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Morphological diversity of coiled planktonic types of the genus *Anabaena* (cyanobacteria) in natural populations – taxonomic consequences

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Abstract – The morphology of 61 planktonic populations of the genus *Anabaena* with coiled trichomes was studied under natural conditions. Samples were collected from Czech water bodies and represent all morphospecies of coiled *Anabaena* that have previously been reported from the Czech Republic. Using the Principal Component Analysis (PCA) and paired t-tests, the existence of clear morphological boundaries of these morphospecies was tested. The only clearly delimited morphospecies found was *A. compacta,* which is defined by the width of vegetative cells, the shape of akinetes and the regularity of coiling. The other morphospecies formed a morphological continuum and no clear-cut boundaries could be observed. Defined groups of morphotypes were thus proposed for practical usage, specifying also the morphological criteria (*A. mendotae* & *A. sigmoidea, A. flos-aquae* & *A. spiroides,* and *A. circinalis* & *A. crassa*). Identification of the studied *Anabaena* morphotypes at a lower taxonomic level is not feasible based only on morphology. Moreover, *A. lemmermannii* taxon appears to be morphologically heterogeneous and requires a thorough taxonomic revision.

***Anabaena* / cyanobacteria / morphological diversity / natural populations / species identification / taxonomy**

Résumé – **Diversité morphologique de formes planctoniques spiralées du genre *Anabaena* (cyanobactéries) en populations naturelles – implications taxinomiques.** La morphologie de 61 populations planctoniques d'*Anabaena* à trichomes spiralés a été étudiée en conditions naturelles. Les échantillons ont été récoltés dans diverses pièces d'eau en République Tchèque. Ils représentent toutes les espèces morphologiques d'*Anabaena* spiralés qui ont été signalées antérieurement de ce pays. L'existence de limites morphologiques nettes a été testée à l'aide d'analyses en composantes principales (PCA) et de test-t pairés. La seule espèce morphologique clairement délimitée est *A. compacta*, définie par la largeur des cellules végétatives, la forme des akinètes et la régularité des spires. Les autres espèces forment un continuum morphologique et aucune limite nette n'a pu être observée. Dans la pratique, des groupes définis de morphotypes sont proposés, en spécifiant aussi leurs critères morphologiques (*A. mendotae* & *A. sigmoidea, A. flos-aquae* & *A. spiroides,*

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A. circinalis & *A. crassa*). La détermination des morphotypes étudiés à un niveau taxinomique inférieur n'est pas possible en se basant uniquement sur la morphologie. De plus, le taxon *A. lemmermannii* paraît morphologiquement hétérogène et nécessiterait probablement une révision taxinomique approfondie.

***Anabaena* / cyanobactéries / diversité morphologique / populations naturelles / déterminations spécifiques / taxinomie**

INTRODUCTION

The genus *Anabaena* is widely accepted to form many morphotypes. Around 80 planktonic freshwater species have been described in the past (Komárek, 1996) belonging to the subgenus *Dolichospermum* Thw. ex Wittr. *et* Nordst. 1889, which comprises all water-bloom-forming morphotypes.

Recently, several molecular studies have been undertaken attempting to clarify the systematics of the genus *Anabaena.* Based on comparisons of the 16S rRNA gene, ITS1 and rbcLX region, the genera *Anabaena* and *Aphanizomenon* appeared to be intermixed (Lyra *et al*., 2001; Gugger *et al*., 2002). This finding was also supported by the results of Rajaniemi *et al*. (2005a, b), who stated that distinct separated clusters at the subgeneric level were not detectable using sequences of 16S rRNA gene, rpoB and rbcLX. These results have been recently confirmed by Willame *et al*. (2006). Thus, as yet no suitable part of the genome has been found that would allow classification of *Anabaena* at the species level.

Current studies, including those mentioned above, deal mainly with the morphology of cultured strains. It is well known, however, that long-term cultivation of cyanobacteria can cause significant morphological changes that do not reflect the situation in natural habitats (Anand, 1988). Thus, confusions and misidentifications can be encountered when cyanobacteria are identified according to the morphology observed in cultivated specimens (Komárek & Anagnostidis, 1989). This confusion is compounded by the questionable status of many species that were established in the past on the basis of their morphology in natural conditions. Many of these are in need of a revision (Komárek, 1996).

Studies on the natural morphology of *Anabaena* that have been published thus far deal only with single populations collected during one field observation (Hill, 1976a, b, c; Hickel, 1982, 1985; Cronberg & Komárková, 1988; Komárková, 1988; Komárková-Legnerová & Cronberg, 1992; Komárková-Legnerová & Eloranta, 1992; Hindák, 2000). No attempt was made to analyse the morphology across the whole spectrum of *Anabaena* populations (morphotypes) in natural conditions and to evaluate the significance of morphological features for species identification. The only exception is a comprehensive study carried out by Li *et al*. (2000) on 50 cultured strains of *Anabaena*, where an identification key to the planktonic species of *Anabaena* was proposed offering morphological features important for morphospecies identification (aggregation of trichomes, character of trichome coiling, position, size and shape of akinetes, size and shape of vegetative cells). In addition, Rajaniemi *et al*. (2005b) attempted to evaluate and discuss the taxonomic significance of selected morphological characteristics. Nevertheless, conclusions derived from these studies are based only on the morphology observed in culture conditions.

The present study aims to complete the missing information on the morphology of planktonic *Anabaena* in natural conditions. The selection was restricted to coiled morphotypes, of which 61 populations were collected from Czech water bodies and their morphology investigated in detail. All coiled morphotypes known from the Czech Republic were included in the selection. Morphological features were compared within and among the populations in order to verify the existence of distinct, morphologically clearly distinguishable morphotypes, and to reveal the populations with intermediate morphology. We attempted to evaluate the validity of the morphological features that are commonly used for identification of *Anabaena* species.

Application of traditional species names can be rather confusing since their concepts were different when interpreted by various authors. Thus to prevent misunderstanding, we summarized the morphotypes and their morphological characteristics together with relevant references in Table 1. We took these morphotype concepts into consideration for the interpretation and discussion of our results. For each morphotype, the original or the oldest available description is given. To provide the information on current concepts and use, morphological descriptions of all morphotypes referred by Komárek (1996) are included (in bold).

MATERIALS AND METHODS

Sampling and morphological parameters

Samples of 61 populations of the genus *Anabaena* with coiled trichomes were collected from Czech fishponds and reservoirs (Table 2) in the years 2003-2006 (May-October, majority of samples in 2004). The entire set of cyanobacterial filaments of the same morphology observed at the same locality and time is considered a population in this study.

Microphotographs of at least 30 non-fixed trichomes per population 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 as possible heterocysts and akinetes in each population). The position of akinetes relative to heterocysts was determined. In addition, coil diameters and distances between coils were measured in regularly coiled trichomes. All size measurements were performed using image analysis (Olympus DP Soft). Length to width ratios of vegetative cells, heterocysts and akinetes were calculated to estimate the cell shape.

