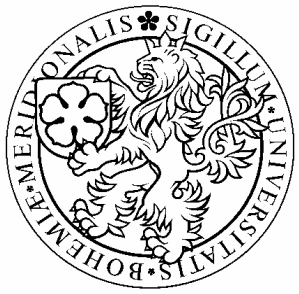
Faculty of Biological Sciences
University of South Bohemia
České Budějovice
Department of Botany



MASTER THESIS

Polyphasic approach to the taxonomy of the selected oscillatorian strains (Cyanobacteria)

Ana Lokmer
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Supervisor: RNDr. Jan Kaštovský, Ph.D.

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Abstract:
Morphology and ultrastructure of 25 oscillatorian strains was examined and phylogenetic analysis of 16S rDNA oscillatorian sequences was conducted. Genera *Phormidium* and *Oscillatoria* were shown to be polyphyletic. Although morphologically similar strains are found in different branches of the phylogenetic tree, considerable correlation between molecular, ultrastructural and some morphological and ecological traits was detected in several lineages.

I declare, that I wrote this master thesis by myself, solely by the use of the listed literature.

In České Budějovice, 23 April, 2007 .....

Děkuji:
- svému školiteli Hanysovi za ochotu, léčivé dávky optimismu a taky za to, že byl na mě hodný
- prof. J. Komárkovi za rady ohledně nejenom určování druhů
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- všem mým kolegům jak z algologické sekce tak těm z Laboratoře molekulární txonomie za to, že s nima byla legrace a práce se pak nezdala tak těžká

Contents

1. Introduction

1.1. Literary review

1.1.1. Short history of cyanobacterial taxonomy

Cyanobacteria or Cyanoprokaryota are oxygen producing, photosynthetising, gram-negative prokaryotes which had a main part in the evolution of the Earth´s atmosphere as we know it today. According to the fossil record, they emerged very early in the Earth´s history. A characteristic cyanobacterial membrane lipid has been extracted from late Archean sedimentary rocks dated to 2.65 Ga (SUMMONS et al. 1999). The microfossils found at the Apex Chert in Western Australia, believed to be cyanoprokaryotes, are even 800 million years older (SCHOPF 1993). A minimum date for the evolution of heterocytic forms is set to 1.5 billion years ago, to the period the oldest fossils interpreted as akinetes have been dated (GOLUBIĆ et al. 1995). Cyanobacteria were also among the initial colonizers of strictly continental habitats, what has been corroborated by the fossil assemblages of the Early Silurian sediments in Virginia, USA (TOMESCU et al. 2006). The enumeration of the habitats they dwell in nowadays is remarkable and their ecological significance is not restricted solely to the production of organic matter (WHITTON & POTTS 2000).
Nevertheless, it is their role in primary production which is apparently accountable for the fact, that the Cyanobacteria have been traditionally studied by botanists, even after the bacterial nature of their cells was recognized. Moreover, their conspicuous (though superficial) resemblance to eukaryotic algae earned them a name "blue-green algae". Although some efforts had been made to classify cyanoprokarytoes under various names (RIPPKA 1988) before in the past, the works of GOMONT (1892) and BORNET & FLAHAULT (1885) are considered the taxonomic starting points for fhe filamentous genera. There have been many attempts to classify cyanoprokaryotes since then (ANAGNOSTIDIS & KOMÁREK 1985), due both to new information gathered and shortcomings of the existing classification schemes. Traditional approach to the problem was based on morphological and ecological traits, with GEITLER (1932) as its most prominent representative. His system, with more or less unambigously defined taxa, is still widely in use, because it enables relatively easy determination of cyanobacteria in natural samples, which is appreciated especially by field phycologists. However, as GEITLER (1932) alone had noticed, it does not reflect evolutionary relations between taxa. Completely different conception was proposed by DROUET (1968, 1973, 1978, 1981), who presumed that the morphological diversity of Cyanobacteria is the result of acting of diverse environmental conditions on the restricted number of genotypes. Therefore, he reduced dramatically number of genera, but it turned out that he had severely underestimated the existing genetic variability (ANAGNOSTIDIS & KOMÁREK 1985, CASTENHOLZ 1992). The bacterial nature of the "blue-green algae" had brought STANIER et al. 1978 to put forward a proposal for their integration under the Bacterial Code of Nomenclature. This concept was realized in the system of RIPPKA et al. (1979) and RIPPKA (1988), which is based both on phenotypic and genotypic characters, yet only on those of the strains brought to culture. The problem of this conception is that it ignores most of the cyanobacterial diversity found in nature, the fact that immediately evoked protests of the ecologically oriented researchers (GEITLER 1979, GOLUBIĆ 1979 and others). The most recent exhaustive reorganization of the system, based on all available type of information (morphological, ecological, genetic, ultrastructural etc) on both cultivated and uncultivated cyanobacteria, was made by ANAGNOSTIDIS & KOMÁREK 1985, 1988, 1990 and KOMÁREK & ANAGNOSTIDIS 1986, 1988. They state that the use of botanical code squares with the tradition

of cyanobacterial systematics and that the acceptance of the bacterial code would only cause more confusion. Furthermore, it enables the determination of cyanoprokaryotes in natural samples. Nevertheless, as soon as the first 16S rRNA gene sequences of Cyanobacteria appeared (GIOVANNONI et al. 1988), it became obvious that many changes in this system would have to be done in order to obtain true image of the diversity and phylogeny of the "blue-green algae".

The state of cyanobacterial systematics is still very complex (for details see KOMÁREK 2006). The system of CASTENHOLZ (2001) is based on the bacteriological code, while that of KOMÁREK & ANAGNOSTIDIS (1999, 2005) was created according to the rules of the botanical code of nomenclature. The proposal has been made for the formation of the consensus nomenclature that would be acceptable for both bacteriologists and botanists (OREN 2004). The worst aspect of the problem is probably that many researchers do not use formal nomenclatoric prescriptions of either of the Codes, not to mention a common habit of assigning arbitrary names from old, unrevised literature to the strains and problems with incorrect taxonomic identification in general (KOMÁREK 2006).

