South African Journal of Botany 123 (2019) 333–340

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

The reclassification of 37 strains from *The Mosonmagyaróvár Algal Culture Collection*, Hungary, which were previously identified as *Anabaena* (Cyanobacteria, Nostocaceae)

N. Horváth [a,*], S. Katona [a], D.E. Berthold [b], Z. Molnár [a], P. Bálint [a], V. Ördög [a,c], B. Pap [d], G. Maróti [d], F. Bánáti [e], K. Szenthe [e], L. Vörös [f], C. Kilgore [b], H.D. Laughinghouse IV [b,g]

[a] Department of Plant Sciences, Faculty of Agricultural and Food Sciences, Széchenyi István University, Vár square 2, H-9200 Mosonmagyaróvár, Hungary
[b] Ft. Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave, Davie, FL 33314, USA
[c] University of KwaZulu-Natal, Research Centre for Plant Growth and Development, Agriculture campus, Carbis Road, Scottsville, Pietermaritzburg, South Africa
[d] Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt. 62, H-6726 Szeged, Hungary
[e] RT-EUROPE Non-profit Ltd., Vár square 2, 'E' Building, H-9200 Mosonmagyaróvár, Hungary
[f] Balaton Limnological Institute, Ecological Research Centre of the Hungarian Academy of Sciences, Klebelsberg Kunó st. 3, H-8237 Tihany, Hungary
[g] Department of Botany, National Museum of Natural History, Smithsonian Institution, PO Box 37012, SI Building, Room 153, MRC 010, Washington, DC 20013-7012, USA

ARTICLE INFO

Article history:
Received 18 July 2018
Received in revised form 23 December 2018
Accepted 6 March 2019
Available online 12 April 2019

Edited by WA Stirk

Key-words:
Molecular phylogenetics
Desmonostoc
Nostoc
Trichormus
Anabaena

ABSTRACT

Study on 37 MACC isolates previously identified as "*Anabaena*," a freshwater filamentous heterocytous taxon, were carried out using the 16S rRNA. The study found that most of the strains were misidentified at genus level. Three clusters of phylogenetically and morphologically similar taxa were identified. The previous determinations were amended with their new taxonomic classifications (partly due to changes in cyanobacterial classification). Some morphological structures could not be found in the cultures (*e.g.* akinetes). Molecular data revealed that 6 of the 37 strains are *Desmonostoc*, 8 are members of the genus *Nostoc*, 19 strains bear genetic resemblance to the genus *Trichormus* and 4 strains remain unresolved. Clades were established by 16S rRNA similarity and p-distances. The goal of this study was to amend the strain designations in this collection. This study reveals the necessity to revisit established culture collections that originally used only morphological classifications for species identification.

1. Introduction

Cyanobacteria are a vast and morphologically diverse group of photo-oxygenic bacteria with wide ecological tolerances and found in most habitats on Earth. Their taxa were traditionally identified based on morphological characters; however, molecular techniques are increasingly incorporated into their study. The combination of molecular (*e.g.* 16S rRNA, ITS, rbclx, rpoC1), morphological and ecological markers (the "polyphasic approach") has become the golden standard in cyanobacterial taxonomy, especially for cryptic species (Fox et al., 1992; Boyer et al., 2001; Malone et al., 2015).

Heterocytous cyanobacteria may be difficult to characterize as their morphology are similar and numerous morphologically well-defined genera appear polyphyletic (Bohunická et al., 2015). One such example is the genus *Anabaena*, which was found polyphyletic by several researchers and currently represents multiple genera (see Komárek, 2013). Several new genera have been described and erected from the original genus and many taxa have been transferred to *Dolichospermum*, *Trichormus*, *Chrysosporum* and *Sphaerospermopsis*. The original genus *Anabaena*, according to the type species, is closer to a large clade containing *Trichormus*, *Nostoc*, *Cylindrospermum* and *Wollea* (Komárek, 2013).

Scrutinizing genera, especially *Anabaena*, is essential with the increasing interest in their toxicology and prevalence through blooms. *Anabaena* is an important genus due to the innumerous chemical compounds they can produce. Certain *Anabaena* species produce harmful toxins with detrimental effects, and projections indicate that these will increase with environmental changes (Lürling et al., 2017). Several *Anabeana* strains found in the MACC culture collection, such as *Anabaena sphaerica* Bornet & Flahault, *Anabaena constricta* (Szafer)

Abbreviations: MACC, the Mosonmagyaróvár Algal Culture Collection; CCALA, Culture Collection of Autotrophic Organisms; MUSCLE, MUltiple Sequence Comparison by Log-Expectation; MEGA, Molecular Evolutionary Genetics Analysis; TIM2 + G + I model, Transition model; RAxML, Randomized Axelerated Maximum Likelihood; OUT, Operational Taxonomic Unit.

* Corresponding author.
E-mail address: horvathnandor@windowslive.com (N. Horváth).

https://doi.org/10.1016/j.sajb.2019.03.014
0254-6299/

Table 1
Summary of phylogenetic results of 37 strains from the MACC collection of Széchenyi István University.

Cluster		MACC strain code	Origin/Source	Previous morphological assignment	New phylogenetic assignment	NCBI (1988) GenBank Accession number
Trichormus	Pond subcluster	63	Fish pond, Hungary	*Anabaena* sp.	*Trichormus* sp.	KY807521
		68	Fish pond, Hungary	*Anabaena* sp.	*Trichormus* sp.	KY807516
		118	Fish pond, Hungary	*Anabaena variabilis*	*Trichormus* sp.	KY807527
		122	Fish pond, Hungary	*Anabaena flos-aquae*	*Trichormus* sp.	KY807524
		248	Fish pond, Hungary	*Anabaena tenericaulis*	*Trichormus* sp.	KY807523
	Soil subcluster	123	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY778000
		140	Soil, Serbia	*Anabaena* sp.	*Trichormus* sp.	KY794657
		141	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY794656
		155	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY794651
		160	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY794652
		217	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY794655
		227	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY794658
		246	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY794653
		264	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY807509
		265	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY807510
		266	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY807507
		267	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY807508
		269	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY807528
		274	Soil, Serbia	*Anabaena constricta*	*Trichormus* sp.	KY807522
Desmonostoc		171	Soil, Serbia	*Anabaena constricta*	*Desmonostoc* sp.	KY807519
		279	Soil, Serbia	*Anabaena constricta*	*Desmonostoc* sp.	KY807512
		282	Soil, Serbia	*Anabaena constricta*	*Desmonostoc* sp.	KY807515
		288	Soil, Serbia	*Anabaena constricta*	*Desmonostoc* sp.	KY807529
		290	Soil, Serbia	*Anabaena variabilis*	*Desmonostoc* sp.	KY807513
		293	Soil, Serbia	*Anabaena constricta*	*Desmonostoc* sp.	KY807520
Nostoc		159	Soil, Serbia	*Anabaena constricta*	*Nostoc* sp.	KY807534
		165	Soil, Serbia	*Anabaena constricta*	*Nostoc* sp.	KY807511
		166	Soil, Serbia	*Anabaena constricta*	*Nostoc* sp.	KY807517
		240	Soil, Serbia	*Anabaena* sp.	*Nostoc* sp.	KY807525
		243	Soil, Serbia	*Anabaena* sp.	*Nostoc* sp.	KY807533
		253	Soil, Serbia	*Anabaena* sp.	*Nostoc* sp.	KY807535
		258	Soil, Serbia	*Anabaena variabilis*	*Nostoc* sp.	KY807536
		268	Soil, Serbia	*Anabaena constricta*	*Nostoc* sp.	KY807531
Unresolved		643	CCALA 005 (Trebon)	*Anabaena* sp.	*Trichormus variabilis*	KY807532
		242	Soil, Serbia	*Anabaena* sp.	*Nostoc sphaericum*	KY807526
		106	Soil, Serbia	*Anabaena constricta*	*Nostoc punctiforme*	KY807518
		262	Soil, Serbia	*Anabaena constricta*	*Nostoc punctiforme*	KY807514