In many populations, the number of akinetes was insufficient for statistical analysis, in some cases they were not present at all. Therefore, the dimensions of akinetes observed during the first year of cultivation were included. Only mature akinetes were measured (those, which possessed fully developed thickened cell wall – recommended by Komárek, 1996). Other morphological features were obtained solely from the natural populations. Those populations that did not show akinete formation in the field or were not successfully isolated into culture were excluded from the study.

Table 1. Survey of *Anabaena* species and their morphological characteristics based on literary sources (minimum – mean value – maximum; outlying values in parenthesis). Abbreviation and symbols: *, *A. lemmermannii* P.Richt.; **, *A. lemmermannii* var. *lemmermannii* P. Richt.; ***, *A. lemmermannii* var. *minor* (Uterm.) Kom.-Legn.; A, akinete; ●, heterocyst; ■■■, vegetative cells; I, irregular; R, regular. In bold – morphological criteria summarized by Komárek as the reflection of the most recent morphospecies concepts. References are indicated as: [1] Hickel, 1982; [2] Komárek, 1999; [3] Geitler, 1932; [4] Komárek, 1996; [5] Nygaard, 1949; [6] Komárková, 1988; [7] Hill, 1976a; [8] Hill, 1976b; [9] Komárková-Legnerová & Cronberg, 1992. *(continued)*

	Vegetative cells		*Heterocysts*		*Akinetes*			*Trichome coiling*			*Reference*
	length [μm]	*width [μm]*	*length [μm]*	*width [μm]*	*length [μm]*	*width [μm]*	*position*	*diameter [μm]*	*distance [μm]*	*regularity*	
A. compacta (Nygaard) Hickel	3.0 - 4.6 - 6.0	3.0 - 4.3 - 5.0	5.0 - 5.4 - 7.5	5.0 - 5.4 - 7.0	8.5 - 10.3 - 12.5	6.5 - 8.3 - 9.0	A■■■●	9.0 - 15.0	5.5 - 11.0	R	[1]
	4.0 - 5.0	**4.0 - 5.0**	**5.5 - 6.0**	**5.5 - 6.0**	**11.0 - 12.5**	**8.0 - 10.5**	**A■■■●**			**R**	**[2]**
A. mendotae Trelease	2.5 - 12.0	2.5 - 5.6	5.4 - 11.0	4.0 - 7.0	16.0 - 30.0	5.5 - 8.5	A■■■●				[3]
	2.5 - 11.0 (12)	**2.5 - 4.5 (5.6?)**	**5.4 - 11.0**	**(2.8) 4.0 - 7.0**	**16.0 - 30.0 (45?)**	**(4.5) 5.5 - 7.0 (8.5)**	**A■■■●**			**I**	**[4]**
A. sigmoidea Nygaard	4.0 - 8.0	3.0 - 4.0	5.5 - 7.5	4.0 - 5.0	16.0 - 21.5	7.5 - 8.5	A■■■●	20.0 - 37.0		I	[5]
	4.0 - 8.5	**(2.5) 3.0 - 4.0 (5)**	**5.0 - 7.5**	**4.0 - 5.0 (7)**	**(10.8?) 16.0 - 21.5**	**7.0 - 8.5**	**A■■■●**			**I**	**[4]**
A. lemmermannii P. Richt.	(2.9) - 6.5 -(12.2)	(2.9) - 4.5 -(6.9)	(3.5) - 5.8 -(7.9)	(3.5) - 5.2 -(6.6)	(13) - 20 -(30)	(6.7) - 9.1 - (10)	A●A			I	[6]*
	4.0 - 8.3 -12.3	3.1 - 3.8 - 4.7					A●A			I	[6]**
	2.9 - 6.0 - 9.1	2.9 - 4.5 - 9.0					A●A			I	[6]***
	(2.5) 4.0 - 9.0 (12.1)	**2.5 - 6.9 (8?)**	**(4.7) 5.5 - 10.9**	**(4) 5.0 - 6.0 (9?)**	**(13) 15 - 34 (37.7?)**	**(6.7) 8 - 11 (13.3)**	**A●A**			**I**	**[4]**

	Vegetative cells		*Heterocysts*		*Akinetes*			*Trichome coiling*			*Reference*
	length [µm]	*width [µm]*	*length [µm]*	*width [µm]*	*length [µm]*	*width [µm]*	*position*	*diameter [µm]*	*distance [µm]*	*regularity*	
A. spiroides Klebahn	6.5 - 8.0	6.5 - 8.0	7,0	7,0	17.6 - 35.0	10.0 - 14.0	A■■■●	45.0 - 54.0	40.0 - 50.0	R	[3]
	(3.5) 4.0 -8.0	**6.0 - 8.0 (9.0)**	**(5.6) 6.5 - 10.0**	**(5.6) 6.5 - 10.0**	**(15) 17.6 - 20.8**	**(9) 10.0 - 14.0**	**A■■■●**	**20.0 - 60.0**	**(10.0) 20.0 - 60.0**	**R**	**[4]**
A. flos-aquae	6.0 - 8.0	4.0 - 5.5 - 8.0	6.0 - 10.0	6.0 - 9.0	20.0 - 35.0 (- 50.0)	6.0 - 13.0	A■■■●			I	[3]
Bréb. ex Born. et Flah.	**(2.5) 6.0 - 8.3 (9.5)**	**(2.5) 6.0 - 8.3 (9.5)**	**5.0 - 10.0**	**(3.5) 4.0 - 8.7 (10)**	**(12) 15.0 - 35.0 (50?)**	**(5) 7.0 - 14.0 (17)**	**A■■■●**	**9.0 - 20.0 (30.0)**		**R, I**	**[4]**
A. curva Hill	7.0 - 10.0 (16.0)	7.0 - 9.0 (10)	8.0 - 10.0	8.0 - 10.0	26.0 - 47.0	9.5 - 11.0 (12)	A■■●	*or* A●A	*or* A●■■■	I	[7]
A. perturbata Hill	6.0 - 8.0 (13.0)	(6) 7.0 - 8.0 (9)	(6) 7 - 8 (9)	(7) 8.0 - 8.5 (10)	(11) 13 - 18 (21)	(9.5)11.0-12.5(14)	A■■●	*or* A●A 28.0 - 34.0	*or* A●■■■ 12.0 - 16.0	R, I	[8]
	(6) 7 - 9 (10-13?)	**(6) 7 - 9 (10-13?)**	**(6) 7.0 - 8.0 (9)**	**(7) 8.0 - 8.5 (10)**	**(11) 13.0 - 18.0 (23)**	**(9.5)11 - 12.5(14)**	**A■■■●**	**28.0 - 34.0 (53.0)**	**(10) 12.0 - 16 (20)**	**R, I**	**[4]**
A. circinalis Rabenh. ex Born. et Flah.	8.0 - 14.0	8.0 - 14.0	8.0 - 10.0	8.0 - 10.1	20.0 - 28.0 (34)	16.0 - 18.0	A■■■●			I, R	[3]
	(4) 6.7 - 8.5 (11?)	**(6.9?) 8 - 11 (15?)**	**(6.5) 9.0 -13.0**	**(6.5) 9.0 -13.0**	**(12.5) 20 - 30 (42)**	**(9) 12.0 - 21.0**	**A■■■●**	**68.0 - 120.0**	**27.0 - 55.0**	**R, I**	**[4]**
A. crassa	9.0 - 12.5 - 15.0	9.0 - 12.5 - 15.0	10.0 - 17.0	10.0 - 17.0	15.0 - 28.0 - 35.0	13.0 - 18.0 - 22.0	A■■■●	50.0 - 70.0	45.0 - 55.0	R	[9]
(Lemm.) Kom.-Legn. et Cronb.	**(4) 8.0 - 12.5 (14)**	**(8) 11.0 - 15.0**	**7.0 - 15.0 (17)**	**7.0 - 15.0 (17)**	**(15) 27.0 - 42.0 (46?)**	**(13) 20 - 25**	**A■■■●**	**40.0 - 70.0 (99?)**	**30.0 - 50.0 (90?)**	**R**	**[4]**