1.1.2. Modern methods in cyanobacterial taxonomy

1.1.2.1. Molecular methods

Molecular methods have become an indispensable tool for characterization of cyanoprokaryotes and the assessment of evolutionary relations among them in recent decades. The direct sequencing of various genes is the most common method used. However, RFLP (Restriction Fragment Length Polymorphism) is also widely applied, especially for more detailed examination of the genetic variability of closely related taxa (ERNST et al. 1995, POSTIUS et al. 1996, BOLCH et al. 1996, LYRA et al. 1997, BOLCH et al. 1999, SCHELDEMAN et al. 1999, COMTE et al. 2007) or to infer the extent of cyanobacterial diversity in nature (LU et al. 1997, FRIAS-LOPEZ et al. 2003, KIM et al. 2004). Also random amplified polymorphic DNA (RAPD) analysis is sometimes used in order to discriminate between genotypes of close relatives (NEILAN 1995, NISHIHARA et al. 1997, BOLCH et al. 1999, CASAMATTA et al. 2003). Much less common are the allozyme (STULP & STAM 1984, KATO et al.1991, NISHIHARA et al. 1997) or the whole-cell protein analysis (PALINSKA et al. 1996, LYRA et al. 1997). Inspite of its fundamental role in determination of bacterial species (WAYNE et al. 1987), DNA-DNA hybridization has been rarely used (STULP & STAM 1984, OTSUKA et al. 2001, SUDA et al. 2002), since it is a very time-consuming procedure. It is also important to realize that the similarity values obtained by DNA-DNA hybridization do not reflect the actual degree of sequence similarity at the primary structure level – the phylogenetic relation for the strains with more than 20% divergence in the genome sequence cannot be determined by this method (ROSSELLÓ-MORA & AMANN 2001). Genomic characteristics, such as the presence and structure of tandem repeats (MAZEL et al. 1990, ASAYAMA et al. 1996, LYRA et al. 1997, RASMUSSEN & SVENNING 1998, CHONUDOMKUL et al. 2004, LYRA et al. 2005) or of a whole gene family (BHAYA et al. 2002), have also been shown to posses some discriminatory power on various taxonomic levels. The growing number of cyanobacterial genomes in public databases (12 finished genome projects at the moment, several others in progress - CyanoBase, JGI) enables the examination of the distribution of genes in a very detailed way. MARTIN et al. 2003 identified 151 uniquely cyanobacterial genes in 8 studied genomes and found a few examples of largely conserved gene order, which could prove useful for solving problems of cyanobacterial evolution on a larger scale.

The small ribosomal subunit gene has been the cornerstone of phylogenetic research for decades for several reasons. SSU rRNAs are universal molecules that contain conserved as

well as rapidly evolving regions. That enables comparison of both closely related taxa and members of different kingdoms. In addition, SSU rRNA genes are long enough to make the statistical evaluation of the results possible (WILMOTTE & GOLUBIĆ 1991). However, SSU rDNA has also a few unpleasant properties. First of all, the evolution of the SSU rRNA genes is subject to the constraints imposed by their function, which may cause, among others, the inability to discriminate between closely related taxa. It has been shown that the two *Bacillus* strains, assigned to different species on the basis of their DNA-DNA hybridization values, have virtually identical 16S rRNA sequence (FOX et al. 1992). In contrast, a case of extremely high intraspecies 16S rDNA diversity has also been documented (HARRINGTON 1999). So, there is no clear threshold value of 16SrDNA identity for species recognition in Bacteria (COENYE et al. 2005), although the strains with less than 97% 16SrDNA sequence similarity can most likely be considered different species (STACKEBRANDT & GOEBEL 1994). In addition, a vast amount of organisms has more than one ribosomal operon, which evokes the question of polymorphism of this gene within the genome (TOUROVA 2003). It seems, though, that the multiple *rrn* operons are usually almost identical in sequence, so the problems would arise only in the case of very closely related species (ACINAS et al. 2004). This intragenomic homogeneity of 16S rDNA can be regarded as the proof of rarity of the Horizontal Gene Transfer (HGT) events (COENYE et al. 2005). However, the problem concerning HGT of 16S rDNA and its impact on the bacterial phylogeny is much more complex (GOGARTEN et al. 2002).

In order to achieve better resolution power between the closely related taxa, another part of the *rrn* operon, the 16S rRNA-23S rRNA internal transcribed spacer region (ITS), has been increasingly used in phylogenetic research. 16S-23S ITS is variable in sequence, length and secondary structure, sometimes even between multiple copies within a single genome (GUGGER et al. 2002). A few conserved regions, though, can be identified (ITEMAN et al. 2000, BOYER et al. 2001). 16S-23S ITS usually contain both $tRNA^{Ile}$ and $tRNA^{Ala}$ genes (WILLIAMSON & DOOLITLE 1983), though there are several examples of 16S-23S ITS with either only $tRNA^{Ile}$ gene (NELISSEN et al. 1994) or completely without tRNA genes (ITEMAN et al. 2000, BOYER et al. 2001, TATON et al. 2003, TATON et al. 2006b). 16S-23S ITS has been successfully used to distinguish the closely related *Arthrospira* (SCHELDEMAN et al. 1999, BAURAIN et al. 2002, BALLOT et al. 2004), *Phormidium* (COMTE et al. 2007), *Microcystis* (OTSUKA et al. 1999) and *Synechococcus*-like strains (ERNST et al. 2003, BECKER et al. 2004). However, TATON et al. 2006b could not detect enough variation in this region to discriminate between closely related cyanobacteria. 16S-23S ITS also revealed the existence of unicellular cyanobacterial ecotypes in different microenvironments of hot springs (FERRIS et al. 2003, WARD et al. 2006). In addition, it has been used to address some questions concerning biogeography of the cyanobacteria (PAPKE et al. 2003, GUGGER et al. 2005, TATON et al. 2006a). In conclusion, it can be said, that the high degree of divergence of ITS makes the phylogenetic analysis possible only for closely related strains, as there is no way to meaningfully align the sequences from more distant relatives (TATON et al. 2006a). Nevertheless, even the sequence configuration can be a useful tool both for understanding population structure of cyanobacteria and studies of higher level phylogeny (BOYER et al. 2002).

Protein-coding gene sequences too have been used to infer phylogenetic relations among cyanobacteria. PALYS et al. 1997 and PALYS et al. 2000 assume that it is protein genes that should be regarded as the primary criterion for demarcating bacterial taxa. They argue that the protein-coding genes evolve faster than 16S rDNA, providing thereby for better resolution between bacterial species. However, there is only one 16S rRNA and the proteins are many, so it will be probably difficult to establish the exact protein-gene based criteria for species delineation. It is possible that a different set of genes will have to be used for the species

demarcation of different groups of bacteria (GEVERS et al. 2005). The protein-coding genes examined in cyanobacteria encompass those for DNA-dependent RNA polymerase – *rpoB, rpoC* and *rpoD* (PALENIK & SWIFT 1996, SEO & YOKOTA 2003, GUGGER et al. 2005, RAJANIEMI et al. 2005, BAKER et al. 2005, LYRA et al. 2005, EVERROAD et al. 2006), RubisCO large subunit and/or chaperonin-like protein X – *rbcL* and *rbcX* (RUDI et al. 1998, GUGGER et al. 2002, SHIMADA et al. 2003, LYRA et al. 2005, RAJANIEMI et al. 2005, TOMITANI et al. 2006), various regions of the phycocyanin operon (BOLCH et al. 1996, NEILAN et al. 1995, BARKER et al., 1999, BOLCH et al. 1999, BITTENCOURT-OLIVEIRA et al. 2001, MANEN & FALQET, 2002, CROSBIE et al. 2003, BALLOT et al. 2004, LIU et al. 2005, TENEVA et al. 2005, PREMANANDH et al. 2006, ABED et al. 2006), nitrogenase complex – *nifD, nifK, nihH* (KALLAS et al. 1985, BEN-PORATH et al. 1993, BEN-PORATH & ZEHR 1994, HENSON et al. 2002, ZEHR et al. 1997, HENSON et al. 2004, GUGGER et al. 2005, ABED et al. 2006), regulatory genes of heterocyte differentiation - *hetR* (JANSON et al. 1999, CARPENTER & JANSON 2001, LUNDGREN et al. 2005, TOMITANI et al. 2006), RNase P RNA gene - *rnpB* (VIOQUE 1997, SCHON et al. 2002), DNA gyrase subunit B – *gyrB* (SEO & YOKOTA 2003), the PSII reaction center protein D1 genes - *psbA* (HESS et al. 1995), the elongation factor Tu gene - *tufA* (DELWICHE et al. 1995), microcystin-coding genes – *mcyA* (HISBERGUES et al. 2003, YOSHIDA et al. 2005, RINTA-KANTO et al. 2006), nodularin synthetase subunit F gene – *ndaF,* intergenic spacer between fas vacuole protein A genes – *gvpA*-IGS (BARKER et al. 1999, LYRA et al. 2005), *hoxH* (ZHANG et al. 2005) and phycoerythrin intergenic spacer (ABED et al. 2006). The tree topologies obtained from various rDNA and protein sequences are congruent in the most cases (e.g. ZEHR et al. 1997, HONDA et al. 1999, ERNST et al. 2003, EVERROAD et al. 2006, TOMITANI et al. 2006), although there is some evidence for HGT in phycocyanin operon of *Athrospira* (MANEN & FALQUET 2002) and recombination of RubisCO genes in several other genera (RUDI et al. 1998). HGT is suspected also for *Synechocystis* sp. PCC 6803 (SEO & YOKOTA 2003) and Nodularia strains from the Baltic Sea (BARKER et al. 1999).