Geitler and *Anabaena miniata* Skuja, have demonstrated ecotoxicological effects against the cabbage root fly as well as fungicidal properties (Ördög, 2015). With the tentative goals of further exploring the ecotoxicology of the MACC isolates and the prevalence of cyanobacterial blooms warranting proper species identification, it is imperative to legitimately identify isolates and provide a molecular framework for future work (Ördög, 2015).

This research focuses on the phylogenetic relationships of 37 MACC strains previously identified as *Anabaena*. Since this part of the MACC collection lacks molecular scrutiny, we evaluated its phylogeny and carried out reclassification of the strains using partial 16S rRNA housekeeping gene.

2. Materials and methods

2.1. Organisms and culture conditions

Thirty-seven strains (Table 1) were selected from the MACC collection, Széchenyi István University, Mosonmagyaróvár, Hungary. Thirty-one strains originated from Serbia (University of Novi Sad), five strains from Hungary (Lepossa, 2003) and one strain is from the Czech Republic (CCALA – Trebon) (Ördög, 2015). The strains were cultured in Z8 medium (Staub, 1961; Kotai, 1972; Niva, 1976) between 24 and 26 °C under a light intensity of 20 μmol m^{-2} s^{-1} provided by cool white light (16 h/8 h light/dark cycle). Cultures were aerated during the light period with 20 L h^{-1} 1.5% CO_2-enriched sterile humidified air (Ördög, 1982).

2.2. Cell morphology

Initial morphological analyses of the strains were carried out at the time of isolation; however, the original taxonomic designation does not comply with current cyanobacterial taxonomy and/or nomenclature. Additionally, more than two decades of cultivation has resulted in ambiguous MACC strains, hardly morphologically distinguishable from one another. In our study, strain morphology was observed using an Olympus BX60 microscope and identified following Komárek (2013). At least 30 trichomes per strain were photographed with a digital camera (Olympus DP 70, magnification 400x). Dimensions of vegetative cells and heterocytes were measured using image analysis software (Olympus DP Soft 3.2). No akinetes were observed at the time of imaging. Most strains have been cultured since the late 1990s, which may have resulted in morphological plasticity that possibly hindered their akinete formation and consequently their identification. The contrast of the photos was enhanced by Adobe Lightroom and collages were made by Fotor 2.0.3.

2.3. DNA extraction, PCR, sequence analyses

For DNA analysis, the partial 16S rRNA gene of 37 MACC strains was sequenced. Total genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific). The 16S rRNA gene was amplified using primers 8F (AGAGTTTGATCCTGGCTCAG) and S8 (TCTACGCATTTCACCGCTAC) (Ezhilarasi and Anand, 2009). The PCR mix contained 10 μL Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher Scientific), 7 μL dH_2O, 1 μL of each primer and 1 μL purified DNA (50–100 ng) to give 20 μL final volume of PCR reaction (0.5 μmol final concentration per primer). Initial denaturation was 98 °C for 30 s, followed by denaturation at 98 °C for 10 s, annealing at 58 °C for 20 s, extension at 72 °C for 30 s, and the final extension at 72 °C for 1 min over 40 cycles. After DNA amplification, products were visualized on a 1.5% agarose gel. The PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific). Purity of the PCR product was tested at 260/280 nm by Nanodrop™. For sequencing, a LifeTech 3500 Genetic Analyzer (Thermo Fisher Scientific) capillary sequencer was used.

Reference sequences of Nostocaceae strains (*Anabaena, Cylindrospermopsis, Desmonostoc, Nostoc*, *Roholtiella*, *Trichormus* and *Wollea*) were downloaded from GenBank (Altschul et al., 1997) and *Chroococcidiopsis thermalis* PCC 7203 was added as a non-heterocytous counterpart. The complete matrix contained 147 sequences. Sequences were aligned using MUSCLE (Castresana, 2000; Edgar, 2004) through MEGA 7 (Kumar et al., 2016). jModelTest 2 was run to determine substitution models for nucleotide evolution with the TIM2 + G + I model as best fit (1000 bootstrap iterations). Phylogenetic relationships among the sequences in Tables 2 and 3 were calculated with Geneious 10.2.3 (Kearse et al., 2012). Maximum Likelihood (ML) analysis was run using RAxML (Stamatakis, 2014). For the Bayesian analysis, two runs of four Markov chains were executed using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) for 2.5×10^7 generations with default parameters, sampling every 100 generations (the final mean standard deviation of split frequencies was lower than 0.01). The first 25% of sampled trees were discarded as burn-in, the rest were used to calculate posterior probabilities of branches (Hrouzek et al., 2013). The final phylogenetic tree was constructed from a concatenated alignment employing Bayesian inference in MrBayes 3.1.2 and maximum likelihood analysis in RAxML 7.3.2. Phylogenetic trees were drawn and edited using Adobe Illustrator CC version 2014.01. Similarity matrix (percentages) for MACC strains comparing partial sequences of the 16S rRNA gene was calculated in Geneious 10.2.3 (Table 2), while p-distances were calculated with MEGA 7 (Table 3)

3. Results

Sequences formed a ~650 bp alignment of 147 OTUs altogether. Three well-supported clades were formed within the order Nostocales (Fig. 1) from which two were monophyletic.

