University of South Bohemia
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ANABAENA

PHENOTYPIC AND GENOTYPIC DIVERSITY OF PLANKTONIC STRAINS IN FISHPONDS AND RESERVOIRS OF THE CZECH REPUBLIC

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Ph.D. dissertation – shortened version

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České Budějovice 2008

Zapomělová, E., 2008: *Anabaena* – Phenotypic and genotypic diversity of planktonic strains in fishponds and reservoirs of the Czech Republic. Ph.D. dissertation, in English – 125 p. (shortened version 13 p.), Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Annotation

Morphological diversity of 61 *Anabaena* populations of 13 morphospecies was described under the field conditions of Czech fishponds and reservoirs. Polyphasic approach was then applied in classification of 45 clonal strains isolated from those populations. Detailed morphological analyses were performed and partial 16S rRNA gene sequences were obtained for 33 of the strains, and secondary metabolite production was evaluated in 20 strains. Plasticity of morphological characteristics under varied conditions of light, temperature, nitrogen and phosphorus was studied in selected strains, as well as their temperature and light growth requirements. The results were then discussed with respect to the delimitation of single *Anabaena* morphospecies. A new genus *Sphaerospermum* was defined for the morphospecies *Anabaena kisseleviana, A. reniformis* and *Aphanizomenon aphanizomenoides,* whose phenotypic and genotypic features differed considerably from all other *Anabaena* morphospecies. Unique information was provided on the occurrence and distribution of *A. reniformis* and *Aph. aphanizomenoides* in the Czech Republic.

Financial support

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 (projects No. IAA 600050704 and AV0Z60170517).

Declaration

I declare that this dissertation was fully worked out by myself using the cited literature only.
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České Budějovice, 1 September 2008

Eliška Zapomělová

Acknowledgement

My first thanks go to my supervisor Dr. Jaroslava Komárková, who guided me to the wonderful world of phytoplankton, provided me with help and advice every time I needed it, and gave me the main inspiration for this work. It was the best school and a great pleasure for me, to listen to her comments always when she sat at the microscope.

I want to express many thanks to my consultant Dr. Klára Řeháková for her support and patience she had with me. She provided me with hundreds of recommendations and advice, and devoted a lot of her time to me. I am very grateful that I was privileged to collaborate with her. I must admire her ability to manage with time and to organize the work, which has been still an unattained goal for me.

Definitely, I also want to thank other members of the Laboratory of Phytoplankton Ecology: Dr. Jitka Jezberová for kind help with phylogenetic analyses and molecular methods, Dr. Petr Znachor for the help with sampling and for critical comments to my manuscripts, and Maruška Kupková for invaluable technical assistance and endless tolerance. I thank B.Sc. Pavel Rychtecký and M.Sc. Kateřina Bernardová for pleasant collaboration during the laboratory or field work, and Martina Vožechová for the chlorophyll-a concentration analyses she always performed quickly and reliably. I am grateful to all of them for the collegiality and nice atmosphere.

My gratitude goes also to other members of the Department of Aquatic Microbial Ecology, headed by Prof. Karel Šimek, and to the director of the Institute of Hydrobiology, Doc. Josef Matěna, for giving me the opportunity to carry out the dissertation at the institute, as well as my B.Sc. and M.Sc. theses. Special thanks belong to Dagmar Šrámková, the secretary of the institute, without whose assistance with the office work I would go crazy very quickly. And I also highly appreciate the help of Jindra Bučková, who takes care of our laboratory dishes.

My deepest thanks go to Prof. Jiří Komárek for his devotion to cyanobacteria and the never-ending enthusiasm, which he has the gift to pass to other people. His lectures, as well as his comments and suggestions to the topic of this dissertation, were invaluable help for me.

All phycological colleagues from the Department of Botany, Faculty of Science, headed by Dr. Hanys Kaštovský, deserve my cordial thanks for keeping the contact and friendliness among all of the people dealing with algae at our faculty.

I wish to express my gratefulness to all the co-authors of the articles. The colleagues M.Sc. Pavel Hrouzek and B.Sc. Daniel Hisem from the Institute of Microbiology, AS CR, are warmly thanked for the data on the secondary metabolites and for the excursion through the HPLC-MS method they arranged for me. I am very grateful to Dr. Stefano Ventura and M.Sc. Elisa Soldati for allowing me to perform the ARDRA analysis in their laboratory at the Institute of Ecosystem Study, CNR, in Florence, and also to their technician Christina Mascalci for her assistance and patience. I highly appreciate the benevolence of other co-authors, M.Sc. Marie Šabacká, M.Sc. Marek Stibal, M.Sc. Lenka Caisová, and Dr. Alena Lukešová, which they had with the long time that the publication of our paper took. I owe special thanks to Dr. Valerie Flechtner from John Carroll University in Cleveland, Ohio, USA, for the language corrections of my manuscripts.

I am deeply indebted to Dr. Pirjo Rajaniemi and Dr. Anne Rantala from University of Helsinki, as well as to the head of their laboratory, Prof. Kaarina Sivonen, who introduced me for the first time to the molecular methods. I also remember kindly my visit in Dr. Muriel Gugger's and Dr. Raphael Willame's laboratory in Luxembourg, where I could learn more about their work.

An important role for my knowledge on cyanobacterial occurrence and distribution in our country played rapturous discussions with other cyanobacteria-lovers: Petr Pumann from the National Institute of Public Health in Prague, Lenka Šejnohová and Radovan Kopp from Masaryk University and Centre for Cyanobacteria and their Toxins in Brno, Emil Janeček from the Ohře River Authority in Teplice, and many others. I also highly appreciate my employment at the Vltava River Authority, where I had an access to phytoplankton samples from all over South Bohemia. Many thanks belong also to Paula de Tezanos Pinto from Argentina for the inspirational discussions via
E-mail. ¡*Estoy feliz, que pudimos encontrarnos este verano en la Republica Checa*!

My greatest debt is to Michal Bílý and Radka Pichlová, who provided me with the earliest insight into the hydrobiology and probably gave me the first impetus to be interested in algae. I will never forget the summer camps in Běstvina.

I wish to cordially thank my close friends from "Telčská kosící četa", Bětka, Ester, Libor, Honza, Verča, Tomáš, and Lucka with Mirek and Matyášek, as well as my flatmates Hanka, Konvalinka, and Káťa (together with our menagerie Brunhilda, Kadlík, Pepík, and Františka) for being by me. Many thanks belong also to our wandering bunch, i.e. Marťa, Olga, Janíček, Aleš, Kulda, Jarda, Irča, Míša, Anička, and other occasional participants. I thank Eva for plenty of hours of good-humoured Spanish conversation, Lenka for her energy, enthusiasm, and the beautiful exotic plants she gave me, Jana from Náchod, Hanka and Zdeňka from Jihlava, and Linda with Jarda and Eliška from Dobříš for staying in touch.

