Hydrobiologia (2010) 639:99–113
DOI 10.1007/s10750-009-0028-y

PHYTOPLANKTON

Polyphasic characterization of eight planktonic *Anabaena* strains (Cyanobacteria) with reference to the variability of 61 *Anabaena* populations observed in the field

Eliška Zapomělová · Klára Řeháková · Jitka Jezberová · Jaroslava Komárková

Published online: 18 December 2009

Abstract The plasticity of morphological features used for single morphospecies identification was studied under varied experimental conditions (temperature, light, nitrogen, phosphorus) in eight planktonic *Anabaena* strains. The strains represented all of the morphospecies with coiled trichomes commonly occurring in Central Europe (two strains of *A. mendotae* & *A. sigmoidea* complex, two *A. lemmermannii* strains, two *A. flos-aquae* strains, and two strains of *A. circinalis* & *A. crassa* complex). Significant effects of the growth conditions on vegetative cell dimensions were observed in seven strains, and P concentration was the main influencing factor in most cases (six strains). Significant effect of an environmental factor (P) on akinete morphology was found in only one strain. Experimentally assessed temperature and light growth optima were specific for each strain and were not consistent with the taxonomic affiliation of the strains. Morphologies of the *Anabaena* strains studied were compared with the field morphologies of 61 *Anabaena* populations of eight morphospecies observed in the Czech Republic. The range of morphological variability of single strains under the experimental conditions spanned the total variability of the populations of relevant morphospecies observed in the field. Delimitations and proper descriptions of the morphospecies are discussed in the light of partial 16S rRNA gene sequences of the studied strains.

Keywords *Anabaena* · Taxonomy · Identification · Morphological variability · 16S rRNA gene

Guest editors: T. Zohary, J. Padisák & L. Naselli-Flores / Phytoplankton in the Physical Environment: Papers from the 15th Workshop of the International Association for Phytoplankton Taxonomy and Ecology (IAP), held at the Ramot Holiday Resort on the Golan Heights, Israel, 23–30 November 2008

E. Zapomělová (✉) · K. Řeháková · J. Jezberová · J. Komárková
Institute of Hydrobiology, Biology Centre of AS CR, Na Sádkách 7, 37005 Ceske Budejovice, Czech Republic
e-mail: eliska.zapomelova@seznam.cz

E. Zapomělová · J. Komárková
Faculty of Science, University of South Bohemia, Branišovská 31, 37005 Ceske Budejovice, Czech Republic

K. Řeháková · J. Komárková
Institute of Botany, AS CR, Dukelská 135, 37982 Trebon, Czech Republic

Introduction

The genus *Anabaena* belongs to the most frequent bloom-forming cyanobacterial genera, affecting water quality worldwide and represents a potential risk due to the production of a range of bioactive toxic compounds (Rapala & Sivonen, 1998; Lyra et al., 2001). Detailed knowledge of the biology, morphological variability, toxicity, and phylogenetic

status of various *Anabaena* morphospecies is therefore required.

Planktonic *Anabaena* species represent a widely diversified group of cyanobacteria, encompassing around 80 freshwater morphospecies (Komárek, 1996). Nevertheless, the proper description and classification of these morphospecies has not yet been satisfactorily resolved and many questions remain unanswered.

So far published studies on morphological features of *Anabaena* have been limited either on morphology in cultures (Stulp, 1982; Stulp & Stam, 1982, 1984a, b, 1985; Li et al., 2000; Zapomělová et al., 2008a) or onto one-shot field observations (e.g., Komárek, 1958; Hill, 1976a, b, c; Hickel, 1982; Cronberg & Komárková, 1988; Komárková, 1988; Komárková-Legnerová & Cronberg, 1992; Komárková-Legnerová & Eloranta, 1992; Padisák & Kovács, 1997; Hindák, 2000; Zapomělová et al., 2007). These approaches, when they are used separately, have serious disadvantages. Observation in the field does not provide complete information on morphological plasticity related to the variety of environmental conditions or growth phases. On the other hand, it has been known for a long time that long-term cultivation can cause substantial morphological changes that do not correspond to the natural conditions (Anand, 1988). Thus, confusions and misidentifications can arise when cyanobacteria are identified according to the morphology later during the cultivation (Komárek & Anagnostidis, 1989). In spite of this, nobody has tried to combine both approaches in the research of *Anabaena*, i.e., field observation, subsequent isolation, and short-term cultivation under varied experimental conditions.

Even the most recent studies applying a polyphasic approach in *Anabaena* classification (Gugger et al., 2002; Rajaniemi et al., 2005a, b; Willame et al., 2006) evaluated morphological features mainly or entirely on strains in cultures. Nevertheless, the enormous value of these studies resides in the combination of morphological and molecular approach that they applied for the first time on a higher number of *Anabaena* isolates. They demonstrated very high 16S rRNA gene, *rpoB* and *rbcLX* sequence similarity between various *Anabaena* morphotypes.

However, the correlation and discussion of morphological plasticity of single *Anabaena* strains under varied environmental conditions and their phylogenetic characteristics are still missing. Consequently, we have described the morphological plasticity of eight planktonic *Anabaena* strains of four morphospecies under a variety of experimental conditions. The results were compared with the field morphologies of 61 *Anabaena* populations observed in the Czech Republic, including the original populations from which the selected strains were isolated. Delimitations of single morphospecies were then discussed in the light of the ecological parameters (temperature and light growth optima) and phylogenetic affiliations based on their 16S rRNA gene sequences.