Table 2. *Anabaena* populations used in this study, their identification codes and sampling localities. F, fishpond; R, reservoir. The populations that were isolated into pure cultures were abbreviated using a code of two plus two digits, separated by a hyphen. The first part of the code symbolizes the sampling year (mostly 2004), the second part is an identification number of the strain in the culture collection. Populations for that no strains were isolated were labelled by the letter P and a code of the sampling locality followed by the date of sampling.

Taxa	*Code*	*Locality*	
A. compacta	04-02	Naděje - Bavorovice	F
	04-07	Březová	R
	04-32	Opatovický	F
	04-41	Svět	F
	04-55	Vajgar	F
	P_Cerni240504	Černiš	F
A. mendotae	04-11	Černiš	F
	P_Orlik220604	Orlík	F
A. sigmoidea	03-01	Stanovice	R
	04-05	Bezdrev	F
	04-06	Březová	R
	04-14	Dehtář	F
	04-27	Koclířov	F
	04-45	Svět	F
	04-54	Velký Tisý	F
	04-61	Domin	F
	04-63	Ženich	F
	P_Rimov240804	Římov	R
	P_Vrano260804	Vranov	R
A. lemmermannii	04-24	Husinec	R
	04-33	Orlík	R
	04-38	Senecký	F
	04-42	Svět	F
A. spiroides	04-51	Svět	F
	P_Homol260504	Homolský	F
	P_Opat-y150904	Opatovický	F
A. flos-aquae	04-01	Naděje - Bavorovice	F
	04-08	Březová	R
	04-09	Břilický	F
	04-10	Byňovský	F
	04-15	Dehtář	F
	04-16	Dehtář	F
	04-19	Hejtman	F
	04-30	Opatovický	F
	04-36	Rožmberk	F
	04-37	Římov	R
	04-40	Skalka	R
	04-50	Svět	F
	04-52	Svět	F
	04-53	Švarcenberk	F

Table 2. *Anabaena* populations used in this study, their identification codes and sampling localities. F, fishpond; R, reservoir. The populations that were isolated into pure cultures were abbreviated using a code of two plus two digits, separated by a hyphen. The first part of the code symbolizes the sampling year (mostly 2004), the second part is an identification number of the strain in the culture collection. Populations for that no strains were isolated were labelled by the letter P and a code of the sampling locality followed by the date of sampling. *(continued)*

Taxa	*Code*	*Locality*	
A. flos-aquae (continued)	04-57	Vajgar	F
	04-60	Valcha	F
	04-62	Žabovřesky	F
	P_Bezdr150904	Bezdrev	F
	P_Dubne180804	Dubnenský	F
	P_Horus150904	Horusický	F
	P_Ratmi150904	Ratmírovický	F
	P_Rozmb150904	Rožmberk	F
A. circinalis +	04-20	Hněvkovice	R
+ *A. crassa*	04-22	Husinec	R
	04-25	Husinec	R
	04-26	Jesenice	R
	04-28	Hodějovický	F
	04-29	Hodějovický	F
	04-34	České údolí	R
	04-46	Svět	F
	04-56	Vajgar	F
	04-58	Vajgar	F
	P_Horak110706	Horák	F
	P_Komor180805	Komorník	F
	P_Skalk230804	Skalka	R

Isolation and culturing

Single trichomes of 43 of the populations studied were isolated using a glass capillary and from these clone strains were grown. For this purpose, we always selected trichomes with morphology typical of the entire populations. The trichomes were transferred repeatedly from a drop of sterile culture medium (WC – Guillard & Lorenzen, 1972) to another one until all other organisms were excluded. The filaments were then inoculated into the wells of microtitrate plates filled with 4 ml of sterile WC medium, one trichome per each well. After one month, if successful growth in the plates was observed, the strains were inoculated into Erlenmeyer's flasks filled with 50 ml of WC medium where they remained during the cultivation. Both the isolates in the microtitrate plates and the strains in Erlenmeyer's flasks were kept at 21°C under 16L:8D light cycle with a photon flux density of 50 $\mu mol.m^{-2}.s^{-1}$ provided by a daylight fluorescent lamps. All strains were clonal (grown from a single filament), free from other cyanobacteria and algae but not axenic.

Statistical analyses

Average, minimum and maximum values of the dimensions of all cell types were computed for each natural population. The existence of clearly delimited *Anabaena* morphotypes was tested using Principal Component Analysis (PCA; Canoco – Ter Braak & Šmilauer, 1998). Besides the parameters mentioned above, information on regularity of coiling and the akinete position relative to the heterocysts was included in the analysis. Diameters of trichome coils and the distances between the coils could not be included since they were not determined for all compared populations. Nevertheless, they were measured in regularly coiled filaments and used for detailed comparison of populations within the *Anabaena circinalis* and *A. crassa* group.

An ordination diagram was created using CanoDraw software (Šmilauer, 1992) to acquire the basic orientation in the data and to reveal distinct groups of similar populations.

Differences between the morphometric parameters of the populations classified as *A. mendotae* and *A. sigmoidea* were tested using two sample t-test (program Statistica; Anonymous, 1996), as well as the differences of the populations of *A. flos-aquae* and *A. spiroides.*

Detailed comparison and discussion of single morphological features was done using box-whisker plots. Box-whisker plots were created by the GraphPad Prism program (GraphPad Software, San Diego California USA, www.graphpad.com).