Finaly, my warmest gratitude goes to my dear family, my parents Magdaléna and Stanislav, my aunt Jana, and my cousin Martin, for their support, encouragement and for keeping us together. I am immensely grateful that I have home where I can whenever come to find understanding and love.

LIST OF ORIGINAL ARTICLES

The dissertation is based on the following articles, which are referred to in the text by their Roman numerals:

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

II **Zapomělová, E.** Hrouzek, P., Řeháková, K., Šabacká, M., Stibal, M., Caisová, L., Komárková, J. & Lukešová, A. (2008): Morphological variability in selected heterocystous cyanobacterial strains as a response to varied temperature, light intensity and medium composition. Folia Microbiologica 53 (4): 333-341.

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

IV **Zapomělová, E.,** Řeháková, K., Jezberová, J. & Komárková J.: Polyphasic characterization of eight planktonic *Anabaena* strains (cyanobacteria) with reference to the variability of 61 *Anabaena* populations observed in the field. (Manuscript).

V **Zapomělová, E.,** Jezberová, J., Hrouzek, P., Hisem, D., Řeháková, K. & Komárková J.: Polyphasic characterization of three strains of *Anabaena reniformis* and *Aphanizomenon aphanizomenoides* (cyanobacteria) and their re-classification to *Sphaerospermum* gen. nov. (incl. *Anabaena kisseleviana)*. Journal of Phycology (submitted).

VI **Zapomělová, E.,** Jezberová, J., Řeháková, K., Hrouzek, P., Soldati, E., Hisem, D., Znachor, P., Komárková, J. & Ventura, S.: Phenotypic and genotypic diversity of planktonic *Anabaena*-like cyanobacteria in fishponds and reservoirs of the Czech Republic. (Manuscript.)

THE AUTHOR'S CONTRIBUTION TO THE ARTICLES

I E. Zapomělová participated in sampling of cyanobacterial water blooms. She isolated and purified all cyanobacterial strains, performed cell size measurements on the original *Anabaena* populations, carried out the statistical analyses, interpreted the results and wrote the article.

II E. Zapomělová participated in the design of the study, carried out the experiments with planktonic strains, and measured morphometric characteristics of the *Anabaena* and *Aphanizomenon* strains. She carried out part of the statistical analyses of the data interpreted the results and wrote the article.

III E.Zapomělová isolated and purified the strains, and designed and performed the experiments on the whole. She carried out the cell-size measurements, the DNA extraction and PCR, and prepared the DNA samples for the sequencing of 16S rRNA gene. Furthermore, she performed the statistical analyses of the data, interpreted the results and wrote the article.

IV E.Zapomělová collected the samples, isolated and purified the strains, and designed and performed the experiments on the whole. She carried out the cell-size measurements, the DNA extraction and PCR of most of the strains, and prepared the DNA samples for the sequencing of 16S rRNA gene. She performed the statistical analyses of the data, interpreted the results and wrote the article.

V E. Zapomělová isolated and purified the strains, evaluated their morphological features, and carried out the DNA extraction and PCR. She participated in the preparation of the DNA samples for the sequencing of 16S rRNA gene. She performed the statistical analyses of the data, interpreted the results and wrote the article.

VI E. Zapomělová collected part of the samples, isolated and purified the strains, evaluated their morphological features, and carried out the DNA extraction. She participated in ARDRA analysis under the supervision of Ms.S. Elisa Soldati and Dr. Stefano Ventura. Furthermore, she performed PCR and preparation of the DNA samples for the sequencing of 16S rRNA gene in 27 *Anabaena* strains. She performed the statistical analyses of the data, interpreted the results and wrote the article.

CONTENTS

ANABAENA

PHENOTYPIC AND GENOTYPIC DIVERSITY OF PLANKTONIC STRAINS IN FISHPONDS AND RESERVOIRS OF THE CZECH REPUBLIC

Introduction

Planktonic cyanobacteria in lakes, reservoirs, fishponds and estuaries all over the world have been recently attracting much publicity due to their tendency to form water blooms of varying intensity (Maršálek et al. 1996, Chorus & Bartram 1999, Oliver & Ganf 2000). Occurrence of water blooms in surface waters represents a potential risk of water quality worsening, since cyanobacteria can produce wide range of toxic or malodorous bioactive compounds (Sivonen & Jones 1999). Moreover, massive decomposition of cyanobacterial biomass is often accompanied by oxygen depletion (Maršálek et al. 1996, Chorus 2002), which may endanger other coexisting organisms. Intensive effort has been therefore focused on possibilities of water-bloom prevention and reduction. Unambiguous classification of bloom-forming cyanobacteria is highly required in this respect, as well as the knowledge on their phylogeny, morphological plasticity and cyanotoxin production under varying environmental conditions. This dissertation is aimed at the diversity within planktonic representatives of the genus *Anabaena*, representing one of the most abundant water-bloom-forming genera in the Czech Republic (Znachor & Komárková 2002, Znachor et al. 2006).

The history of *Anabaena* classification

Cyanobacteria were originally classified mostly on the basis of morphological features. The progress of molecular methods initiated a combined application of both morphological and molecular approaches. Current taxonomic revisions tend to be conducted by a multidisciplinary (polyphasic) approach including molecular, morphological, physiological, cytological, toxicological and ecological data.

The genus *Anabaena* was established by Bory in 1822 (Geitler 1932) and has recently been classified into the filamentous heterocystous cyanobacteria, subsection IV, family I (Rippka et al. 2001). Under the Botanical Code the genus *Anabaena* Bory ex Born. et Flah. belongs to the order Nostocales (Komárek & Anagnostidis 1989). For many years the descriptions of the 57 *Anabaena* species given by Geitler (1932) were the major point of reference for the classification of the genus. Later Drouet (1978) criticized this classification: in his opinion only two *Anabaena* species existed. From many species that were established in the past only some are well known and accepted at present, others need a revision (Komárek 1996).

From the morphological point of view, the genus *Anabaena* is distinguished from the genus *Aphanizomenon* on the basis of botanical species *Anabaena oscillarioides* Bory and *Aphanizomenon flos-aquae* (L.) Ralfs. These two species are clearly distinguishable, however, both genera comprise a lot of “transitional” species in which the traditional diacritical features are not strictly defined (Komárek & Anagnostidis 1989). Moreover, recent phylogenetic studies have shown that the genera *Anabaena* and *Aphanizomenon* are intermixed also on the basis of 16S rRNA, *rpoB* and *rbcLX* genes (Gugger et al. 2002 a, Rajaniemi et al. 2005 a, Rajaniemi et al. 2005 b). Suitable part of genome which would allow reliable classification of these cyanobacteria at subgeneric level has not been yet found.