1.1.2.2. Impact of molecular methods on cyanobacterial taxonomy

Molecular methods have had a great impact on every level of cyanobacterial taxonomy. Prochlorophytes, which were considered a special group of oxyphototroph prokaryotes for the lack of phycobiliproteins and the presence of chlorophyll b, have been shown to be polyphyletic according to the 16S rDNA and scattered throughout the cyanobacterial lineage (GIOVANNONI et al. 1988, WILMOTTE 1994). In addition, a phycobiliprotein gene, similar to that of the marine *Synechococcus*, has been detected in *Prochlorococcus marinus* CCMP 1375 strain (HESS et al. 1996). Another important task solved by 16S rDNA sequencing was the origin of plastids that were proven to have descended from cyanobacteria (GIOVANNONI et al. 1988, DOUGLAS & TURNER 1991). This was later confirmed also by *tufA* gene sequence (DELWICHE et al. 1995). Plastids form a monophyletic group within cyanobacterial lineage, but no strong candidate for the sister taxon to plastids exists at present (TURNER 1997, TURNER et al. 1999). In addition, monophyly of heterocytic cyanobacteria was confirmed and the orders Chroococcales and Oscillatoriales were shown to be polyphyletic (GIOVANNONI et al. 1988, WILMOTTE & GOLUBIĆ 1991, LITVAITIS 2002). The baeocyte-forming order Pleurocapsales seemed to be monophyletic at first (GIOVANNONI et al. 1988), but it turned out to be polyphyletic too, with *Chroococcidiopsis thermalis* being a close relative of heterocytous cyanobacteria (TURNER 1997, ISHIDA et al. 2001, FEWER et al. 2002). The same may be stated for heterocytous order Stigonematales - it has been shown recently, on the basis of both 16S rDNA and *nif* genes, that it is polyphyletic as well (ZEHR et al. 1997, GUGGER & HOFFMANN 2004, HENSON et al. 2004).

The question of how many evolutionary lineages within cyanobacteria exist remains unanswered. WILMOTTE & GOLUBIĆ (1991) and TURNER (1997) detected 10 clusters,

WILMOTTE (1994) proposed an alternative of 8 clusters, HONDA et al. 1999 found 7 and LITVAITIS (2002) was able to distinguish 9 groups of cyanobacterial 16S rDNA sequences. However, the groups are inconsistent and supported merely by low bootstrap values, which might suggest that rapid adaptive radiation occurred after the invention of oxygenic photosynthesis (HONDA et al. 1999). Moreover, topology of the phylogenetic tree made by WILMOTTE & HERDMAN 2001 is "fan-like", so nothing can be said about the relations between most "groups" (there are many "loner" sequences, i.e. sequences without close relatives). For these reasons as well as for the lack of data, CASAMATTA et al. 2005 suggest, that it is too early to make conclusions about how many cyanobacterial lineages there are.
The situation on the generic and subgeneric level is even more confused. The ecological studies concerning diversity of cyanoprokaryotes in various habitats are many (e.g. WILLAME et al. 2006, TATON et al. 2006b, KOMÁREK et al. 2005, ZWART et al. 2005, FRIAS-LOPEZ et al. 2003, BECKER et al. 2004), but it is often not clear what is meant under the taxonomic designations assigned to the studied organisms. In most studies only molecular diversity is being disscussed (e.g. GEISS et al. 2004, KIM et al. 2004, FOURÇANS et al. 2004, NAGY et al. 2005), commonly with no reference to morphology or to determination literature used. In addition, there are many misidentified strains in culture collections (KOMÁREK 1994) and no morphological data for a bulk of available cyanobacterial sequences (WILMOTTE & HERDMAN 2001). However, some work has been done on the cytomorphological and polyphasic characterization of chroococcalian "*Synechocystis*", "*Synechococcus*" and a few other unicellular strains (e.g. KOMÁREK 1996, KOMÁREK 1999a, KOMÁREK et al. 2004, KORELUSOVÁ 2005) as well as of hetercytous *Aphanizomenon/Anabaena/Nostoc* strains (e.g. GUGGER et al. 2002, RAJANIEMI et al. 2005). The studies on filamentous "*Phormidium*" and "*Oscillatoria*" genera are few (PFFEIFER & PALINSKA 2002, CASAMATTA et al. 2003, TENEVA et al. 2005, MARQUARDT & PALINSKA 2007). Although there is often no correlation between morphological and molecular traits, especially for taxa with very simple morphology (WILMOTTE et al. 1992, LEE & BAE 2001, MARGHERI et al. 2003), some morphologically well defined genera were shown to be monophyletic. These include *Microcystis* (NEILAN et al. 1997, OTSUKA et al. 1998), *Arthrospira* (NELISSEN et al. 1994, MANEN & FALQUET 2002, ZHANG et al. 2005), *Planktothrix* (SUDA et al. 2002) or marine *Trichodesmium* species (BEN-PORATH et al. 1993, ABED et al. 2006). Nothwithstanding the unreliability of traditional morphological criteria, some cytomorphological and ultrastructural characters were found to correlate well with molecular data. This concerns e.g. the cell division type (PALINSKA et al. 1996, CASAMATTA et al. 2003, KOMÁREK et al. 2004) and especially the thylakoid arrangement, which seems to have substantial taxonomic value (KOMÁREK & KAŠTOVSKÝ 2003, KORELUSOVÁ 2005). Some other traits, such as perforation-patterns in the cell wall of cyanobacteria may prove useful on certain taxonomic levels (PALINSKA & KRUMBEIN 2000).