RESULTS

Six morphotypes / "species" and one intermixed group of two species were preliminarily identified according to currently used species-defining morphological criteria within the group of the populations studied. Their morphological and morphometric parameters are summarized in Table 3. The morphotypes are *A. compacta* (Nygaard) Hickel 1985, *A. flos-aquae* Brébisson ex Bornet *et* Flahault 1888, *A. lemmermannii* P. Richt. 1903, *A. mendotae* Trelease 1889, *A. sigmoidea* Nygaard 1949, and *A. spiroides* Klebahn 1895. The intermixed group comprises morphotypes of *A. circinalis* Rabenh. ex Bornet *et* Flahaut 1888 and *A. crassa* (Lemm.) Kom.-Legn. *et* Cronb. 1992, where no distinguishing characteristics were found.

The existence of morphological boundaries of these morphotypes was tested by the Principal Component Analysis (PCA, Fig. 1).

A. compacta. The only well defined cluster in the PCA diagram (Fig. 1) is formed by the populations of *A. compacta.* The morphotype is clearly identified by the combination of three morphological features: the width of vegetative cells, the shape of akinetes (widely ovoid, length: width ratio 1.1-1.3) and the regularity of coiling (Figs 2-3; Table 3).

Other populations formed a rather continuous group of morphotypes.

A. mendotae* & *A. sigmoidea. Two populations were preliminarily classified as *A.* cf. *mendotae* (04-11, P_Orlik220604) because their morphology corresponded exactly to the taxon description (Table 1).

The null hypothesis (H_0) that the morphological parameters of populations classified as *A. mendotae* do not differ from those of *A. sigmoidea* can

Table 3. Morphological characteristics of the *Anabaena* morphotypes and morphological groups studied (minimum – mean value – maximum).

		A. compacta	*A. mendotae*	*A. sigmoidea*	*A. mend./ sigm. group*	*A. lemmermannii*	*A. spiroides*	*A. flos-aquae*	*A. fl.-aq./ spir. group*	*A. circinalis / crassa* group
Number of populations		6	2	11	13	4	3	22	25	13
Veg. cells	length [µm]	3.5 - 5.0 - 6.0	4.0 - 6.6 - 10.6	3.7 - 6.0 - 9.3	3.8 - 6.1 - 9.5	4.5 - 7.5 - 11.1	3.9 - 6.1 - 8.9	4.3 - 6.4 - 9.1	4.3 - 6.4 - 9.0	5.0 - 7.7 - 11.2
	width [µm]	3.8 - 4.8 - 5.9	2.7 - 3.5 - 4.3	3.1 - 4.2 - 5.3	3.1- 4.1 - 5.1	4.0 - 5.2 - 6.5	5.8 - 7.4 - 9.3	5.3 - 6.6 - 8.0	5.4 - 6.7 - 8.1	8.4 - 10.4 - 12.1
	l:w ratio	0.7 - 1.0 - 1.4	1.1 - 1.9 - 3.2	0.9 - 1.5 - 2.4	0.9 - 1.5 - 2.6	0.9 - 1.5 - 2.4	0.5 - 0.8 - 1.2	0.7 - 1.0 - 1.4	0.7 - 1.0 - 1.3	0.5 - 0.7 - 1.1
Heterocysts	length [µm]	4.7 - 5.6 - 6.5	5.3 - 6.3 - 8.3	5.1 - 6.3 - 7.9	5.1 - 6.3 - 7.9	5.8 - 6.9 - 8.2	5.8 - 7.2 - 8.4	5.9 - 7.2 - 8.6	5.9 - 7.2 - 8.5	9.2 - 10.3 - 11.6
	width [µm]	4.7 - 5.6 - 6.3	4.2 - 5.2 - 5.8	4.4 - 5.5 - 6.5	4.4 - 5.5 - 6.4	5.4 - 6.2 - 7.3	6.6 - 7.8 - 8.8	6.1 - 7.3 - 8.7	6.1 - 7.4 - 8.7	9.4 - 10.6 - 11.7
	l:w ratio	0.9 - 1.0 - 1.1	1.0 - 1.2 - 1.7	0.9 - 1.1 - 1.5	0.9 - 1.2 - 1.6	1.0 - 1.1 - 1.3	0.8 - 0.9 - 1.0	0.8 - 1.0 - 1.2	0.8 - 1.0 - 1.2	0.9 - 1.0 - 1.1
Akinetes	length [µm]	8.2 - 8.9 - 9.8	13.5 - 20.9 - 26.6	18.2 - 22.6 - 27.4	17.5 - 22.3 - 27.3	13.8 - 19.2 - 25.6	16.1 - 18.5 - 22.8	16.1 - 19.6 - 24.0	16.1 - 19.5 - 23.9	20.0 - 23.4 - 28.1
	width [µm]	7.0 - 7.6 - 8.3	5.4 - 6.7 - 7.8	5.7 - 6.8 - 7.8	5.7 - 6.8 - 7.8	6.3 - 7.9 - 9.9	10.7 - 11.7 - 13.2	8.8 - 10.5 - 12.7	9.0 - 10.6 - 12.7	13.5 - 15.3 - 16.5
	l:w ratio	1.1 - 1.2 - 1.3	1.8 - 3.2 - 4.4	2.7 - 3.4 - 4.3	2.5 - 3.3 - 4.3	1.7 - 2.5 - 3.5	1.3 - 1.6 - 2.0	1.5 - 1.9 - 2.4	1.5 - 1.8 - 2.3	1.4 - 1.6 - 1.9
	position	A■■■●	A■■■●	A■■■●	A■■■●	A●A	A■■■●	A■■■●	A■■■●	A■■■●
Trichome coiling	diameter [µm]	6.6 - 10.6 - 15.0					19.4 - 26.0 - 35.7	16.7 - 23.3 - 30.7	17.7 - 24.3 - 32.6	39.4 - 52.0 - 73.3
	distance [µm]	3.5 - 6.8 - 11.5					12.9 - 20.4 33.1	4.8 - 9.5 - 16.2	7.8 - 13.6 - 22.5	27.6 - 39.8 - 58.1
	diam:dist ratio	1.6					1.3	2.5	2.1	1.3
	regularity	regular	irregular	irregular	irregular	irregular	regular	5 regular 17 irregular	8 regular 17 irregular	regular

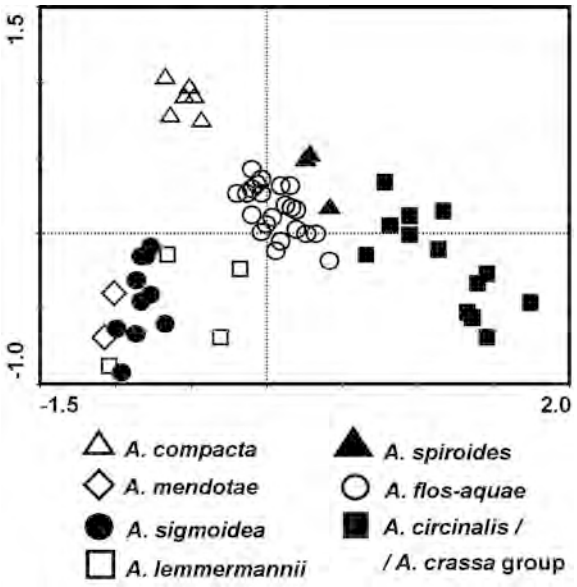


Fig. 1. PCA diagram based on the morphological characteristics of the *Anabaena* populations observed in natural conditions. Each symbol represents a single population, shapes symbolize a preliminary morphospecies identification. Using the PCA, the existence of clear morphological boundaries of these morphotypes was tested. The first and the second canonical axes explain together 74.7 % of the total variance.