Morphological studies on *Anabaena* populations / strains

So far published studies dealing with morphological characteristics of *Anabaena* were limited either on morphology in culture conditions (Stulp 1982, Stulp & Stam 1982, Stulp & Stam 1984 a, b, Stulp & Stam 1985, Li et al. 2000, Rajaniemi et al. 2005 a, b, Willame et al. 2006) or on one-shot field observations (Hill 1976 a, b, c, Hickel 1982, Hickel 1985, Cronberg & Komárková 1988, Komárková 1988, Komárková-Legnerová & Cronberg 1992, Komárková-Legnerová & Eloranta 1992, Padisák & Kovács 1997, Hindák 2000, Lepistö & Holopainen 2008). These approaches have serious disadvantages, when they are used separately. Observation in field does not provide complete information on morphological variability in relation to various environmental conditions or growth phases. On the other hand, it has been known for a long time that long-term cultivation can cause serious morphological changes, which do not correspond to the situation in 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).

For all that, in the research of *Anabaena* nobody has tried to combine both approaches, that means field observation and short-term cultivation of higher number of *Anabaena* representatives under various growth conditions. Even also the most recent studies applying polyphasic approach in *Anabaena* classification (Gugger et al. 2002 a, Rajaniemi et al. 2005 a, b) evaluated morphological features mainly or entirely on strains in cultures.

Another weak point of some works, including the recent ones, is the use of classical species names accompanied by the lack of morphological data (descriptions, measurements, microphotographs or drawings). Traditional names can be rather confusing, since their concepts may differ when they are interpreted by different authors. For instance, ambiguous is the attitude of authors to distinguishing the morphospecies *A. circinalis* and *A. flos-aquae* (Beltran & Neilan 2000). In these cases, the trichome spiral breadth and akinete characteristics are often quoted as the main differentiating criteria. However, according to the original descriptions of *A. flos-aquae* Bréb. ex Born. et Flah. 1888 and *A. circinalis* Rabenh. ex Born. et Flah. 1888, trichome width is the main identification criterion. Trichome width of *A. flos-aquae* should range from 6.0 to 8.3 μm while the trichome width of *A. circinalis* should be 8.0–11.0 μm (Komárek 1958, Komárková-Legnerová & Cronberg 1992, Komárek 1996). It is evident that *A. circinalis* can be hardly misinterpreted for *A. flos-aquae*. A similar shift in the species concept can be observed also in some other *Anabaena* species.

Phylogenetic analyses of *Anabaena* strains

Only several parts of genome have been sequenced repeatedly in various strains of *Anabaena* (www.ncbi.nlm.nih.gov). From these sequences, 16S rRNA, ITS1, *rbcLX* and *rpoB* genes were investigated in detail and taxonomic conclusions have been deduced in the most recent studies (Gugger et al. 2002 a, Rajaniemi et al. 2005 a, b, Willame et al. 2006).

These works demonstrated that *Anabaena* strains were intermixed with *Aphanizomenon* on the basis of both morphological and molecular features and their assignment to a single genus was suggested (Rajaniemi et al. 2005 a). The subgenus *Dolichospermum* (Ralfs ex Born. et Flah.) Kom. et Anagn. 1989 was proposed for the group of planktonic *Anabaena* excl. *Aphanizomenon* (Rajaniemi et al. 2005 b).

None of the above-mentioned sequences appears to be suitable for distinguishing the taxa of planktonic *Anabaena / Aphanizomenon* complex at lower than generic level. Nevertheless, the studies of Rajaniemi et al. (2005 a, b) have suggested that the differences in 16S rRNA gene sequences, however low, correlate highly with morphological similarities of planktonic *Anabaena* strains (except trichome coiling). Thus, from the sequences available for the present, the 16S rRNA gene seems to be rather convenient to confront morphological and molecular characteristics, at least within the group of planktonic *Anabaena*.

The number of *Anabaena* strains in the most recent studies is either not very high (Gugger et al. 2002 a; Willame et al. 2006) or the most of the strains come from the same locality, and thus duplicates or triplicates of the same strain can appear under different strain codes in the analyses (Rajaniemi et al. 2005 a, b). The results, therefore, cannot be simply generalized and general conclusions on the overall infrageneric diversity and phylogenetic relationhips can be hardly deduced. More *Anabaena* strains from various separated localities are highly required not only for molecular but also for the overall polyphasic studies.

Fatty acids as chemotaxonomic markers in planktonic *Anabaena*

Possible evolutionary significance of cyanobacterial fatty acids was for the first time pointed out by Kenyon & Stanier (1970). Their chemotaxonomic value was studied several times within the group of planktonic *Anabaena* (Li & Watanabe 2001, Gugger et al. 2002 b, Li & Watanabe 2004). While fatty acid composition of *Anabaena* strains with straight trichomes (Li & Watanabe 2001) seems not to match with their morphology and phylogenetic affiliation as is currently known (Rajaniemi et al. 2005 a, b), fatty acid profiles of *Anabaena* strains with coiled trichomes (Li & Watanabe 2004) correspond almost exactly to the recently published phylogenetic analyses (Gugger et al. 2002 a, Rajaniemi et al. 2005 a, b). Gugger et al. (2002 b) demonstrated that fatty acid composition of planktonic *Anabaena* strains agreed with their toxicity. All hepatotoxic strains grouped together, whereas the non-toxic and neurotoxic strains grouped with the non-toxic *Aphanizomenon* strains. Nevertheless, the interconnection of toxicological and morphological approaches would be useful in this respect, since Li and Watanabe (Li & Watanabe 2001, Li & Watanabe 2004) did not evaluated the toxicity of the studied *Anabaena* strains and, on the contrary, most of the strains of Gugger et al. (2005 b) were identified simply as *Anabaena* sp.

Ecology of planktonic representatives of the genus *Anabaena*

Many studies have addressed the question of why cynanobacteria are able to achieve the dominance in a wide range of environmental conditions. Numerous hypotheses on cyanobacterial success in plankton have been suggested, from which the following ones may be relevant for *Anabaena* morphospecies (detailed reviews were given by Hyenstrand et al. 1998, Dokulil & Teubner 2000, and Cronberg & Annadotter 2006):

(1) Higher temperature optima in comparison to other algal groups (Robarts & Zohary 1987).
(2) Low light-energy requirements (Niklisch & Kohl 1989). Regarding *Anabaena* morphospecies, the temperature and light requirements have been evaluated in only individual strains (e.g. Stulp 1982,

Stulp & Stam 1985, Rapala & Sivonen 1998, Li & Watanabe 2002, Tsujimura & Okubo 2003). The deduction of general conclusions is therefore problematic.

(3) More effective uptake kinetics for inorganic carbon (Shapiro 1997).

(4) Ability to regulate the buoyancy and thus the position in the water column (Reynolds et al. 1987).

