

Biogeographically interesting planktonic Nostocales (Cyanobacteria) in the Czech Republic and their polyphasic evaluation resulting in taxonomic revisions of *Anabaena bergii* Ostenfeld 1908 (*Chrysosporum* gen. nov.) and *A. tenericaulis* Nygaard 1949 (*Dolichospermum tenericaule* comb. nova)

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Abstract Questions of biogeography of freshwater cyanobacteria and their ability to colonize new areas have been recently discussed in connection with increasing occurrence of some formerly rare morpho-species in temperate zones. Nevertheless, the general

knowledge about the distribution of cyanobacterial species is still fragmentary, and any new findings on cyanobacterial biogeography and spread are valuable. In this study, we provide updated information on the occurrence of *Anabaena bergii*, *Raphidiopsis mediterranea*, and *Sphaerospermopsis aphanizomenoides* in the Czech Republic. In addition, more nostocacean morphospecies are newly reported from the Czech Republic (*A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, and *S. reniformis*). All of these morphospecies were characterized from a morphological point of view, and their phylogenetic affiliations were assessed on the basis of their 16S rRNA gene sequences. Based on these results, *Anabaena bergii* was reclassified into *Chrysosporum* gen. nov., and *D. tenericaule* comb. nova was established.

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Introduction

Questions on the biogeography of freshwater cyanobacteria and their ability to colonize new areas have been recently discussed in connection with the increasing occurrence of some formerly rare

morphospecies in temperate zones. The most studied in this respect were *Cylindrospermopsis raciborskii* (Hindák, 1988; Padisák, 1991, 1997; Maršálek et al., 2000; Stefaniak & Kokociński, 2005; Stüken et al., 2006; Kaštovský et al., 2010) and *Sphaerospermopsis aphanizomenoides* (Padisák & Kovács, 1997; Hindák, 2000; Stefaniak & Kokociński, 2005; Stüken et al., 2006; Zapomělová et al., 2009, 2010; Kaštovský et al., 2010).

Nevertheless, in general, knowledge about the distribution of cyanobacterial species is still fragmentary and also its driving factors remain unclear. There are several reasons of this lack of information: floristic lists are missing or insufficient for many regions of the world, and the level of taxonomic knowledge is low in many groups (Hoffmann, 1996). In addition to this, the absence of akinetes in many nostocacean populations hampers the identification at species level as their size, shape, and position are important taxonomic criteria. Any piece of knowledge on cyanobacterial biogeography and spread is therefore valuable.

The only synoptic study summarizing the occurrence of alien cyanobacteria in the Czech Republic is by Kaštovský et al. (2010). Before it, several minor taxonomic studies mentioned potential non-native species but did not refer on their distribution (Gardavský, 1989; Marvan et al., 1997).

In this study, we provide updated information on the occurrence of *Anabaena bergii*, *Raphidiopsis*

mediterranea, and *Sphaerospermopsis aphanizomenoides* in the Czech Republic. In addition, more nostocacean morphospecies are newly reported from the Czech Republic (*A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, and *S. reniformis*). Further goals were to characterize these morphospecies from a morphological point of view and to assess their phylogenetic affiliations on the basis of their 16S rRNA gene sequences.

Materials and methods

Sampling, isolation, and cultivation of strains

Samples of phytoplankton were collected in 2000–2011 from various localities all over the Czech Republic using a 20-μm mesh plankton net. Occurrences of *Anabaena bergii*, *A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, *Raphidiopsis mediterranea*, *Sphaerospermopsis aphanizomenoides*, and *S. reniformis* were recorded, and morphologies of these morphospecies were evaluated from the fresh material as described below. Single trichomes were isolated from the samples using a glass capillary and an inverted microscope (Olympus IX 71), and clonal strains were grown (Table 1). The strains were maintained in WC medium (Guillard & Lorenzen,

Table 1 Cyanobacterial morphospecies used in this study, corresponding strain codes, their origin, and year of isolation

Taxonomic assignment	Strain code	Geographical origin	Year of isolation
<i>A. bergii</i>	09-02	Očko sandpit lake, Czech Republic	2009
<i>A. fusca</i>	no ^a	Hubenov reservoir, Czech Republic	2008
<i>A. tenericaulis</i>	08-10	Máchovo fishpond, Czech Republic	2008
<i>A. tenericaulis</i>	08-11	Máchovo fishpond, Czech Republic	2008
<i>D. curvum</i>	04-19	Hejtman fishpond, Czech Republic	2004
<i>D. mucosum</i>	06-04	Horák fishpond, Czech Republic	2006
<i>D. mucosum</i>	06-05	Horák fishpond, Czech Republic	2006
<i>D. mucosum</i>	08-03	Mařka fishpond, Czech Republic	2008
<i>D. mucosum</i>	08-09	Mařka fishpond, Czech Republic	2008
<i>R. mediterranea</i>	07-04	Papež fishpond, Czech Republic	2007
<i>S. aphanizomenoides</i>	04-43	Svět fishpond, Czech Republic	2004
<i>S. aphanizomenoides</i>	09-03	Hostivař fishpond, Czech Republic	2009
<i>S. reniformis</i>	06-01	Pěšák fishpond, Czech Republic	2006
<i>S. reniformis</i>	07-01	Vyšehrad fishpond, Czech Republic	2007

^a Only natural population was observed and described in this article

1972) under constant culture conditions (21°C, 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 16 h/8 h light–dark cycle). The exception was *A. fusca* where no clonal strain was isolated, and only natural population was described in this article.