3.1. Nostoc cluster (Fig. 1):

The monophyly of the genus *Nostoc* was well delimited and supported (0.9 posterior probability). The *Nostoc* cluster was sister to *Nostoc piscinale* CENA21, *Trichormus azollae* and four OTUs of *Gloeotrichia*. In our phylogenetic approach, eight MACC strains fell into the cluster "*Nostoc*" (shaded gray). This clade was identified as *Nostoc sensu stricto*, since it includes clearly established *Nostoc* strains including *Nostoc commune* and *Nostoc punctiforme* PCC 73102 and terrestrial representatives of *Nostoc*, together with *Nostoc calcicola* III. Similar clustering has been

Table 2
Similarity matrix (percentages) of 24 strains. Six MACC strains represent the four clusters/groups (*Desmonostoc*, *Nostoc*, *Trichormus*, Unresolved). Reference strain accession numbers are: 1. *Cylindrospermum stagnale* PCC7417 (AJ133163), 2. *Cylindrospermopsis raciborskii* (straight) (AF067819), 3. *Dolichospermum flos-aquae* UTEX LB2338 (DQ234823), 4. *Desmonostoc muscorum* I (AJ630451), 5. *Desmonostoc muscorum* II (AJ630452), 6. *Desmonostoc muscorum* CENA61 (AY218828), 7. *Fischerella muscicola* (KF417427), 8. *Halotia longispora* CENA420 (KJ843313), 9. *Mojavia pulchra* JT2-VF2 (AY577534), 10. *Nodularia harveyana* Lukesová 18 94 (AM711554), 11. *Nostoc punctiforme* PCC 73102(AF027655), 12. *Sphaerospermopsis reniformis* 06-01 (FM161348), 13. *Tolypothrix* sp. IAM M-259 (AB093486), 14. *Trichormus* sp. PCC 7120 (BA000019), 15. *Trichormus variabilis* NIES23 (AF247593), 16. *Trichormus variabilis* Hindák (AJ630456), 17. *Wollea saccata* ACCS 045 (GU434226) and 18. *Chroococcidiopsis thermalis* (AB039005) non-heterocyte group.

	106	165	242	274	282	643	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
MACC 106 (Unresolved)																							
MACC 165 (*Nostoc*)	94.1																						
MACC 242 (Unresolved)	92.0	89.7																					
MACC 274 (*Trichormus*)	95.8	92.5	92.8																				
MACC 282 (*Desmonostoc*)	94.0	92.3	90.3	94.4																			
MACC 643 (Unresolved)	92.5	91.4	91.2	92.7	93.8																		
1. *Cylindrospermum stagnale*	93.5	92.7	92.5	95.5	93.1	89.3																	
2. *Cylindrospermopsis raciborskii*	89.8	89.1	91.6	89.3	86.9	85.0	92.4																
3. *Dolichospermum flos-aquae*	92.8	91.7	90.2	93.2	95.1	88.2	95.8	91.0															
4. *Desmonostoc muscorum* I	93.0	93.5	90.7	93.0	95.3	89.3	95.9	91.5	97.7														
5. *Desmonostoc muscorum* II	93.0	93.5	90.7	93.0	95.3	89.3	95.9	91.5	97.7	100													
6. *Desmonostoc muscorum* CENA61	90.4	88.9	95.1	91.9	89.7	85.8	94.7	92.2	94.2	94.7	94.7												
7. *Fischerella muscicola*	93.0	90.9	90.8	92.4	90.4	84.8	93.7	90.6	91.6	92.6	92.6	79.2											
8. *Halotia longispora*	93.7	91.2	92.7	93.3	92.6	88.2	95.4	91.4	94.9	95.6	95.6	83.2	72.7										
9. *Mojavia pulchra*	95.4	94.5	92.3	93.9	92.8	87.1	95.0	91.9	94.3	94.9	94.9	94.4	91.0	95.3									
10. *Nodularia harveyana*	93.2	92.3	91.9	93.8	91.5	87.5	95.2	92.2	94.4	95.1	95.1	93.5	92.3	96.4	93.6								
11. *Nostoc punctiforme*	93.2	95.9	89.8	91.4	91.4	87.2	94.9	92.0	94.8	95.4	95.4	94.2	91.6	94.2	95.5	94.5							
12. *Sphaerospermopsis reniformis*	92.0	90.5	92.6	92.3	90.5	86.6	93.1	95.3	92.1	92.2	92.2	91.8	90.0	93.0	92.4	92.6	91.2						
13. *Tolypothrix* sp.	96.1	94.8	92.8	94.3	92.3	88.7	96.0	92.9	96.0	96.3	96.3	95.5	92.5	95.6	96.3	95.1	96.0	93.4					
14. *Trichormus* sp.	93.8	91.5	93.2	97.4	92.3	88.1	96.6	92.3	95.8	96.4	96.4	96.2	92.3	95.4	95.7	95.3	94.5	92.7	96.7				
15. *Trichormus variabilis* NIES23	93.8	91.4	92.7	97.4	92.3	87.9	96.1	92.2	95.2	95.8	95.8	95.6	92.0	94.8	95.7	94.7	94.1	93.0	96.3	99.5			
16. *Trichormus variabilis* HINDAK	91.1	90.6	93.0	91.4	92.1	91.4	95.4	93.4	94.3	95.5	ö5.5	94.3	92.7	95.5	94.6	95.0	93.3	94.1	94.8	94.7	94.1		
17. *Wollea saccata*	89.8	89.1	91.1	90.1	88.8	86.7	92.1	93.7	91.5	91.7	91.7	91.1	88.9	91.6	91.7	91.6	90.8	93.2	92.5	92.5	91.9	93.9	
18. *Chroococcidiopsis thermalis*	84.4	86.5	85.1	85.3	85.3	81.5	89.5	87.2	89.6	89.2	89.2	88.5	89.6	88.1	88.4	89.3	88.8	87.5	89.5	89.8	89.2	88.8	86.2

Table 3
Estimates of evolutionary divergence between sequences. The number of base differences per site between sequences are shown. The analysis involved 24 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 320 positions in the final dataset. Six MACC strains represent the four clusters/groups (*Desmonostoc*, *Nostoc*, *Trichormus*, Unresolved). Reference strain accession numbers are: 1. *Cylindrospermum stagnale* PCC7417 (AJ133163), 2. *Cylindrospermopsis raciborskii* (straight) (AF067819), 3. *Dolichospermum flos-aquae* UTEX LB2338 (DQ234823), 4. *Desmonostoc muscorum* I (AJ630451), 5. *Desmonostoc muscorum* II (AJ630452), 6. *Desmonostoc muscorum* CENA61 (AY218828), 7. *Fischerella muscicola* (KF417427), 8. *Halotia longispora* CENA420 (KJ843313), 9. *Mojavia pulchra* JT2-VF2 (AY577534), 10. *Nodularia harveyana* Lukesova 18 94 (AM711554), 11. *Nostoc punctiforme* PCC 73102 (AF027655), 12. *Sphaerospermopsis reniformis* 06–01 (FM161348), 13. *Tolypothrix sp.* IAM M-259 (AB093486), 14. *Trichormus sp.* PCC 7120 (BA000019), 15. *Trichormus variabilis* NIES23 (AF247593), 16. *Trichormus variabilis* HINDAK (AJ630456), 17. *Wollea saccata* ACCS 045 (GU434226), and 18. *Chroococcidiopsis thermalis* (AB039005) non-heterocyte group.