Materials and methods

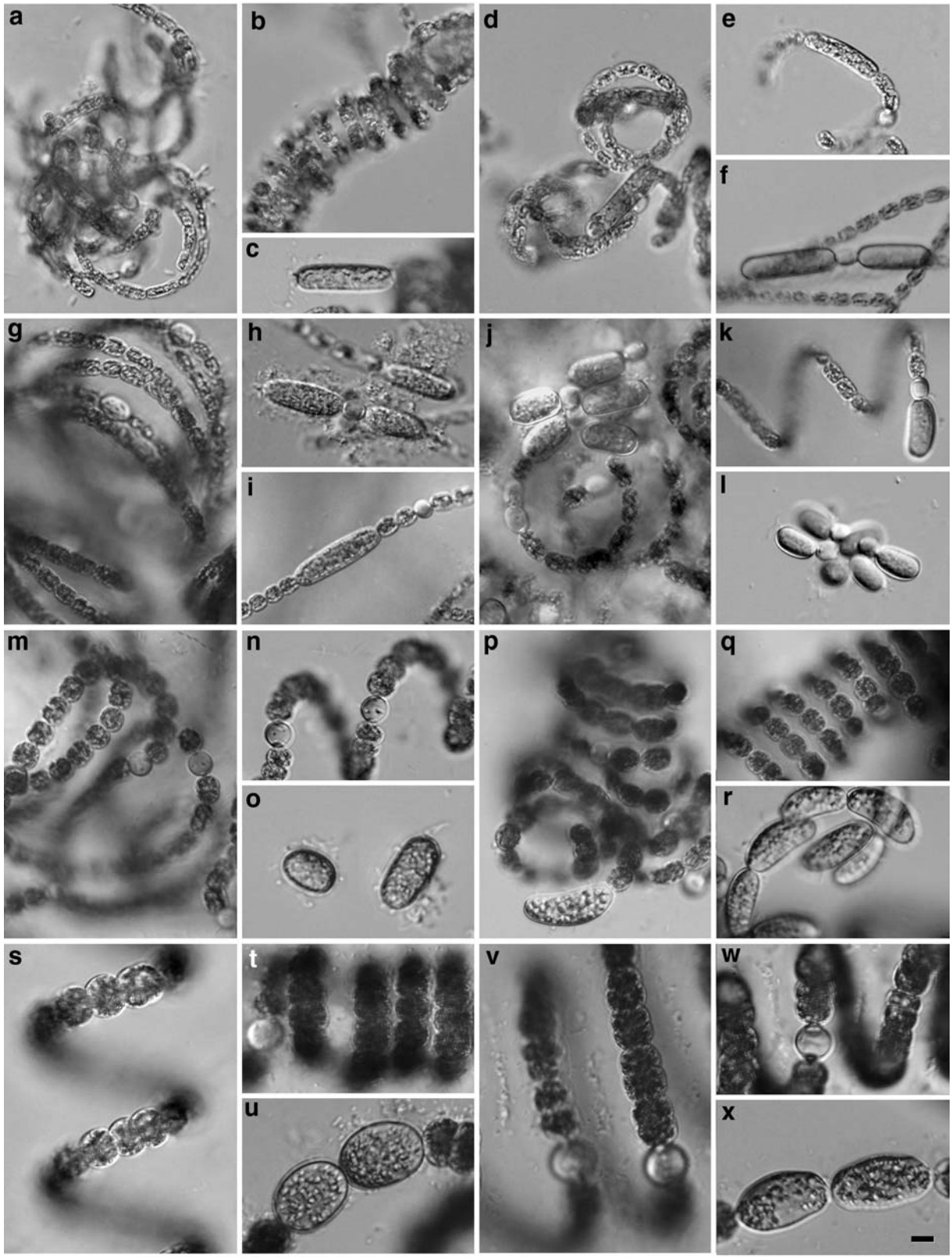
Strains and cultivation

The eight *Anabaena* strains studied (Fig. 1) were unialgal, clonal, but not axenic. They were isolated in 2004 from different localities in the Czech Republic and cultivated in WC medium (Guillard & Lorenzen, 1972) at 21°C and the light intensity of 70 μmol m^{-2} s^{-1} (16:8 L:D cycle).

Cross gradients of light and temperature

Cross gradients (Kvíderová & Lukavský, 2001) were used to test the effect of light and temperature on the cyanobacterial morphology and to determine the growth optima of the strains studied. For the morphological experiments, the strains were exposed in sterile culture plates (9 × 12 cm^2, 12 wells, 6.5 ml each) to nine different combinations of light and temperature of cross gradients (Fig. 2a) for 10 days. The temperature ranged from 10 to 28°C and the

Fig. 1 Microphotographs of the studied *Anabaena* strains. **a–c** ▶ *A. mendotae/A. sigmoidea* strain 04-11 from the fishpond Černiš. **d–f** *A. mendotae/A. sigmoidea* strain 04-45 from the fishpond Svět. **g–i** *A. lemmermannii* strain 04-33 from the Orlík reservoir. **j–l** *A. lemmermannii* strain 04-42 from the fishpond Svět. **m–o** *A. flos-aquae* strain 04-40 from the Skalka reservoir. **p–r** *A. flos-aquae* strain 04-53 from the fishpond Švarcenberk. **s–u** *A. circinalis/A. crassa* strain 04-26 from the Jesenice reservoir. **v–x** *A. circinalis/A. crassa* strain 04-28 from the fishpond Hodějovický. Images **a**, **d**, **g**, **j**, **m**, **p**, **s**, and **v** illustrate the morphology of the strains in the original populations in the field. Magnification of all microphotographs is identical; the *scale bar* in the lower right corner represents 10 μm


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x

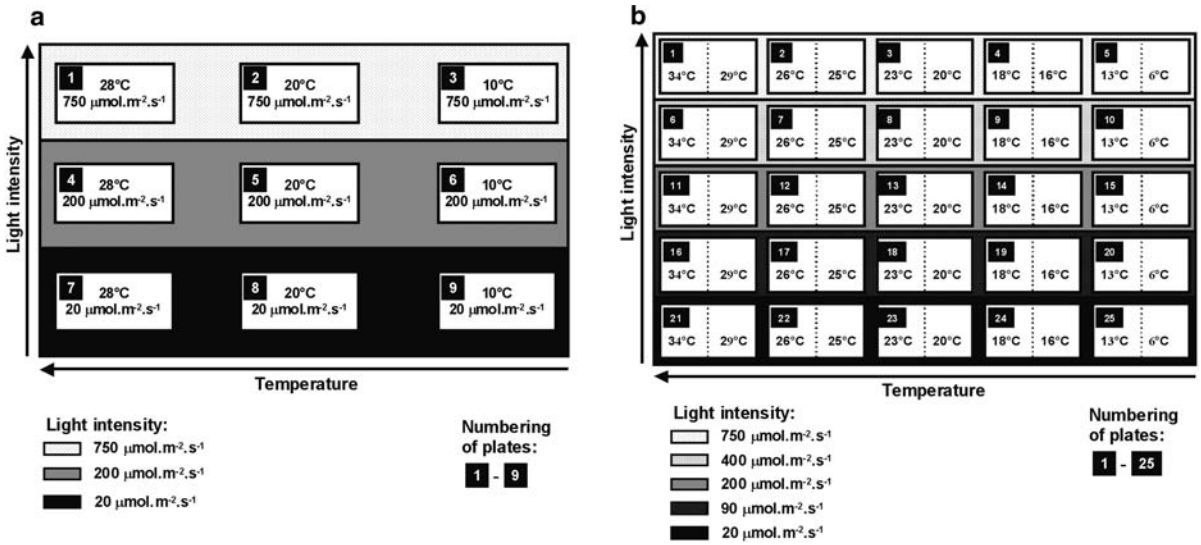


Fig. 2 Design of the cross-gradient experiments. **a** Evaluation of morphological variability of the strains in relation to light and temperature. **b** Determination of light and temperature growth optima of the strains. Gradients of light intensity and temperature are indicated with *arrows*

range of light intensity, provided by sodium vapor lamps, was 20–750 µmol m^{-2} s^{-1}. To estimate the temperature and light growth optima of the strains, a modified design of the cross-gradient experiments was used (Fig. 2b). Identical volumes of stirred dense batch culture were inoculated into sterile culture plates containing fresh WC medium (9 × 12 cm^2, 6 wells, 16 ml each). The plates were then exposed to 25 positions of the cross-table. The temperature range was 6–34°C, and the light intensity was 20–750 µmol m^{-2} s^{-1}. The experiments were terminated in the exponential phase of growth of the fastest growing cultures. Chlorophyll *a* concentrations were determined spectrophotometrically after acetone extraction (Lorenzen, 1967) and compared among the positions of cross gradients.

Table 1 Modifications of WC medium used in the experiments

Medium type	P (µg l^{-1})	N (µg l^{-1})
$WC_{0\times P}$	0	8×10^3
$WC_{0.001\times P}$	8×10^{-1}	8×10^3
$WC_{0.1\times P}$	8×10^1	8×10^3
$WC_{1\times P} = WC_{1\times N} = WC$	8×10^2	8×10^3
$WC_{10\times P}$	8×10^3	8×10^3
$WC_{0\times N}$	8×10^2	0
$WC_{0.001\times N}$	8×10^2	8×10^0
$WC_{0.1\times N}$	8×10^2	8×10^2
$WC_{10\times N}$	8×10^2	8×10^4
"Starvation medium"	0	0

Concentration series of nitrogen and phosphorus

Modified types of WC medium containing different concentrations of nitrogen (N) and phosphorus (P) (Table 1) were used to test the effect of nutrients on the cyanobacterial morphology. The concentration series were designed with respect to N and P concentrations commonly occurring in fishponds and reservoirs of the Czech Republic (Znachor et al., 2006). To force the strains to deplete their intracellular nutrient reserves, cyanobacterial biomass was incubated in a modified WC medium without N and P ("starvation medium," Table 1) for 7 days prior to the experiment. Equimolar concentrations of KCl were added to the "starvation medium," $WC_{0\times P}$, $WC_{0.001\times P}$, and $WC_{0.1\times P}$, to retain the original K^+ concentration.

Morphometry

Microphotographs of at least 30 fresh trichomes per each natural population or per each experimental

treatment were taken with a digital camera (Olympus DP 70, magnification 400×). Dimensions of all cell types were measured (five vegetative cells per trichome measured in 30 trichomes and as many heterocytes and akinetes as was possible to find in each sample). Length:width ratios of vegetative cells, heterocytes, and akinetes were computed to roughly characterize the cell shapes. All size measurements were performed using image analysis (Olympus DP Soft).