Evaluation of morphological characteristics

Field morphologies of all of the studied strains were evaluated. Microphotographs of at least 30 fresh trichomes per each population were taken with a digital camera (Olympus DP 70, magnification 400 $\times$). Dimensions of all cell types were measured. Five vegetative cells per trichome were measured in 30 trichomes and as many heterocytes and akinetes as it was possible to find in each sample. Akinetes were missing in the population of *A. tenericaulis*, and their dimensions were acquired from the cultivated strains during the first year of cultivation. Length:width ratios of vegetative cells, heterocytes, and akinetes were used as a rough approximation of the cell shapes. All size measurements were performed using image analysis (Olympus DP Soft).

Statistical analyses of morphological data

Basic statistical characteristics such as mean values, 25 and 75% percentiles and extreme values were computed for the morphological parameters of the field populations.

DNA extraction, PCR and sequencing

The biomass of the studied strains was harvested in the exponential phase of growth by repeated centrifugation, during which the trichomes were washed several times by NaCl solution (concentration 1 g l⁻¹) to remove mucilaginous substances. The biomass samples were stored at -20°C until DNA extractions. DNA was extracted using UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). The 16S rRNA gene and ITS region were amplified with primers 16S27F and 23S30R (Taton et al., 2003). Amplification was carried out as follows: one cycle of 5 min at 94°C; 10 cycles of 45 s at 94°C, 45 s at 57°C, and 2 min at 72°C; 25 cycles of 45 s at 94°C, 45 s at 54°C, and 2 min at 72°C; and a final elongation step of 7 min at 72°C. PCR product

was used as a template for sequencing with primers 16S27F, 23S30R (Taton et al., 2003), primer CYA781F(a) (Nübel et al., 1997), and the reverse complement of Primer 14 (Wilmotte et al., 1993).

Phylogenetic analyses

Partial sequences of the 16S rRNA gene (1357 bp) were aligned using the program BioEdit version 7.0.9.0 (Hall, 1999), and the alignment was edited manually. Phylogenetic trees were constructed by maximum-likelihood (ML), maximum parsimony (MP), and neighbor-joining (NJ) (Saitou & Nei, 1987) algorithms in the program PAUP* version 4.0b10 (Swofford, 2003). The topology for the phylogenetic tree was derived from ML. The GTR + I + G evolutionary model of substitution was found for the best fit to the data using ModelTest 3.7 (Posada, 2008). The parameters (base frequencies, rate matrix of substitution types, and shape of gamma distribution) were estimated from the data. 100, 1000 and 1000 bootstrap replicates were performed for ML, MP and NJ analysis, respectively. Nucleotide sequences were deposited at GenBank under the accession numbers FM161348-1350, FN691914, FN691919, FN691920, and JQ237768-JQ237774.

Results

Occurrence of the studied nostocacean morphospecies in the Czech Republic

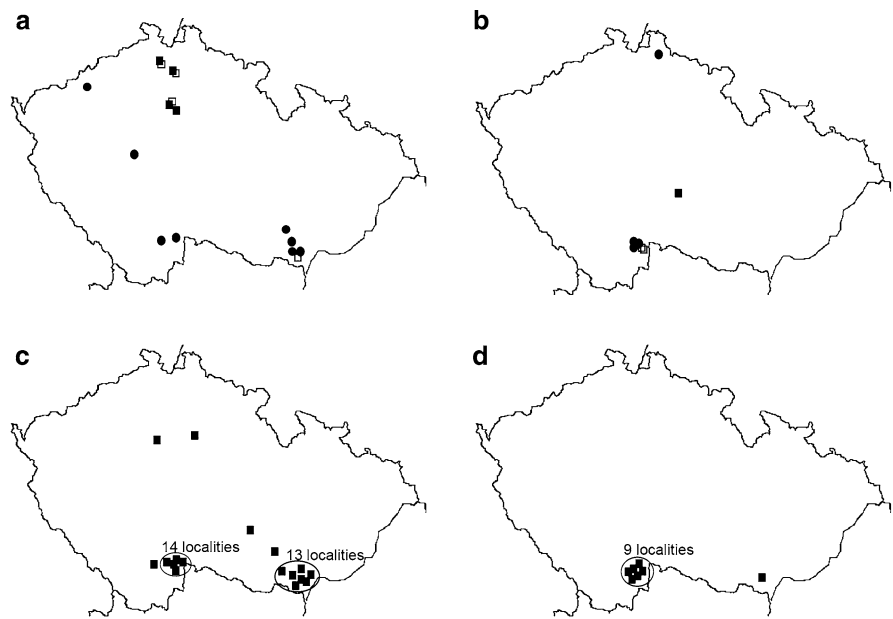
All of the studied nostocacean morphospecies are rather rare and sparsely distributed in the Czech Republic. Their occurrence is demonstrated in Fig. 1.

Anabaena bergii was only found at four localities in the Czech Republic (Máchovo jezero recreation pond, Dubice reservoir, Očko sandpit lake, and sandpit lake near Křenek; Fig. 1a).

Anabaena fusca was observed in Hubenov reservoir near Jihlava (Fig. 1b) in 2008, but no clonal strain was acquired.

Anabaena tenericaulis has been recently noticed at three localities (Máchovo jezero recreation pond, Dubice reservoir, and Očko sandpit lake). Besides that, it was reported from Mlýnský fishpond in South Moravia in the past (Fig. 1a).

Fig. 1 Distribution of the studied cyanobacterial morphospecies in the Czech Republic: **a**—*C. bergii* (filled square), *D. tenericaule* (open square), and *R. mediterranea* (filled circle); **b**—*A. fusca* (filled square), *D. curvum* (open square), and *D. mucosum* (filled circle); **c**—*S. aphanizomenoides* (filled square); **d**—*S. reniformis* (filled square)



Occurrence of *Dolichospermum curvum* was noticed at two localities, Staňkovský and Hejtman fishponds, in South Bohemia (Fig. 1b).

