

Problem of the taxonomic category “species” in cyanobacteria

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With 6 figures and 2 tables in the text

Abstract: In Cyanobacteria (cyanophytes, cyanoprokaryotes) “species” has been traditionally defined on the basis of morphological characters (“Geitlerian system”) and these same criteria are still commonly used for identification of natural populations. Modern investigations, based on EM-methods and molecular sequencing have proven the differences between the genotypes which correspond more or less with the traditional phenotypic genera (revised after re-evaluation of morphological characters). However, within genotypes enormous variability of natural morphotypes and ecotypes has been found and the same diversity has been confirmed in strains in cultures. Genotypically distinct species have not been confirmed, and the separation of them according to morphological criteria is problematic with respect to wide morphological variability. – Because the purely traditional (morphological) concept of cyanobacterial species is frequently in disagreement with modern knowledge of cyanobacteria, the criteria for species delimitation should be changed. Taxonomic classification is always conventional; the definition of “species” in cyanobacteria is possible, if it will be based on rules developed in concordance with modern knowledge of morphology (phenotype variability and stability of different features), physiology, ecology and molecular bases of individual morpho- and ecotypes (e.g. morphospecies belonging to proved and distinct genotypes and characterised by ecological characterization).

Key words: Cyanoprokaryotes, cyanobacteria, taxonomy, nomenclature, genus delimitation, species problem.

Introduction

Taxonomic classification is always conventional to a certain degree, but necessary for orientation in natural diversity. Species is traditionally considered as the basic taxonomic category for all organisms, however this taxon is problematic in cyanobacteria (cyanoprokaryotes, cyanophytes) in the light of modern classification criteria.

The traditional classification of cyanophytes was based on morphology and phenotypic variation as in other groups of all phototropic organisms (from simple algae to vascular plants). The variable and sometimes unstable criteria like cell-shape and cell-size, width of filaments and presence of sheaths, were often over-estimated. In addition, taxa based on several selected impressive characters were found to be heterogeneous and not well delimited. For example, the traditional complex of the genera *Oscillatoria/Phormidium/Lyngbya* is based on the frequency of sheaths, and does not correspond to molecular data; therefore it has to be drastically re-evaluated.

The modern taxonomy of cyanoprokaryotes was substantially influenced by the recent application of the following methodological approaches:

- Paleobotanical research: Cyanoprokaryotes developed in Early Precambrian and have survived up to now without substantial change in their common appearance and vitality. The numerous morphological forms, which occur during the long existence of these microorganisms, indicate the continual occurrence of similar repeated morphotypes, but not the existence of unchanged genotypes. Instead they support the theory of the special mutual and continual diversification strategy (“static evolution” of SCHOPF and GOLUBIĆ in PENNISI 1994).
- Phenotype (morphological) approach: Widely inducible variation, dependent on environmental conditions and variability within genotypes was recognised. This indicates that the importance and stability of several phenotype characters must be re-evaluated.
- Ecological investigations: The species (morpho- and ecotypes) are always ecologically delimited, and therefore ubiquitous species do not exist. Certainly, the ecological demands and ecophysiological characters must be a part of a taxonomic evaluation. The geographic distribution of species depends on the distribution of corresponding environmental conditions.
- Electron microscopy: This technique has explained numerous enigmatic characters of cyanobacteria and has yielded a great deal of stable phenotypic features. Electron microscopy helps also to elucidate phylogenetic relationships between cyanobacterial types on all taxonomic levels.
- Molecular approach: Information provided by molecular sequencing and DNA/DNA hybridization has become the basic criteria for re-evaluation of the cyanobacterial classification system at all taxonomic levels.

Molecular background

Taxonomic classification should be in agreement with phylogenetic relations. Recent studies have found surprising coincidences between traditional genera (limited to “small” phenotype genera re-evaluated, e.g., by ANAGNOSTIDIS & KOMÁREK 1988 and KOMÁREK & ANAGNOSTIDIS 1989, 1998) and clusters derived from molecular sequencing (Fig. 1).

Numerous genera defined by combined molecular and traditional morphological evaluation appear also in the most recent edition of Bergey’s Manual