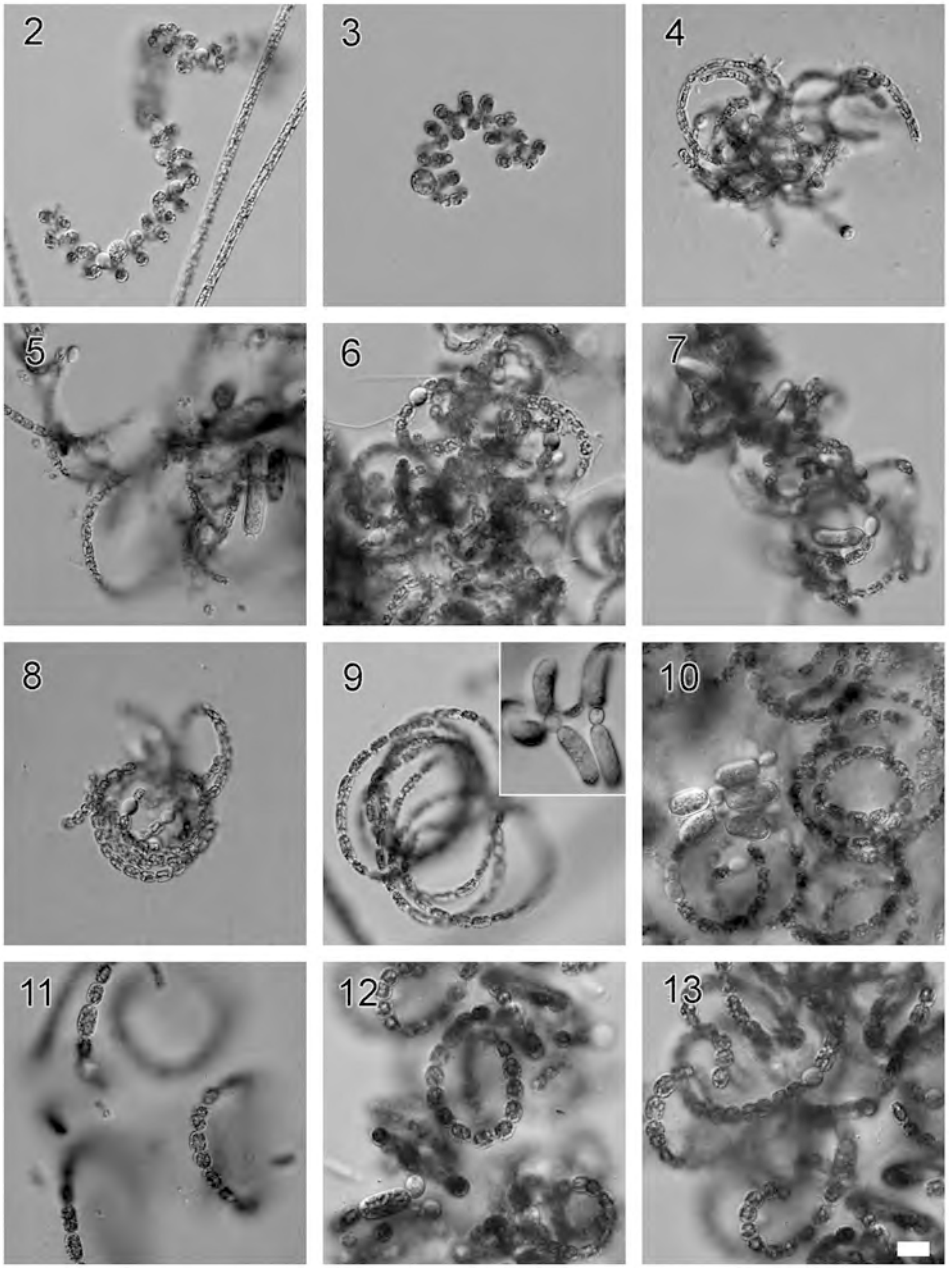
Table 4. Parameters of two sample t-tests comparing the morphology of *A. mendotae* and *A. sigmoidea* or *A. flos-aquae* and *A. spiroides.*

	A. mendotae vs. *A. sigmoidea* t-value	p-value	*A. flos-aquae* vs. *A. spiroides* t-value	p-value
Vegetative cell length	1.477581	0.167571	– 1.566550	0.130875
Vegetative cell width	**– 6.314220**	**0.000057**	**2.850976**	**0.009043**
Heterocyst length	– 0.259254	0.800227	0.048912	0.961412
Heterocyst width	– 1.356320	0.202183	1.465384	0.156354
Akinete length	– 1.564130	0.146082	– 0.625471	0.537819
Akinete width	– 0.648074	0.530232	**2.180541**	**0.039702**

be rejected only when mean values of the width of vegetative cells were compared (two sample t-test, Table 4).

In the PCA diagram (Fig. 1), the populations of *A. mendotae* are tightly adjacent to the group of *A. sigmoidea* populations. Populations of *A. sigmoidea* generated a cluster that is clearly separated from the group of *A. flos-aquae* populations.

Therefore, we suggest classification of these morphotypes as a joint morphological group. The main morphological characteristics defining this group are the width and length: width ratio (i.e. the shape) of vegetative cells, length: width ratio (i.e. the shape) of akinetes, and irregularity of trichome coiling (Figs 4-8; Table 3). According to the Botanical Code (Greuter *et al.*, 2000),



Figs 2-13. Microphotographs of selected *Anabaena* populations of the morphospecies *A. compacta* (2-3), *A. mendotae* & *A. sigmoidea* (4-8), and *A. lemmermannii* (9-13). Population codes: (2) 04-32; (3) P_Cerni240504; (4) 04-11; (5) P_Orlik220604; (6) 04-05; (7) 04-06; (8) 04-27; (9) 04-24; (10) 04-42; (11) 04-33; (12-13) 04-38. Scale = 10 µm (right bottom corner).