1.1.2.3. Biochemical characters

Various biochemical characters have also been examined in order to assess their value for cyanobacterial taxonomy. Each species of tropical marine *Lyngbya* spp. and *Symploca* spp. had a distinct chemotype on each locality, but some compounds were specific either for *Symploca* or *Lyngbya* (THACKER & PAUL 2004). No correlation was detected between bioactivity profiles and genetic characteristics in Antarctic cyanobacterial strains (TATON et al. 2006b). Toxin production sometimes correlates with phylogenetic proximity, as shown for neurotoxic *Anabaena* strains (LYRA et al. 1997, OTSUKA et al. 1999), but usually the toxic and non-toxic strains are intermixed (NEILAN et al. 1997, LYRA et al. 1997, OTSUKA et al. 1999, GUGGER et al. 2002, CHONUDOMKUL et al. 2004, YOSHIDA et al. 2005). The presence or absence of phycoerythrin may also have some taxonomic value. Closely related *Hydrocoleum* and *Trichodesmium* genera have very similar pigment profiles (ABED et al. 2006) and the tight

cluster of *Leptolyngbya* PCC 7375 and "*Phormidium persicinum*" related strains can be characterized by the presence of PE (WILMOTTE et al. 1992, MARQUARDT & PALINSKA 2007). The separation of PE and non-PE closely related *Planktothrix agardhii* strains into two groups was additionally supported by DNA-DNA hybridization values (SUDA et al. 2002). However, the relation between the PE content and evolutionary propinquity is usually not so clear (OTSUKA et al. 1999, CROSBIE et al. 2003, EVERROAD et al. 2006). No correlation between carotenoid content and colour was detected in studied *Spirulina* and "*Oscillatoria*" strains (AAKERMANN et al. 1992). On the other hand, *Microcoleus chthonoplastes* could easily be distinguished from other strains of similar morphotypes by carotenoid and MAA content (KARSTEN & GARCIA-PICHEL, 1996). Fatty acid composition can be used in order to characterize closely related taxa, but it is difficult to interprete its taxonomic meaning (LI & WATANABE 2001, 2004, SUDA et al. 2002). The same holds, at least for the present, for all mentioned characters. The value and significance of these characters depends on the taxonomic level examined.

1.1.3. Relationship between genotypic and phenotypic characters

Cyanobacteria are morphologically diverse in comparison to the rest of bacteria. Nevertheless, only a quite restricted number of morphotypes can be recognized. Molecular methods enabled revelation of cryptic genetic, physiological and ecological diversity among them. Rather broadly defined species *Phormidium retzii* was shown to be quite variable on molecular level. Although there were some morphological distinctions between individual populations, they did not correlate with genetic similarity (CASAMATTA et al. 2003). Strains morphologically corresponding to the genus *Geitlerinema* generated very different restriction patterns (MARGHERI et al. 2003). The studies on cyanobacterial communities of both geothermal springs in the Philippines (LACAP et al. 2005) and Lake Fryxell in Antarctica (TATON et al. 2003) revealed significantly higher degree of diversity by molecular methods than by light microscopy. Genetically distinct toxic *Microcystis* and *Planktothrix* populations were found in different parts of the same lake (RINTA-KANTO & WILHELM 2006). However, it is also possible that the strains, closely related on molecular level, show substantial phenotypic variability, as shown for several *Merismopedia* isolates (PALINSKA et al. 1996). MOORE et al. 1998 detected co-existing *Prochlorococcus* ecotypes, almost identical genetically, but possesing very different light-dependent physiologies.
Interesting topic is the correlation between phylogenetic relatedness and ecological or ecophysiological characters. Unicellular cyanobacteria from hypersalinne habitats in various geographical regions form a well defined cluster on the basis of their 16S rDNA that was denominated *Halothece* cluster (GARCIA-PICHEL et al. 1998). This was later confirmed by other researchers (MARGHERI et al. 1999). Moderately halophilic, benthic strains with very thin trichomes also form a distinct cluster in the phylogenetic tree and were assigned to a new genus *Halomicronema* (ABED et al. 2002). In addition, correlation between molecular and ecophysiological traits was demonstrated for several marine *Phormidium* isolates (PFEIFFER & PALINSKA 2002), for Antarctic psychrophilic *Phormidium* strains (NADEAU et al. 2001) and for Antarctic *Phormidium* strains from ornithogenic substrate (COMTE et al. 2007). It is interesting that the only available 16S rDNA sequences of cyanobacteria from stone surfaces of buildings group with those of desert strains from distant geographic region, while sequence homology with the strains from other habitats is quite low. The authors suggest that the sequence similarity somehow reflects the capacity to survive in such extreme environments (CRISPIM & GAYLARDE 2003). The analysis of the *hli* gene family, which has to do with adaptation to high light intensities, revealed that some groups of this gene family are specific

either for marine or freshwater cyanobacteria (BHAYA et al. 2002). A nice example of ecological divergence of morphologically and phylogenetically closely related, but distinct genera *Trichodesmium* and *Hydrocoleum* (*Blennothrix*) was documented by ABED et al. 2006. The first one is planktonic, the latter is the most common mat-forming cyanobacterium in tropical oceans.

1.1.4. Endemism in Cyanobacteria

Little is known about endemism in cyanobacteria. The study of this subject is complicated by the fact that the reference literature designed for temperate region is usually used for determination of cyanobacteria in other geographic regions as well. In addition, the propriety of taxonomic assignment of many strains is questionable (KOMÁREK 1999b). He added that according to morphological and ecological characters more than 60% of Antarctic species could be regarded endemic. Molecular methods can be a useful tool for solving question of endemic or cosmopolitan distribution of cyanobacteria and some work on this matter has already been done. Nonmarine picocyanobacteria fall in several different clusters, yet the same genotypes were found in various geographic regions and it can be concluded that they are cosmopolitan in distribution (CROSBIE et al. 2003). *Microcoleus chthonoplastes*, the inhabitant of various hypersalinne habitats is probably also cosmopolitan according to the molecular data (GARCIA-PICHEL et al. 1996), as well as various *Prochlorococcus* ecotypes (SCHON et al. 2002). On the contrary, Antarctic saline lakes differed one from another in the composition of the cyanobacterial microflora (TATON et al. 2006a). NADEAU et al. 2001 and CASAMATTA et al. 2003 suggest that Antarctic cyanobacterial flora has origin in many different lineages of temperate regions according to the results of 16S rDNA analysis. There is no discrepancy between this theory and the existence of endemism, as the subsequent adaptation of these temperate taxa to the polar conditions could result in the nascence of the new morpho/genotypes. In addition to the already mentioned case, 3 more exclusively Antarctic phylotypes were detected by 16S rDNA sequencing (TATON et al. 2006b). Significant molecular differences were detected also in hot springs of different geographic regions, at least for the portion of studied genotypes (PAPKE et al. 2003, JING et al. 2005). Endemic species are probably common in tropical alkaline marshes, according to both morphological and molecular data (KOMÁREK et al. 2005). *Nodularia* strains from Australia group separately from other related strains from different regions. In addition, Australian *Nodularia* from different lakes are genetically diverse too (BOLCH et al. 1999). The interesting situation is encountered in tropical *Cylindrospermopsis raciborski* species: while there is no clear geographical distinction between Japanese and Thai and Thai and Australian strains (CHONUDOMKUL et al. 2004), there is clear grouping of its strains on a more global scale - Australian/African, European and North American population can be clearly distinguished by 16S rDNA (NEILAN et al. 2003) or 16S-23S ITS (GUGGER et al. 2005).