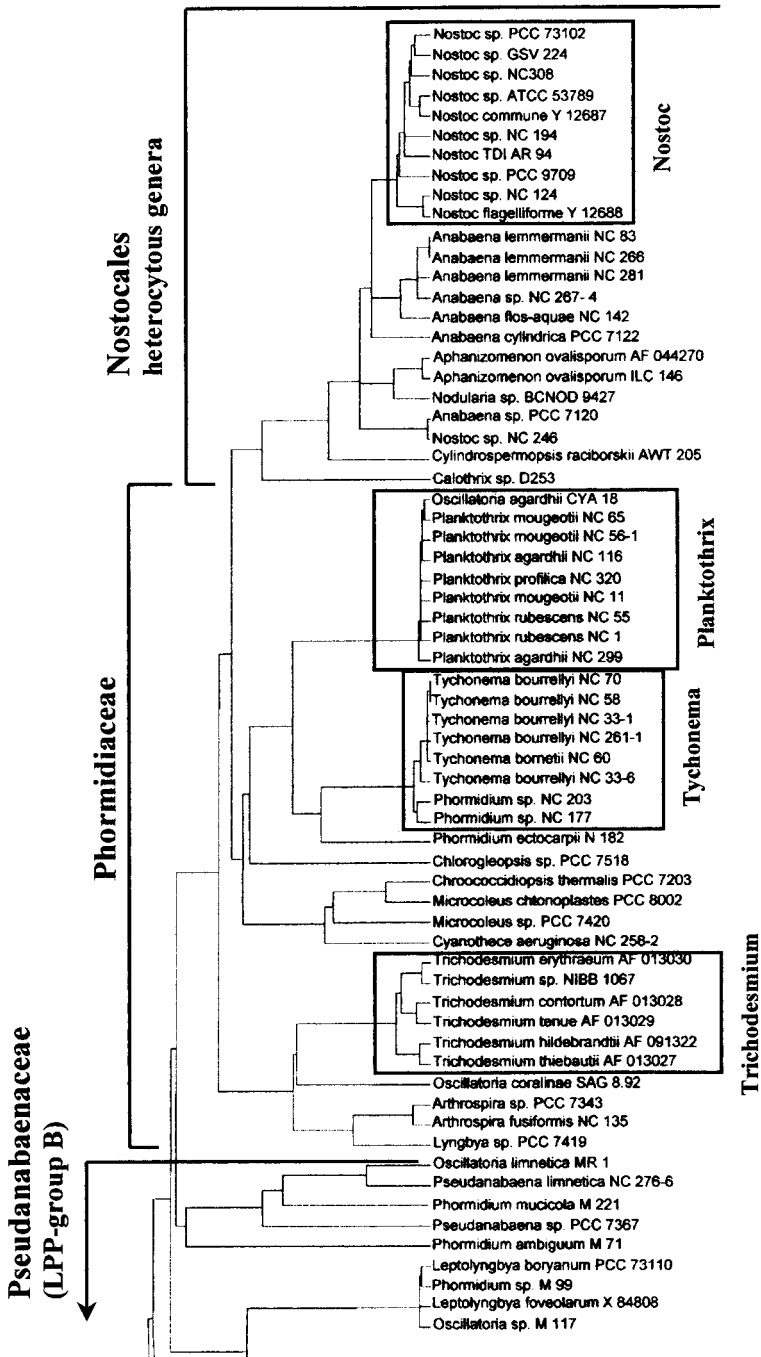


Fig. 1. Part of the phylogenetic tree derived by Megalign from GeneBank of NCBI with separated genera (*Nostoc*, *Anabaena*, *Planktothrix*, *Tychonema*, *Trichodesmium*, *Arthrospira* and others).

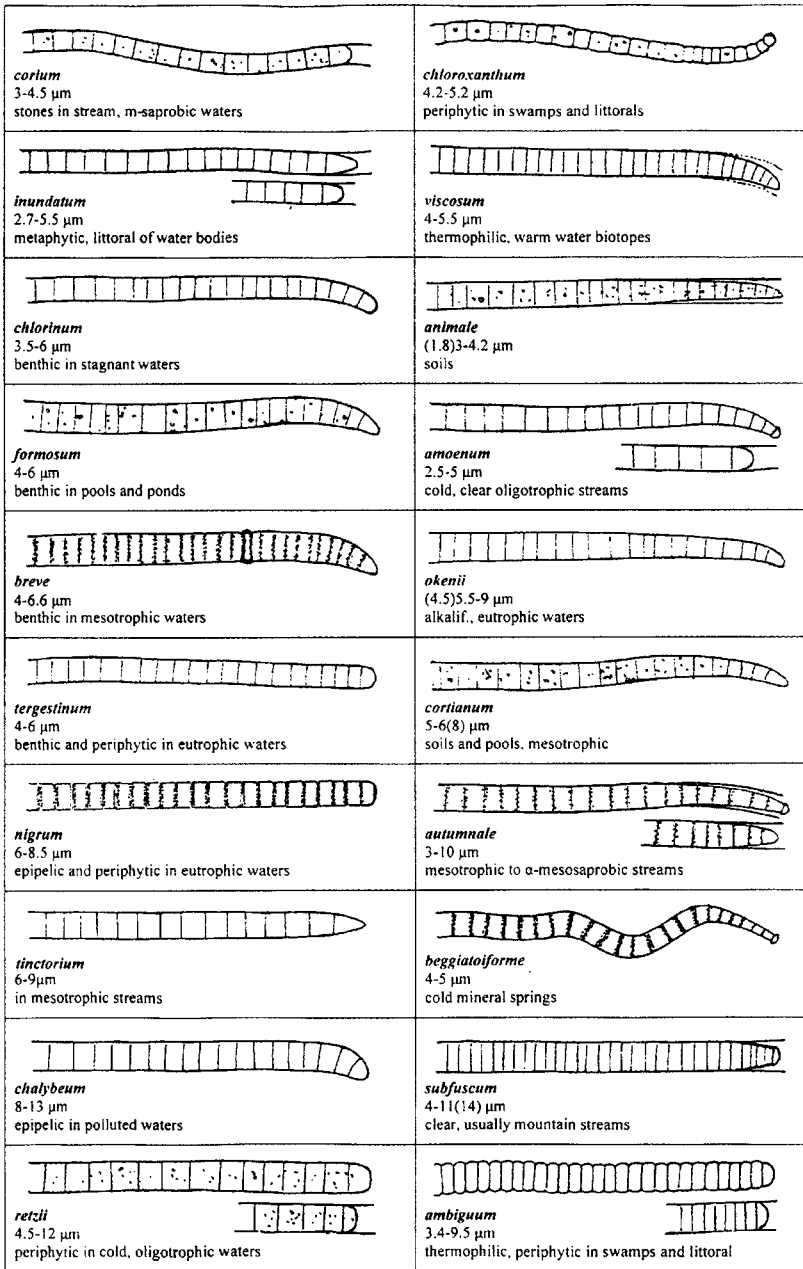
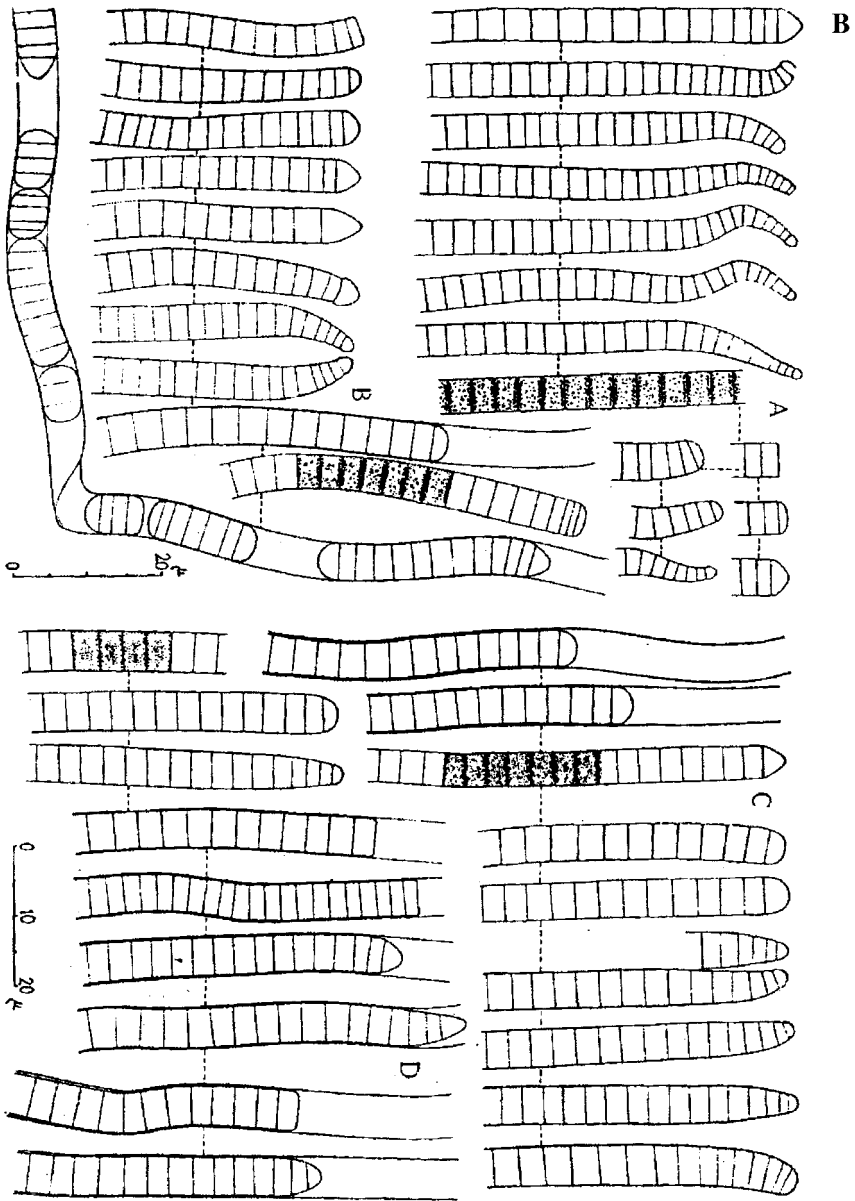