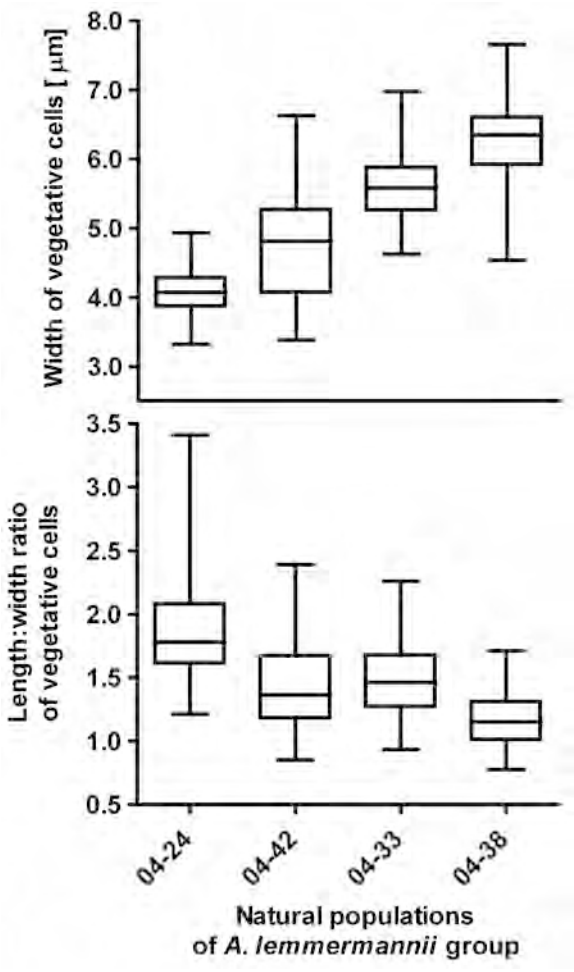
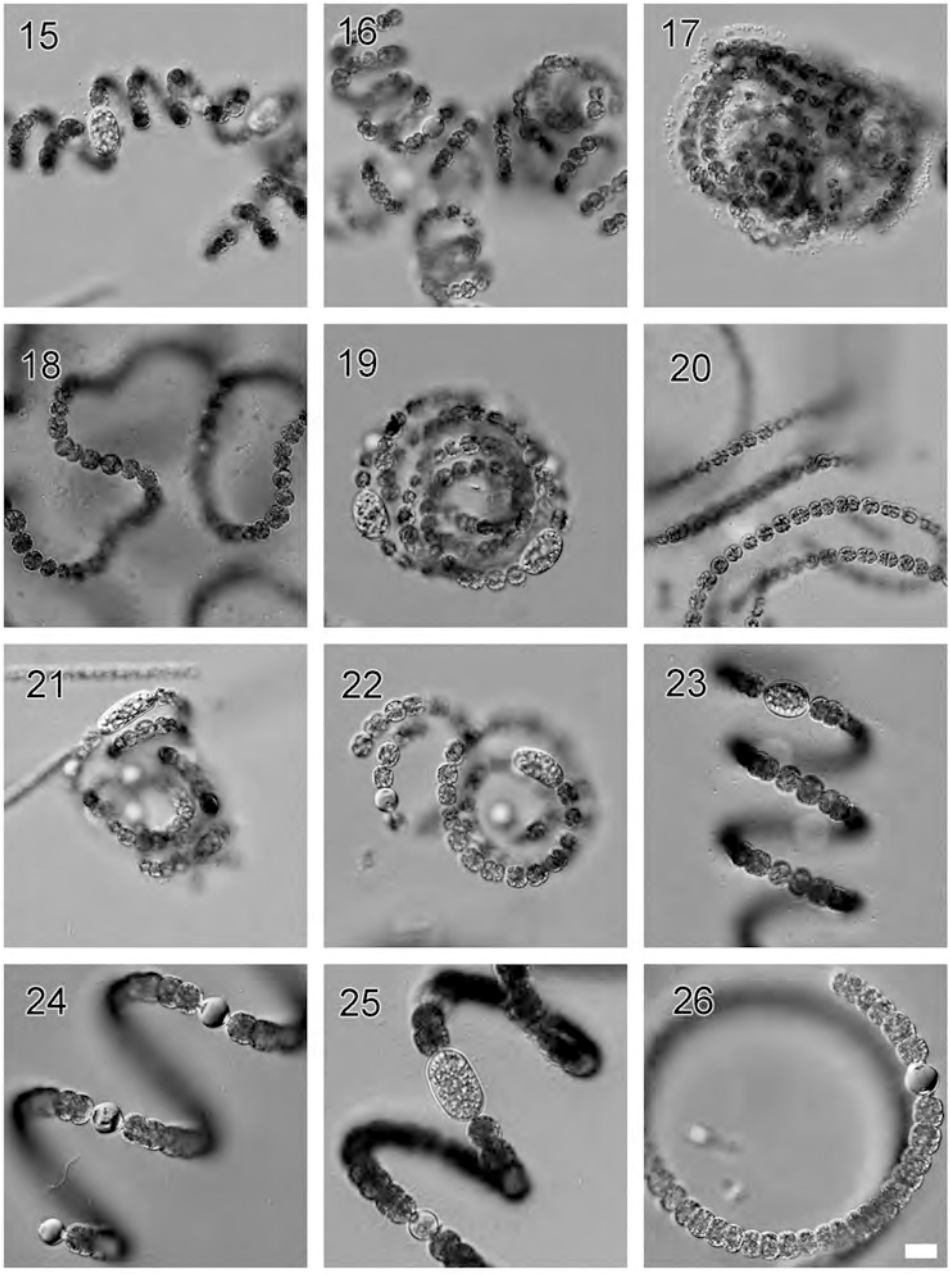


Fig. 14. Box-whisker plots of width and length:width ratio of vegetative cells of four populations classified as *Anabaena lemmermannii* according to the akinete position. Whiskers represent minimal and maximal values, boxes symbolize ± standard deviation and lines inside boxes mean values.

A. mendotae Trelease 1889 possesses a priority over *A. sigmoidea* Nyg. 1949, and thus populations fitting these two morphotypes should all received the name *A. mendotae*.

A. lemmermannii. Four populations of *A. lemmermannii,* represent a continuous transition between *A. sigmoidea* and *A. flos-aquae* groups in our study (Fig. 1). The main criterion for identification of these populations as *A. lemmermannii* was the position of akinetes (two akinetes, each at one side of a heterocyst). However, other morphological characters, especially the vegetative cell width and length: width ratio, were highly variable among particular populations (Figs 9-14). The variance of mean values of vegetative cell width of single populations was 10.4 (whereas *A. flos-aquae* & *A. spiroides* group showed 0.30, *A. mendotae* & *A. sigmoidea* group 0.08 and *A. compacta* 0.06, respectively). The taxon seems to be apparently heterogeneous and requires a taxonomic revision (see Discussion).



Figs 15-26. Microphotographs of selected *Anabaena* populations of the morphospecies *A. flos-aquae* & *A. spiroides* (15-22), and *A. circinalis* & *crassa* (23-26). Population codes: (15) P_Opat150904; (16) 04-15; (17) 04-16; (18) 04-19; (19) 04-30; (20) 04-37; (21) P_Horus150904; (22) P_Ratmi150904); (23) P_Horak110706; (24) P_Komor180804; (25) 04-26; (26) 04-46. Scale = 10 µm (right bottom corner).