It is apparent that much work will have to be done in future, before it will be possible to create a realistic image of cyanobacterial diversity and evolutionary relations between them.

1.2. Aims of the thesis

- Detection of contingent correlation between morphological, ultrastructural, molecular and ecological traits in the examined oscillatorian strains
- Determination of the causes of the polyphyly of *Phormidium* and *Oscillatoria* genera on the basis of the obtained data.
- Evaluation of the phylogenetic position of oscillatorian cyanobacteria in the global context of cyanobacterial phylogeny.

2. Materials and Methods

2.1 Origin of cyanobacterial strains used in this study

Cyanobacterial strains used in this study are listed in Table 1. Strains *Geitlerinema* cf. *splendidum* KLABOUCHOVA 1987/1, *Phormidium animale* HINDAK 1967/39, GROWTHER/1459-6, HINDAK 1963/108, *Oscillatoria sancta* KOCH 1970/SAG 74.79 and *Oscillatoria limosa* KOVACIK 1987/5 were obtained from Culture Collection of Algal Laboratory (CCALA) in Trebon, Czech Republic. Strains *Oscillatoria* sp. BJ1, SA, G, FW, O were obtained from the Department of Botany of the University of Punjab, Pakistan. Strain *Phormidium* sp. B-Tom was collected by Jan Kaštovský. The remaining strains were collected by the author.
Taxonomic designations of the studied strains were revised in accordance with information gathered during the research. The system of KOMÁREK & ANAGNOSTIDIS 2005 was used as the basis for genus and species determination.

2.2 Isolation and cultivation conditions

The strains collected by the autor were isolated by picking a single filament from the original sample using a glass Pasteur pipette. The filament was placed into a small sterile plastic tube containing liquid BG 11medium then and cultivated under the conditions described below. At least 15 replicates were conducted for each sample, resulting in the establishment of 1-3 clonal cultures per sample.
All strains were cultivated on 1,5% agar plates amended with BG 11 medium (STANIER et al. 1971). The light regime was set at 12 h day and 12 h night.

2.3. Morphological characterization

The morphology of the studied Cyanobacteria was examined with an Olympus BX51 light microscope and the photographs were taken with an Olympus Camedia digital camera C5050 ZOOM. Qualitative characteristics examined comprise the shape of the cells in a filament, the shape of the end cell, the presence/absence of calyptra, hormogonia and mucilage, the form of that mucilage, mat colour and the motiliy of filaments. The quantitative traits measured were filament width, cell length and the end cell length and width. Subsequently, the median, 25% and 75% quantiles and the range of the measured traits (including cell width/length ratio for both filament and end cells) were calculated in Statistica 7.1 (STATSOFT Inc. 2005).

2.4. Ultrastructural characterization

The strains were examined by TEM in order to determine the thylakoid pattern. Specimen preparation was as follows:
Cells were fixed with 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, and later post-fixed with 2% osmium tetroxide. The fixed material was dehydrated in an acetone series (30, 50, 70, 80, 90, 95 and 100%) and embedded in Spurr's resin (SPURR 1969). Ultrathin cross-sections were stained with uranylacetate and Pb-citrate and examined in a JEOL 1010 electron microscope and documented with a Lhesa 72WA CCD camera.

2.5. Molecular characterization

2.5.1.DNA isolation and partial 16S rDNA + 16S-23S ITS sequencing

DNA was isolated with Invisorb® Spin Plant Mini Kit according to the protocol and stored at -20˚C, except that the initial desintegration of cells was preformed by breaking up fresh filaments in a mini-beadbeater using a mixture of 0.1, 0.5 and 1.00 mm diameter glass beads. The presence of DNA was checked by agarose gel electrophoresis.
The PCR was performed in the Biometra® T3 thermocycler as desribed in Table 2. Each PCR mixture contained 1µl of DNA, 16.5 µl dH_2O, 10x *Taq* reaction buffer, 1.5 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase, 0.2 mM dNTP mixture, 1µg/µl of BSA and 0.5 µM of each primer (Table 3). Final volume of the reaction mixture was 25 µl. The PCR products were separated by agarose gel electrophoresis, cut out and repurified with JetQuick Gel extraction Spin Kit.
The products were subsequently cloned with Invitrogen TOPO TA Cloning® Kit for Sequencing. DH5α^{TM} –T1^{R} Competent Cells were used for transformation. 3-4 white colonies were picked from each plate and cultivated in LB medium containing 10 mg/ml bacto-tryptone, 5 mg/ml bacto- yeast extract, 10 mg/ml NaCl and 12 µl AMP. The plasmids were repurified with JetQuick Plasmid Miniprep Spin Kit and digested with EcoR1 enzyme to chcek for the presence of the insert.
BigDye® Terminator Cycle Sequencing Kit v. 3.1 was used for sequencing. The amount of the plasmid added to the reaction mixture was adjusted depending on the concentration of the samples, which was calculated from the absorbance values at λ=260 nm. The cycle sequencing reaction was preformed as desribed in Table 2. The primers are listed in Table 3. Automated sequencing was conducted on ABI 3130 Genetic Analyzer at the Institute of Plant Molecular Biology of the Czech Academy of Sciences in České Budějovice.

2.5.2. Sequence assembly

The sequences were assembled using SeqMan^{TM} II software, DNASTAR ver 4.0 (DNASTAR Inc. 1999). Each clone was assembled separately. The sequence chromatograms were checked by eye to solve the ambiguities if possible. Subsequently the consensus sequence was made if the individual clone sequences were not too divergent. In that case the divergent sequence was treated separately in further analyses.

2.5.3. Selection of the oscillatorian 16S rDNA sequences from the GenBank

The following procedure was conducted in order to reduce the number of sequences used in the actual analysis in the most objective manner. The amount of potentially usable sequences is large (more than 400), which makes the phylogenetic analysis virtually infeasible. It was not possible to simply download a selected portion of the sequences, because many of them are found under an incorrect taxonomic designation.
All availiable oscillatorian 16S rDNA sequences longer than 1000 bp were downloaded from GenBank (shorter sequences were also included if considered to be important for some reason). These were aligned in ClustalX (THOMPSON et al. 1997, HIGGINS & SHARP 1988) using default parameters, except that the "delay divergent sequence" option was set to 95%. After minor adjustments of the alignments performed in BioEdit (HALL, 1999), the distances between the sequences were calculated in DnaDist programme of Phylip 3.2 software package (FELSENSTEIN 2005) using the F84 model. Only one or two members of the groups of

completely identical sequences were included into further analyses. Subsequently, a new alignment was created in the way described above, which was then examined in detail in BioEdit (HALL 1999). A few alternative versions of the alignment were made. The optimal model for the phylogenetic analysis of each version was calculated using Modeltest (POSADA &CRANDALL 1998) with the initial NJ tree calculated in PAUP (SWOFFORD 1998). The parameters, selected on the basis of Akaike Information Criterion (AIC) were used in the following computations. The ML trees were built up using PhyML (GUINDON &GASCUEL 2003) with 100 bootstrap replicates conducted. MP tree was constructed in the PAUP programme from only one of the alignments, just to check if the tree buliding algorythm would radically change the resulting topology. Swapping algorithm used was TBR (Tree Bisection and Reconnection) and again 100 boostrap replicates were carried out. The trees were then examined in order to detect statistically highly supported clusters that are present in all trees. All availiable morphological, ultrastructural, ecological and biogeographical data about the taxons found in these clusters were searched out in order to decide which ones will be included in succeeding analyses. 101 oscillatorian 16rDNA sequences were chosen for the analysis.
Furthermore, 16S rDNA sequences of the chroococcalian strains with well known morphology and ultrasturcture (*Synechocystis* sp. PCC 7202, *Synechococcus* PCC 6307, *Cyanothece* sp. PCC 7424, *Halothece* strain MPI 96P605, *Gloeocapsa* PCC 73106, *Microcystis aeruginosa* PCC 7941, *Synechocystis* sp. PCC 6803, *Synechococcus elongatus* PCC 6301) were chosen for analysis. Two heterocytous strains (*Nodularia spumigena* PCC 73104 and *Fischerella muscicola* PCC 7414) were also included. *Gloeobacter violaceus* PCC 7421 and *Lactobacillus casei* I-5 were used as outgroups.