(5) Migration of cyanobacteria from the sediment to the water column, providing a competitive advantage by storage of internal phosphorus reserves (Ganf & Oliver 1982, Pettersson et al. 1993). Vertical migration related to light and nutrient availability was described several times from *Anabaena* morphospecies (Oliver & Walsby 1984, Spencer & King 1989, Brookes et al. 1999).

(6) Low TN:TP ratios have been reported to be beneficial for both nitrogen- and non-nitrogen fixing cyanobacterial species (Smith 1983). The preference of low N:P ratios by *Anabaena* was reported by Stockner & Shortreed (1988). They referred on manipulation of N:P ratios in a lake fertilization experiment, where the increase of the molar N:P ratio resulted in elimination of *Anabaena* bloom and the development of picoplanktonic cyanobacteria and algae.

(7) The inorganic nitrogen hypothesis postulates that nitrogen fixing-species are favoured by the lack of nitrogen, whereas non-nitrogen-fixing cyanobacteria by ammonium-nitrogen, and nitrate-nitrogen availability induces the development of eukaryotic microalgae (Blomqvist et al. 1994).

(8) Resistance against zooplankton grazing (Haney 1987). Although filamentous cyanobacteria are mostly inedible for consumers due to their size, some cases of grazing of *Anabaena* trichomes by amoebas (Cook et al. 1974), protozoa (Canter et al. 1990) and crustaceans (Epp 1996) were reported.

(9) Suppression of other algae and grazers through the excretion of organic compounds, including cyanotoxins (Keating 1977, Sivonen & Jones 1999, Leflaive & Ten-Hage 2007). *Anabaena* strains were referred to produce microcystins, anatoxin-a, anatoxin-a(S), saxitoxins, paralytic shellfish poisons (PSPs) and lipopolysaccharides (Sivonen & Jones 1999, Beltran & Neilan 2000, Singh et al. 2005).

Little attention has been paid so far to the coexistence and competitive relationships of single components of phytoplankton assemblages. Regarding the genus *Anabaena*, several studies were carried out. Alternating dominance of cyanobacteria *Anabaena* spp. and the diatoms *Aulacoseira* spp. in a turbid-river weir pool in Australia was shown to depend directly on the establishment or destruction of persistent thermal stratification. *Aulacoseira* sank out of the euphotic zone during the stratification of the water column and *Anabaena* occupied the illuminated surface layer. On the contrary, *Aulacoseira* tended to dominate the phytoplankton during the periods of water column mixing (Sherman et al. 1998). Other authors also confirmed the importance of persistent thermal stratification for the occurrence of *Anabaena* (Maier et al. 2001, Mitrovic et al. 2003, Westwood & Ganf 2004 a, b). Laboratory experiments aimed at competitive interactions of *Anabaena* (unidentified strain) and *Aphanizomenon flos-aquae* demonstrated that growth demands of even these closely related cyanobacteria differed considerably, which was manifested by their different competitive abilities under various environmental conditions (De Nobel et al. 1997, De Nobel et al. 1998).

Numerous studies have been focused on the akinetes of *Anabaena* morphospecies, their ecological role and the driving factors for their formation and germination. Light, temperature and nutrients are considered triggering the akinete development, however the significance of each of them appears to be different for various *Anabaena* morphospecies and strains (Rother & Fay 1979, Cmiech et al. 1984, Li et al. 1997, Hori et al. 2003, Olli et al. 2005). The ecological function of akinetes also has not yet been satisfactorily clarified. The authors either observed akinetes germinating immediately after their formation (and therefore regenerating the existing population), or sinking to the sediment as resting cells (Cmiech et al. 1984, Rother & Fay 1977, Reynolds 1972, Kravchuk et al. 2002). Recruitment of *Anabaena* populations from the sediment to the water column can be divided in two to three phases: (1) akinete germination, (2) growth at the sediment surface (this phase is sometimes missing), and (3) migration to the pelagic zone (Karlsson-Elfgren & Brunberg 2004). Light intensity appears to be the main triggering factor for akinete germination, though the requirements of various *Anabaena* morphospecies or strains can vary (Herdman 1987). The ability of akinetes to perennate for many years in sediments led to the belief that their main role is to survive adverse conditions. However, some authors pointed out that the akinetes in sediments need not be the main reservoir of viable cyanobacterial cells. Many nostocacean cyanobacteria have been shown to survive the winter as vegetative filaments (Reynolds 1972, Barbiero & Welch 1992), which are then the primary inoculum for the development of new water blooms (Head et al. 1999, Karlsson-Elfgren 2003).

Scope and outline

This dissertation was focused on interconnection and discussion of three approaches (phenotypical, molecular, and ecophysiological) applied to the classification of planktonic *Anabaena* morphospecies. An innovation brought with the project was the main accent on morphology in natural conditions, as well as the investigation on the relationships between morphological features and varied growth conditions. Phylogenetic analysis based on 16S rRNA gene sequences was performed for the first time in strains from many different localities and the strains represented the whole size spectrum of *Anabaena* morphospecies.

The main aims of this study were:

(1) to examine morphological diversity of planktonic *Anabaena* in natural populations, and to evaluate the range of variability of single morphospecies with respect to their delimitation (paper I).

(2) to describe the effects of varied factors (light intensity, temperature, concentration of N and P) on morphometric features of selected *Anabaena* strains, representing wide spectrum of morphotypes commonly occurring in the Czech Republic (papers II, III, and IV).

(3) to compare the range of morphological variability of these strains with the total morphological diversity of the natural *Anabaena* populations of relevant morphospecies (papers III and IV).

(4) to determine some ecophysiological features of the studied *Anabaena* strains (temperature and light growth optima, production of secondary metabolites) and to examine their consistency with morphological and genotypic characteristics of the isolates (papers III, IV, V, and VI).

(5) to gain a deeper understanding of the genotypic and phenotypic diversity of planktonic *Anabaena* morphospecies in the Czech Republic (papers V and VI).

Results

This dissertation consists of six papers – two papers already published (paper I and II), one paper accepted for publication (III), one paper submitted (paper V) and two manuscripts in progress (paper IV and VI).

Paper I

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

Zapomělová, E., Řeháková, K., Znachor, P. & Komárková, J. *Cryptogamie Algologie* 28: 353-371 (2007).

Morphology of 61 planktonic populations of the genus *Anabaena* with coiled trichomes was studied under natural conditions. The samples were collected from the Czech water bodies, representing 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 lower taxonomic level is not feasible based only on morphology. Moreover, *A. lemmermannii* taxon appears to be morphologically heterogeneous and probably needs a thorough taxonomic revision.