Fig. 2. A – Review of main traditional *Phormidium* species (after KOMÁREK 1988). B – Variability of *Phormidium autumnale* from one locality (after KANN & KOMÁREK 1970).



(CASTENHOLZ ed. 2001). All cases in which phenotypic evaluations disagree with molecular evaluations must be complexly revised. Further revision of existing traditional genera (separation of “new” genotypes – genera, or unification of related types) is also necessary. We can conclude, that genetic characters derived from molecular 16S rRNA sequencing are (or should be) in agreement with phenotypic markers used for modern definitions of traditional genera (Figs 1, 3, 5).

Problems arise with classification of cyanobacteria on subgeneric level, specifically with species concept. The traditional morphological features used exclusively in the characterization of infrageneric diversity, such as size and shape of cells, presence of sheaths, pigmentation, and type of branching have been found frequently unstable and variable. An example of wide morphological variation within one genotype is in the simple and polymorphic genus *Phormidium*, which has variable traditional species that are recognizable only if they are developed in typical morphotypes (Fig. 2). From the recent results follows (e.g., CASTENHOLZ 2001), that the species delimitation by traditional morphological criteria has not been commonly supported by molecular sequencing. This conclusion was accented perhaps also due to the omission of ecophysiological specificity of taxonomic units in traditional taxonomy. Morphological, ecophysiological, biochemical and molecular analyses have demonstrated, that the enormous number of single deviations and/or wide variability at the strain-level within each genetically stable genotype (genus) prohibits the definition and delimitation of suitable clusters (species). Good examples of this infrageneric diversity can be found in the studies of KONDRATEVA (1968) about *Microcystis* from Ukraine, KOMÁREK (1972) about *Phormidium autumnale* strains, KOHL & NICKLISCH (1981) about *Limnothrix* (“*Oscillatoria*”) *redekei* from one lake near Berlin, Germany, WATERBURY et al. (1986) about *Synechococcus/Cyanobium* populations from North Atlantic, WATERBURY & RIPPKA (1989) about *Synechococcus*-like cyanobacteria, KATO et al. (1991) about *Microcystis* from central Japan (Fig. 4), and BARKER et al. (2000) about *Nodularia spumigena* from Baltic Sea, etc. These numerous and geographically widespread examples caused several authors to be sceptical in the definition of any species in the case of cyanobacteria (OTSUKA et al. 2000). CASTENHOLZ (2001) avoids this category arguing that genetic comparisons are necessary in order to characterize species of cyanobacteria (“it is much too early characterise and catalog species of cyanobacteria” and “all of ... classifications will have to be revised almost completely when genetic comparisons become available”; p. 480). Numerous other authorities hesitate to use “species” in cyanobacteria without molecular support.