A. flos-aquae* & *A. spiroides. Based on the PCA analysis, the group of *A. flos-aquae* seems to be uniform (Fig. 1). Nevertheless, obvious differences in trichome coiling patterns were detected within this group (Figs 15-22). The regularity of coiling in cultures was often unstable and alternant dominance of regularly and irregularly coiled filaments was then observed for the same strain. This was not the case of other *Anabaena* morphotypes, where the irregularity / regularity of coiling was strictly determined. Furthermore, the inter-population variability in the dimensions of all cell types was also rather high (e.g. the variance of mean values of vegetative cell width was 0.30). Although the vegetative cell dimensions were different among different populations, their cell shape (expressed as length: width ratio) was alike. The same results were obtained for heterocysts. The akinete dimensions and their length: width ratios were highly variable among different populations (data not shown).

Three populations that were preliminarily classified as *A.* cf. *spiroides* (04-51, P_Homol260504, P_Opat-y150904) are placed in a cluster with *A. flos-aquae* populations (Fig. 1). The null hypothesis (H_0) that the morphological parameters of the populations classified as *A. flos-aquae* do not differ from those of *A. spiroides* can be rejected when mean values of the width of vegetative cells and mean values of the akinete width were compared (two sample t-test, Table 4). The real differences are, nevertheless, insignificant for reliable identification of these morphotypes (Table 3).

For practical usage, we propose regarding all these types as a combined morphological group. The main defining morphological characteristics of the group are the width of vegetative cells, shape of vegetative cells (more or less spherical, length: width ratio 1.0 on average), and length: width ratio of akinetes (Table 3). According to the Botanical code (Greuter *et al.*, 2000), *A. flos-aquae* Rabenh. ex Born. *et* Flah.1888 has priority over *A. spiroides* Kleb. 1895, and thus populations fitting this two morphotypes should all received the name *A. flos-aquae*.

A. circinalis* & *A. crassa. No clear morphological subgroups were found within the group of populations that were classified as *A. circinalis* & *A. crassa* group. In the PCA diagram the populations form a co-cluster (Fig. 1) and also the detailed comparison of single morphometric characteristics does not show any clear differences (Figs 23-27). Therefore we suggest reclassification of these morphotypes as a joint morphological group. The major defining morphological characteristics of the group are the width of vegetative cells and the regularity of trichome coiling (Table 3). Following the Botanical Code rules (Greuter *et al.*, 2000), the priority name of the group is *A. circinalis*.

DISCUSSION

For the first time, the morphological diversity of coiled planktonic *Anabaena* species has been studied within a wide spectrum of morphotypes based on morphology observed in natural habitats. Morphological parameters of all morphotypes commonly occurring in the standing waters of the Czech Republic were assessed.

It can be concluded from the presented study that *A. compacta* (studied and described by Hickel, 1982; 1985) is the only morphotype clearly delimited

from the others. All drawings and photographs available in the literature also confirm small morphological variability within this species.

Other coiled *Anabaena* morphotypes represent a morphological continuum rather than a single well defined species. However, groups of populations displaying similar morphology can be found. They show broader morphological plasticity than the current view of the species concept and we suggest them to be used for practical determination in special cases when combined morphological and molecular analyses cannot be carried out.

Recent studies, exploring mainly the structure of 16S rRNA, have shown that individual planktonic species (morphospecies) within the genus *Anabaena* are highly similar (Gugger *et al*., 2002; Rajaniemi *et al*., 2005a; Willame *et al*., 2006), with the exception of *A. compacta.* This is in perfect agreement with our conclusions based exclusively on the morphological approach.

One of the biggest unsolved issues of classification within the *Anabaena* genus is the ambiguous definition of many species. Concepts of the same species often differ when interpreted by different authors. Furthermore, numerous species established in the past were not properly distinguished from others and their morphological parameters overlapped with descriptions of other species (see below).

Thus, the concepts of *A. mendotae* and *A. sigmoidea* have not been satisfactorily clarified. Komárek (1996) recommended identification of these two types using the shape of vegetative cells (in *A. mendotae* long, cylindrical, cell walls only slightly constricted, whereas in *A. sigmoidea* shorter, barrel-shaped with obvious constrictions between cells) and the inclination to trichome fragmentation in *A. sigmoidea* as the main taxonomic criteria. On the other hand, Li *et al.* (2000) reported the latter morphotype (barrel-shaped cells with constrictions) under the name of *A. mendotae*. Available molecular analyses do not offer any conclusions since molecular characteristics of these morphotypes have not as yet been compared. When comparing single morphological features of the studied populations (Table 4), only the width and length: width ratios of vegetative cells were found to be slightly different. The real values are, nevertheless, irrelevant for the sound identification (the difference in mean width of vegetative cells is less than 1 μm; Table 3).

Another example of vague species definition is the complex of species related to *A. flos-aquae*. The descriptions of *A. flos-aquae* and *A. spiroides* overlap in all parameters (Table 1) and precise identification is therefore hardly feasible.

Similarly, the unclear definition of *A. perturbata* Hill 1976 (Hill, 1976b) hampers the clear identification of this morphospecies. As shown in Table 1, its morphological characteristics strongly overlap with the description of *A. flos-aquae* and *A. spiroides.* According to the original description and photographic documentation, the akinetes of *A. perturbata* should be nearly spherical. On the other hand, later concepts of this taxon (summarized by Komárek, 1996) are wider and include morphotypes with longer and kidney-shaped akinetes (Table 1). Further revision of *A. perturbata* is therefore necessary.

An analogous shift in the species concept can be observed also in *A. curva* Hill 1976. The morphology of the strain 04-19 (particularly the coiling pattern and accumulation of filaments in thick mucilage; Fig. 18) strongly resembles *A. curva* as presented by Li *et al.* (2000). On the contrary, according to the original description of Hill (1976a), akinetes of this morphospecies should be markedly curved, not kidney-shaped. Thus we suggest the revision of the taxon both on molecular and also morphological level.

Marked inconsistencies in trichome coiling patterns were observed among the populations of the *A. flos-aquae* complex. However, recent molecular results based on the structure of 16S rRNA (Gugger *et al*., 2002; Rajaniemi *et al*., 2005a; Willame *et al*., 2006) have shown that *Anabaena* taxa with coiled trichomes are clustered tightly together with the taxa with straight trichomes. Therefore, it is likely that trichome coiling itself cannot be regarded as a taxonomically distinguishing feature.

In *A. circinalis* and *A. crassa*, shifts in species concepts can also be noticed. Two morphological characteristics (width of vegetative cells and coil diameter) were pointed out by several authors (Komárková-Legnerová & Cronberg, 1992; Komárková-Legnerová & Eloranta, 1992; Komárek, 1996) as suitable for distinguishing these species. However, according to the original descriptions of both species (Geitler, 1932; Komárková-Legnerová & Cronberg, 1992), the ranges of vegetative cell width overlap markedly. On the other hand, ranges of vegetative cell width presented by Komárek (1996) almost do not overlap and define quite clearly these two morphospecies. The present study has shown that neither the trichome width nor the coil diameter is a reliable morphological criterion for distinguishing morphospecies of *A. circinalis* from *A. crassa* (Fig. 27).