Morfologie 61 planktonních populací sinic rodu *Anabaena* se spiralizovanými vlákny byla studována v přírodních podmínkách. Vzorky byly odebírány z českých stojatých vod a reprezentovaly všechny druhy rodu *Anabaena* se spiralizovanými vlákny, které byly v minulosti v České republice zaznamenány. Existence jasných morfologických hranic mezi jednotlivými druhy byla testována pomocí analýzy hlavních komponent (PCA) a párových t-testů. Jediným jasně vymezeným morfotypem byla *A. compacta*, která je definována šířkou vegetativních buněk, tvarem akinet a pravidelností spiralizace vláken. Ostatní druhy tvořily morfologické kontinuum a nebylo mezi nimi možné vymezit jasné hranice. Pro praktické použití byly proto navrženy skupiny morfotypů společně s morfologickými kritérii, které je definují (*A. mendotae* & *A. sigmoidea, A. flos-aquae* & *A. spiroides,* and *A. circinalis* & *A. crassa).* Identifikace studovaných morfotypů rodu *Anabaena* na nižší taxonomické úrovni pouze na základě morfologických vlastností se jeví jako nemožná. Taxon *A. lemmermannii* se navíc jeví jako morfologicky heterogenní a pravděpodobně bude vyžadovat důkladnou taxonomickou revizi.

Paper II

Morphological variability in selected heterocystous cyanobacterial strains as a response to varied temperature, light intensity and medium composition

Zapomělová, E. Hrouzek, P., Řeháková, K., Šabacká, M., Stibal, M., Caisová, L., Komárková, J. & Lukešová, A. *Folia Microbiologica* 53 (4): 333-341 (2008).

The effect of temperature, light and nutrient composition on morphological traits was examined in seven nostocacean cyanobacteria *(Anabaena planctonica, A. sphaerica* var. *conoidea, A. spiroides, Aphanizomenon gracile, Nostoc* sp., *Scytonema* sp., and *Tolypothrix* sp.). Their morphological variability was high; however, only some of the features showed changes reflecting varied growth conditions. The frequency of heterocyst occurrence decreased with increasing nitrogen concentration. Within the range studied, the effect of temperature on heterocyst frequency of *Tolypothrix* sp. and planktonic *Anabaena* strains could be fitted by a normal curve with a clear optimum while linear correlation was found in *Aphanizomenon gracile*. T- and S-type branching was observed in both *Scytonema* sp. and *Tolypothrix* sp. strains. T-type branching was found markedly dependent on nitrogen concentration. The abundance of necridic cells of *Tolypothrix* sp. increased linearly with temperature and light intensity. Regularity of trichome coiling of *A. spiroides* depended on culture medium suggesting that nutrient composition may be the main controlling factor. By contrast, the effect of the experimental conditions on the dimensions of vegetative cells and heterocysts was weak. Their variability was markedly higher within each experimental treatment than among treatments.

Vlivy teploty, světla a živin na morfologické vlastnosti byly studovány u s sedmi kmenů nostokálních sinic (*Anabaena planctonica, A. sphaerica* var. *conoidea, A. spiroides, Aphanizomenon gracile, Nostoc* sp., *Scytonema* sp., and *Tolypothrix* sp.). Morfologická variabilita těchto kmenů byla vysoká, avšak pouze některé z jejich morfologických znaků vykazovaly změny závislé na měnících se podmínkách prostředí. Frekvence výskytu heterocytů klesala s rostoucí koncentrací dusíku. V rozmezí experimentálních teplot použitých ve studii byla závislost frekvence heterocytů kmenu *Tolypothrix* sp. a planktonních kmenů *Anabaena* na teplotě fitována normální křivkou s jasným optimem, zatímco u kmenu *Aphanizomenon gracile* byla nalezena lineární korelace. U kmenů *Scytonema* sp. a *Tolypothrix* sp. bylo pozorováno jak větvení typu T, tak větvení typu S. Větvení typu T silně záviselo na koncentraci dusíku. Abundance nekroidních buněk kmenu *Tolypothrix* sp. vzrůstala lineárně s teplotou a světelnou intenzitou. Pravidelnost spiralizace vláken kmenu *A. spiroides* závisela na druhu použitého kultivačního média, což naznačuje, že hlavním řídícím faktorem by v tomto ohledu mohlo být živinové složení. Naopak vliv experimentálních podmínek na rozměry vegetativních buněk a heterocytů byl slabý. Jejich variabilita byla výrazně vyšší v rámci každé experimentální varianty, než mezi variantami navzájem.

Paper III

Experimental comparison of phenotypical plasticity and growth demands of two strains from the *Anabaena circinalis / A. crassa* complex (cyanobacteria)

Zapomělová, E., Hisem, D., Řeháková, K., Hrouzek, P., Jezberová, J., Komárková, J., Korelusová, J. & Znachor, P. *Journal of Plankton Research* (in press).

Two cyanobacterial strains were isolated in 2004 from different localities in the Czech Republic. Field morphology of the strain 04-26 (Jesenice reservoir) matched with the species description of *A. crassa* (Lemm.) Kom.-Legn. et Cronb. 1992, while the strain 04-28 (Hodějovický fishpond) was identified as *A. circinalis* Rabenh. ex Born. et Flah. 1888. Both of these strains, exposed to various experimental conditions (temperature, light intensity, nitrogen and phosphorus concentration), displayed highly similar morphologies and were able to span the morphological variability of both of the above-mentioned species. Significant relationships between environmental conditions (temperature, phosphorus) and morphological characteristics (vegetative cell and heterocyte dimensions, trichome coiling parameters) have been recorded for the first time within the genus *Anabaena*. The studied strains differed in their temperature and light growth optima and in secondary metabolite contents. However, both of them were identical (100% similarity) on the 16S rRNA gene sequence and showed 99.9–100% similarity to the published 16S rRNA sequences of *A. circinalis* strains from northern Europe.

V roce 2004 byly ze dvou různých lokalit v České republice izolovány dva kmeny sinic. Morfologie kmenu 04-26 (přehradní nádrž Jesenice) pozorovaná v přírodních podmínkách odpovídala popisu druhu *Anabaena crassa* (Lemm.) Kom.-Legn. et Cronb. 1992, zatímco kmen 04-28 (Hodějovický rybník) byl identifikován jako *A. circinalis* Rabenh. ex Born. et Flah. 1888. Oba tyto kmeny, když byly vystaveny různým experimentálním podmínkám (teplota, intenzita světla, koncentrace dusíku a fosforu), vykazovaly vysoce podobné morfologické vlastnosti a byly schopné pokrýt morfologickou variabilitu obou výše zmíněných druhů. Poprvé v rodě

Anabaena byly zjištěny signifikantní vlivy podmínek prostředí (teplota, fosfor) na morfologické charakteristiky (rozměry vegetativních buněk a heterocytů, parametry spiralizace vláken). Studované kmeny se lišily ve svých teplotních a světelných růstových optimech a v obsahu sekundárních metabolitů. Sekvence jejich 16S rRNA genu však byly identické (100% similarita) a vykazovaly 99,9–100% similaritu s publikovanými sekvencemi 16S rRNA genu kmenů *A. circinalis* ze severní Evropy.