	106	165	242	274	282	643	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
MACC 106 (Unresolved)																							
MACC 165 (*Nostoc*)	0.04																						
MACC 242 (Unresolved)	0.04	0.08																					
MACC 274 (*Trichormus*)	0.03	0.05	0.05																				
MACC 282 (*Desmonostoc*)	0.04	0.05	0.07	0.04																			
MACC 643 (Unresolved)	0.03	0.04	0.06	0.04	0.05																		
1. *Cylindrospermum stagnale*	0.04	0.06	0.06	0.02	0.04	0.04																	
2. *Cylindrospermopsis raciborskii*	0.07	0.08	0.07	0.08	0.10	0.08	0.08																
3. *Dolichospermum flos-aquae*	0.05	0.06	0.08	0.04	0.02	0.05	0.04	0.10															
4. *Desmonostoc muscorum* I	0.04	0.03	0.08	0.06	0.03	0.03	0.05	0.08	0.03														
5. *Desmonostoc muscorum* II	0.04	0.03	0.08	0.06	0.03	0.03	0.05	0.08	0.03	0.00													
6. *Desmonostoc muscorum* CENA61	0.06	0.08	0.03	0.06	0.08	0.07	0.06	0.09	0.08	0.08	0.08												
7. *Fischerella muscicola*	0.06	0.08	0.08	0.07	0.08	0.08	0.07	0.08	0.08	0.06	0.06	0.09											
8. *Halotia longispora*	0.03	0.05	0.05	0.05	0.04	0.04	0.04	0.08	0.04	0.04	0.04	0.07	0.08										
9. *Mojavia pulchra*	0.04	0.05	0.08	0.06	0.06	0.06	0.05	0.09	0.07	0.06	0.06	0.08	0.09	0.04									
10. *Nodularia harveyana*	0.05	0.05	0.08	0.06	0.05	0.05	0.05	0.07	0.05	0.04	0.04	0.08	0.08	0.05	0.07								
11. *Nostoc punctiforme*	0.04	0.01	0.08	0.05	0.05	0.04	0.05	0.07	0.05	0.03	0.03	0.08	0.08	0.05	0.05	0.05							
12. *Sphaerospermopsis reniformis*	0.08	0.08	0.08	0.07	0.09	0.09	0.07	0.04	0.08	0.10	0.10	0.09	0.12	0.07	0.09	0.09	0.09						
13. *Tolypothrix sp.*	0.02	0.04	0.05	0.04	0.05	0.03	0.03	0.08	0.05	0.04	0.04	0.06	0.07	0.02	0.03	0.06	0.04	0.08					
14. *Trichormus sp.*	0.03	0.06	0.05	0.00	0.05	0.05	0.02	0.08	0.04	0.05	0.05	0.06	0.06	0.05	0.06	0.06	0.05	0.07	0.03				
15. *Trichormus variabilis* NIES23	0.03	0.06	0.05	0.00	0.05	0.05	0.02	0.08	0.04	0.05	0.05	0.06	0.06	0.05	0.06	0.06	0.05	0.07	0.03	0.00			
16. *Trichormus variabilis* HINDAK	0.05	0.05	0.07	0.05	0.05	0.02	0.04	0.08	0.5	0.04	0.04	0.07	0.08	0.04	0.06	0.04	0.05	0.09	0.04	0.05	0.05		
17. *Wollea saccata*	0.07	0.07	0.09	0.08	0.08	0.06	0.08	0.07	0.08	0.07	0.07	0.10	0.11	0.06	0.07	0.07	0.07	0.06	0.06	0.08	0.08	0.06	
18. *Chroococcidiopsis thermalis*	0.11	0.10	0.13	0.11	0.09	0.11	0.11	0.12	0.09	0.09	0.09	0.13	0.13	0.12	0.13	0.10	0.09	0.12	0.12	0.10	0.10	0.12	0.13

found in other *Nostoc* studies (Ramírez et al., 2011). MACC 268 fell into a separate smaller clade with similarity ranging from 93.8 to 95.1% to the other seven strains. Those seven strains were more similar to each other (94.8–99.2%). MACC 165 and 258 could possibly be the same species (99.2%). However, further molecular research (Table 2) should be carried out, because this finding cannot be based solely on sequence similarity.

All isolates originated from soils (Serbia). In MACC 253, the vegetative cells are barrel-shaped to ± spherical, 4.9–5.2 μm wide and 5.1–5.5 μm long. Heterocytes are ± spherical, 4.0–4.2 μm wide and 5.7–6 μm long. As for the other seven strains, vegetative cells are barrel-shaped, irregularly spherical to ellipsoidal, 2.1–4.2 μm wide and 2.9–5.3 μm long. Heterocytes are barrel-shaped to subspherical, 2.4–6.1 μm wide and 3.3–6.4 μm long (Table 1). The heterocytes are positioned intercalary and solitary (Table 1). The description of MACC 253 agrees with the description of *Nostoc punctiforme* (Komárek, 2013). These isolates are examples that under laboratory conditions *Nostoc* and *Anabaena* (see previous assignment in Table 1) can be easily confused. Additional molecular studies on these isolates are necessary in order to properly generate species inferences.

3.2. Trichormus cluster (Fig. 1):

The *Trichormus* cluster was highly supported (posterior probability of 1.00 and ML bootstrap support of 82%). The current clade contains PCC7120 and NIES23, which are referenced as *Trichormus* (Bohunická et al., 2015; Genuário et al., 2015; Miscoe et al., 2016; Hentschke et al., 2016). This clade also contains *Trichormus variabilis* ATCC 29413. *Trichormus azollae* Kom-BAI-1983 (formerly *Anabaena azollae*) usually falls outside of the *Trichormus variabilis* clade. In our strains, no akinetes were observed, but the vegetative cells are barrel-shaped and the straight or mildly bent trichomes have spherical and oval heterocytes. According to the 16S rRNA similarity analysis, MACC strains in the *Trichormus* clade range from 94.2 to 99.7% similarity. When the reference sequences are included (such as *Trichormus fertilissimus* RPAN 47, *Trichormus variabilis* IAM M3, *Trichormus* sp. PCC 7120, *Trichormus variabilis* NIES 23, *Trichormus variabilis* ATCC 29413), this value changes to 96.4–98.5%. A well-supported subcluster (1.00 posterior probability and 99% ML bootstrap) was identified with five strains (MACC 63, 68, 118, 122 and 248) collected from a fish pond (Hungary). These strains show more than 97% similarity with *Trichormus variabilis* ATCC 29413. As for the 14 other terrestrial strains (from Serbia), their similarity with *Trichormus variabilis* IAM M3 and *Trichormus* sp. PCC 7120 is more than 97% (Tables 1 and 2).

3.3. Desmonostoc cluster (Fig. 1):

For the *Desmonostoc* cluster, many of the reference strains for *Desmonostoc* (*e.g.* isolates in Hrouzek et al., 2013, such as *Desmonostoc muscorum* I and *Desmonostoc muscorum* II) were included in our analysis, resulting in a well-supported clade (posterior probability 0.95). Two recently published species of *Desmonostoc*: *D. geniculatum* and *D. vinosum* (Miscoe et al., 2016) fall into this clade along with the reference strains. *Nostoc muscorum* has been shown to be polyphyletic, incorporating those "*Nostoc*" species with soft mucilage. The MACC strains (MACC 171, 279, 282, 288, 290, and 293) found in this cluster