Dolichospermum mucosum was found in three fishponds in South Bohemia (Horák, Mařka, Nový Vdovec) and at a locality in North Bohemia (Mšeno reservoir in Jablonec nad Nisou; Fig. 1b).

Sphaerospermopsis aphanizomenoides has been displaying an increasing occurrence frequency in the Czech Republic during the last decade. It has been found at 32 localities so far (Fig. 1c): 15 sites in South Bohemia (fishponds Baštýř, Dvořiště, Koclířov, Malý Tisý, Naděje, Nový Vdovec, Opatovický, Podsedek, Rožmberk, Stolec, Stříbřec, Svět, Velký Tisý, Víra, and Ženich), 2 sites in Central Bohemia (Poděbrady reservoir, Hostivař reservoir), and 15 localities in South Moravia (reservoirs Brno and Mostiště, fishponds Dolní Písečný, Dvorský, Hlohovecký, Horní Lužický, Horní Písečný, Mlýnský, Nad Sádkami, Novodvorský, Novoveský, Prostřední, Velký Martinec, Vrkoč, Výtopa, and Zámecký).

Sphaerospermopsis reniformis was found at nine localities in South Bohemia (fishponds Dobrá Vůle, Fišmistr, Horák, Klec, Láska, Naděje, Pěšák, Rožmberk, and Vyšehrad) and in Hlohovecký fishpond in South Moravia (Fig. 1d).

Raphidiopsis mediterranea was observed at seven localities: fishponds Bagr and Fišmistr in South Bohemia, fishpond Papež in Central Bohemia, coalpit

lakes near Droužkovice in North-West Bohemia, and fishponds Františkův, Hlohovecký, and Zámecký in South Moravia (Fig. 1a).

Morphology of the studied nostocacean morphospecies

Morphometric characteristics of the studied morphospecies (Table 2) were measured directly from living water-bloom samples, except for akinetes of *A. tenericaulis* whose data were acquired from cultivated strains. General morphologies of these cyanobacteria are demonstrated in microphotographs (Fig. 2). Akinetes of *Anabaena bergii* from Očko sandpit were widely ovoid, with brownish epispor, vegetative cells were cylindrical, more-or-less isodiametric (length:width ratio around 0.9), and terminal cells were conical (Fig. 2a–d). Only proakinetes (immature stages of akinetes) were observed in the natural population of *A. fusca*. The missing data cannot be completed from culture conditions, as no clonal strain was isolated from this population. Nevertheless, the position of proakinetes was in accordance with the taxon description (near heterocytes, with 2–3 vegetative cells in between), as well as lemon-shaped vegetative cells and the pattern of trichome coiling (Fig. 2l–n). Trichomes of *A. tenericaulis* were very tiny, around 4 µm wide, and cross-walls between vegetative cells were almost invisible without

Table 2 Morphometric parameters of the studied nostocacean morphospecies measured from the original natural populations (living samples). The order of the strains in the table follows Table 1 where the strains are organized in alphabetical order. Values are means (minimum, maximum)

Strain code	Vegetative cells			Heterocytes			Akinetes		
	Length (μm)	Width (μm)	Length:width	Length (μm)	Width (μm)	Length:width	Length (μm)	Width (μm)	Length:width
09-02	4.2 (2.9, 6.0)	4.8 (3.8, 6.0)	0.9 (0.6, 1.4)	6.6 (5.6, 8.0)	6.2 (4.9, 7.5)	1.1 (0.9, 1.4)	17.1 (13.7, 22.7)	10.8 (8.5, 12.0)	1.6 (1.2, 2.5)
<i>A. fusca</i>	5.8 (3.8, 9.3)	5.3 (4.4, 6.0)	1.1 (0.8, 1.6)	7.5 (6.9, 8.1)	7.1 (6.6, 7.4)	1.1 (1.0, 1.2)	Only proakinetes present		
08-10, 08-11	9.6 (4.5, 18.0)	3.6 (2.6, 4.7)	2.8 (1.0, 5.1)	9.3 (4.2, 13.4)	3.9 (2.7, 5.1)	2.5 (1.1, 4.1)	28.6 (20.1, 39.7)	5.9 (5.0, 6.9)	4.9 (3.2, 6.8)
04-19	6.0 (4.2, 7.7)	7.0 (6.0, 8.2)	0.9 (0.7, 1.1)	7.2 (6.4, 8.9)	7.4 (6.3, 8.9)	1.0 (0.8, 1.1)	21.0 (20.6, 21.4)	10.7 (10.1, 11.3)	2.0 (1.8, 2.1)
06-04, 06-05	5.5 (3.3, 7.7)	6.2 (4.8, 7.4)	0.9 (0.6, 1.2)	8.9 (7.3, 11.3)	8.1 (6.5, 9.4)	1.1 (0.9, 1.3)	15.5 (11.7, 26.5)	13.7 (11.4, 16.9)	1.1 (0.9, 1.6)
08-03, 08-09	7.9 (4.2, 12.5)	8.7 (7.0, 11.2)	0.9 (0.5, 1.3)	10.1 (8.8, 11.6)	9.9 (8.8, 11.5)	1.0 (0.9, 1.2)	17.5 (14.7, 21.4)	15.7 (12.4, 18.8)	1.1 (1.0, 1.3)
07-04	9.5 (5.5, 16.7)	3.5 (2.8, 4.2)	2.7 (1.4, 4.8)	No	No	No	14.3 (9.5, 19.8)	4.0 (3.4, 4.6)	3.6 (2.3, 5.1)
04-43	5.5 (3.3, 11.2)	4.4 (3.1, 7.2)	1.3 (0.7, 2.3)	6.3 (5.2, 8.5)	5.9 (4.8, 7.1)	1.1 (0.9, 1.3)	10.0 (7.5, 11.4)	10.3 (8.9, 11.4)	1.0 (0.8, 1.2)
09-03	5.1 (2.1, 7.5)	3.8 (2.8, 6.1)	1.3 (0.6, 2.2)	6.2 (4.8, 8.7)	5.2 (3.3, 6.3)	1.2 (1.0, 2.1)	10.2 (7.8, 14.0)	9.9 (7.6, 12.7)	1.0 (0.9, 1.2)
06-01	5.3 (4.1, 7.7)	5.2 (4.3, 6.0)	1.0 (0.7, 1.4)	6.9 (5.8, 7.6)	7.1 (6.3, 8.2)	1.0 (0.9, 1.1)	10.0 (8.5, 12.3)	9.5 (8.0, 10.5)	1.1 (0.9, 1.2)
07-01	6.1 (3.9, 9.5)	4.8 (3.9, 5.6)	1.3 (0.8, 2.1)	7.0 (6.0, 7.9)	6.5 (5.4, 7.6)	1.1 (0.9, 1.3)	9.5 (8.7, 10.5)	9.4 (9.0, 9.8)	1.0 (0.9, 1.1)