2.5.4. Phylogenetic analysis

The phylogenetic analysis including the author´s 16S rDNA sequences was conducted in basically the same way as described above. The alignment was made in MAFFT (KATOH et al. 2005) and was subsequently checked by eye in BioEdit (HALL, 1999) in order to exclude hypervariable and ambigous sites. Again, several different versions of the alignment were made. As some sequences were only cca 600 bp long, they were excluded from some alignments in order to check if that would siginificantly change the tree topology. The missing characters were replaced by N´s in the alignments including these short sequences. This was done in order to discriminate between indels and missing traits. Parameters for the maximum likelihood analysis were chosen on the basis of AIC, again calculated in the Modeltest programme (POSADA &CRANDALL 1998).
Maximum likelihood trees were built using the PhyML software (GUINDON &GASCUEL 2003). The model proposed by Modeltest was used: GTR+I+Γ with 6 nucleotide substitution categories, proportion of invariable sites set to 0.5032 and gamma shape parameter value 0.6405. Bootstrap replicates were set to 1000.
Maximum parsimony trees were created in PAUP (SWOFFORD 1998). Gaps were treated as fifth character and sequence addition was set to random. The tree swapping algorythm was again set to TBR. The resulting phylograms were examined in order to get the values of the homoplasy, consistency and retention indices. Then a 50% major rule consensus tree was created and 1000 bootstrap replicates were conducted.
MrBayes 3.1 software (RONQUIST & HUELSENBECK 2003) was used to infer bayesian phylogeny. 1 000 000 generations were run and the burn-in value was set to 10%.
All trees were subsequently checked by eye in order to see if there were some substantial incongruities between the trees constructed by different methods.

2.5.5. 16S-23S ITS characterization

The 16S-23S ITS sequences were aligned with MAFFT and checked for the presence of the tRNA genes. The length of the sequences was also recorded. The phylogenetic analysis was not conducted as the 16S-23S ITS sequences differed substantially and the meaningful alignment of the large part of the sequences was impossible.

3. Results and Discussion

3.1 Morphology

More than a half of the studied strains can be assigned to the genus *Phormidium* sensu KOMÁREK & ANAGNOSTIDIS 2005. The exceptions to the rule are the strains P-G, P-SA, HINDAK 1967/39, KLABOUCHOVA 1987/1 and GROWTHER/1459-6, which were recognized as members of the genus *Geitlerinema* sensu KOMÁREK & ANAGNOSTIDIS 2005. Strain P-BJ1 belongs to the genus *Leptolyngbya* sensu KOMÁREK & ANAGNOSTIDIS 2005, while the strains KOCH 1970/SAG 74.79, Fkv-3 and Fkv-4 fit to the description of the genus *Oscillatoria* sensu KOMÁREK & ANAGNOSTIDIS 2005.
Filament width, cell length/width ratio and the apical cell width and length/width ratio are listed in Table 1. Cell length was rather variable depending on the state and the age of the culture, but it changed in a more or less predictable manner in each strain. The same holds for the apical cell proportions and shape. Although usually more than 100 measurements were carried out for each quantivative trait, it is possible that the values are slightly biased, as the measurements were not equally distributed among different stages of the life cycle (growth phase vs. death phase). In addition, only about 60 measurements were realized on *Phormidium* cf. *aerugineo-coeruleum* R-aq and *Oscillatoria* cf. *curviceps* Fkv-3 (the strain Fkv-4 was not measured at all).
Deformations, such as swollen cells in the filament or variously misshaped apical cells, were observed occasionally.

3.1.1. *Phormdium* strains

Strains assigned to the *Phormidium* genus fall into 5 different groups sensu KOMÁREK & ANAGNOSTIDIS 2005:

- *Phormidium* group II (*Phormidium animale* HINDAK 1963/108 and *Phormidium* cf. *okenii* Led-Z)
- *Phormidium* group III (*Phormidium* cf. *formosum* P-FW and *Phormidium* cf. *formosum* P-O)
- *Phormidium* group V (*Phormidium* cf. *tergestinum* Drak and *Phormidium* cf. *aerugineo-coeruleum* R-aq)
- *Phormidium* group VII (*Phormidium autumnale* CB-G, CB-V, Kvet-0, Kvet-1, Kvet-2 and *Phormidium* cf. *amoenum* BW-0, BW-1, I-Sab)
  The strains of this group are referred to as "*Phormidium autumnale*" group.
- *Phormidium* group VIII (*Phormdium* cf. *subfuscum* I-Roc, *Phormidium* cf. *irriguum* KOVACIK 1987/5)

Strain *Phormidium* sp. B-Tom is a special case and therefore it was not assigned to any of these groups (see below).

3.1.1.1. *Phormidium* group II strains

This group encompasses *Phormidium animale* HINDAK 1963/108 and *Phormidium* cf. *okenii* Led-Z strains (Fig. 4). The strains have no calyptra and are highly motile. Hormogonia are of different length, but mostly about 10 cells long.

- *Phormidium animale* HINDAK 1963/108 strain morphology is in agreement with the description of the *Phormidium animale* species, except for the fact that the trichomes are commonly constricted at the cross-walls. Cell content is divided in thin

chromatoplasma and broader centroplasma. One or two not too prominent granules may also be present. Cells in the older trichomes are usually shorter than those in growing young trichomes. They may also contain a larger amount of smaller, yet more conspicuous granules. These are often arranged along the cross-walls. Dying trichomes have distinctly constricted, relatively short cells and rather sharply pointed and sometimes elongated apical cells. They are almost colourless and often contain variously swollen cells.

- *Phormidium* cf. *okenii* Led-Z mat is dark blue-green in colour. The trichomes are gradually narrowed towards ends and distinctly constricted at cross-walls. The difference between chromatoplasma and centroplasma is noticeable. The chromatoplasma varies in thickness and its edge is more or less "toothed". Mucilage is either diffluent of in the form of a delicate colourless sheath. The cells are slightly shorter than wide or isodiametrical, sometimes even longer than wide (again the older trichomes tend to have shorter cells).The apical cells are often not developed. Developed apical cells are bent, elongated and usually rounded. Hormogonia and necridic cells were rarely observed. The strain is morphologically even more similar to *Phormidium cortianum* than to *Phormidium okenii*, yet the *Phormdium cortianum* species occurs in thermal and mineral springs, while Led-Z strain was isolated from a pond.