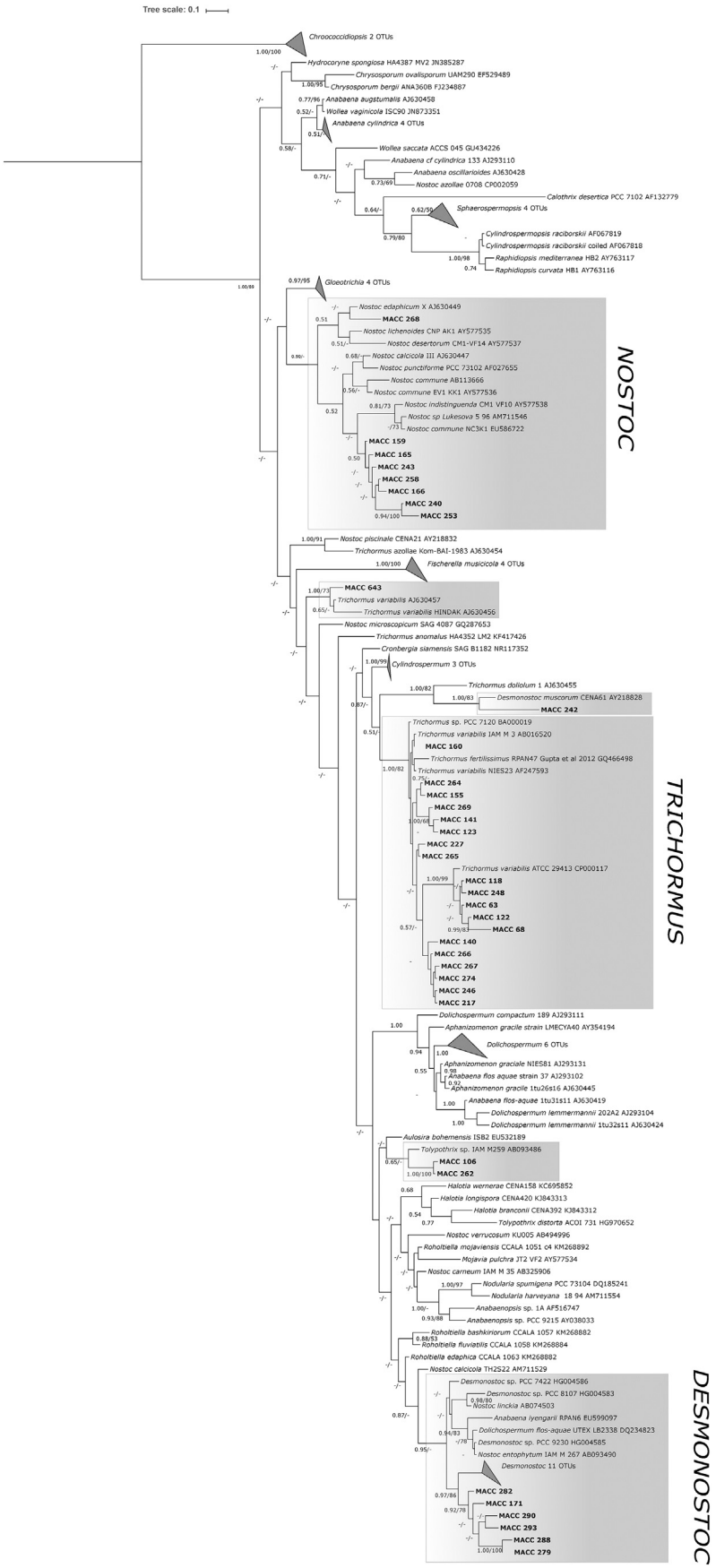

Fig. 1. 16S rRNA gene phylogenetic tree based on 147 OTUs demonstrating the position of 37 MACC strains. The tree is based on Bayesian topology and the support values are given for Bayesian posterior probabilities and maximum likelihood (BI/ML). The cut-off values for bootstrap and probability are 50 and 0.5, respectively. *Chroococcidiopsis thermalis* PCC 7203 was used as the outgroup.

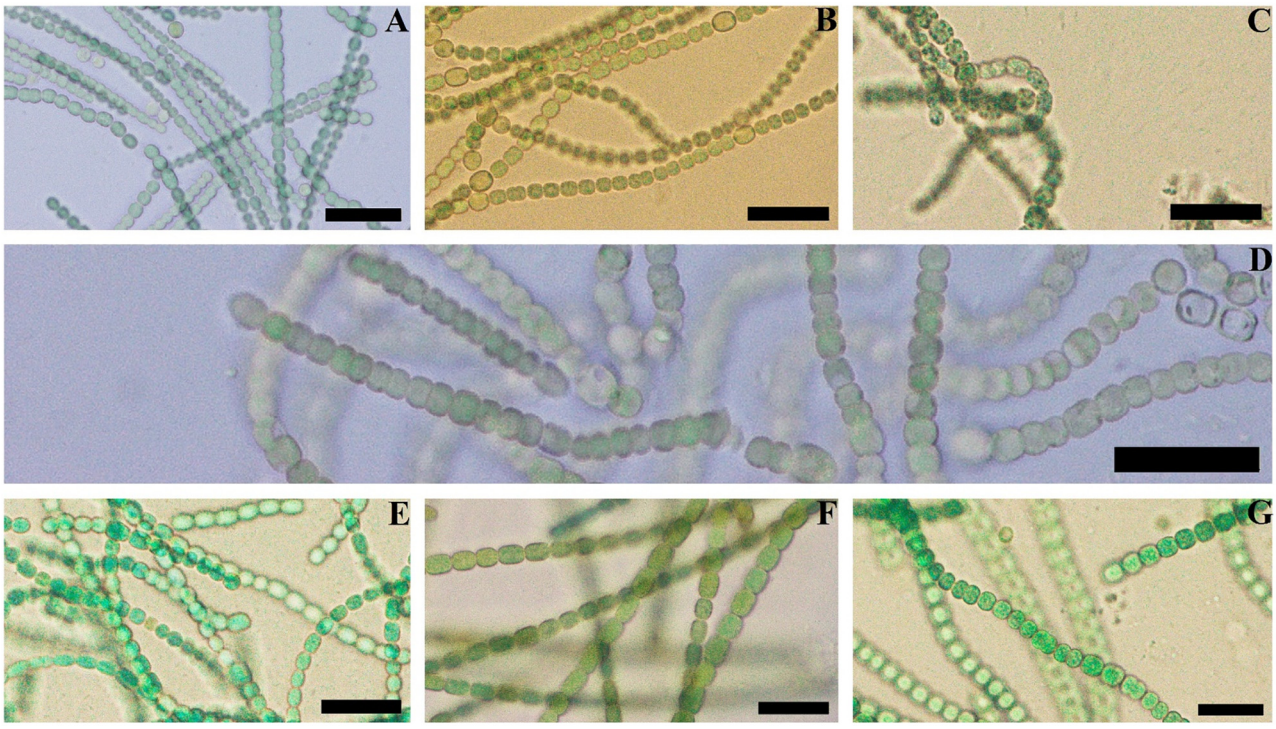

Fig. 2. Bright field images of the studied MACC strains representing the four clusters/groups (*Desmonostoc*, *Nostoc*, *Trichormus* soil and *Trichormus* pond subcluster plus three unresolved groups). A: MACC 118 from the *Trichormus* pond subcluster. B: MACC 274 from *Trichormus* soil cluster. C: MACC 282 from *Desmonostoc* cluster. D: MACC 165 from the *Nostoc sensu stricto* cluster. E: MACC 106 from the *Tolypothrix*-type unresolved group. F: MACC 643, which is grouped with *Trichormus* species but outside of the true *Trichormus* cluster. G: MACC 242, which is likely a new, cryptic genus. Scale bar = 20 μm.

were initially identified as *Anabaena constricta* and *A. variabilis* and originated from Serbian soil samples (Table 1). This molecular analysis modified the previous taxonomic delimitation based on morphology. In culture conditions, isolates of the genus *Desmonostoc* are often confused with old cultures of *Trichormus*, *Anabaena* or *Nostoc*. The microphotographs show the barrel-shaped cells and heterocytes, but these characteristics do not distinguish them from other genera (*e.g. Trichormus* and *Anabaena*). The high resemblance between the cells in the trichomes was noted (Fig. 2, Table 1). Akinetes were not formed by these strains either. Similarity among the six strains ranged from 95.2 to 97.5% and all originated from Serbian soils (Tables 2 and 3).