Natural diversity

In contrast to the above described variability within all genetically delimited genera, there are found in nature recognisable distinct deviations (morphotypes). These are stable, can occur sometimes in different areas, and persist for years in biotopes with more or less stable or slowly changing ecological conditions.

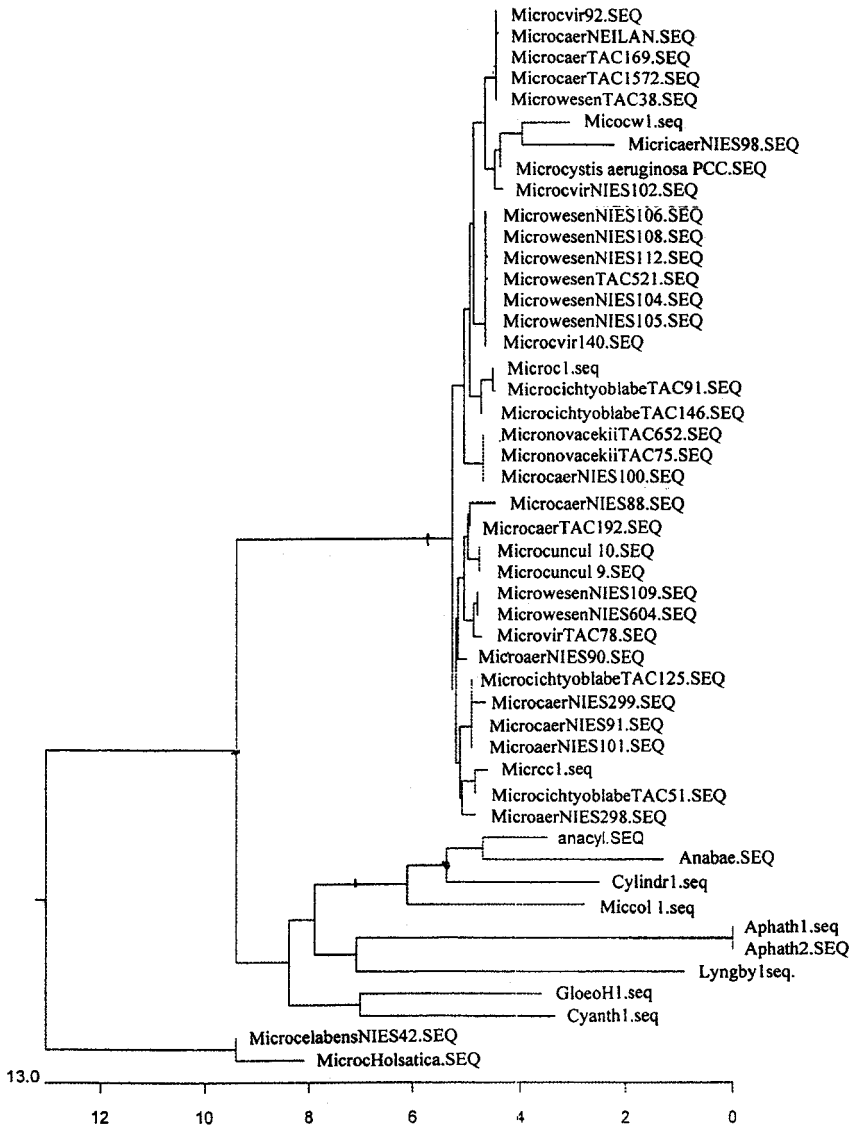


Fig. 3. Part of a phylogenetic tree of cyanobacteria concerning the genus *Microcystis*, derived by Megalign from GeneBank of NCBI.

Frequently undetectable by the standard genetic methods (16S rRNA sequencing), these morphotypes represent coherent clusters of variable strains as described in the previous subchapter, and can be characterised by other stable ecophysiological, biochemical or cytomorphological features (*Aphanothece stagnina*, *Anabaena crassa*, *Aphanizomenon flos-aquae*, *Nostoc commune*, *Gloeotrichia echinulata*, *Geitleria calcarea* among others). The genetic coding of these stable eco- and

morphotypes is evidently more complicated, connected with different segments of genomes, and can not be detected by the uniform method of 16S rRNA sequencing. Elucidation of this type of cyanobacterial diversity is at the beginning, and the question arises, if the genetic approach is the best for identification of cyanobacterial entities in nature. It does not exist the reason, why not to use other stable determination criteria (conform with genetic markers) to practical classification of natural populations.

Examples of several genera:

1. The genus *Microcystis* (Figs 3, 4, Tab. 1)

Following recent phenotype taxonomic revision (which eliminated not-aerotopated morphospecies “*M. holsatica*”, “*M. elabens*”, “*M. incerta*”, etc.), the genus *Microcystis* represents distinct and delimited genotype cluster comprising only planktic species with gas vesicles in cells (Fig. 3). However, the genetic differences between morphospecies within the genus are very small and transient, and do not allow for the delimitation of species. The whole genus comprises an endless number of small deviations (Fig. 4), not coinciding with traditional morphospecies (KONDRATEVA 1968, KATO et al. 1991, OTSUKA et al. 2000, 2001). However, several distinct morphospecies occur repeatedly in eutrophic freshwaters (e.g., in Table 1, *M. viridis* and *M. wesenbergii*). These morphospecies are more or less stable (KOMÁREK 2002), and additionally characterised by their life cycles (REYNOLDS et al. 1981, KOMÁREK et al. 2002) and sometimes by ecological demands. The morphospecies must be therefore related to segments of the genome other than those highlighted by 16S rRNA sequencing.