Statistical analyses

The effects of light, temperature, P, and N on the morphometric characteristics were tested by redundancy analysis (RDA) with Forward selection. Monte-Carlo permutation test was used for calculation of *P*-values. The data were centered and standardized. Morphologies of the studied strains under varied experimental conditions and field morphologies of the 61 *Anabaena* populations (including those from which the studied strains were isolated) were compared by principal component analysis (PCA). These statistical analyses were performed using the program CANOCO (Ter Braak & Šmilauer, 1998), and ordination diagrams were created using CanoDraw software (Šmilauer, 1992). Basic statistical characteristics such as average values, 25 and 75% percentiles, and extreme values were computed for each morphological feature. Box-whisker plots were created by the GraphPad Prism program (GraphPad Software, San Diego, CA, USA, www.graphpad.com). The light and temperature growth optima of the strains were derived from the surface plots, which were created using the program Statistica (Anonymous, 1996).

Phylogenetic study

The biomass was harvested in the exponential phase of growth by repeated centrifugation, during which the trichomes were washed several times with the physiological solution (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 cAlaR (Wilmotte et al., 1994), primers WAW1486R and Primer 14 (Wilmotte et al., 1993), and primer CYA781F(a) (Nübel et al., 1997). Sequences were aligned in the program ARB (http://www.arb-home.de). The alignment was edited manually and ambiguous bases were removed. For the phylogenetic analysis, trees were built with the Neighbor-joining method (NJ) (Saitou & Nei, 1987) and the Maximum Parsimony (MP) algorithm in the PHYLIP program (Felsenstein, 2004). Five-hundred bootstrap replicates were performed both for NJ and MP analyses. Nucleotide sequences were deposited at GenBank under the accession numbers FM242083–FM242088. In addition to the strains whose accession numbers are given in the phylogenetic tree, following sequences were used for the construction of the phylogenetic trees: AJ293112, AJ293115, AJ293116, AJ630413, AJ630415, AJ630417, AJ630430, AJ630432, AF247572, AF247575, AF247576, AF247584, AJ 293126, AJ293127, AJ630441, AJ630418, AJ293111, AJ293107, AJ630409, AJ630445, AJ133154, AJ293124, AJ293131, AJ293104, AJ133159, EF568912, EF547196, AJ293106, AJ293113, AJ133156, AJ630419, AJ630422, AJ630423, AY196087, AY196088, AJ630457, AJ630458, AJ630428, AJ133161, AF160256, AJ133181, AY038033, and AY038036.

Results

Morphological plasticity of the strains with reference to the variability of 61 *Anabaena* populations observed in the field

Morphometrical characteristics of the eight studied *Anabaena* strains were measured under various temperature, light intensity, and concentration of N and P (Table 2). These data were compared with field morphologies of 61 *Anabaena* populations, including those from which the studied strains were isolated. PCA has demonstrated that the studied strains were able to span the total variability of the populations of

Table 2 Summary of morphometric parameters of the *Anabaena* strains observed during the experiments

Strain code	Vegetative cells			Heterocytes			Akinetes	
	Length (μm)	Width (μm)	Length:width	Length (μm)	Width (μm)	Length:width	Length (μm)	Width (μm)	Length:width
04-11	8.5 (5.8, 11.5)	3.5 (2.9, 5.4)	2.5 (1.4, 3.8)	8.0 (5.8, 11.6)	4.8 (3.9, 6.1)	1.7 (1.2, 2.8)	21.5 (16.8, 27.5)	5.8 (4.5, 7.3)	3.6 (1.7, 5.2)
04-26	9.1 (6.0, 12.0)	11.2 (8.5, 13.5)	0.9 (0.7, 1.2)	11.3 (7.5, 13.0)	11.5 (8.5, 13.0)	1.0 (0.9, 1.1)	25.2 (20.0, 30.0)	15.6 (12.5, 18.5)	1.6 (1.3, 1.9)
04-28	12.3 (5.5, 17.0)	10.2 (7.0, 13.0)	1.3 (0.6, 2.1)	10.6 (8.5, 12.6)	11.8 (9.0, 13.0)	1.1 (0.9, 1.2)	23.5 (18.0, 29.0)	15.5 (13.0, 18.5)	1.2 (1.4, 2.0)
04-33	6.5 (4.7, 8.3)	4.8 (4.0, 5.6)	1.4 (1.0, 1.7)	6.7 (5.6, 8.2)	5.8 (4.2, 7.0)	1.3 (1.0, 1.6)	25.2 (20.3, 28.0)	8.2 (7.1, 9.3)	3.0 (1.8, 4.0)
04-40	6.5 (4.6, 8.6)	7.3 (5.9, 8.7)	0.9 (0.7, 1.1)	7.7 (6.0, 9.3)	7.6 (6.1, 9.1)	1.0 (0.9, 1.1)	16.8 (11.7, 20.4)	11.2 (9.2, 12.3)	1.5 (1.2, 1.8)
04-42	6.8 (4.0, 8.7)	4.8 (3.8, 5.6)	1.4 (0.9, 2.0)	6.4 (5.1, 8.0)	5.3 (4.2, 6.1)	1.2 (1.0, 1.4)	15.6 (11.7, 18.4)	7.6 (6.6, 8.4)	2.0 (1.7, 2.4)
04-45	7.8 (4.6, 11.8)	4.3 (3.3, 5.3)	1.7 (1.1, 2.6)	6.2 (5.2, 8.3)	5.1 (4.5, 6.0)	1.3 (1.1, 1.5)	25.0 (17.5, 29.3)	6.6 (5.7, 8.1)	3.8 (2.3, 4.6)
04-53	7.8 (5.3, 11.0)	7.0 (5.5, 8.4)	1.1 (0.9, 1.8)	8.2 (6.9, 11.7)	8.0 (6.8, 10.4)	1.1 (1.0, 1.2)	20.8 (16.0, 27.8)	9.5 (7.8, 12.0)	2.1 (1.7, 2.8)

Values are means (25% percentile, 75% percentile), outlying values were omitted

relevant morphospecies commonly observed in the field (Fig. 3). Detailed morphological characterization of the 61 *Anabaena* populations was published elsewhere (Zapomělová et al., 2007).

Effects of environmental conditions on the morphologies of the strains

Redundancy analyses were employed to evaluate the effects of varied temperature, light intensity, and concentration of N and P on the morphologies of the studied *Anabaena* strains.

Vegetative cells

Significant effects of experimental conditions on vegetative cell dimensions were demonstrated in seven strains (Table 3). Phosphorus concentration was the main factor, since its effect was significant in six strains. The response of vegetative cell dimensions to varied P concentration was consistent in all six strains and is demonstrated on the strain 04-45 (Fig. 4). The longest vegetative cells were observed in the lowest P concentrations ($WC_{0\times P}$, $WC_{0.001\times P}$) and their width decreased with increasing P. Together with enlarged length of vegetative cells in low P concentrations, their width decreased. Therefore, length:width ratios of vegetative cells were higher in lower P concentrations and the cells were obviously elongated. The vegetative cell elongation of the strain 04-26 resulted in the straightening of its trichomes. In this strain, the combined effect of P concentration and temperature on vegetative cell dimensions was demonstrated. The dimensions of vegetative cells of the strain 04-53 were significantly influenced by the light intensity. Both the width and the length of vegetative cells of this strain increased with the light intensity while their length:width ratio (i.e., the cell shape) remained more or less constant (Fig. 5).

Heterocytes

Significant effects of the experimental conditions on the dimensions of heterocytes were demonstrated in only two strains (Table 3). The significant factors were the temperature for the strain *A. circinalis* 04-26 and the light intensity for the strain *A. flos-aquae* 04-53.