3.4. Unresolved clades (Fig. 1)

MACC 643 is assigned to *Trichormus* as it falls together with *Trichormus variabilis* and *Trichormus variabilis* 2001/4, though out of the *sensu stricto Trichormus* cluster. It is distant to *Anabaena augstumalis* and *Anabaena cylindrica* PCC7122. MACC 643 was 91.4% similar with *Trichormus variabilis* 2001/4 (Tables 1 and 2).

Despite being grouped with *Desmonostoc muscorum* CENA61, MACC 242 is closer to the true *Trichormus* and supported in its position (1.00 posterior probability). CENA61 is unresolved, sister to the true *Trichormus variabilis. Trichormus* Greifswald and *Trichormus* Hindák 2001/4 also fall far from this strain. MACC 242 is likely a new, cryptic genus. The similarity between MACC 242 and *Desmonostoc muscorum* is 95.1% (Tables 1 and 2).

MACC 106 and 262 form the last group of unresolved taxa. Although they are placed in a small cluster with *Tolypothrix*, they are not well supported (0.65 posterior probability) compared to the *Nostoc*, *Trichormus* and *Desmonostoc* clusters. The type for *Tolypothrix* is *Trichormus distorta*, and the reference strain for this taxon is *Trichormus distorta* ACOI 731 (Hauer et al., 2014). MACC 106 and 262 did not group together with the reference strain but with *Tolypothrix* IAM-259. Reháková et al. (2007) also confirmed the divergence in the position of *Tolypothrix* IAM-259 compared to other *Tolypothrix* reference sequences. Bravakos et al. (2016) suggested that IAM-259 might be *Halotia*, but this remark remains unverified. Similarity between these two strains (MACC 106 and 262) and *Tolypothrix* IAM-259 is 96.1% (Tables 1 and 2). This cluster needs further taxonomic revision and might be a putative new genus.

3.5. Similarity matrix and p-distance

Similarity matrix (percentages) for MACC strains comparing partial sequences of the 16S rRNA gene and p-distances (evolutionary divergence between sequences) are shown below in Tables 2 and 3. Due to shortage of space in this publication, only a few representatives of each cluster were shown. The results of these calculations confirmed the results originated from the phylogenetic analysis shown on Fig. 1 and helped the establishment of clades in which the MACC strains were grouped.

4. Discussion

The goal of this study was to correctly designate the strains at genus level in the MACC collection that lacked molecular phylogenetic interpretations. Molecular analyses in this study corrected the genus designation of 33 strains within the MACC collection and highlights the necessity for accurate identification of culture collections. Correcting these designations contributes to refining taxonomic models and methods and provides a proper platform for future work.

A key finding was that performing molecular analyses (based on 16S rRNA) allowed reclassification of 37 strains (previously assigned to the genus *Anabaena*) into various genera including *Desmonostoc*, *Nostoc* and *Trichormus*. Species of the genus *Trichormus* are similar to *Anabaena* in appearance, but akinete formation is more similar to the genus *Nostoc* (Komárek and Anagnostidis, 1989; Hindák, 2000). Previous molecular analyses have also confirmed the difference between the above-mentioned three genera and showed *Trichormus* is polyphyletic but more

precise taxonomic classification is required (Rajaniemi et al., 2005; Kastovsky and Johansen, 2008; Papaefthimiou et al., 2008). The genus *Nostoc* is also polyphyletic, and phylogenetic analyses based on 16S rRNA sequences have revealed that several genotypes fall outside of the "true *Nostoc*" cluster (Novis and Smissen, 2006; Lukesová et al., 2009; Mateo et al., 2011; Ramírez et al., 2011; Osorio-Santos et al., 2014; Silva et al., 2014; Shalygin et al., 2017). Consequently, some *Nostoc*-related morphotypes have been placed in new genera, such as *Mojavia* and *Desmonostoc* (Reháková et al., 2007; Hrouzek et al., 2013; Genuário et al., 2015). *Desmonostoc* forms a sister group with *Nostoc* and their phylogenetic placement has been confirmed by several authors (Hrouzek et al., 2003, 2005; Reháková et al., 2007; Papaefthimiou et al., 2008; Mateo et al., 2011; Komárek, 2013; Genuário et al., 2015). This group consistently fell outside of *Nostoc sensu stricto* in their 16S rRNA phylogenies and until it was separated from *Nostoc*, it was referred to as "*Nostoc* Group II" (Reháková et al., 2007; Vaccarino and Johansen, 2011; Johansen et al., 2014). Morphologically, the species of the genera *Mojavia*, *Desmonostoc* and *Halotia* are morphologically very similar to species of the true *Nostoc* genus, even their life cycle. It is difficult to separate groups within the *Nostoc sensu lato* solely based on morphological characteristics and thus the use of genetic markers proves indispensable (Genuário et al., 2015). Since our analyses were carried out on partial 16S rRNA gene, we are reclassifying our strains at the genus-level. We advocate that culture collections update their strain designations with phylogenetic data. Although some strains were highly similar in their 16S rRNA gene sequences and in morphology, as in the case of MACC 165 and 258, their lumping to the same taxon is not recommended due to the lack of resolution within a single partial gene sequence. To properly delineate cryptic species, it is pertinent to scrutinize these isolates with additional genetic or biochemical assays (Perkerson et al., 2011; Hentschke et al., 2016; Sciuto and Moro, 2016).

Another problem is that most of the morphologically described *Anabaena, Desmonostoc*, *Nostoc* and *Trichormus* species have either no reference sequences available in databases or those sequences that are available are limited to a few genetic markers (Perkerson et al., 2011; Hentschke et al., 2016; Sciuto and Moro, 2016). There are also many misidentifications in Genbank complicating cyanobacterial taxonomy and phylogenetic comparisons. In many cases, new sequence information is added incorrectly to public databases resulting in misleading identifications. Despite the huge amount of information available in molecular databases, researchers should approach data with caution.