constrictions. Akinetes in cultures were long cylindrical (Fig. 2e–h). Trichomes of *Dolichospermum curvum* were gathered in a massive mucilaginous mass both in nature and in cultured strains. Trichome coiling pattern was very specific: organization of trichomes into half-circles and trichome coiling direction is changed during the switch between the half-circles. Vegetative cells were spherical and akinetes kidney shaped (Fig. 2i–k). *D. mucosum* displayed irregular trichome coiling, spherical akinetes and thick mucilaginous envelope of trichomes (visible after staining with Indian ink; Fig. 2o–p). *Sphaerospermopsis aphanizomenoides* had spherical akinetes adjacent to heterocytes and narrowed but not pointed ends of trichomes (Fig. 2q–t). Trichomes of *S. reniformis* were regularly coiled but the compactness of coiling differed in the two populations studied (fishponds Pěšák and Vyšehrad). Nevertheless, both of these populations displayed an autapomorphic feature of the genus, i.e., spherical akinetes next to heterocytes (Fig. 2u–v). Trichomes of *Raphidiopsis mediterranea* were very thin (3–4 μm), with pointed terminal cells, invisible cross-walls, and relatively short cylindrical akinetes (Fig. 2w–z).

16S rRNA gene phylogeny of the studied nostocacean morphospecies

The studied nostocacean strains were spread in six different clusters A–F (Fig. 3) of ML, MP, and NJ phylogenetic trees based on partial 16S rRNA gene sequences (1357 bp). Cluster A contained two highly supported subclusters, *A. bergii* strains, including our strain 09-02, and *Aphanizomenon ovalisporum* strains. *Dolichospermum curvum*, *D. mucosum*, and *Anabaena tenericaulis* all appeared in the big clade of the genus *Dolichospermum*, but each of these morphospecies was situated in a different subclade. *D. curvum* 04-19 was in cluster B together with a Finnish strain of *D. flos-aquae* 0tu33s15, receiving a high-bootstrap support. *A. tenericaulis* 08-10 and 08-11 were both in cluster C, close to morphospecies *D. mendotae* and *Aphanizomenon gracile*. All these morphospecies display similar morphologies (vegetative cells longer than wide and elongated cylindrical akinetes). *D. mucosum* 06-04, 06-05, 08-03, and 08-09 appeared in cluster D together with *D. planctonicum*, *D. smithii*, *D. spiroides*, and *D. viguieri*. Common morphological features of the cyanobacteria of cluster D are the width

