molecular Biology and Evolution* 18: 1057–1069.
- BOYER, S.L.; JOHANSEN, J.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. – *Journal of Phycology* 38: 1222–1225.
- CASAMATTA, D.A.; STANIĆ, M.; GANTAR & RICHARDSON, L.L. (2012): Characterization of *Roseonema reptotaenium* (Oscillatoriales, Cyanobacteria) gen. et sp. nov. isolated from Caribbean black band disease. – *Phycologia* 51:489–499.

- CASAMATTA, D.A.; VIS, M.L. & R.G. SHEATH. (2003): Cryptic species in cyanobacterial systematics: a case study of *Phormidium retzii* (Oscillatoriales) using 16S rDNA and RAPD analyses. – *Aquatic Botany* 77: 295–309.
- 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). – *Journal of Phycology* 41: 421–438.
- DVOŘÁK, P.; HAŠLER, P. & POULÍČKOVÁ, A. (2012): Phylogeography of the *Microcoleus vaginatus* (cyanobacteria) from three continents – a spatial and temporal characterization. – *PLoS ONE* 7: e40153. doi:10.1371/journal.pone.0040153
- DVOŘÁK, P.; HINDÁK, F.; HAŠLER, P.; HINDÁKOVÁ, A. & POULÍČKOVÁ, A. (2014): Morphological and molecular studies of *Neosynechococcus sphagnicola*, gen. et sp. nov. (Cyanobacteria, Chroococcales). – *Phytotaxa* 170: 24–34.
- ENGENE, N.; GUNASEKERA, S. P.; GERWICK, W. H. & PAUL, V. J. (2013): Phylogenetic Inferences Reveal Large Extent of Novel Biodiversity in Chemically Rich Tropical Marine Cyanobacteria. – *Appl. Env. Microbiol.* 79: 1882–1888.
- ENGENE, N.; ROTTACKER, E. C.; CHOI, H.; BYRUM, T.; KAŠTOVSKÝ, J. H.; KOMÁREK, J.; ELLISMAN, M. H. & GERWICK W. H. (2012): *Moorea producta* gen. nov., sp. nov. and *Moorea bouillonii* comb. nov., tropical marine cyanobacteria rich in bioactive secondary metabolites. – *Int. J. Syst. Evol. Microbiol.* 62: 1172–1179
- HAŠLER, P.; DVOŘÁK, P.; JOHANSEN, J.R.; KITNER, M.; ONDŘEJ, V. & POULÍČKOVÁ, A. (2012): Morphological and molecular study of epipellic filamentous genera *Phormidium*, *Microcoleus* and *Geitlerinema* (Oscillatoriales, Cyanophyta/Cyanobacteria). – *Fottea* 12: 341–356.
- HAŠLER, P.; DVOŘÁK, P. & POULÍČKOVÁ, P. (2014): A new genus of filamentous epipellic cyanobacteria, *Johansenia*. – *Preslia* 86: 81–94.
- JENSEN, T. E. (1993): Cyanobacterial Ultrastructure. – In: BERNER, T. (ed.): *Ultrastructure of Microalgae*. – pp. 7–51, CRC Press, Boca Raton, Florida.
- JOHANSEN, J.R. & CASAMATTA, D.A. (2005): Recognizing cyanobacterial diversity through adoption of a new species paradigm. – *Algological Studies* 117: 71–93.
- JOHANSEN, J.R.; KOVÁČIK, L.; CASAMATTA, D.A. & KAŠTOVSKÝ, J. (2011): *Leptolyngbya corticola* sp. nov. (Pseudanabaenaceae, Cyanobacteria), an aerophytic, heteropolar taxon from the Czech Republic. – *Nova Hedwigia* 92: 283–302.
- KOMÁREK, J. & ANAGNOSTIDIS, K. (2005): Cyanoprokaryota. 2. Teil: Oscillatoriales. – In: BÜDEL, B.; GÄRDNER, G.; KRINENITZ, L. & SCHAGERL, M. (eds): *Süßwasserflora von Mitteleuropa*, Vol. 19/2. – 759 pp., Elsevier, München.