Rajaniemi *et al*. (2005a) analysed two types of *A. circinalis* strains, classified as *A. circinalis* and *A.* cf. *circinalis* var. *macrospora*. According to the cell dimensions, only the strains of *A. circinalis* fell within the populations classified as *A. circinalis* & *A. crassa* group in our study. These strains were commonly clustered with the strains of *A. crassa* in the neighbour-joining tree based on 16S rRNA gene, which is in perfect agreement with our conclusions.

Morphological features of the population 04-24 (Fig. 9) matched precisely the description of *A. lemmermannii* var. *lemmermannii* P. Richt., whereas the population 04-38 (Figs 12-13) fitted the description of *A. lemmermannii* var. *minor* (Uterm.) Kom.-Legn. (= *A. utermoehlii* Geitl.) (Table 1, 3). Nonetheless, two populations with intermediate morphology were noticed (04-33, 04-42; Figs 10-11) suggesting rather the existence of a morphological continuum between these types of the *A. lemmermannii* taxon (found also by Komárková, 1988 and Li *et al*., 2000).

As for *A. lemmermannii,* the position of akinetes is regarded as the most important feature distinguishing unquestionably the taxon (Komárková, 1988; Komárek, 1996). Nevertheless, in the light of molecular results (Gugger *et al*., 2002) and wide morphological variability observed (Komárková, 1988; present results), it seems rather irrelevant. The populations 04-24 and 04-42 showed high similarity with *A. mendotae* & *A. sigmoidea* group in all morphological features whereas morphology of the population 04-38 resembled *A. flos-aquae* & *A. spiroides* group (Figs 9-13). The results of Gugger *et al*. (2002) also demonstrated that *A. lemmermannii* is diversified at the molecular level. In consensus parsimony trees based on 16S rDNA and ITS1-S sequences, most of *A. lemmermannii* strains belonged to a subcluster 3 together with *A. flos-aquae* strains whereas one strain was placed in a distant subcluster 1 together with *A. mendotae*. Thus, the position of akinetes seems to be a taxonomically unimportant feature. Presumably, two groups could be found within the taxon *A. lemmermannii,* one belonging to the group of *A. mendotae* & *A. sigmoidea* and the second one to the group of *A. flos-aquae* & *A. spiroides*. To confirm this hypothesis, detailed studies involving more populations are required both on molecular and morphological level.

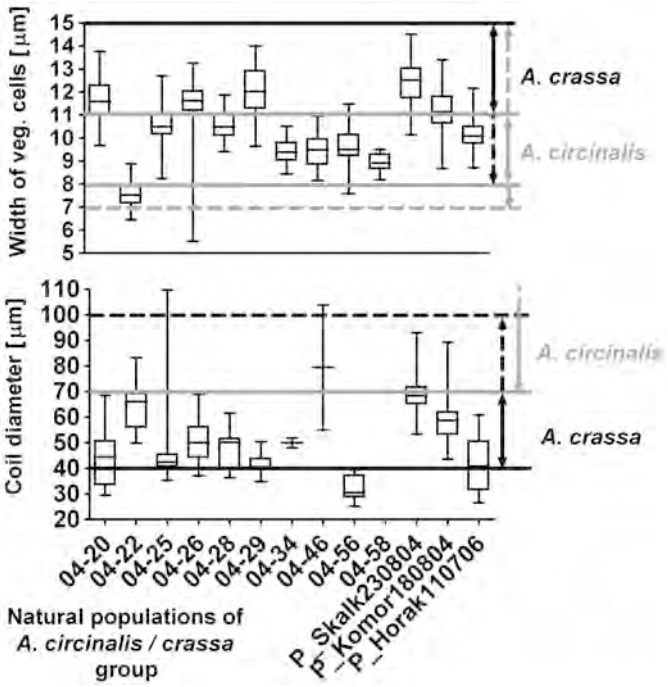


Fig. 27. Box-whisker plots of vegetative cell widths and coil diameters of *Anabaena circinalis* & *A. crassa* group. Whiskers represent minimal and maximal values, boxes symbolize ± standard deviation and lines inside boxes mean values. Limit values for both species after Komárek (1996) are included (solid line – common values, dashed line – extreme values).

Another question should also be discussed here, namely the stability of morphological features in dependence on environmental conditions. Stulp (1982) showed that deviations in the morphology of *Anabaena* strains occurs only under extreme temperature conditions. These deviations were observed either for taxonomically unimportant morphological characteristics or under a temperature that is hardly attainable in the normal planktonic conditions (35°C). Komárek (1996) is in agreement with these conclusions and regards single species of *Anabaena* as morphologically constant: their typical forms repeatedly occur in different localities and time.

We did not investigate either the development of morphology of single populations during the season or the influence of environmental factors. Nevertheless, the relatively high number of sampling sites and the number of populations studied are supposedly sufficient to give a true picture on *Anabaena* variability. The samples were collected during the growing season (May-October) and a relatively wide range of environmental conditions (light, temperature, water column stratification, nutrient limitation) can thus be expected. Moreover, all morphotypes studied encompassed populations collected both from fishponds, where the water column is mixed during the whole year and limitation by nutrients scarcely occurs, and from reservoirs, where the temperature stratification is observed in summer and limitation by nutrients, especially P, is frequently encountered. We therefore suppose that a wide range of morphological variability in the *Anabaena* populations in natural conditions was satisfactorily covered in the study.

If akinetes were not found in the natural populations, their dimensions were measured in the cultured strains. Nevertheless, our data were obtained

during a short-time cultivation (not later than one year after isolation) when the morphological changes of the strains were negligible. It seems reasonable to assume that the size and shape of akinetes were not modified significantly in comparison to those in the natural waters.

WC medium (Guillard & Lorenzen, 1972) is routinely used for cultivation of bloom-forming cyanobacteria in our culture collection. According to our long-term experience, *Anabaena* strains prosper much better in WC than in the commonly used BG11 medium (Stanier *et al*., 1971). When WC medium is used, the strains are green, not yellowish as in BG11, and fewer morphological abnormalities occur (Zapomělová, 2004). The nitrogen and phosphorus concentrations in WC medium span approximately the same range as in the fishponds sampled. The water column of Czech reservoirs is usually stratified during the summer and nutrient concentrations are therefore lower. In our data set, most populations were taken from fishponds (45, i.e. 74%) but all morphotypes studied also comprised populations collected from reservoirs (16 in total, i.e. 26%) – see Table 2. The PCA diagram convincingly showed that locality did not influence morphological features markedly since the populations of the same morphotype formed clusters regardless of the type of locality.

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