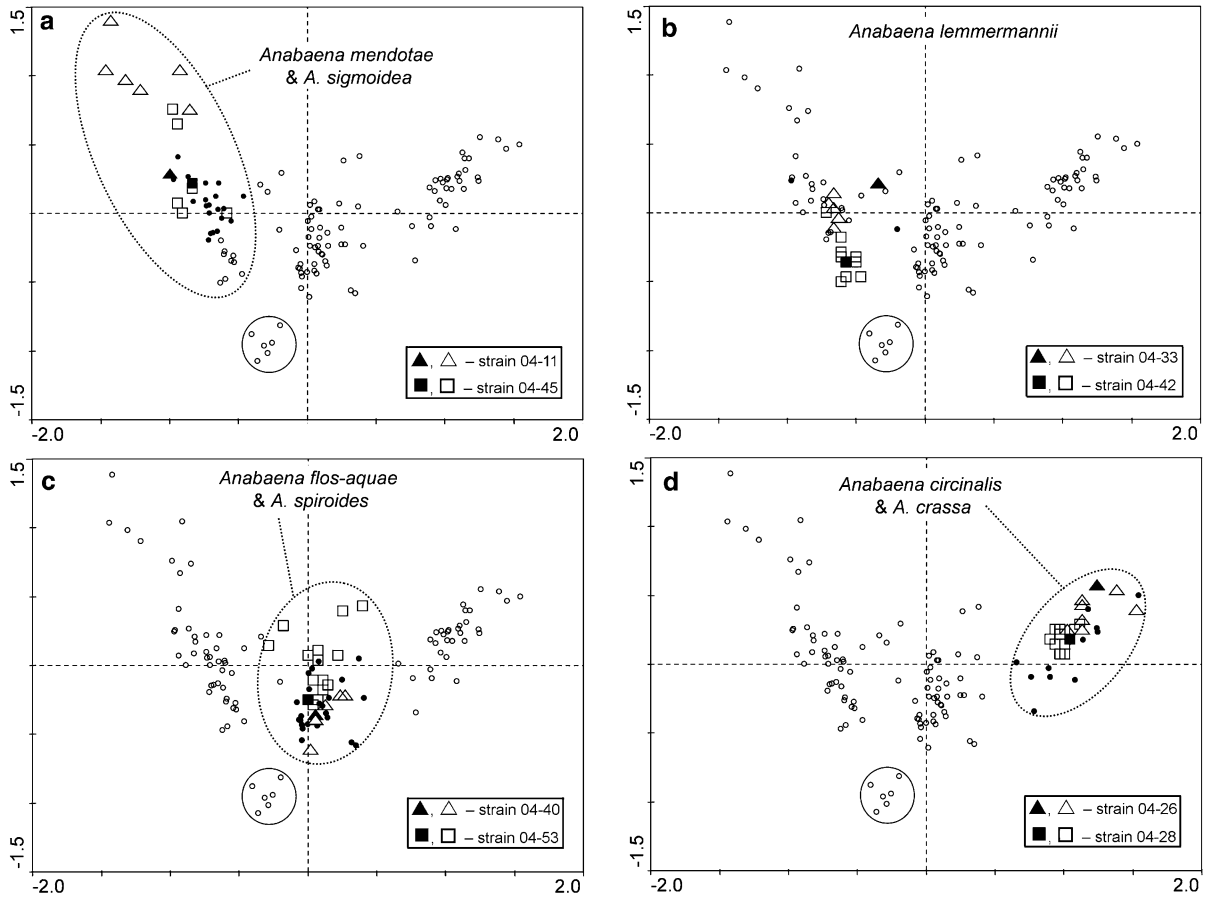


Fig. 3 PCA diagrams based on the morphologies of the eight studied strains and of 61 planktonic *Anabaena* populations under the field conditions. The first and the second canonical axes explain together 72.2% of the total variance. The diagrams **a**–**d** represent identical analysis, in which different parts are highlighted. **a** The complex of *A. mendotae* and *A. sigmoidea* morphospecies. **b** *A. lemmermannii*. **c** The complex of *A. flos-aquae* and *A. spiroides* morphospecies. **d** The complex of *A. circinalis* and *A. crassa* morphospecies. *White circles* represent single field populations or single experimental treatment of the studied strains. *Black circles* represent field populations of the relevant morphospecies or morphospecies complex. *Squares* and *triangles* indicate the studied strains—*black symbols* represent the original field morphologies and *white symbols* the morphological features under various experimental conditions. Six *white circles* at the bottom part of the diagrams, which are indicated by the *cyclic solid line*, illustrate clearly delimited *A. compacta* morphospecies, demonstrated by Zapomělová et al. (2007)

Akinetes

The dimensions of akinetes were the most stable morphometric features. The effect of P concentration was demonstrated only in the strain 04-53 (Table 3). Both the width and the length of akinetes of this strain increased with the P concentration while the length:width ratio (i.e., the akinete shape) remained constant (Fig. 6).

In the strain *A. lemmermannii* 04-33, where the position of akinetes was important for the morphospecies identification, variations in this morphological trait occurred. The akinetes of this strain were usually adjacent to heterocytes and also the original population in the field displayed this akinete position. However, the dominance of akinetes remote from heterocytes was observed in some batch cultures of the strain 04-33, both during the routine cultivation and the experiments, regardless of the environmental conditions. On the contrary, akinetes of the strain *A. lemmermannii* 04-42, as well as its original field population, were steadily adjacent to heterocytes, both during the experiments and the 4-year cultivation.

Table 3 Parameters of redundancy analyses (RDA) and morphological characteristics of the *Anabaena* strains for which significant effects of environmental factors were proved

Morphological parameter	Influencing factor	Strain code	Variability explained by the model (%)/the factor (%)	*F*-value	*P*-value
Vegetative cells	Phosphorus	04-11	49.4/45.7	5.900	0.0400
		04-26	80.1/75.4	21.469	0.0080
		04-28	75.9/70.9	17.073	0.0260
		04-40	55.7/52.7	7.798	0.0180
		04-42	70.5/58.3	9.792	0.0040
		04-45	53.7/42.1	5.093	0.0340
	Temperature	04-26	52.5/44.8	4.871	0.0260
	Light	04-53	61.1/60.8	10.850	0.0020
Heterocytes	Temperature	04-26	85.4/82.8	28.985	0.0020
	Light	04-53	68.1/66.4	13.845	0.0020
Akinetes	Phosphorus	04-53	33.5/32.8	3.422	0.0420

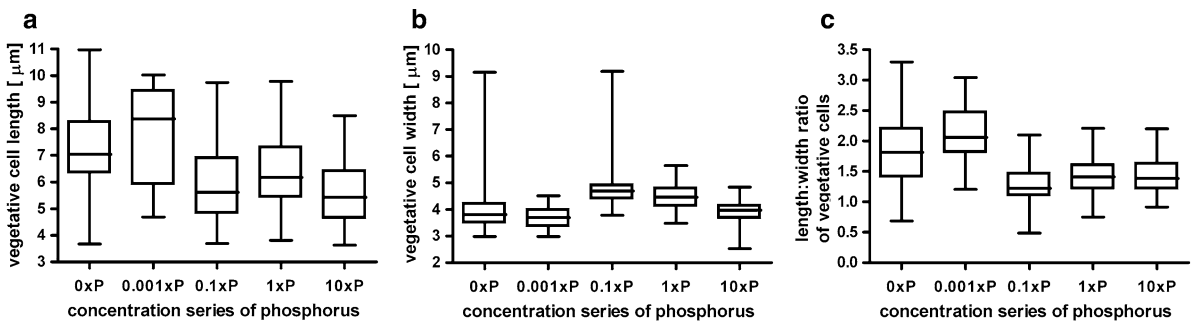


Fig. 4 Vegetative cell dimensions of the *A. mendotae/A. sigmoidea* strain 04-45 under various P concentrations. **a** Vegetative cell length. **b** Vegetative cell width. **c** Length:width ratio of vegetative cells. *Whiskers* represent minimal and maximal values, *boxes* symbolize 25 and 75% percentiles, and *lines inside boxes* show mean values

Analogously, batch cultures in the strain *A. mendotae/A. sigmoidea* 04-45 were observed several times, where only akinetes adjacent to heterocytes formed, although the prevailing akinete position of this strain and of its original field population was remote from heterocytes. Akinetes of the strain *A. mendotae/A. sigmoidea* 04-11 and of its original field population were steadily remote from heterocytes, both during the experiments and the 4-year cultivation.