- LARKIN, M.A.; BLACKSHIELDS, G.; BROWN, N.P.; DUENNA, R.; MCGETTIGAN, P.A.; MCWILLIAM, H.; VALENTIN, F.; WALLACE, I.M.; WILM, A.; LOPEZ, R.; THOMPSON, J.D.; GIBBON, T.J. & HIGGINS, D.G. (2007): Clustal W and Clustal X version 2.0. – *Bioinformatics* 23: 2947–2948.
- LI, Z. & BRAND, J. (2007): *Leptolyngbya nodulosa* sp. nov. (Oscillatoriales), a subtropical marine cyanobacterium that produces a unique multicellular structure. – *Phycologia* 46: 396–401.
- LOKMER, A. (2007): Polyphasic approach to the taxonomy of the selected oscillatorian strains (Cyanobacteria) [Ms. thesis]. – 61 pp., University of South Bohemia, České Budějovice, Czech Republic.
- MARQUARDT, J. & PALINSKA, K.A. (2007): Genotypic and phenotypic variability of *Phormidium*-like cyanobacteria from different habitats and geographical sites. – *Archives of Microbiology* 187: 397–413.
- PERKERSON, R.B.; JOHANSEN, J.R.; KOVÁČIK, L.; BRAND, J.; KAŠTOVSKÝ, J. & CASAMATTA, D.A. (2011): A unique Pseudanabaenacean (Cyanobacteria) genus *Nodosilinea* gen. nov. based on morphological and molecular data. – *Journal of Phycology* 47: 1397–1412.
- Posada, D. (2008): jModelTest: phylogenetic model averaging. – *Molecular Biology and Evolution* 25: 1253–1256.
- POULÍČKOVÁ, A.; HAŠLER, P.; LYSÁKOVÁ, M. & SPEARS, B. (2008): The ecology of freshwater epipellic algae: an update. – *Phycologia* 47: 437–450.
- POULÍČKOVÁ, A.; DVOŘÁK, P.; MAZALOVÁ, P. & HAŠLER, P. (2014): Epipellic microphototrophs: an overlooked assemblage in lake ecosystems. – *Freshwater Science* 33: 513–523.
- Quiblier, C.; Wood, S.; Echenique-Subiabre, I.; Heath, M.; Villeneuve, A. & Humbert, J.F. (2013): A review of current knowledge on toxic benthic freshwater cyanobacteria – Ecology, toxin production and risk management. – *Water Research* 47: 5464–5479.
- RONQUIST, F. & HUELSENBECK, J. P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics* 19: 1572–1574.
- SHEATH, R.G. & COLE, K.M. (1992): Biogeography of stream macroalgae in North America. – *J. Phycol.* 28: 448–460.
- STAMATAKIS, A. (2014): RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. – *Bioinformatics*, DOI: 10.1093/bioinformatics/btu033
- STAUB, R. (1961): Research on physiology of nutrients of the planktonic cyanobacterium *Oscillatoria rubescens*. – *Schweizerische Zeitschrift Fur Hydrologie* 23: 83–198.
- STRUŠNEČKÝ, O.; KOMÁREK, J.; JOHANSEN, J.; LUKEŠOVÁ, A. & ELSTER, J. (2013): Molecular and morphological criteria for revision of the genus *Microcoleus* (Oscillatoriales, Cyanobacteria). – *J. Phycol.* 49: 1167–1180.
- STRUŠNEČKÝ, O.; KOMÁREK, J. & ŠMARD, J. (2014): *Kamptomena* (Microcoleaceae, Cyanobacteria), a new genus derived from the polyphyletic *Phormidium* on the basis of combined molecular and cytological markers. – *Preslia* 86: 193–207.
- SWOFFORD, D. L. (2001): PAUP*. Phylogenetic analysis usány parsimony (*and other methods). – Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Tamura, K.; Stecher, G.; Peterson, D.; Filipiński, A. & Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. – *Molecular Biology and Evolution* 30: 2725–2729.
- WHITTON, B.A. & POTTS, M. (2000): The ecology of cyanobacteria. Their diversity in time and space. – 669 pp., Springer, Berlin.
- ZUCKER, M. (2003): Mfold web server for nucleic acid folding and hybridization prediction. – *Nucleic Acids Res.* 31: 3406–3415.