3.1.1.2. *Phormidium* group III strains

This group comprises *Phormidium* cf. *formosum* P-FW and P-O strains (Fig. 4). The mats of these strains are blue-green in colour. The trichomes are motile and constricted at the occasionally translucent cross-walls. The sheaths are very thin, colourless, or only diffluent slime is present. Cells of young growing trichomes are usually distinctly shorter than wide, while those of the old trichomes are very variable in length. Chromatoplasma fills almost the whole cell volume in young trichomes, but as the trichome grows older pale centroplasma takes over. Apical cells are without calyptra, mostly elongated, round-conical in shape and bent. However, they were often not developed. Hormogonia are variable in size, cca 2-20 cells long.

3.1.1.3. *Phormidium* group V strains

Phormidium cf. *tergestinum* Drak and *Phormidium* cf. *aerugineo-coeruleum* R-aq belong to this group, characterized by rounded apical cells and the absence of calyptra. Both strains are motile.

- *Phormidium* cf. *tergestinum* Drak (Fig. 2) was not examined in detail (it was collected only recently), but a few basic characteristics of the strain were recorded. The mat is blue-green and so are the trichomes as well. The trichomes are equally wide along the whole length and distinctly constricted at cross-walls. The cells are shorter than wide and the cell content is more or less homogenous. Mucilage is often present, in the form of very delicate and almost imperceptible sheaths.
- *Phormidium* cf. *aerugineo-coeruleum* (Fig. 3) was also not examined in detail (it did not grow well in culture). The mat is brown and it appeared as the soft carpet covering the stones in the aquarium where it was collected. The sheaths are either absent or rather thick. Brown trichomes are distinctly constricted at cross-walls, with cells shorter than wider and with somewhat keritomized cell content. Very thin chromatoplasma is observable in young trichomes. The cross-walls of the older trichomes become inconspicuous and the cell content becomes more homogenous and granulated. The trichomes do not break easily, probably because of the firm colourless sheaths.

3.1.1.4. "*Phormidium autumnale*" group

All members of this group have a calyptra and are motile. Hormogonia, separated by necridia, are usually 7-15 cells long, although some 2-3-celled hormogonia were also noticed. The strains somewhat vary in cell proportions, shape of the end cell and the sheath appearance. However, significant intrastrain variability is also present.

- *Phormidium autumnale* CB-G and *Phormidium autumnale* CB-V (Fig. 5) were assigned to this particular species especially on the basis of their ecology. They were isolated from a cyanobacterial mat in a puddle near a dump yard. An interesting observation was made during the isolation of the strains. The original mat was of typical cyanobacterial blue-green colour. The inspection of the field sample by light microscope revealed only blue-green trichomes of this morphotype in addition to few thin filamentous cyanobacteria. However, after a week of cultivation greyish trichomes began to dominate the Petri dish. So, the green and greyish trichomes were isolated separately and the colour of the trichomes proved to be stable in culture. Two strains are otherwise almost identical. The trichomes are not constricted or slightly constricted at the cross-walls. Cell content of young growing trichomes is either homogenous or distinguished into not very conspicuos chromatoplasma and centroplasma. The cells are mostly shorter than wide. Sheaths are thin and colourless. Trichomes are only shortly narrowed towards ends and usually have a calyptra. However, as the culture grows older, the cells are getting longer and the fine but distinct granulation at the cross-walls usually appears. Also the sheath becomes thicker and the trichomes become gradually narrowed towards ends. Apical cells become distinctly capitate and have a conspicuous calyptra of variable shape. Apical cells in general are much smaller than those in a trichome. Very old cultures of *Phormidium autumnale* CB-G, the greyish isolate, lose their greyish colour and become yellow-green.
- *Phormidium autumnale* Kvet-0, Kvet-1 and Kvet-2 (Fig. 6) were assigned to the same species as previously described strains, although some slight differences were noticed between those groups of strains. The sheaths are overall thinner and typical gradual attenuation of trichomes found in CB strains was observed only exceptionally. Instead, trichomes are mostly shortly narrowed towards ends, regadless of the culture´s age. The conical-rounded calyptra is not so conspicuous and it usually reminds a common trichome cell, apart from having no thylakoids. Small granules can often be seen in centroplasma. The border between centroplasma and chromatoplasma looks like that described for *Phormidium* cf. *okenii* Led-Z. In very old trichomes a coarse granulation sometimes appears at the cross-walls and the cells may become longer than wide. The mat is of dark blue-green colour. No particular difference was noticed between those three strains (isolated from the same benthic cyanobacterial mat), although the strain Kvet-1 was more vital than the other two, what might indicate the existence of some physiological variability between the strains. However, it is only a hypothesis and no experiments were undertaken in order to confirm this.
- *Phormidium* cf. *amoenum* BW-0, BW-1 and I-Sab (Fig.6) are somewhat different than *Phormidium autumnale* strains. All three were isolated from puddles situated in woods. The mat is bluish grey. Although there is no statistical diferrence between cell proportions of *Phormidium* cf. *amoenum* and *Phormidium autumnale* strains, the cells of the former are often almost isodiametrical and are usually granulated at the cross-walls. Constrictions at the cross-walls are more common than in previously described strains. Centroplasma and chromatoplasma can be distinguished in the cells of younger trichomes, but the distinction is not very conspicuous. The sheaths are thin and the trichomes are gradually attenuated towards the end. The calyptra is more or

less rounded. This description holds for what had been commonly observed. However, it seems that the culture conditions are somehow inappropriate for *Phormidium* cf. *amoenum* strains, as their phenotype resembles those of old cultures of *Phormidium autumnale* strains. Indeed, the trichomes with rather short cells and ungranulated cell content were observed on several occasions, so it is not clear whether the recorded morphological traits are also common and representative for field populations of these strains or the commonly observed phenotype is just a reaction to suboptimal culture conditions (unfortunately it was not possible to microscopically examine these strains immediately after collection).

3.1.1.5. *Phormidium* group VIII

Two strains are assigned to this group - *Phormidium* cf. *subfuscum* I-Roc and *Phormdium* cf. *irriguum* KOVACIK 1987/5.

- *Phormdium* cf. *subfuscum* I-Roc (Fig. 3) was isolated from a cyanobacterial mat in a small waterfall. Situation similar to that described for *Phormidium autumnale* CB-G and CB-V strains occurred during the isolation. The original mat was blue-green, but after a few days in culture brown filaments appeared. Unfortunately, blue-green filaments did not grow in culture. Neither *Phormdium* cf. *subfuscum* I-Roc grows well in culture, so it was difficult to examine its morphology. Nevertheless, the mat is brown, and the motile trichomes are long, unconstricted at the granulated cross-walls and shortly narrowed towards ends. Gradually narrowed trichomes were observed on several occasions. The cells are shorter than wide. The calyptra is usually obtuse or widely conical. Mucilage is often present, either in the form of thin and firm sheath or as diffluent slime.
- *Phormidium* cf. *irriguum* KOVACIK 1987/5 (Fig. 2) was previously assigned to *Oscillatoria limosa* species. Although morphologically very similar, *Phormidium* cf. *irriguum* KOVACIK 1987/5 trichomes are substanitally thinner than those of *Oscillatoria limosa.* The thallus is dark green in colour. The trichomes are blue-green or even yellow-green in older cultures. Growing trichomes are usually distinctly constricted at cross walls and the cell-content is usually finely granulated. Centroplasma and chromatoplasma can be distinguished sometimes, usually in younger cells. Older trichomes are less conspicuously constricted or unconstricted at the cross-walls, have more homogenous cell content and more distinct sheaths. Thickened cell wall was observed in the apical cells of the older trichomes several times.