Trichome coiling

Alternating occurrence of regularly (helixes) and irregularly (clumps) coiled trichomes was observed in the majority of the strains (Fig. 1), except for the strains *A. circinalis/A. crassa* 04-26 and 04-28, where the predominant type of the trichome coiling was regular. Proportional abundance of regularly and irregularly coiled trichomes differed both among the strains and among various experimental treatments of the same strain. Nevertheless, the effect of experimental conditions on the regularity of trichome coiling was not demonstrated in any of the strains studied.

Temperature and light preferences of the strains

The temperature and light growth optima of the *Anabaena* strains studied were rather diverse, ranging from 13.5 to 28°C and from 80 to 360 µmol m^{-2} s^{-1}, respectively. All of the strains were able to grow in the whole range of experimental temperature (10–28°C), but some of them did not survive extreme light

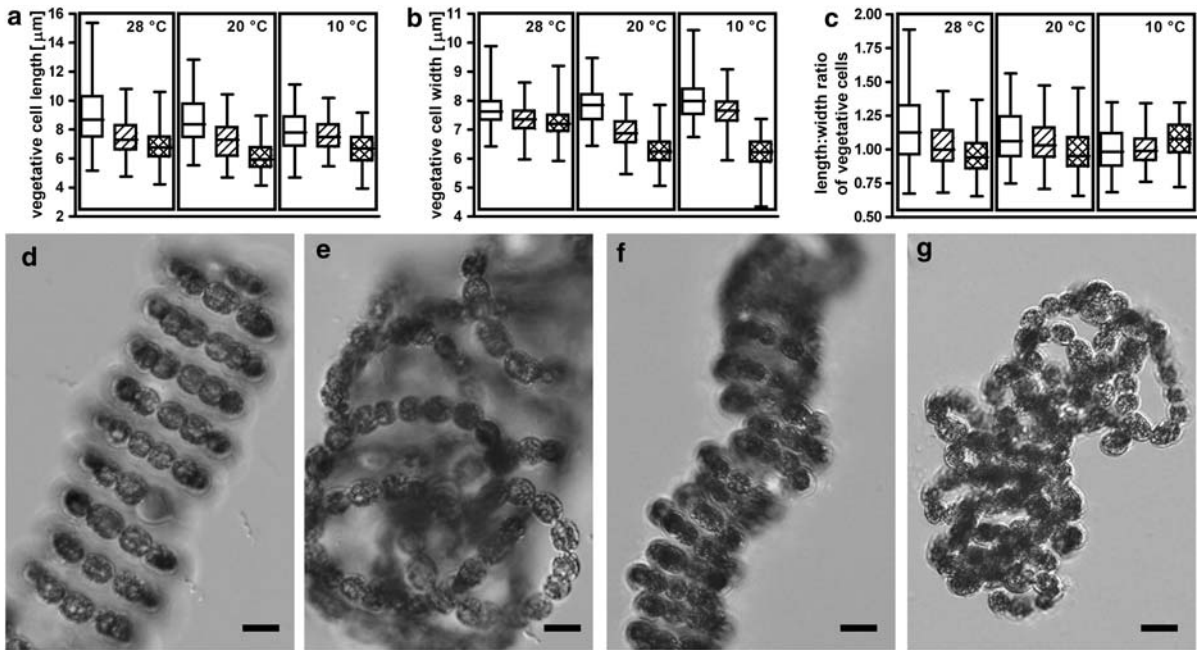


Fig. 5 Vegetative cell dimensions of the *A. flos-aquae* strain 04-53 in various positions of cross gradients of light and temperature. Light intensity is symbolized by *shading* (750 µmol m^{-2} s^{-1}, *plain boxes*; 200 µmol m^{-2} s^{-1}, *simple shading*; 20 µmol m^{-2} s^{-1}, *double shading*). *Whiskers* represent minimal and maximal values, *boxes* symbolize 25 and 75% percentiles, and *lines inside boxes* show mean values. **a** Vegetative cell length. **b** Vegetative cell width. **c** Length:width ratio of vegetative cells. Microphotographs show differences in vegetative cell dimensions of the strain 04-53 grown at 750 µmol m^{-2} s^{-1} (**d–e**) and 20 µmol m^{-2} s^{-1} (**f–g**). *Scale bars* represent 10 µm

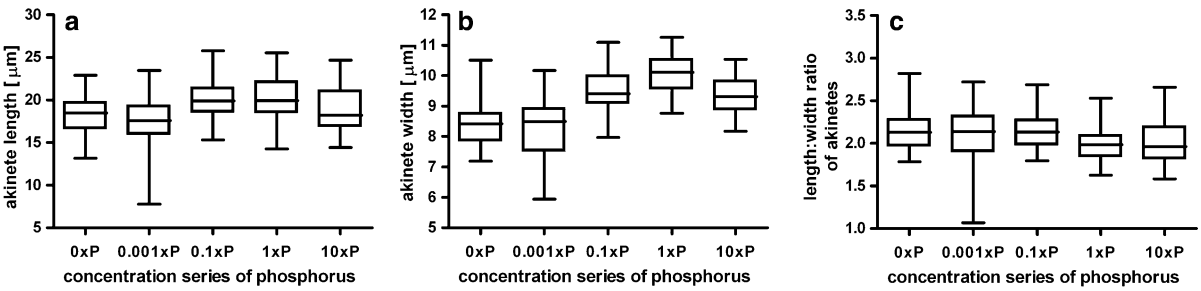


Fig. 6 Akinete dimensions of the *A. flos-aquae* strain 04-53 under various P concentrations. **a** Akinete length. **b** Akinete width. **c** Length:width ratio of akinetes. *Whiskers* represent minimal and maximal values, *boxes* symbolize 25 and 75% percentiles, and *lines inside boxes* show mean values

intensities (20 and 750 µmol m^{-2} s^{-1}). The temperature and light preferences were specific for each strain and were not consistent with the taxonomic affiliation of the strains (Table 4).

Phylogenetic relationships of the strains

The eight studied *Anabaena* strains were placed into three different clusters in the distance tree of partial 16S rRNA gene sequences (Fig. 7). Cluster A contained both *A. circinalis/A. crassa strains* (04-26 and 04-28), showing 100% identity. Besides, *A. flos-aquae* 04-53 was also affiliated to cluster A. The strains *A. medotae/A. sigmoidea* 04-11 and 04-45 and *A. lemmermannii* 04-33 appeared together in cluster B with high bootstrap support. The strains *A. lemmermannii* 04-42 and *A. flos-aquae* 04-40 were situated in cluster C.

Table 4 Temperature and light optima of the studied *Anabaena* strains

Morphospecies affiliation	Strain	Temperature (°C)	Light (μmol m^{-2} s^{-1})
Anabaena mendotae/A. sigmoidea	04-11	18.5–**21.0**–23.5	80–**150**–220
A. mendotae/A. sigmoidea	04-45	16.0–**18.5**–21.0	140–**200**–260
A. lemmermannii	04-33	18.5–**22.0**–25.5	120–**210**–300
A. lemmermannii	04-42	13.5–**16.0**–18.5	190–**230**–270
A. flos-aquae	04-40	19.5–**22.5**–25.5	220–**250**–280
A. flos-aquae	04-53	25.0–**27.0**–29.0	110–**140**–170
A. circinalis/A. crassa	04-26	17.5–**20.0**–22.5	220–**290**–360
A. circinalis/A. crassa	04-28	22.0–**25.0**–28.0	100–**160**–210

The range of the values represents the experimentally determined growth optima; their median values are in *bold*