Paper IV

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

Zapomělová, E., Řeháková, K., Jezberová, J. & Komárková J. (manuscript)

Plasticity of morphological features of eight planktonic *Anabaena* strains was studied under varied experimental conditions (temperature, light, nitrogen, phosphorus), with respect to the delimitation of single morphospecies. 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 proved 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 studied *Anabaena* strains were compared with 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, which have been also provided.

U osmi planktonních kmenů sinic rodu *Anabaena* byla studována plasticita morfologických znaků v různých experimentálních podmínkách (teplota, světlo, dusík, fosfor), a to s ohledem na vymezení jednotlivých morfotypů (druhů). Kmeny reprezentovaly všechny morfotypy se spiralizovanými vlákny, které jsou běžně nalézány ve střední Evropě (dva kmeny z morfologického komplexu *A. mendotae* & *A. sigmoidea*, dva kmeny *A. lemmermanni*, dva kmeny *A. flos-aquae* a dva kmeny z morfologického komplexu *A. circinalis* & *A. crassa*). Signifikantní vliv růstových podmínek na rozměry vegetativních buněk byl prokázán u sedmi z těchto kmenů a ve většině případů (u šesti kmenů) byla hlavním ovlivňujícím faktorem koncentrace P. Signifikantní vliv nějakého faktoru prostředí (P) na rozměry akinet byl zjištěn pouze u jednoho kmenu. Experimentálně stanovená teplotní a světelná optima byla specifická pro každý kmen a neodrážela taxonomické zařazení kmenů. Morfologické charakteristiky studovaných kmenů r. *Anabaena* byly srovnány s morfologickými znaky 61 populací r. *Anabaena* z České republiky, které příslušely k osmi různým morfotypům a byly pozorovány v přírodních podmínkách. Vymezení a jasný popis jednotlivých morfotypů jsou diskutovány s ohledem na částečné sekvence 16S rRNA genu studovaných kmenů, které jsou rovněž k dispozici.

Paper V

Polyphasic characterization of three strains of *Anabaena reniformis* and *Aphanizomenon aphanizomenoides* (cyanobacteria) and their re-classification to *Sphaerospermum* gen. nov. (incl. *Anabaena kisseleviana)*

Zapomělová, E., Jezberová, J., Hrouzek, P., Hisem, D., Řeháková, K. & Komárková J. *Journal of Phycology* (submitted)

Occurrences of rare cyanobacteria *Anabaena reniformis* Lemmermann and *Aphanizomenon aphanizomenoides* (Forti) Horecká et Komárek were recently detected at several localities in the Czech Republic. Two monoclonal strains of *An. reniformis* and one strain of *Aph. aphanizomenoides* were isolated from distant localities and different sampling years. They were characterized by a combination of morphological, genetic and biochemical approaches. For the first time, partial 16S rRNA gene sequences were obtained for these morphospecies. Based on this gene, all of these strains clustered separately from the other planktonic *Anabaena* and *Aphanizomenon* strains. They appeared in a cluster with *Cylindrospermopsis* Seenaya & Subba Raju and *Raphidiopsis* Fritsch & Rich, clustered closely together with two *An. kisseleviana* Elenkin strains available from GenBank. A new generic entity was defined (*Sphaerospermum* genus novum, with the type species *S. reniformis,* based on the traditional species *Anabaena reniformis*). These results contribute significantly to the knowledge base about genetic heterogeneity among planktonic *Anabaena* and *Aphanizomenon* morphospecies since this group has been regarded monophyletic, up until now. Accordingly, the subgenus *Dolichospermum,* previously proposed for the group of planktonic *Anabaena,* should be revaluated. Secondary metabolite profiles of the *An. reniformis* and *Aph. aphanizomenoides* strains differed considerably from 17 other planktonic *Anabaena* strains of eight morphospecies isolated from Czech water bodies. Production of puwainaphycin A was found in both of the *An. reniformis* strains. Despite the relatively short phylogenetic distance from *Cylidrospermopsis,* the production of cylindrospermopsin was not detected in any of our strains.

Výskyt vzácných sinic *Anabaena reniformis* Lemmermann and *Aphanizomenon aphanizomenoides* (Forti) Horecká et Komárek byl v posledních letech zaznamenán na několika lokalitách v České republice. Dva monoklonální kmeny *An. reniformis* a jeden kmen *Aph. aphanizomenoides* byly izolovány v různých letech z různých lokalit. Tyto kmeny byly charakterizovány za pomoci kombinace morfologického, genetického a biochemického přístupu. Vůbec poprvé byly pro tyto morfotypy sinic získány částečné sekvence 16S rRNA genu. Na základě tohoto genu klastrovaly všechny studované kmeny odděleně od všech ostatních planktonních kmenů r. Anabaena a Aphanizomenon. Objevily se v klastru společně s *Cylindrospermopsis* Seenaya & Subba Raju a *Raphidiopsis* Fritsch & Rich, velmi blízko dvěma kmenům *An. kisseleviana* Elenkin, jejichž sekvence 16S rRNA genu byly získané z databáze GenBank. Byl definován nový rod *(Sphaerospermum* genus novum, s typovým druhem *S. reniformis,* založeným na tradičním druhu *Anabaena reniformis*). Tyto výsledky přinášejí nové informace k poznání genetické heterogenity mezi planktonními morfotypy sinic r. *Anabaena* a *Aphanizomenon*, neboť tato skupina byla dosud považována za monofyletickou. Podrod *Dolichospermum*, který byl již dříve navržen pro skupinu všech planktonních sinic r. *Anabaena*, bude tedy muset být přehodnocen. Profily sekundárních metabolitů studovaných kmenů *An. reniformis* a *Aph. aphanizomenoides* se výrazně lišily od profilů dalších 17 planktonních kmenů r. *Anabaena* osmi morfotypů, izolovaných z České republiky. Navzdory relativně krátké fylogenetické vzdálenosti našich kmenů od *Cylindrospermopsis* nebyla u žádného z nich zjištěna produkce cylindrospermopsinu.