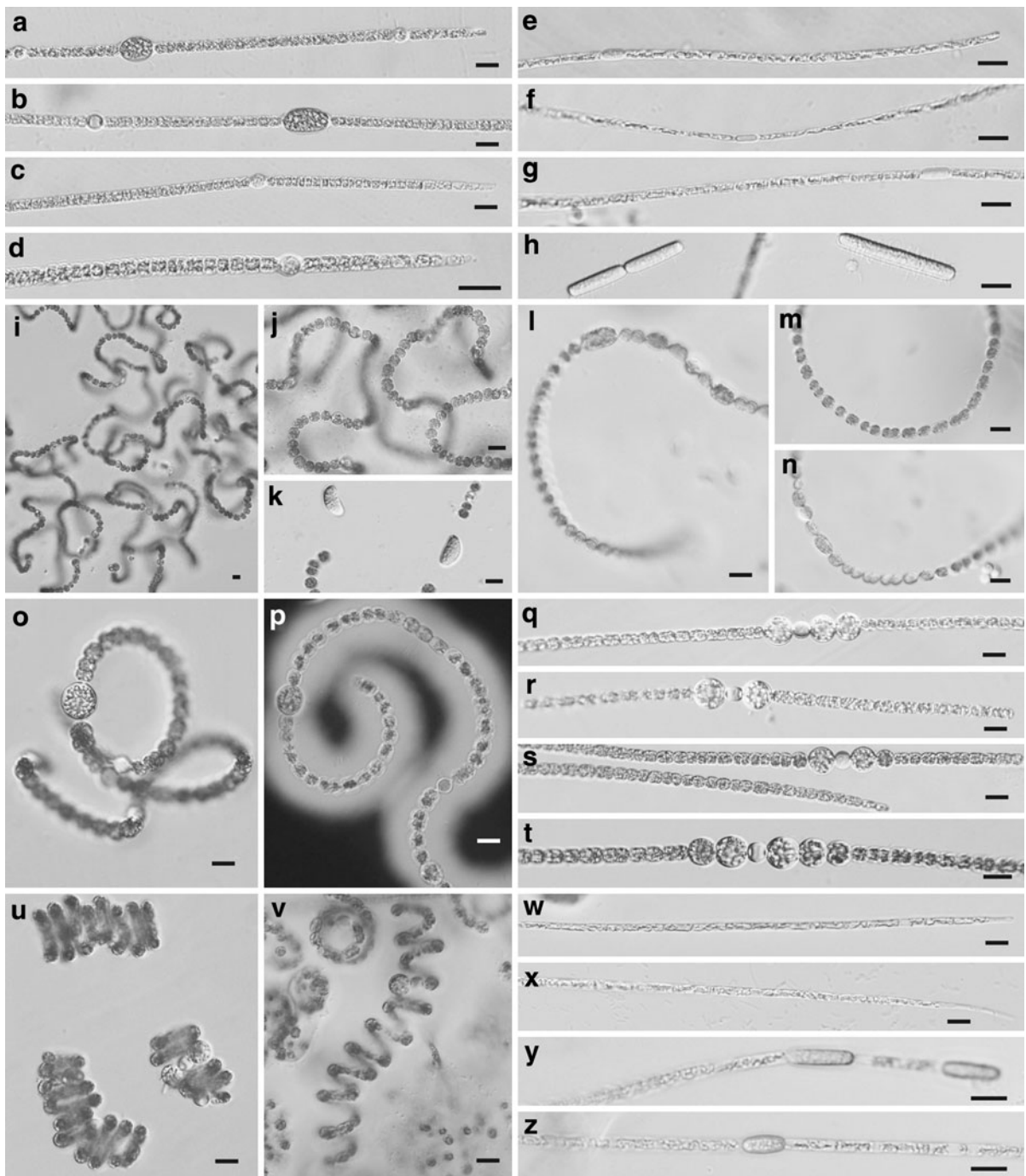


Fig. 2 Microphotographs of studied cyanobacterial morphospecies. *C. bergii* (a–d); *D. tenericaule* (e–h); *D. curvum* (i–k); *A. fusca* (l–n); *D. mucosum* (o–p); *S. aphanizomenoides* (q–t); *S. reniformis* (u–v); *R. mediterranea* (w–z). Scale bars represent 10 μ m

of trichomes ($\geq 8 \mu$ m) and length-to-width ratios of vegetative cells (usually <1 , i.e., the cells are barrel shaped, shorter than wide). The strains of the genus *Sphaerospermopsis* (*S. aphanizomenoides* 04-43 and

09-03, and *S. reniformis* 06-01 and 07-01) clustered together in a highly supported cluster E. *Raphidiopsis mediterranea* 07-04 appeared in cluster F, together with *Cylindrospermopsis raciborskii*.

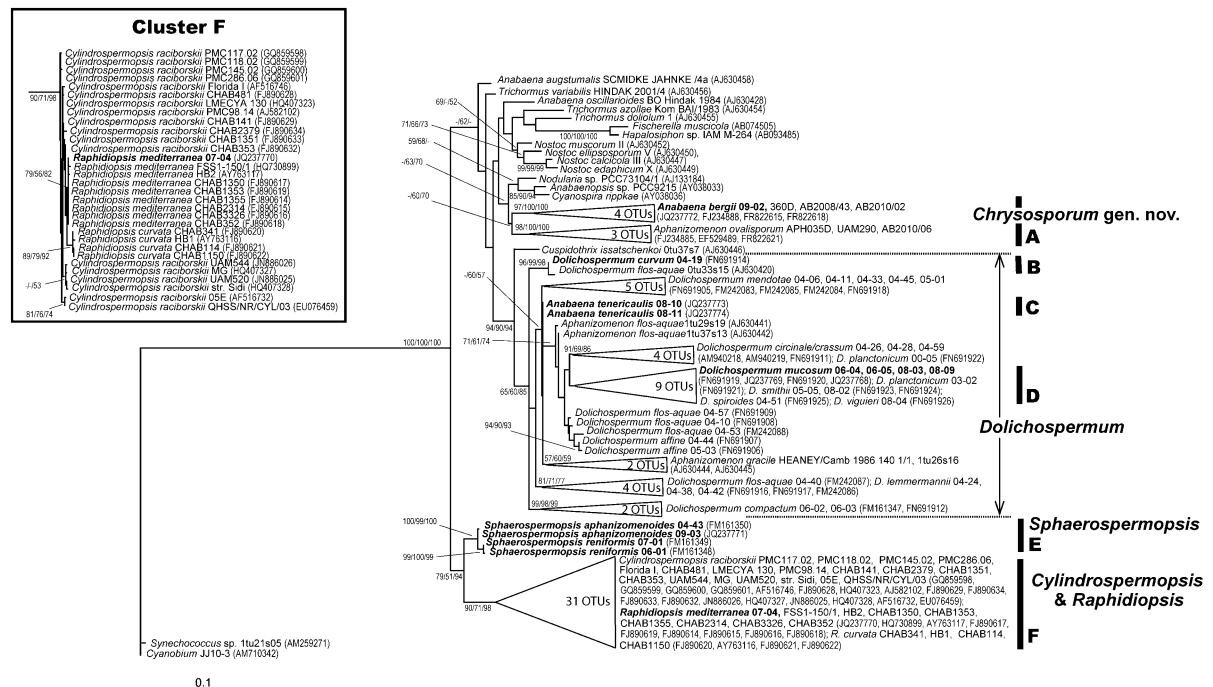


Fig. 3 Maximum-likelihood tree based on 16S rRNA gene sequences (1357 bp) showing the clustering of studied *Anabaena* strains. Numbers near the nodes indicate the bootstrap