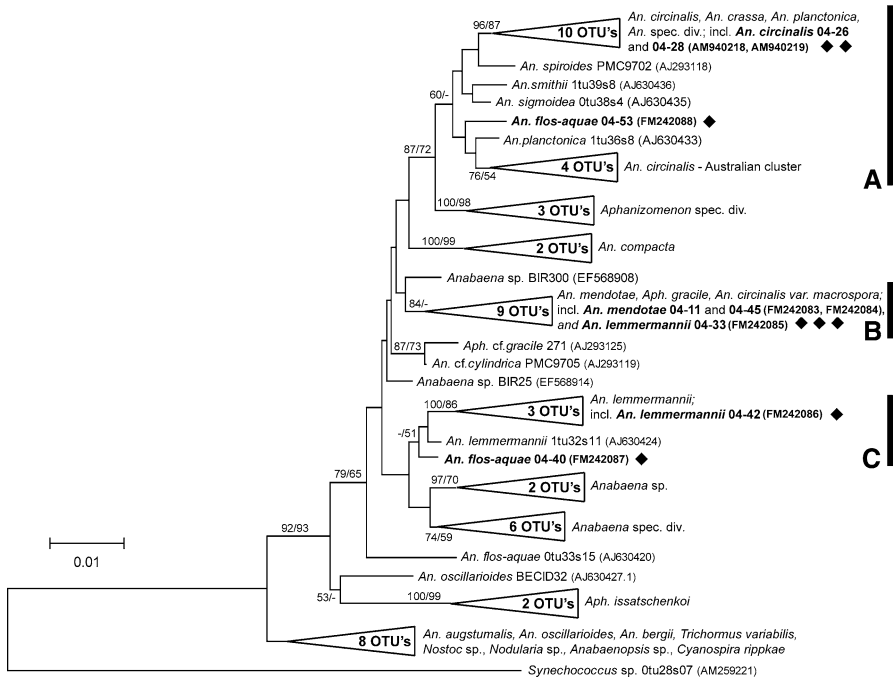


Fig. 7 Neighbor-joining tree based on 16S rRNA gene sequences (1,340 bp) showing the clustering of studied *Anabaena* strains. *Numbers* near the nodes indicate the bootstrap values over 50% for NJ and MP analyses. *An.* *Anabaena*, *Aph.* *Aphanizomenon*, *incl.* including, *OTU* operational taxonomic unit, *spec. div.* different species, *filled diamond* studied *Anabaena* strains

Discussion

The range of morphological variability of single *Anabaena* strains

A comparison was drawn between the morphologies of *Anabaena* isolates exposed to varied experimental conditions and the populations from the field conditions. This is the first direct evidence for the potential of a single cyanobacterial strain to span the total variability of all relevant morphospecies or morphospecies complexes. These findings were consistent in all of the eight strains studied, representing distinctly different *Anabaena* morphospecies, which possibly indicates that they may be generalized throughout the entire group of the planktonic *Anabaena*.

Our present conclusions are in a good agreement with previous suggestions that only larger morphological complexes can be delimited within the group of planktonic *Anabaena* since the traditional morphospecies are not distinctly defined by their morphological characteristics (Zapomělová et al., 2007). The only reported morphospecies that appears to be clearly defined by both the morphological and phylogenetic characteristics was *A. compacta* (Nygaard) Hickel (Rajaniemi et al., 2005a, b). It was

therefore excluded from this study, since no transitions to other morphospecies were expected.

Despite this, there may be a reason for keeping the narrow traditional morphospecies and their names as ecomorphs. More studies are to be conducted to clarify the relationships between single morphological features and environmental factors. The ecomorphs could be then used for estimation of environmental conditions in the field.

Taxonomic implications

Significant relationships between environmental factors and taxonomically important morphological features were demonstrated and described for the first time within the genus *Anabaena*. Only a few studies focusing on these problems within the genus *Anabaena* have been published so far (Stulp, 1982; Stulp & Stam, 1984b, 1985; Zapomělová et al., 2008a); however, none of them detected significant responses in *Anabaena* morphology related to variable growth conditions. On the contrary, it was demonstrated on other genera that some of the features which have been traditionally used in cyanobacterial classification varied under different culture conditions (e.g., dimensions and length:width ratios of vegetative cells and appearance of colonies of chroococcacean cyanobacteria; Chang, 1988; Doers & Parker, 1988; Wilmotte, 1988; Palinska et al., 1996; Saker & Neilan, 2001).

This study has revealed some limitations in the classification of planktonic *Anabaena* and has indicated future directions for research of particular morphological groups within this genus.

Anabaena mendotae Trelease 1889 vs. A. sigmoidea Nygaard 1949

Modifications of vegetative cell morphology under various P concentrations are important mainly in connection with the morphospecies complex *A. sigmoidea* & *A. mendotae*. Vegetative cell length, the length: width ratio, and their general shape are traditionally regarded as distinguishing between these two taxa, together with the dimensions of the akinetes (Komárek, 1996; Komárek & Zapomělová, 2007). Morphometric parameters observed in the strains 04-11 and 04-45 during the experiments spanned the ranges of both *A. mendotae* and *A. sigmoidea* (Table 2), which was supported also by the genetic data. Both these strains displayed very high 16S rRNA gene sequence similarity and appeared together in one cluster, although the field morphology of the strain 04-11 corresponded more with the description of *A. mendotae*, and the strain 04-45 was identified as *A. sigmoidea* (Fig. 1, a vs. d). Investigation of higher number of *A. mendotae* and *A. sigmoidea* strains would be required to clarify the status of these morphospecies.

Anabaena circinalis Rabenhorst ex Bornet et Flahault 1888 vs. A. crassa (Lemmermann) Komárková-Legnerová et Cronberg 1992

Similarly, our experiments demonstrated that the studied *A. circinalis* strains (04-26, 04-28) were able to span the variability of both *A. circinalis* and *A. crassa*, as they were originally described. Trichome widths and coil diameters are considered the main distinguishing criteria for these species (Komárek, 1958; Komárková-Legnerová & Cronberg, 1992; Komárková-Legnerová & Eloranta, 1992). The two strains studied displayed a wide range of features, corresponding with the description of both *A. circinalis* and *A. crassa* (Table 2; in detail described in Zapomělová et al., 2008b). Continuous variability of trichome widths and coil diameters was reported also from 13 field populations of *A. circinalis* and *A. crassa* studied in the Czech Republic (Zapomělová et al., 2007). Broader morphological and molecular revision of this morphospecies complex would be necessary to elucidate the taxonomic validity of the taxa *A. circinalis* and *A. crassa*.

Anabaena flos-aquae complex

The two studied *A. flos-aquae* strains (04-40, 04-53) exhibited marked differences in 16S rRNA gene sequences. They were located in distinctly separate subclusters in the phylogenetic tree based on the 16S rRNA gene. From a morphological point of view, these strains differed mainly in the shape and size of the akinetes and in the trichome coiling morphology (Table 2; Fig. 1, m–o vs. p–r). Ambiguities in the definition of the morphospecies *A. flos-aquae* were previously pointed out by Zapomělová et al. (2007). The concepts of *A. flos-aquae* (Lyngbye) Brébisson ex Bornet et Flahault 1888, *A. spiroides* Klebahn

1895, *A. perturbata* Hill 1976, and similar morphospecies differ among various authors and the shifts in these concepts can also be observed during the history of the cyanobacterial taxonomy. The definition of *A. flos-aquae* is rather vague, encompassing wide range of morphotypes with rounded vegetative cells, approximately 4–8 μm in diameter, and with kidney shaped or cylindrical akinetes (Komárek & Zapomělová, 2007), i.e., also *A. perturbata* and *A. spiroides*. Morphological heterogeneity within *A. flos-aquae* complex is evident also from the herein presented PCA (Fig. 3). Furthermore, obvious differences among various *A. flos-aquae* strains were previously shown by Rajaniemi et al. (2005a) at the genetic level (16S rRNA gene, *rpoB* and *rbcLX* sequences). Both the morphological and molecular analyses of more strains from this species complex are highly required to revise the status of the *A. flos-aquae* taxon.