2. The genus *Planktothrix* (Figs 1, 5, Tab. 2)

The similar situation occurs in the genus *Planktothrix* as in *Microcystis*. Genetic uniformity within the genus (Fig. 1) is not in agreement with analyses of natural populations, from which several morpho- and ecospecies have been characterised (SKULBERG & SKULBERG 1985 under *Oscillatoria*, ANAGNOSTIDIS & KOMÁREK 1988, Tab. 2). Several authors consider the main traditional species (especially *P. agardhii* and *P. rubescens*) to be conspecific (BEARD et al. 1999) due to genetic similarity. However, SUDA et al. (2001) recently recognised several taxonomic types on the bases of combined ecophysiological and genetic markers, which correspond well with traditional species (Fig. 5).

3. The genus *Anabaena* (Figs 1, 6)

The genus *Anabaena* is obviously heterogeneous. The genus is comprised of several genetically distinguishable clusters: planktic types with gas vesicles and living mainly in solitary trichomes (type *Neospira* = *Anabaena* subg. *Dolichospermum*); benthic mat-forming types (typical *Anabaena*); the recently re-defined genus *Trichormus* with special strategy of akinete formation; the groups of transitional *Anabaena/Aphanizomenon* morphospecies with narrowed trichome ends

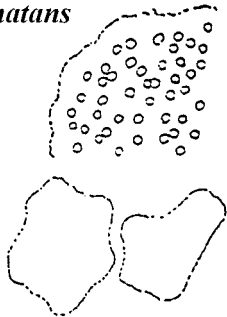
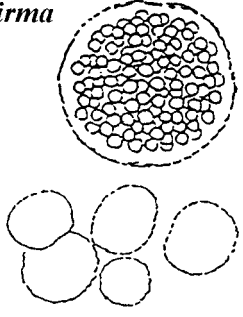
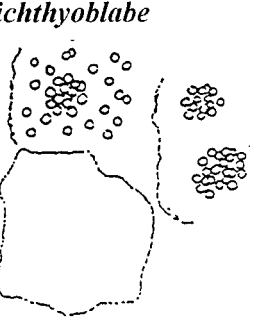
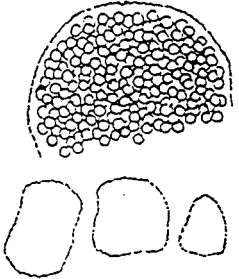

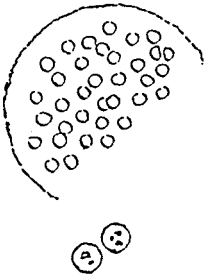
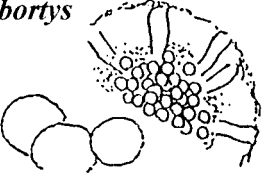
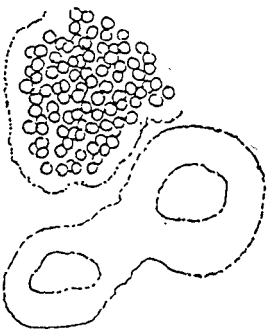
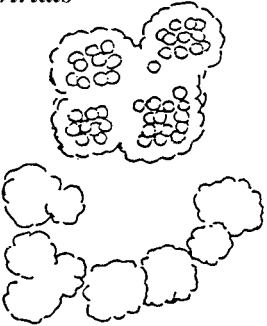
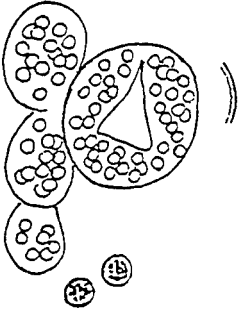
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<p><i>natans</i></p> 	<p><i>firma</i></p> 	<p><i>ichthyoblabe</i></p> 
$\varnothing B = > 3 \mu\text{m}$		
<p><i>flos-aquae</i></p> 	<p><i>novacekii</i></p> 	<p><i>smithii</i></p> 
	<p><i>bortys</i></p> 	
<p><i>aeruginosa</i></p> 	<p><i>viridis</i></p> 	<p><i>wesenbergii</i></p> 

Table 1. Main distinct morphotypes of the genus *Microcystis* occurring repeatedly in European stagnant waters. (From KOMÁREK 2001)