An important factor that hampered our identification was the lack of akinetes. Akinete length is a key morphological character that best differentiates isolates on a species level. Unfortunately, in our case, there were no akinetes in the cultures and generating them has not been successful thus far. Furthermore, cultivated strains of the same species (morphospecies) are often very morphologically plastic, reflecting the effect of varied growth conditions and could also result in erroneous identifications (Zapomelova et al., 2008). Komárek and Anagnostidis (1989) estimated that more than 50% of strains in collections do not correspond to the diagnoses of the taxa to which they are assigned. Of course, other metric characters, such as width and length of vegetative cells and heterocytes, are useful for taxonomic differentiation.

This study examined 37 and systematically resolved 33 MACC strains at genus level based on morphology and molecular methods. This collection offers an unexploited potential as a repository of taxonomic data for algal diversity in relation to unexamined public algal collections.

5. Conclusion

This study emphasizes the necessity for correct strain designations in the MACC collection and possibly other established culture collections. This part of the collection had never been studied using molecular methods and there was a need for revision due to constantly evolving taxonomy. The relationships among the species within the genus do not fully agree with the previous morphology based classifications. This study revealed that the strains belong to at least three different genera. The use of molecular procedures lead to a more reliable taxonomic delimitation and will provide a more comprehensive understanding of the diversity of the MACC cyanobacteria and cyanobacteria found in other culture collections.

Conflict of interest

All authors declare that there is no conflict of interest regarding the publication of this paper.

Formatting of funding sources

This research was funded by SABANA (Grant No. 727874) from the EU Horizon 2020 Research and Innovation program and by the NKFI-FK-123899 grant. Gergely Maróti thanks the János Bolyai Research Scholarship of the Hungarian Academy of Sciences for support. Haywood Dail Laughinghouse acknowledges the USDA NIFA, Hatch project # FLA-FTL-005697 for additional support.

Acknowledgements

The authors highly appreciate the valuable contribution of Dr. Wendy Ann Stirk to the improvement of the manuscript. The authors are also indebted to anonymous reviewers for their valuable comments and suggestions leading to the improvement of this manuscript.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389–3402. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC146917/pdf/253389.pdf.

Bohunická, M., Pietrasiak, N., Johansen, J.R., Berrendero-Gomez, E., Hauer, T., Gaysina, L.A., Lukešová, A., 2015. *Roholtiella*, gen. Nov. (Nostocales, cyanobacteria) – a tapering and branching member of the Nostocaceae. Phytotaxa 197, 84–103. https://doi.org/10.11646/phytotaxa.197.2.2.

Boyer, S.L., Fletcher, V.R., Johansen, J.R., 2001. Is the 16S-23S internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. Molecular Biology and Evolution 18, 1057–1069. https://doi.org/10.1093/oxfordjournals.molbev.a003877.

Bravakos, P., Kotoulas, G., Skaraki, K., Pantizadou, A., Economou-Amilli, A., 2016. A polyphasic taxonomic approach in isolated strains of cyanobacteria from thermal springs of Greece. Molecular Phylogenetics and Evolution 98, 147–160. https://doi.org/10.1016/j.ympev.2016.02.009.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.

Ezhilarasi, A., Anand, N., 2009. Phylogenetic analysis of *Anabaena* spp. (cyanobacteria) using sequences of 16S rRNA gene. Australian Journal of Basic and Applied Sciences 3, 4026–4031. https://pdfs.semanticscholar.org/f747/45c3c86bdeee9a5f7269e3ccc31fd3ddacef.pdf.

Fox, G.E., Wisotzkey, J.D., Jurtshuk Jr., P., 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. International Journal of Systematic Bacteriology 42, 166–170. http://www.microbiologyresearch.org/docserver/fulltext/ijsem/42/1/ijs-42-1-166.pdf?expires=1529841357&id=id&accname=guest&checksum=14F2FB78001F93153912D78130C4CEF9.

Genuário, D.B., Vas, M.G.M.V., Hentschke, G.S., Sant'Anna, C.L., Fiore, M.F., 2015. *Halotia* gen. nov., a phylogenetically and physiologically coherent cyanobacterial genus isolated from marine coastal environments. International Journal of Systematic and Evolutionary Microbiology 65, 663–675. https://doi.org/10.1099/ijs.0.070078-0.

Hauer, T., Bohunická, M., Johansen, J.R., Mareš, J., Berrendero-Gomez, E., 2014. Reassessment of the family Microchaetaceae and establishment of new families Tolypothrichaceae and Godleyaceae. Journal of Phycology 50, 1089–1100. https://doi.org/10.1111/jpy.12241.

Hentschke, G.S., Johansen, J.R., Pietrasiak, N., Fiore, M.F., Rigonato, J., Sant'Anna, C.L., Komárek, J., 2016. Phylogenetic placement of *Dapisostemon* gen. nov. and *Streptostemon*, two tropical heterocytous genera (cyanobacteria). Phytotaxa 245, 129–143. https://doi.org/10.11646/phytotaxa.245.2.4.

Hindák, F., 2000. Morphological variation of four planktic nostocalean cyanophytes – members of the genus *Aphanizomenon* or *Anabaena*? Hydrobiologia 438, 107–116. https://doi.org/10.1023/A:1004118213936.

Hrouzek, P., Simek, M., Komarek, J., 2003. Nitrogenase activity (acetylene reduction activity) and diversity of six soil *Nostoc* stains. Algological Studies/Archiv für Hydrobiologie 108, 87–101. https://www.schweizerbart.de/papers/archiv_algolstud/detail/108/50180/Nitrogenase_activity_acetylene_reduction_activity_and_diversity_of_six_soil_iNostoc_i_stains.

Hrouzek, P., Ventura, S., Lukesova, A., Mugnai, M.A., Turicchia, S., Komárek, J., 2005. Diversity of soil *Nostoc* strains: phylogenetic and phenotypic variability. Algological Studies 117, 251–264. https://doi.org/10.1127/1864-1318/2005/0117-0251.

Hrouzek, P., Lukesová, A., Mares, J., Ventura, S., 2013. Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*. Fottea 13, 201–213. https://doi.org/10.5507/fot.2013.016.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755. http://webpages.icav.up.pt/PTDC/BIA-BEC/104097/2008/23.pdf.

Johansen, J.R., Bohunická, M., Lukešová, A., Hrčková, K., Vaccarino, M.A., Chesarino, N.M., 2014. Morphological and molecular characterization within 26 strains of the genus *Cylindrospermum* (Nostocaceae, cyanobacteria), with descriptions of three new species. Journal of Phycology 50, 187–202. https://doi.org/10.1111/jpy.12150.

Kastovsky, J., Johansen, J.R., 2008. *Mastigocladus laminosus* (Stigonematales, cyanobacteria): phylogenetic relationship of strains from thermal springs to soil-inhabiting genera of the order and taxonomic implications for the genus. Phycologia 47, 307–320. https://doi.org/10.2216/PH07-69.1.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Komárek, J., 2013. Süswasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota 3. Teil / 3rd Part: Heterocytous Genera. Springer Spektrum https://www.springer.com/la/book/9783827409324.

Komárek, J., Anagnostidis, K., 1989. Modern approach to the classification system of Cyanophytes, 4. Nostocales. Archiv für Hydrobiologie 82, 247–345. https://www.schweizerbart.de/papers/algol_stud/detail/56/66018/Modern_approach_to_the_classification_system_of_Cyanophytes_4_Nostocales.