Taxonomic revisions resulting from the herein presented phylogenetic analyses

Chrysochloris genus novum

Diagnosis Trichomes of varying length, solitary, free-floating, straight, more-or-less constricted at the cell-walls. Terminal cells conical. Vegetative cells with aerotopes, cylindrical, compressed during division. Heterocytes only intercalary, solitary, spherical, or slightly elongated. Akinetes widely ovoid (length:width ratio 1.2–2.5), with brownish or yellow–brown epispor, distant heterocytes. Planktonic in stagnant freshwater, usually sandpit lakes, halophilic lakes, reservoirs with higher conductivity, relict-lakes and estuaries of big rivers (Danube, Don).

Type species *Chrysochloris bergii* (Ostenfeld) comb. nova.

Basionym *Anabaena bergii* Ostenfeld, Izv. Turkestansk. Otd. Imp. Russk. Geogr. Obshch. 5 [Wiss. Ergebn. Aralsee-Exped. 8]: 142, pl. V: figs. 3, 4. 1908. (L)

values over 50% for NJ, ML, and MP analyses (in format NJ/ML/MP). OTUs, operational taxonomic units; studied strains are in bold

Autapomorphic characteristics Brownish or yellow–brown epispor of akinetes, conical terminal cells.

Etymology The name of the genus is derived from a Greek word *chryso* (gold) that reflects the characteristic color of the akinete epispor. The suffix *spor* is derived from a Greek word *spora* (seed), which is a symbolic designation of akinetes commonly used in cyanobacterial nomenclature.

On the basis of its morphological and the 16S rRNA gene sequence similarities, the following taxon is also transferred to *Chrysochloris*:

Chrysochloris ovalisporum (Forti) comb. nova [basionym: Accad. Agr. Sc. Lett. Verona, Ser. 4, 12: 1911].

Affiliation of the following taxa to the genus *Chrysochloris* is to be confirmed using analysis of their 16S rRNA gene sequences:

Anabaena hatueyi Komárek [basionym: Preslia, Praha 77: 219, 2005]

Anabaena minderi Huber-Pestalozzi [basionym: Das Phytoplankton des Süsswassers: Systematik

und Biologie, Schweizerbart'sche Verlagsbuchhandlung, Stuttgart: figs. 10, 11, 19, 20. 1938]
Anabaena recta Geitler et Ruttner [basionym: Arch. Hydrobiol. suppl. 14: 459, 1936]
Anabaena salina Liebetanz [basionym: Bull. Int. Acad. Polon. Sc. et Lett., Cl. Sc. Math. et Nat., Ser. B, 1925: 109, pl. 1: Fig. 5. 1925].

Dolichospermum tenericaule comb. nov

Both ML and NJ phylogenetic analyses of the 16S rRNA gene sequences proved the affiliation of *A. tenericaulis* strains (08-10, 08-11) within the genus *Dolichospermum*. We thus suggest reclassification of *Anabaena tenericaulis* Nygaard (1949) as *Dolichospermum tenericaule* comb. nov.

Discussion

This study refers on the occurrence of eight potentially non-indigenous nostocacean morphospecies in the Czech Republic: *Anabaena bergii*, *A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, *Raphidiopsis mediterranea*, *Sphaerospermopsis aphanizomenoides*, and *S. reniformis*. For the species *D. curvum* and *A. fusca*, this is the first report on their occurrence in the Czech Republic. *A. tenericaulis* was previously found only once (Losos & Heteša, 1971) here. Three more nostocacean species were referred by Kaštovský et al. (2010) to be alien or potentially expansive in this region: *Cuspidothrix issatschenkoi*, *Cylindrospermopsis raciborski*, and *Dolichospermum compactum*.

Chrysosporum bergii comb. nova (former *Anabaena bergii*)

The herein presented localities of occurrence of *A. bergii* were all also reported by Kaštovský et al. (2010). The species was originally described from Lake Aral and still occurs there (Orlova & Rusakova, 1999). First records from the Czech Republic were by Heteša et al. (1997) from Košarská and Stulíková oxbow pool in Dyje river alluvium. Since 2001 to date, it has been repeatedly found in North Bohemia as was summarized in the “Results” section. This species has recently been reported also from other regions of

Central Europe, such as Slovakia (Hindák, 1992, 2000) or northeast Germany (Stüken et al., 2006).

This morphospecies has been classified into the genus *Anabaena* so far but it displays several specific morphological features that are different both from this genus (currently containing species without gas vesicles) and from the genus *Dolichospermum* (planktonic representatives, separated from the traditional genus *Anabaena* by Wacklin et al. (2009)). These features are mainly the conical shape of terminal cells and brownish to yellow–brown epispor of akinetes. And also the cylindrical vegetative cells with little pronounced constriction at cross-walls is something different from typical *Anabaena* or *Dolichospermum*.