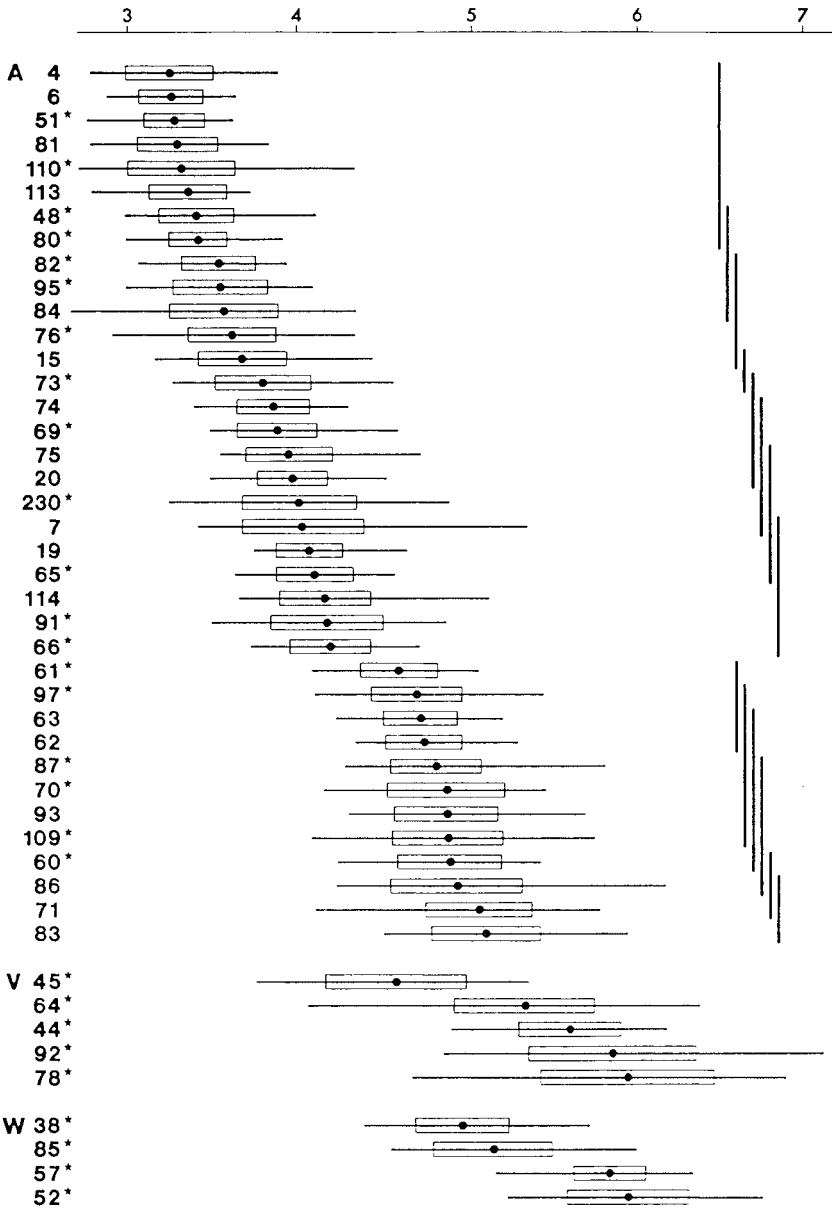


Fig. 4. Intraspecific variation in *Microcystis* strains (cell size) from stagnant waters in central Japan; **A** = *M. aeruginosa*-complex, **V** = *M. viridis* cluster, **W** = *M. wesenbergii* cluster (after KATO et al. 1991).

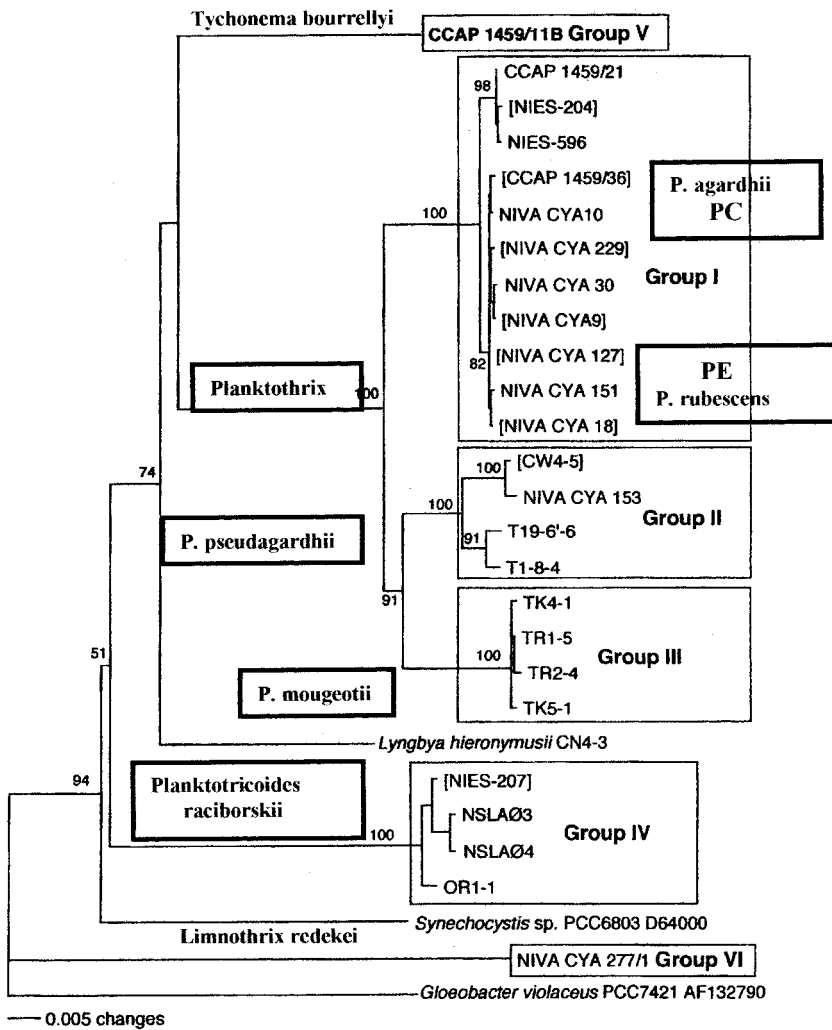


Fig. 5. Part of phylogenetic tree of cyanobacterial genus *Planktothrix* combined with biochemical and ecophysiological characters (after SUDA et al. 2002).