Anabaena lemmermannii Richter in Lemmermann 1903 vs. A. mendotae & A. sigmoidea

Very important finding of this study is the relation between the akinete position stability and the phylogenetic affiliation of the *A. lemmermannii*-like strains 04-33 and 04-42. Based on the 16S rRNA gene sequences, the strain 04-33 with variable akinete position clustered together with the strains from *A. mendotae* & *A. sigmoidea* group. From those strains, occasional dominance of akinetes adjacent to the heterocytes was observed in the strain 04-45. On the other hand, the strain 04-42, with the akinetes steadily adjacent to the heterocytes, grouped together with some other *A. lemmermannii* strains from Genbank into a separate cluster. The validity of *A. lemmermannii* was challenged by Zapomělová et al. (2007), who pointed out the enormous morphological heterogeneity within this taxon. The only identification feature of this morphospecies considered is the position of akinetes adjacent to heterocytes, while the shape and dimensions of the vegetative cells are highly variable (Komárková, 1988; Zapomělová et al., 2007). Nevertheless, the genetic cluster comprising the strain 04-42 appears to be clearly separate and corresponds exactly to the main *A. lemmermannii* cluster of Gugger et al. (2002) and Rajaniemi et al. (2005a, b). *A. mendotae* and *A. sigmoidea* strains constitute a distinct, highly consistent cluster together with the strain 04-33. This cluster agrees exactly with the *A.* cf. *lemmermannii* strain PH262 of Gugger et al. (2002), which exhibited outlying position in the phylogenetic tree and probably represented the *A. lemmermannii*-like strain with unstable akinete position. For the present, *A. lemmermannii* appears to be a valid taxon, which should be, however, verified by thorough analyses of more *A. lemmermannii* strains. Moreover, some other phenotypic features will then have to be found allowing its identification, since the determination of the stability of akinete position would be extremely problematic in natural populations.

Experimental conditions and the ecological context

Phosphorus

Phosphorus concentration was the main factor influencing the morphology of vegetative cells, which can be easily explained by reduced division rate and consequential cell elongation under the P limitation. Significant differences in the trichome width were previously reported also from P-limited and non-limited populations of *Cylindrospermopsis raciborskii* (Komárková et al., 1999; Shafik et al., 2003). Extreme modification of morphology under the P-limiting conditions was observed in the strain 04-26, where the elongation of vegetative cells resulted in the trichome straightening (Zapomělová et al., 2008b).

Light intensity

Anabena flos-aquae 04-53 was the only strain that showed significant response of the vegetative cell dimensions toward varied light intensity. The majority of the strains studied did not exhibit any response to the light, which corresponded well with the conclusions of Stulp & Stam (1985). Variations in cell sizes and shapes induced by changes in light intensity were reported from different cyanobacterial strains (Wyman & Fay, 1997), but their trends were not consistent, indicating that the response may be strain specific. Influence of light on trichome coiling was reported from the oscillatoriacean cyanobacterium *Arthrospira fusiformis* (Bai & Seshadri, 1980) but has never been demonstrated in Nostocales,

although cases of trichome straightening are known from *Anabaena* cultures (Booker & Walsby, 1979; Hickel, 1982; Zapomělová, 2004; Zapomělová et al., 2008a).

Temperature

A significant effect of temperature was found in the strain 04-26, whose vegetative cells and heterocytes were bigger (both lengths and widths) at higher temperature values. A potential explanation of these cells' enlargement may be the accumulation of metabolic products resulting from enhanced metabolic rates (Robarts & Zohary, 1987).

Nitrogen

Insignificant effect of low N concentrations can be easily explained by the N_2 fixing ability of *Anabaena* (heterocytes). However, our study also demonstrated that the high N concentrations achievable in the field had no effect on *Anabaena* morphology. This is in disagreement with the results of Saker & Neilan (2001), who found significant morphological variability (dimensions of vegetative cells and heterocytes) in heterocytous cyanobacterium *Cylindrospermopsis raciborskii* exposed to different sources and concentrations of N.

The growth demands

These experiments suggest that *Anabaena* strains of even the same morphospecies are able to prosper in a wide range of temperature and light conditions. The temperature and light preferences were specific for each strain and did not reflect the taxonomic affiliations of the *Anabaena* strains studied. Our results are supported by similar conclusions of Rapala & Sivonen (1998), who focused on strain-specific differences in growth rates of various *Anabaena* strains as a function of temperature and light. Thus, interstrain variability may be expected in temperature and light preferences. On the contrary, Stulp & Stam (1985) demonstrated similar growth response of various representatives of one and the same group of *Anabaena* morphospecies to temperature and light.

Acknowledgments We would like to thank Marie Kupková, Martina Vožechová, and Jindra Bučková from the Biology Centre of AS CR, Institute of Hydrobiology for the valuable technical assistance. This study was largely supported by the GA ASCR (Project No. KJB600960703) and by Grant Agency of the Czech Republic (No. 206/06/0462). Partial financial assistance for this study was provided by Project FRVŠ No. 3492/2005 and by GA ASCR (Project Nos. IAA 600050704 and AV0Z60170517). This work was presented as a contributed paper at the Bat Sheva de Rothschild seminar on Phytoplankton in the Physical Environment—The 15th Workshop of the International Association of Phytoplankton Taxonomy and Ecology, IAP (Israel).

References

Anand, N., 1988. Culture studies and taxonomy of blue-green algae—certain identification problems. Archiv für Hydrobiologie Supplement 80: 141–147.

Anonymous, 1996. Statistica for Windows [Computer Program Manual]. Statsoft, Tulsa, OK.

Bai, N. J. & C. V. Seshadri, 1980. On coiling and uncoiling of trichomes in the genus *Spirulina*. Archiv für Hydrobiologie Supplement 60: 32–47.

Booker, M. J. & A. E. Walsby, 1979. The relative form resistance of straight and helical blue-green algal filaments. British Phycological Journal 14: 141–150.

Chang, T. P., 1988. Morphological remarks on *Pseudanabaena mucicola* (Huber-Pestalozzi et Naumann) (Bourrelly) Chang. Algological Studies 50–53: 59–70.

Cronberg, G. & J. Komárková, 1988. *Anabaena farciminiformis*, a new nostocacean blue-green alga from Scania, South Sweden. Archiv für Hydrobiologie Supplement 80: 277–282.

Doers, M. P. & D. L. Parker, 1988. Properties of *Microcystis aeruginosa* and *M. flos-aquae* (cyanobacteria) in culture: taxonomic implications. Journal of Phycology 24: 502–508.

Felsenstein, J., 2004. PHYLIP (Phylogeny Inference Package) Version 3.6—Computer Program, distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Gugger, M., C. Lyra, P. Henriksen, A. Couté, J.-F. Humbert & K. Sivonen, 2002. Phylogenetic comparison of the cyanobacterial genera *Anabaena* and *Aphanizomenon*. International Journal of Systematic and Evolutionary Microbiology 52: 1–14.

Guillard, R. R. & C. J. Lorenzen, 1972. Yellow-green algae with chlorophyllide c. Journal of Phycology 8: 10–14.

Hickel, B., 1982. A helical, bloom forming *Anabaena*-like blue-green alga (Cyanophyta) from hypertrophic lakes. Archiv für Hydrobiologie 95: 115–124.

Hill, H., 1976a. A new species of *Anabaena* (Cyanophyta, Nostocaceae) from a Minnesota lake II. Phycologia 15: 65–68.

Hill, H., 1976b. A new species of *Anabaena* (Cyanophyta, Nostocaceae) from a Minnesota lake III. Phycologia 15: 69–71.

Hill, H., 1976c. A new species of *Anabaena* (Cyanophyta, Nostocaceae) from a Minnesota lake I. Phycologia 15: 61–64.

Hindák, F., 2000. Morphological variation of four planktic nostocalean cyanophytes – members of the genus *Aphanizomenon* or *Anabaena*? Hydrobiologia 438: 107–116.

Komárek, J., 1958. Die taxonomische Revision der planktischen Blaualgen der Tschechoslowakei. In Komárek, J. & H. Ettl (eds), Algologische Studien. Verlag der Tschechoslowakischen Akademie der Wissenschaften, Prag: 10–206.

Komárek, J., 1996. Klíč k určování vodních květů sinic v České republice [A key for determination of water-bloom-forming cyanobacteria in the Czech Republic]. In Maršálek, B., V. Keršner & P. Marvan (eds), Vodní květy sinic [Cyanobacterial Water Blooms]. Nadatio flos-aquae, Brno: 22–85 (in Czech).

Komárek, J. & K. Anagnostidis, 1989. Modern approach to the classification system of Cyanophytes, 4 – Nostocales. Archiv für Hydrobiologie Supplement 82: 247–345.