Kotai, J., 1972. Instructions for Preparation of Modified Nutrient Solution Z8 for Algae (NIVA B-11/69).

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Lepossa, A., 2003. Talajalgák mennyiségi vizsgálata a Balaton-felvidéki Nemzeti Parkban, valamint talajból izolált algatenyészetek növényi növekedést befolyásoló hatásainak kimutatása. PhD értekezés. Veszprémi Egyetem, Növénytermesztési és Kertészeti Tudományok Doktori Iskola, Keszthely http://konyvtar.uni-pannon.hu/doktori/2003/Lepossa_Anita_dissertation.pdf.

Lukesová, A., Johansen, J.R., Martin, M.P., Casamatta, D.A., 2009. *Aulosira bohemensis* sp. nov.: further phylogenetic uncertainty at the base of the Nostocales (cyanobacteria). Phycologia 48, 118–129. https://doi.org/10.2216/08-56.1.

Lürling, M., Van Oosterhout, F., Faassen, E., 2017. Eutrophication and warming boost cyano-bacterial biomass and microcystins. Toxins 9, 64–80. https://doi.org/10.3390/toxins9020064.

Malone, C.F.S., Rigonato, J., Laughinghouse IV, H.D., Schmidt, E.C., Bouzon, Z.L., Wilmotte, A., Fiore, M.F., Sant'Anna, C.L., 2015. *Cephalothrix* gen. nov. (cyanobacteria): towards an intraspecific phylogenetic evaluation by multilocus analyses. International Journal of Systematic and Evolutionary Microbiology 65, 2993–3007. https://doi.org/10.1099/ijs.0.000369.

Mateo, P., Perona, E., Berrendero, E., Leganés, F., Martín, M., Golubić, S., 2011. Life cycle as stable trait in the evaluation of diversity of *Nostoc* from biofilms in rivers. FEMS Microbial Ecology 76, 185–198. https://doi.org/10.1111/j.1574-6941.2010.01040.x.

Miscoe, L.H., Johansen, J.R., Kociolek, J.P., Lowe, R.L., Vaccarino, M.A., Pietrasiak, N., Sherwood, A.R., 2016. Novel cyanobacteria from caves on Kauai. Hawaii. Bibliotheca Phycologica 120, 152. https://www.schweizerbart.de/publications/detail/isbn/9783443600471/Bibliotheca_Phycologica_Band_120.

National Center for Biotechnology Information (NCBI) [Internet], 1988. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. [cited 2018 Jun 25]. https://www.ncbi.nlm.nih.gov/.

Niva, 1976. Estimation of Algal Growth Potential. Norwegian Institute for Water Research, Publ, pp. D2–25.

Novis, P.M., Smissen, R.D., 2006. Two genetic and ecological groups of *Nostoc commune* in Victoria Land, Antarctica, revealed by AFLP analysis. Antarctic Science 18, 573–581. https://doi.org/10.1017/S0954102006000617.

Ördög, V., 1982. Apparatus for laboratory algal bioassays. Internationale Revue der Gesamten Hydrobiologie 67, 127–136.

Ördög, V., 2015. Dissertation: Mikroalgák biotechnológiai alkalmazása a növénytermesztésben és növényvédelemben. Hungarian Academy of Sciences http://real-d.mtak.hu/765/42/dc_881_14_doktori_mu.pdf.

Osorio-Santos, K., Pietrasiak, N., Bohunická, M., Miscoe, L.H., Kovacik, L., Martin, M.P., Johansen, J.R., 2014. Seven new species of *Oculatella* (Pseudanabaenales, cyanobacteria): taxonomically recognizing cryptic diversification. European Journal of Phycology 49, 450–470. https://doi.org/10.1080/09670262.2014.976843.

Papaefthimiou, D., Hrouzek, P., Mugnai, M.A., Lukesova, A., Turicchia, S., Rasmussen, U., Ventura, S., 2008. Differential patterns of evolution and distribution of the symbiotic behaviour in Nostocacean cyanobacteria. International Journal of Systematic and Evolutionary Microbiology 58, 553–564. https://doi.org/10.1099/ijs.0.65312-0.

Perkerson, R., Johansen, J.R., Kovacik, L., Brand, J., Casamatta, D.A., 2011. A unique Pseudanbaenalean (cyanobacteria) genus *Nodosilinea* gen. nov. based on morphological and molecular data. Journal of Phycology 47, 1397–1412. https://doi.org/10.1111/j.1529-8817.2011.01077.x.

Rajaniemi, P., Hrouzek, P., Kastovská, K., Willame, R., Rantala, A., Hoffmann, L., Komárek, J., Sivonen, K., 2005. Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, cyanobacteria). International Journal of Systematic and Evolutionary Microbiology 55, 11–26. https://doi.org/10.1099/ijs.0.63276-0.

Ramírez, M., Hernández-Mariné, M., Mateo, P., Berrendero, E., Roldán, M., 2011. Polyphasic approach and adaptative strategies of *Nostoc* cf. *commune* (Nostocales, Nostocaceae) growing on Mayan monument. Fottea 11, 73–86. https://doi.org/10.5507/fot.2011.008.

Reháková, 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. https://doi.org/10.2216/06-92.1.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180.

Sciuto, K., Moro, I., 2016. Detection of the new cosmopolitan genus *Thermoletolyngbya* (cyanobacteria, Leptolyngbyaceae) using the 16S rRNA and 16S-23S ITS region. Molecular Phylogenetics and Evolution 105, 15–35. https://doi.org/10.1016/j.ympev.2016.08.010.

Shalygin, S., Shalygina, R., Johansen, J.R., Pietrasiak, N., Gómez, B.E., Bohunická, M., Mareš, J., Sheil, C.A., 2017. *Cyanomargarita* gen. nov. (Nostocales, cyanobacteria): convergent evolution resulting in a cryptic genus. Journal of Phycology 53, 762–777. https://doi.org/10.1111/jpy.12542.

Silva, C.S.P., Genuário, D.B., Vaz, M.G.M.V., Fiore, M.F., 2014. Phylogeny of culturable cyanobacteria from Brazilian mangroves. Systematic and Applied Microbiology 37, 100–112. https://doi.org/10.1016/j.syapm.2013.12.003.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Staub, R., 1961. Ernährungsphysiologisch-autökologische Untersuchungen an der planktischen Blaualge *Oscillatoria rubescens* D.C. Schweizer Zeitschrift für Hydrobiologie 23, 82–198. https://doi.org/10.3929/ethz-a-000092335.

Vaccarino, M.A., Johansen, J.R., 2011. *Scytonematopsis contorta* sp. nov. (Nostocales), a new species from the Hawaiian Islands. Fottea 11, 149–161. https://doi.org/10.5507/fot.2011.015.

Zapomelova, E., Hrouzek, P., Řeháková, K., Šabacká, M., Stibal, M., Caisová, L., Komárková, J., Lukešová, A., 2008. Morphological variability in selected heterocystous cyanobacterial strains as a response to varied temperature, light intensity and medium composition. Folia Microbiologica 53, 333–341. https://doi.org/10.1007/s12223-008-0052-8.