(types of *Aphanizomenon gracile*, *Anabaena recta* and others); the *Aphanizomenon*-like types with pointed apical cells (type of *Aphanizomenon issatschenkoi*). This variability demands separation on the generic level. Thus, the main two “*Anabaena*-types” (planktic as solitary trichomes, and benthic in mats) represent two genetic clusters, and they both contain many subgeneric stable morphotypes, recognisable in natural populations. The stability of the individual morphotypes from the “planktic cluster” has been documented and reviewed several times in modern studies (WATANABE, M. 1971, LI & WATANABE 1998, LI et al. 1998, KOMÁREK 1999, LI & WATANABE 2001).

Table 2. List of main morphospecies of the genus *Planktothrix*.

Species	Distribution	Trichome width [µm]	Note
<i>zahidii</i>	Pakistan	2.5–3.5	straight trichomes
<i>suspensa</i>	temperate zones	2.4–4.0	calyptrate
<i>prolifica</i>	northern Europe, central North America	2.0–5.8	red (“red agardhii”), calyptrate
<i>clathrata</i>	northern parts of temperate zone	5.6–6.0	cylindrical trichomes, constricted
<i>agardhii</i>	temperate and tropical zone	(2.8?)4.0–6.0(9.5?)	narrowed ends, calyptrate
<i>cryptovaginata</i>	eastern Europe	(4.0)5.0–8.5	facultative sheaths
<i>rubescens</i>	central and north Europe, central North America	(4.0)6.0–8.2(9.0)	red, calyptrate
<i>mougeotii</i>	cosmopolitan (excl. polar regions)	(5.0)5.5–9.7(10?)	cylindrical ends
<i>planctonica</i>	eastern Europe	8.0–10.0	slightly coiled, cylindrical trichomes
<i>geitleri</i>	scarcely in tropical and southern temperate zone	(6.0)7.0–10.0(11.0)	narrowed straight ends
<i>raciborskii</i>	eastern Asia, tropical and subtropical Asia, Africa	6.0–9.0(11.0)	narrowed and curved ends

Conclusions

(Possible generic and species concepts)

It is possible to summarise that genotypic characterisation of genera by 16S rRNA sequencing can be (and should be) commonly accepted, and corresponds almost perfectly to revised morphological traditional genera. For numerous genera revised according to current phenotypic criteria (*Stania*, *Pseudanabaena*, *Planktothrix*, *Limnothrix*, *Leptolyngbya*, *Geitlerinema*, *Tychonema* and others) there exist corresponding genetic markers. These characterizations are robust enough to be accepted in Bergey’s Manual (CASTENHOLZ 2001), and this concept of genera can be now a good basis for taxonomic, ecological, as well as molecular research in cyanobacteria. However, numerous current genera will have to be divided into several other genotypic units on the generic level, following further elucidation of the spectrum of cyanobacterial genera. The definition of new (small) generic taxa (clusters characterised by 16S rRNA analyses) is also expected, e.g., in heterogeneous genera *Leptolyngbya*, *Anabaena* and others (as an example see description of the genus *Planktothricoides* – SUDA et al. 2002).

The concept of the category “species” is more complicated. A subgeneric category is necessary for orientation in diversity and ecological (ecophysiological)