Komárek, J. & E. Zapomělová, 2007. Planktic morphospecies of the cyanobacterial genus *Anabaena* = subg. *Dolichospermum* – 1. Part: coiled types. Fottea 7: 1–31.

Komárková, J., 1988. Morphological variation in natural populations of *Anabaena lemmermannii* in respect to existence of *Anabaena utermoehlii*. Archiv für Hydrobiologie Supplement 80: 93–108.

Komárková, J., R. Laudares-Silva & P. A. C. Senna, 1999. Extreme morphology of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) in the Lagoa do Petri, a freshwater coastal lagoon, Santa Catarina, Brazil. Algological Studies 94: 207–222.

Komárková-Legnerová, J. & G. Cronberg, 1992. New and recombined filamentous Cyanophytes from lakes in South Scania, Sweden. Algological Studies 67: 21–31.

Komárková-Legnerová, J. & P. Eloranta, 1992. Planktic blue-green algae (Cyanophyta) from Central Finland (Jyväskylä region) with special reference to the genus *Anabaena*. Algological Studies 67: 103–133.

Kvíderová, J. & J. Lukavský, 2001. A new unit for crossed gradients of temperature and light. Nova Hedwigia (Beiheft: Algae and Extreme Environments) 123: 541–550.

Li, R., M. Watanabe & M. M. Watanabe, 2000. Taxonomic studies of planktic species of *Anabaena* based on morphological characteristics in cultured strains. Hydrobiologia 438: 117–138.

Lorenzen, C. J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equation. Limnology and Oceanography 12: 343–346.

Lyra, C., S. Suomalainen, M. Gugger, C. Vezie, P. Sundman, L. Paulin & K. Sivonen, 2001. Molecular characterization of planktic cyanobacteria of *Anabaena*, *Aphanizomenon*, *Microcystis* and *Planktothrix* genera. International Journal of Systematic and Evolutionary Microbiology 51: 513–526.

Nübel, U., F. Garcia-Pichel & G. Muyzer, 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied and Environmental Microbiology 63: 3327–3332.

Padisák, J. & A. Kovács, 1997. *Anabaena compacta* (Nygaard) Hickel – Új kékalga faj a Balaton üledékélben és plankton jában. [*Anabaena compacta* (Nygaard) Hickel – a new blue-green algal spedies in the sediments and plankton of lake Balaton.] (in Hungarian with English summary). Hidrológiai Közlöny 77: 29–32.

Palinska, K., W. Liesack, E. Rhiel & W. E. Krumbein, 1996. Phenotype variability of identical genotypes: the need for a combined approach in cyanobacterial taxonomy demonstrated on *Merismopedia*-like isolates. Archives of Microbiology 166: 224–233.

Rajaniemi, P., P. Hrouzek, K. Kaštovská, R. Willame, A. Rantala, L. Hoffmann, J. Komárek & K. Sivonen, 2005a. Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). International Journal of Systematic and Evolutionary Microbiology 55: 11–26.

Rajaniemi, P., J. Komárek, R. Willame, P. Hrouzek, K. Kaštovská, L. Hoffmann & K. Sivonen, 2005b. Taxonomic consequences from the combined molecular and phenotype evaluation of selected *Anabaena* and *Aphanizomenon* strains. Algological Studies 117: 371–391.

Rapala, J. & K. Sivonen, 1998. Assessment of environmental conditions that favor hepatotoxic and neurotoxic *Anabaena* spp. strains cultured under light limitation at different temperatures. Microbial Ecology 36: 181–192.

Robarts, R. D. & T. Zohary, 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. New Zealand Journal of Freshwater Research 21: 391–399.

Saitou, N. & M. Nei, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Saker, M. L. & B. A. Neilan, 2001. Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from Northern Australia. Applied and Environmental Microbiology 67: 1839–1845.

Shafik, H. M., L. Vörös, P. Sprőber, M. Présing & A. W. Kovács, 2003. Some special morphological features of *Cylindrospermopsis raciborskii* in batch and continuous cultures. Hydrobiologia 506–509: 163–167.

Šmilauer, P., 1992. CANODRAW Users Guide v. 3.0. Microcomputer Power, Ithaca, NY.

Stulp, B. K., 1982. Morphological variability of *Anabaena* strains (Cyanophyceae) under different culture conditions. Archiv für Hydrobiologie Supplement 63(Algological Studies 30): 165–176.

Stulp, B. K. & W. T. Stam, 1982. General morphology and akinete germination of a number of *Anabaena* strains (Cyanophyceae). Archiv für Hydrobiologie Supplement 63(Algological Studies 30): 35–52.

Stulp, B. K. & W. T. Stam, 1984a. Genotypic relationships between strains of *Anabaena* (Cyanophyceae) and their correlation with morphological affinities. British Phycological Journal 19: 287–301.

Stulp, B. K. & W. T. Stam, 1984b. Growth and morphology of *Anabaena* strains (Cyanophyceae, Cyanobacteria) in cultures under different salinities. British Phycological Journal 19: 281–286.

Stulp B. K. & W. T. Stam, 1985. Taxonomy of the genus *Anabaena* (Cyanophyceae) based on morphological and genotypic criteria. Archiv für Hydrobiologie Supplement 71 (Algological Studies, 38/39): 257–268.

Taton, A., S. Grubisic, E. Brambilla, R. De Wit & A. Wilmotte, 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo dry valleys,

Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology 69: 5157–5169.

Ter Braak, C. J. F. & P. Šmilauer, 1998. CANOCO Reference Manual. Microcomputer Power, Ithaca, NY.

Willame, R., C. Boutte, S. Grubisic, A. Wilmotte, J. Komárek & L. Hoffmann, 2006. Morphological and molecular characterization of planktonic cyanobacteria from Belgium and Luxembourg. Journal of Phycology 42: 1312–1332.

Wilmotte, A., 1988. Growth and morphological variability of six strains of *Phormidium* cf. *ectocarpi* Gomont (Cyanophyceae) cultivated under different temperatures and light intensities. Algological Studies 50–53: 35–46.

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

Wilmotte, A., J. M. Neefs & R. De Wachter, 1994. Evolutionary affiliation of the marine nitrogen-fixing cyanobacterium *Trichodesmium* sp. strain NIBB 1067, derived by 16S ribosomal RNA sequence analysis. Microbiology 140: 2159–2164.

Wyman, M. & P. Fay, 1997. The Cyanobacteria. In Fay, P. & C. Van Baalen (eds), Acclimation to the natural light climate. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam: 347–376.

Zapomělová, E., 2004. Morfologická variabilita a růst vybraných kmenů sinic rodu *Anabaena* a *Aphanizomenon* v závislosti na podmínkách prostředí [Morphological variability and growth of chosen cyanobacterial strains of genera *Anabaena* and *Aphanizomenon* in the dependence on environmental conditions]. MSc thesis, University of South Bohemia, Czech Republic (in Czech).

Zapomělová, E., K. Řeháková, P. Znachor & J. Komárková, 2007. Morphological diversity of coiled planktonic types of the genus *Anabaena* (cyanobacteria) in natural populations – taxonomic consequences. Cryptogamie/Algologie 28: 353–371.

Zapomělová, E., P. Hrouzek, K. Řeháková, M. Šabacká, M. Stibal, L. Caisová, J. Komárková & A. Lukešová, 2008a. Morphological variability in selected heterocystous cyanobacterial strains as a response to varied temperature, light intensity and medium composition. Folia Microbiologica 53: 333–341.

Zapomělová, E., D. Hisem, K. Řeháková, P. Hrouzek, J. Jezberová, J. Komárková, J. Korelusová & P. Znachor, 2008b. Experimental comparison of phenotypical plasticity and growth demands of two strains from the *Anabaena circinalis/A. crassa* complex (cyanobacteria). Journal of Plankton Research 30: 1257–1269.

Znachor, P., T. Jurczak, J. Komárková, J. Jezberová, J. Mankiewicz, K. Kaštovská & E. Zapomělová, 2006. Summer changes of cyanobacterial bloom composition and microcystin concentration in eutrophic Czech reservoirs. Environmental Toxicology 21: 236–243.