Based on the 16S rRNA gene sequence, a distinct and highly supported cluster was detected using maximum-likelihood, maximum parsimony, and neighbor joining phylogenetic analyses. This cluster (A) is markedly distant from the big *Dolichospermum* cluster (containing subclusters B, C, and D, Fig. 3). Also the type species of the genus *Anabaena*, *A. oscillarioides*, represented by the strain BO HINDAK 1984, appeared in a clearly different position in the phylogenetic tree, as well as another representative of benthic *Anabaena*, *A. augstumalis* SCMDKE JAH-NKE 4/a (Fig. 3). These findings are in agreement with results by Rajaniemi et al. (2005) and mainly by Stüken et al. (2009).

Regarding these specific morphological and molecular features of *Anabaena bergii*, we erected the new genus *Chrysosporum*. Besides *A. bergii*, we also proposed re-classification of *Aphanizomenon ovalisporum* into *Chrysosporum*. Stüken et al. (2009) demonstrated that *A. bergii* and *A. ovalisporum* form two sister phylogenetic clusters based on several genomic regions (16 s RNA, *cpcA* and *cpcB* genes). One of these clusters contained exclusively *A. bergii* strains, while two *A. bergii* were admixed to *A. ovalisporum* strains in the second cluster. Morphologies of these strains are not available, and reliability of species identification of these strains cannot be therefore assessed. Bootstrap supports of each of these clusters were higher (around 100) than clustering of these two clusters together (54–80, depending on genomic region and phylogenetic analysis), so the existence of a second genus containing *Aphanizomenon ovalisporum* might be discussed here. Nevertheless, we suggest an establishment of just one genus *Chrysosporum* for now because the species

identification of the strains by Stüken et al. (2009) cannot be verified. Furthermore, the original descriptions of *Anabaena bergii* and *Aphanizomenon ovalisporum* both encompass the important identification features (conical terminal cells, brownish epispore of akinetes), proposed autapomorphic for *Chrysosporum*. On the other hand, the only morphological difference between *A. bergii* and *A. ovalisporum* is the length:width ratio of vegetative cells (longer than wide in *A. ovalisporum*) that appears to be rather questionable (also depends on growth rate and division frequency of cells). A thorough revision of both these species is needed, and a special genus for *A. ovalisporum* might be separated from *Chrysosporum* if it will be proved well founded.

Anabaena fusca

Anabaena fusca has been found for the first time in the Czech Republic at the only locality of Hubenov reservoir near Jihlava. The species was described from a lake in Minnesota, USA (Hill, 1976b), and has not yet been reported from anywhere else than North America.

All morphological features observed in the Czech population fitted well to the original description of the taxon.

Nevertheless, the phylogenetic affiliation of this species remains unclear, because its 16S rRNA gene has not yet been sequenced. In this study, we only present the first observation of its occurrence, and the isolation of a strain is intended in the future. Wacklin et al. (2009) performed a substantial revision of the traditional genus *Anabaena* and reclassified the majority of planktonic representatives into the new genus *Dolichospermum*. *Anabaena fusca*, although morphologically similar to some *Dolichospermum* morphospecies, was excluded from this revision because of the lack of molecular data. Detailed polyphasic evaluation of this morphospecies is therefore highly desirable.

Dolichospermum tenericaule comb. nova (former *Anabaena tenericaulis*)

This is the first report on the occurrence of *D. tenericaule* in the Czech Republic since the finding by Losos & Heteša (1971). The species was described under the name *Anabaena tenericaulis* from Jægerbakke Dam, Selandia, Denmark (Nygaard, 1949) and was sparsely distributed in Nordic localities

(Komárek, pers. comm.; Vuorio, pers. comm.; Willén, pers. comm.). Little is known about its distribution outside the Nordic countries. To our knowledge, it has never been reported from another country in Central Europe.

All morphological and morphometric parameters of the herein described strains (08-10, 08-11) are in agreement with the original description and drawings by Nygaard (1949).

The 16S rRNA gene of representatives of this species was sequenced for the first time. The herein presented phylogenetic analyses demonstrated that the strains 08-10 and 08-11 formed a tight cluster C (Fig. 3), close to *Dolichospermum mendotae* and *Aphanizomenon gracile* strains, whose sequences were acquired from Genbank. Cluster C is a part of a big clade of the genus *Dolichospermum*. It is possible to find morphological characteristics that are common for cluster C, *D. mendotae* and *A. gracile*: long cylindrical akinetes, elongated vegetative cells (length:width ratio > 1), and relatively thin trichomes (ca. 3–5 µm). Reclassification of *Anabaena tenericaulis* Nygaard 1949 as *Dolichospermum tenericaule* comb. nova was proposed based on these findings.

Dolichospermum curvum

This study reports on the occurrence of *D. curvum* at two sites in South Bohemia, fishponds Hejtman and Staňkovský. They are neighboring hypertrophic fishponds, interconnected by a channel, and therefore the two populations of *D. curvum* in these fishponds are presumably not isolated one from another. The species was described from a lake in Minnesota, USA, (Hill, 1976a) and later reported from Finland (Komárková-Legnerová & Eloranta, 1992) and Japan (Li et al., 2000).

From morphological point of view, our strain 04-19 is more similar to the Japanese (Li et al., 2000) population than to the population from USA (Hill, 1976a). According to the original description, the akinetes should be distinctly bent, while our population, as well as that one from Japan, displays kidney-shaped akinetes. Shape of akinetes of the Finnish population (Komárková-Legnerová & Eloranta, 1992) was somewhere in between the American and Japanese populations. Nevertheless, all of these populations displayed similar pattern of trichome coiling and a massive mucilaginous mass around trichomes.

Detailed polyphasic evaluation would be necessary to confirm that these populations from different parts of the world represent a tight phylogenetic cluster with the above-mentioned range of variability in the shape of akinetes.