Paper VI

Phenotypic and genotypic diversity of planktonic *Anabaena*-like cyanobacteria in fishponds and reservoirs of the Czech Republic

Zapomělová, E., Jezberová, J., Řeháková, K., Hrouzek, P., Soldati, E., Hisem, D., Znachor, P., Komárková, J. & Ventura, S. (manuscript)

Planktonic cyanobacteria from the genus *Anabaena* and related morphospecies were studied using a combination of morphological, molecular and biochemical approaches. Both the field morphologies and morphological features under the culture conditions were described in 45 strains of 13 morphospecies *(Anabaena affinis, An. compacta, An. circinalis, An. crassa, An. curva, An. flos-aquae, An. lemmermannii* var. *lemmermannii, An. lemmermannii* var. *minor, An. mendotae, An. reniformis, An. sigmoidea, An. spiroides,* and *Aphanizomenon aphanizomenoides)* and compared with 24 other *Anabaena* populations observed in the field. Partial 16S rRNA gene sequences (1340 bp) have been compared among 33 selected strains. *An. circinalis* and *An. crassa* strains clustered together in the phylogenetic tree and another separate tight cluster was formed by *An. mendotae* and *An. sigmoidea* strains. Most *An. lemmermannii* strains appeared in another distinct cluster. However, three *An. lemmermannii*-like strains, which demonstrated only occasional dominance of akinetes adjacent to heterocytes in batch cultures, appeared in the cluster of *An. mendotae* and *An. sigmoidea*. *An. flos-aquae* strains were spread throughout the whole planktonic-*Anabaena* section of the phylogenetic tree based on 16S rRNA gene. Present study confirmed previously reported clear morphological and molecular delimitation of *An. compacta,* as well as the separation of *An. reniformis* and *Aph. aphanizomenoides* (previously reclassified into a new genus *Sphaerospermum).* Preliminary ARDRA of 16S rRNA gene plus ITS regions of the strains supported these findings. Secondary metabolite contents have been determined for 20 selected strains using HPLC-MS analysis. The secondary metabolite profiles were consistent neither with morphological, nor phylogenetic affiliation of the strains and also did not reflect geographical origin of the strains and trophic status of the original sampling localities.

Planktonní sinice r. *Anabaena* a příbuzných morfotypů byly studovány pomocí kombinace morfologického, molekulárně-biologického a biochemického přístupu. Morfologické charakteristiky v přírodních podmínkách i během kultivace byly popsány u 45 kmenů příslušejících k 13 různým morfotypům (*Anabaena affinis, An. compacta, An. circinalis, An. crassa, An. curva, An. flos-aquae, An. lemmermannii* var. *lemmermannii, An. lemmermannii* var. *minor, An. mendotae, An. reniformis, An. sigmoidea, An. spiroide,* a *Aphanizomenon aphanizomenoides)* a byly porovnány s 24 dalšími populacemi r. *Anabaena* pozorovanými v přírodě. Částečné sekvence 16S rRNA genu (1340 bp) byly srovnány u 33 vybraných kmenů. Kmeny *An. circinalis* a *An. crassa* se ve fylogenetickém stromečku objevily ve společném klastru a další oddělený a soudržný klastr byl tvořen kmeny *An. mendotae* a *An. sigmoidea*. Většina kmenů *An. lemmermannii* se nacházela v dalším jasně vymezeném klastru, avšak tři kmeny připomínající morfotyp *An. lemmermannii* s pouze příležitostnou dominancí akinet přilehlých k heterocytům se objevily v klastru morfotypů *An. mendotae* a *An. sigmoidea*. Kmeny *An. flos-aquae* byly rozptýlené po celé části fylogenetického stromečku 16S rRNA genu zahrnující planktonní *Anabaena*. Tato studie potvrdila již dříve zjištěné jasné morfologické i molekulární vymezení morfotypu *An. compacta*, stejně jako oddělení *An. reniformis* a *Aph. aphanizomenoides* (dříve reklasifikováno do nového rodu *Sphaerospermum*). Předběžná analýza ARDRA úseku 16S rRNA genu a přilehlého ITS u studovaných kmenů byla v souladu s těmito zjištěními. U 20 vybraných kmenů byl metodou HPLC-MS stanoven obsah sekundárních metabolitů. Profily sekundárních metabolitů nebyly v souladu ani s morfologickými charakteristikami kmenů, ani s jejich fylogenetickou příslušností, a rovněž tak nereflektovaly geografický původ kmenů či trofii původních odběrových lokalit.

General discussion, conclusions and perspectives

The submitted dissertation deals with 13 planktonic morphospecies of *Anabaena*-like cyanobacteria: *Anabaena affinis* Lemmermann 1897*, An. compacta* (Nygaard) Hickel 1985*, An. circinalis* Rabenhorst ex Bornet et Flahault 1888*, An. crassa* (Lemmermann) Komárková-Legnerová et Cronberg 1992*, An. curva* Hill 1976*, An. flos-aquae* (Lyngbye) Brébisson ex Bornet et Flahault 1888*, An. lemmermannii* var. *lemmermannii* Richter 1903*, An. lemmermannii* var. *minor* (Utermoehl) Komárková-Legnerová 1988*, An. mendotae* Trelease 1889*, An. reniformis* Lemmermann 1898 (newly suggested the reclassification into *Sphaerospermum reniformis* (Lemmermann) Zapomělová et al. submitted), *An. sigmoidea* Nygaard 1949*, An. spiroides* Klebahn 1895*,* and *Aphanizomenon aphanizomenoides* (Forti) Horecká et Komárek 1979 (newly *Sphaerospermum aphanizomenoides* (Forti) Zapomělová et al. submitted). This dissertation represents the first polyphasic assessment of planktonic *Anabaena* diversity in the Czech Republic. The interconnection of the knowledge on natural *Anabaena* morphologies and the response of single morphometric features to varying environmental factors is unique, providing new basis for the discussion on *Anabaena* morphospecies delimitation.

Principal component analysis (PCA) of morphometric features of 61 natural populations (65 populations incl. *Sphaerospermum* morphospecies and two additional *An. lemmermannii* populations in **paper VI)** has demonstrated that only larger groups of *Anabaena* morphospecies are reliably distinguishable in phytoplankton communities on the basis of their morphology **(paper I).** The groups identified by the PCA were: *Anabaena mendotae* & *An. sigmoidea* complex, *An. flos-aquae* & *An. spiroides* & *An.* cf. *curva* complex, and *An. circinalis* & *An. crassa* complex.

The only up to now clearly defined morphospecies were found *An. compacta* and *Sphaerospermum* spp. (*An. reniformis* and *Aph. aphanizomenoides*). *An. compacta* was characterized by its width of trichomes, subspherical shape of akinetes remote from heterocytes, and the density of its filament coiling. The delimitation of *An. compacta* was also supported by its clustering based on 16S rRNA gene and was in a good agreement with previously published findings of Rajaniemi et al. (2005 a, b). *Sphaerospermum* morphospecies were characterized by the width and shape of their vegetative cells, and by the position of their spherical or subspherical akinetes next to the heterocytes. The clear delimitation of the *Sphaerospermum* morphospecies was supported by their 16S rRNA sequences, which were considerably different from all other planktonic *Anabaena* strains **(papers V and VI)**