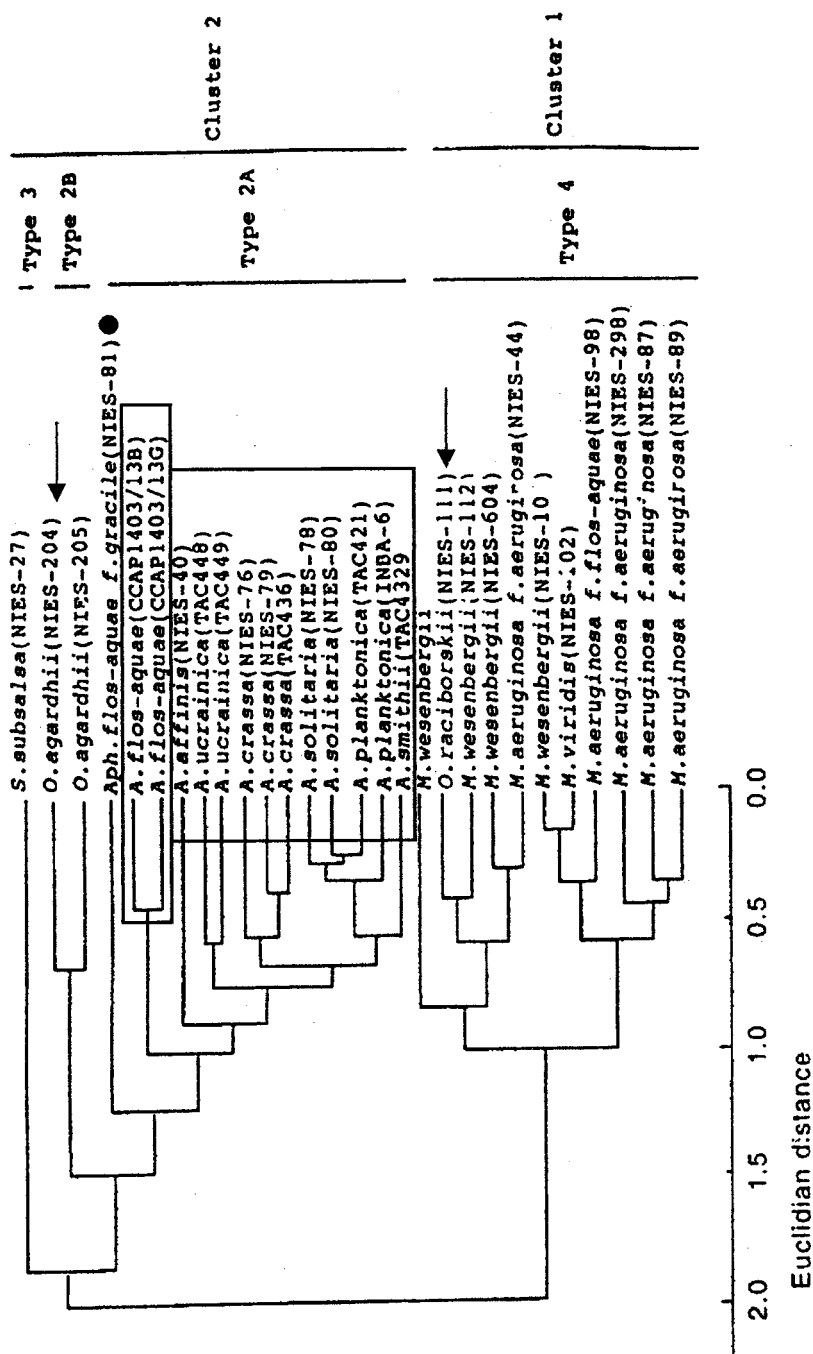


Fig. 6. Relationships among planktic *Anabaena* species on the bases of traditional taxonomic characters and non-polar fatty acids composition (after LI & WATANABE 1998, LI et al. 1998).

variability within genera – genotypes. The classification of developmental phases and steps between strains and stabilized genotypes (genera) is necessary also for the explanation of cyanobacterial evolution. Variable populations within a genus are able to be classified into clusters (species) only using combined biochemical, ecophysiological, ecological and partly morphological characters, not strictly in genetic terms. In order to define conventional species, we must respect together phenotype stability (in row of generations), ecological characters and genotype criteria as much as possible.

The definition of the taxonomic rank “species” in cyanobacteria is therefore unclear and a source of confusions. The problem also is, that the acceptance of “species” concept depends, to certain degree, on conventional agreement, and it is not possible to suppose that all cyanobacteriologists (both “experimental” and “field”) will be willing to consider any conventional proposal as acceptable. However, it is not possible to wait on the future satisfactory final solution. The continual work is necessary, and the more or less conventional unified concept of species, which enables to work also with literary data from past decades is highly desirable. There are now 3 main possibilities for species definitions in cyanobacteriology:

1. The use of currently described species (“Geitlerian concepts”) with restriction of descriptions of new species (morpho/ecospecies). This approach follows from the present unclear species concept, and it is based in principle on the incorrect thesis, that all cyanoprokaryotes are more or less ubiquitous, with cosmopolitan distribution, and mostly known and described. However, it omits the modern knowledge of ecology, modern EM and molecular data, and ignores the diversity of cyanobacteria in unknown biotopes. This approach causes many misinterpretations and mistakes in the literature.
2. The elimination of the taxonomic level “species”, because it is not yet possible to find satisfactory and uniform genetic criteria for this category. However, this proposal is probably not acceptable for ecologists, who need to be able to identify different eco- and morphotypes between taxonomic categories “genotypes” (genera) and “strains” (populations, respectively). Moreover, this simplified approach does not express satisfactorily the existing complicate diversity in nature. All slight stable deviations (or clonal strains) within genera (also inside of morphologically uniform populations) could be designated in this case as “species”, as a basic taxonomical unit (without binomial nomenclature, of course). Many such deviations are well recognizable in cultures, but quite undetectable in natural samples.

Registration of cyanobacterial strains, clusters and related assemblages (groups, subgroups etc.) only on the basis of exactly defined experimentally discovered criteria, without binomial nomenclature and possibly without morphological criteria is used mainly in bacteriological literature (see, e.g., CASTENHOLZ ed. 2001, *Bergey’s Manual*). This approach is the only which is based purely on exact experimental data, but without other markers, comparison with phenotype stable units in nature and described in previous literature,

and without binomial nomenclature is applicable for ecological research only with difficulties.

3. Conventional compromise with respect to morphology, ecology, physiology and molecular biology (“polyphasic approach”). The species definition corresponding with this concept should be as follows: “Group of populations (+ strains) which belongs to one genotype (genus), is characterized by stabilized phenotypic features (definable and recognizable, with distinct limits of variation), and by identical ecological demands. These characters should occur repeatedly (in time) in various localities with the same ecological conditions.” Advantages of this definition are the integration of current phenotypic variability with modern molecular and EM data, coincidence of cytomorphological, ecological and molecular biological characters, and the wide applicability of existing traditional names connected with known morphotypes in nature. The disadvantage, however, is that almost all genera and species must be revised and re-evaluated in future. Moreover, the diversity of cyanobacteria particularly from tropical and extreme biotopes is almost unknown, and numerous new species from these biotopes must be described. The close collaboration of “botanical” and “bacteriological” oriented cyanoprokaryota-researchers would be essential.

I prefer the last (3rd) concept. Of course, it requires numerous revisions, changes in the system and descriptions of many new types from currently unknown biotopes, which is not a very popular approach. However, any of the concepts demands many changes, and the last one seems to be most congruent with the existing cyanobacterial diversity. The danger always exists that many “new species” will be described without satisfactory documentation and characterization. Only by outlining special rules for description of new species such an undesirable practice can be prevented.

The proposed approach assumes also the respect and acceptance of selected combined “botanical” and “bacteriological” rules for classification, which are not in contrary to experimental results. The system of clusters, subclusters, groups and subgroups (CASTENHOLZ 2001) is different from botanical species mainly by elimination of nomenclature. The binomial designation of taxonomic units could be possibly exchanged with another method, but only in the case that it will be more convenient for common use in experimental as well as ecological studies, and better suited for orientation in cyanobacterial diversity.

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