For the present, our strain 04-19 represents the only *D. curvum* strain whose 16S rRNA gene has been sequenced. The herein published phylogenetic analyses have indicated that *D. curvum* belongs to *Dolichospermum* cluster where it represents a specific, highly supported subcluster (cluster B in Fig. 3). Besides our strain 04-19, a strain Otu33s15 from Finland designated as *D. flos-aquae* also appeared in cluster B. Morphology of this strain was evaluated by Rajaniemi et al. (2005) but the information about trichome coiling pattern and presence of mucilage is missing in this publication and a microphotograph is not provided. As morphometric features of *D. curvum* are highly similar to those of *D. flos-aquae*, this Finnish population might have been misidentified and its affiliation to the species *D. curvum* cannot be excluded.

Dolichospermum mucosum

Occurrence of *D. mucosum* in the Czech Republic has only been noticed at four localities. This is the first finding from Central Europe as it has been reported from North Europe Komárková-Legnerová & Eloranta, 1992; Rajaniemi et al., 2005), Japan (Li et al., 2000), and Ukraine (Komárek & Zapomělová, 2007) so far.

Sequencing of the 16S rRNA gene of four Czech strains (06-04, 06-05, 08-03, and 08-09) confirmed that this species belongs to the genus *Dolichospermum* (revision performed by Wacklin et al., 2009). Its clustering together with *D. smithii* strains (straight trichomes of similar cell dimensions, spherical akinetes) is in accordance with results by Rajaniemi et al. (2005). These authors also presented a close phylogenetic affinity between coiled and straight morphospecies of *Dolichospermum* of similar cell dimensions and identical akinete shapes.

Sphaerospermopsis aphanizomenoides

The occurrence frequency of *S. aphanizomenoides* in the Czech Republic has been continuously increasing. For the first time, it was found by Horecká & Komárek

(1979) from Central Moravia. Later findings were not until 1994 (Heteša et al., 1997) and then 2004 (Zapomělová et al., 2009). Kaštovský et al. (2010) referred on 20 sites where this species has recently occurred and since the publication of this study it has been newly found at more than 10 localities. Most of these localities are eutrophic to hypertrophic fishponds. An updated list of these localities has been provided above. This species was originally described from a lake in Anatolia (Geitler, 1932) and has predominantly been reported from the tropical and subtropical regions. Nevertheless, it appears to have expanded to the temperate zones of Central Europe within the last few years: Hungary (Padisák & Kovács, 1997), Slovakia (Hindák, 2000), Poland (Stefaniak & Kokociński, 2005), northeast Germany (Stüken et al., 2006), and the Czech Republic (Zapomělová et al., 2009). Reliable identification of *S. aphanizomenoides* is only possible when akinetes are present. Misidentification or oversight of populations without akinetes is therefore highly probable, and this species might be even more frequent than has been reported so far.

The first polyphasic evaluation of Czech strains of *S. aphanizomenoides* was provided by Zapomělová et al. (2009). A distinct highly supported molecular cluster was recognized based on the 16S rRNA gene, which resulted in erection of the new genus *Sphaerospermopsis* and reclassification of former *Aphanizomenon aphanizomenoides* (Zapomělová et al., 2009, 2010). Identical results were concurrently obtained from German and Portuguese strains, and were furthermore supported by analyses of some other genomic regions (Stüken et al., 2009; de Figueiredo et al., 2010).

Sphaerospermopsis reniformis

This article provides an information on nine localities of *S. reniformis* occurrence in the Czech Republic. For the first time, this species was noticed by Keršner (1997) in South Moravia and later Zapomělová et al. (2009) reported on occurrences in South Bohemia in 2006 and 2007. Little is known about the biogeography of this species. It appears to be sparsely distributed at very distant localities around the world: Germany, Ukraine, Japan, Cuba, and Africa (Lemmermann, 1898; Aptekar in Elenkin, 1938; Watanabe et al., 2004; Komárek, 2005; Cronberg & Annadotter, 2006; Komárek & Zapomělová, 2007). Similar to *S.*

aphanizomenoides, the absence of akinetes might hamper the recognition and identification of this species in natural populations and thus this taxon might well have been overlooked.

The herein described strains of *S. reniformis* (06-01, 07-01) have already been studied by Zapomělová et al. (2009). Based on this polyphasic evaluation, *S. reniformis* was established the type species of the newly erected genus *Sphaerospermopsis* (Zapomělová et al., 2009, 2010). Results of our present phylogenetic analyses are in accordance with that.

Raphidiopsis mediterranea

This study informs about the occurrence of *R. mediterranea* at seven localities in the Czech Republic. Kaštovský et al. (2010) mentioned four of them but alerted that this species is probably more widespread but previous findings might have confused it with a similar and better-known species *Cylindrospermopsis raciborskii*. *R. mediterranea* is considered a subtropical species but later has also spread to the temperate zone, as was summarized by Kaštovský et al. (2010). Regarding Central Europe, its occurrence has been recently reported from Slovakia (Maršálek et al. 2000).

Based on phylogenetic analyses of the 16S rRNA gene sequence, our *R. mediterranea* strain 07-04 clustered together with other *Raphidiopsis* and *Cylindrospermopsis raciborskii* strains from GenBank (cluster F, Fig. 3). The first evidence of the close phylogenetic relationship of these genera was published by Li et al. (2008). Later studies confirmed these findings and even declared that *R. mediterranea* represents a non-heterocytous life-cycle stage of *Cylindrospermopsis raciborskii* (Moustaka-Gouni et al., 2009, 2010).

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