Special attention was paid to *An. lemmermannii.* The validity of the taxon was challenged in **paper I,** since the only identifying feature was the position of akinetes next to the heterocytes, while the shape and dimensions of vegetative cells highly varied among various *An. lemmermannii* strains **(papers I and VI;** previously pointed out by Komárková 1988). Nevertheless, the genetic cluster D **(paper VI),** comprising five *An. lemmermannii* strains studied (04-24, 04-38, 04-42 05-02, 08-01), appears to be clearly separate and corresponds exactly with the main *An. lemmermannii* cluster of Gugger et al. (2002 a) and Rajaniemi et al. (2005 a, b). Thus for present, *An. lemmermannii* appears to be a valid taxon. Its reliable identification is, however, hampered by the occasional predominance of akinetes adjacent to heterocytes that has been proved in several strains from different genetic cluster B of *An. mendotae* & *An. sigmoidea* complex **(papers IV and VI).** Based on our measurements of morphometric parameters of various *An. lemmermannii* and *An. mendotae / An. sigmoidea* strains, we have suggested additional morphometric criteria for distinguishing cyanobacteria of these two genetic clusters. Majority of the *An. lemmermannii* strains from cluster D displayed vegetative cells wider than 5 μm and longer than 7 μm, and akinetes shorter than 20 μm, while the situation was inverse within the cluster B of *An. mendotae / An. sigmoidea* **(paper VI).** However, the reliability of these criteria should be verified by thorough analyses of numerous *An. lemmermannii* and *An. mendotae / An. sigmoidea* strains from different parts of the world.

Laboratory experiments aimed at the effects of environmental factors on *Anabaena* morphologies **(papers II, III, and IV)** supported our hypothesis of larger morphospecies complexes within the genus *Anabaena.* Majority of the investigated strains exhibited high variability of vegetative cell dimensions related to changing growth conditions, mainly phosphorus concentration, but sometimes also temperature or light intensity **(paper IV).** This finding may have also taxonomic consequences, since the dimensions of vegetative cells are quoted to be important distinguishing criteria between the morphospecies *An. circinalis* and *An. crassa*, *An. mendotae* and *An. sigmoidea*, and *An. flos-aquae* and *An. spiroides* (Komárek 1958, Komárková-Legnerová & Cronberg 1992, Komárková-Legnerová & Eloranta 1992, Komárek 1996, Komárek & Zapomělová 2007). The responses of morphologies to the environmental factors were more or less consistent in both strains from the *An. circinalis* & *An. crassa* complex **(paper III)**, but appeared to be strain-specific, when we compared all of the studied *Anabaena* strains **(papers II and IV).** General conclusions are therefore hardly deducible.

Two strains of *An. circinalis* & *An. crassa* complex, exposed to varying experimental conditions, were shown to span the morphological variability of both *An. circinalis* and *An. crassa,* as they were originally described (Komárek 1958, Komárková-Legnerová & Cronberg 1992, Komárková-Legnerová & Eloranta 1992; **papers III and IV).** This finding was supported by our previous results on continuous transitions of vegetative cell width and trichome coil diameters observed in 13 An. *circinalis / An. crassa* populations in the field **(paper I)**, and also by the molecular analyses **(papers III, IV, and VI).**

Our results revealed enormous genotypic heterogeneity of the cyanobacteria from *An. flos-aquae* complex **(paper VI)**, which has been already indicated by Rajaniemi et al. (2005 a, b). Six of the eight *An. flos-aquae*-like strains, whose 16S rRNA gene was sequenced, were intermixed with *An. circinalis* / *An. crassa* strains in cluster A, while two other *An. flos-aquae*-like strains (04-40 and 04-19) appeared separately, each of them in different cluster (clusters D and E; **paper VI).** Different phylogenetic affiliations of the strains 04-40 and 04-19 were not clearly supported by their morphologies, since they matched exactly with the characteristics given for *A. flos-aquae* (Geitler 1932, Komárek 1996). Thus, the delimitation of the taxon *An. flos-aquae* appears to be rather complicated and thorough taxonomic revision of this cyanobacterial complex is highly required for the future.

The *Sphaerospermum* cluster **(papers V and VI)** represents a newly discovered group of planktonic *Anabaena*-like cyanobacteria, which has never been reported before. Our strains of *An. reniformis* and *Aph. aphanizomenoides* clustered into a separate, highly supported cluster with *An. kisseleviana* strains from Genbank. This clade was located markedly apart from all other planktonic *Anabaena* strains, and with high bootstrap support clustered close to the *Cylindrospermopsis* and *Raphidiopsis* clusters, reported by Saker & Neilan (2001) and Li et al. (2008). Consequently, a new generic entity *Sphaerospermum* was defined, with the type species *S. reniformis,* based on the traditional species *Anabaena reniformis* **(paper V).** These results contributed significantly to the knowledge base about genetic heterogeneity among planktonic *Anabaena* and *Aphanizomenon* morphospecies since this group has been regarded monophyletic, up until now. Accordingly, the subgenus *Dolichospermum,* previously proposed for the group of planktonic *Anabaena* (Rajaniemi et al. 2005 b), should be revaluated. The clustering of *An. reniformis* with coiled filaments close to *An. kisseleviana* and *Aph. aphanizomenoides* with straight filaments supported the previous indications that the occurrence of trichome coiling does not reflect the phylogeny (Rajaniemi et al. 2005 b). *Anabaena* strains with coiled and straight trichomes, but of similar morphometric characteristics, often appear in the same clusters in phylogenetic trees, based on 16S rRNA gene sequences (Beltran & Neilan 2000, Rajaniemi et al. 2005 a, b).

Our studies on *Spherospermum* **(paper V)** provided also important data on the occurrence and distribution of *An. reniformis* and *Aph. aphanizomenoides*. Scanty records exist on the occurrence of *An. reniformis* around the world. It appears to be sparsely distributed, at very distant localities: Germany, Ukraine, Japan, Cuba, and Africa (Lemmermann 1898, Aptekar ex Elenkin 1938, Watanabe et al. 2004, Komárek 2005, Cronberg & Annadotter 2006, Komárek & Zapomělová 2007), and it has been reported only three times from the Czech Republic so far (Keršner 1997). *Aph. aphanizomenoides* have predominantly been described from the tropical and subtropical regions, but within the last few years it seems to have expanded to the temperate zones of Central Europe such as Hungary (Padisák & Kovács 1997), Slovakia (Hindák 2000), Poland (Stefaniak & Kokociński 2005), and northeast Germany (Stüken et al. 2006). Our paper V is the first report on its occurrence in the Czech Republic in almost 30 years, since it was reported by Horecká & Komárek (1979) from central Moravia.

Neither the HPLC-MS spectra of secondary metabolites, nor the temperature and light preferences matched with the phylogenetic and morphospecies affiliations of our strains **(papers III, IV and VI)**. These findings were consistent with published results on discrepancies between phylogenetic features and protein or toxin production (Palinska et al. 1996, Bolch et al. 1999, Iteman et al. 2002). Rapala and Sivonen (Rapala & Sivonen 1998) indicated that the differences in growth rates of various *Anabaena* strains as a function of light and temperature are probably strain-specific. Our results even demonstrated considerable differences of temperature and light preferences in various strains of the same morphospecies **(paper III, IV)**.

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