3.1.1.5. Phormidium sp. B-Tom

Phormidium sp. B-Tom (Fig. 7) forms a wiry blue-green mat and grows rather slowly. The trichomes are immotile, usually with firm conspicuous sheaths. Growing trichomes are usually composed of almost isodiametrical cells that become shorter as the trichome grows older. Also the constrictions at the cross-walls become deeper in the aging cultures. The apical cell is either rounded or conical in shape. The cell content is either homogenous or the centroplasma, fringed by very thin chromatoplasma may be observed. This strain is probably a member of the new, so far not formally described genus *Phormidesmis* (KOMÁREK, personal communication).

3.1.2. *Geitlerinema* strains

The following strains were assigned to this genus: *Geitlerinema* cf. *lemmermannii* P-G, P-SA, *Geitlerinema* cf. *pseudacutissimum* KLABOUCHOVA 1987/1, *Geitlerinema* cf.*acuminatum* HINDAK 1967/39 and *Getlerinema* sp. GROWTHER/1459-6. All are motile and form hormogonia without necridic cells.

- *Geitlerinema* cf. *lemmermannii* P-G and P-SA (Fig. 8) form blue-green mats. The trichomes are unconstricted at the cross-walls and equally wide along the whole length. There is a small granule on each side of the cross-wall. Thin though distinct chromatoplasma fringes pale centroplasma. Cells are longer than wide as well as the apical cells, which are mostly rounded and sometimes slightly narrowed. Thin sheaths are usually present, but not always. As the culture grows older, the cell content becomes coarsely granulated.
- *Geitlerinema* cf. *pseudacutissimum* KLABOUCHOVA 1987/1 (Fig. 8) forms a brownish green mat which can be easily detached from the surface. However, there are additional filaments under surface, which intergrow the agar. The cells are barrel-shaped and their content is distinguished into a thin fringe of chromatoplasma and light centroplasma. The apical cells are usually a bit elongated and bent. Firm sheaths were never observed, but diffluent mucilage is quite common. The cells occasionally contain small orange granules near the cross-walls. The older trichomes become coarsely granulated. The strain is intensively motile.
- *Geitlerinema* cf.*acuminatum* HINDAK 1967/39 (Fig. 7) forms a blue-green mat on the agar surface. The trichomes are unconstricted or slightly constricted at the cross-walls. Only in older cultures do these constrictions become more apparent. Centroplasma and chromatoplasma can be distinguished occasionally. This difference is, however, slight and the cell content appearance, at least in young trichomes, is more similar to that described for *Phormidium* cf. *formosum* than for other *Geitlerinema* strains. The apical cells are elongated and sharply pointed. As the culture grows older, the trichomes acquire yellow-green colouration.
- *Getlerinema* sp. GROWTHER/1459-6 (Fig. 7) forms a blue green leathery mat. Trichomes are slightly constricted at distinctly granulated cross-walls. Older trichomes often contain swollen cells without any cell content. Sheaths are very thin and almost imperceptible, but there is often a considerable amount of diffluent slime, especially in the aging cultures. The apical cells are slightly elongated, conical-rounded and bent.

3.1.3. *Leptolyngbya* strains

The only studied strain assigned to this genus was P-BJ1. *Leptolyngbya* cf. *boryana* P-BJ1 thallus is bright blue-green. The trichomes are long, distinctly constricted at the cross-walls. Pseudobranches were not observed. Cells are shorter than wider. There is usually one small central granule in each cell and a thin chromatoplasma is distinguishable from the centroplasma. The apical cells are rounded. Sheaths are thin and colourless, but firm and conspicuous.

3.1.4. *Oscillatoria* strains

Oscillatoria sancta 1970/SAG 74.79 and *Oscillatoria* cf. *curviceps* Fkv-3 and Fkv-4 both have a typical oscillatorian cell division type. Their cells are distinctly shorter than wide and both are brownish in colour.

- *Oscillatoria sancta* 1970/SAG 74.79 (Fig. 1) has usually short filaments, which is not typical for this species. However, this could be due to a long maintenance in culture. The trichomes are constricted at the cross-walls. The healthy growing trichomes are composed of cells with homogenous brown content, often finely granulated at the cross-walls, but as the culture grows older the whole cells become granulated. The trichomes are somewhat narrowed towards the ends and the apical cell has a more or less flattened calyptra. Hormogonia are variable in size, but usually they are very short (2-6 cells).Various amount of slime, either diffluent or in a form of a thin sheath, may be present.
- *Oscillatoria* cf. *curviceps* Fkv-3 and Fkv-4 (Fig. 1) were not examined to detail because they were collected only recently. Trichomes are reddish-brown and constricted at the coarsely granulated cross-walls. They are not attenuated towards ends. Sheaths are thin. Calyptra was not observed.

3.1.5. Intrastrain variability

Substantial intrastrain variability may be observed, as already mentioned, in most strains. The strains vary in cell shape and size, mucilage production, trichome width and cell content appearance. If seen only once, some strains may have been assigned to completely different species. Although the existence of phenotypic variability of cyanobacterial strains both in nature and culture is well-known to those who work with them, the causes and range of this variability have been studied only exceptionally. It has been shown that light intensity, temperature (ŠABACKÁ, personal communication) and salinity (GARCIA-PICHEL et al. 1998) can significantly influence cell proportions. Changes in slime production and colony shape have been reported for *Merismopedia* strains under standard cultivation conditions (PALINSKA et al. 1996). However, cell division type seems to be a constant character. The cell width also seems to be much less variable than the cell length in general. So, the cell width is probably genetically fixed to a greater extent, while the cell length variability is mostly the result of the effect of particular growth conditions on the rate and frequency of cell division (in addition to cell length variation which is due to the cell division type itself). It is interesting that in some strains (e.g. those of *Phormidium autumnale*) cells tend to be longer in older cultures, while in others (e.g. *Phormidium animale* HINDAK 1963/108) the opposite is the case. So, the relation between the life cycle stage and the rate of cell division and growth may also be determined genetically. Reliability of the apical cell shape for species determination was confirmed in the studied strains. At least some trichomes had typically developed apical cells in all life cycle stages. They served as confirmation that the considerable morphological changes observed are not the result of the replacement of originally isolated strain by a new one.

3.1.6. Variability among differenet isolates from the same locality

Morphological differences between the isolates from Květné lake are negligable, the same holds for the isolates *Phormidium* cf. *amoenum* BW. Moreover, *Phormidium* cf. *amoenum* I-Sab, found more than 1000 km away is indistinguishable from *Phormidium* cf. *amoenum* BW

strains. On the other hand, two *Phormdium autumnale* CB strains are each of different colour. The same situation occurred in *Phormdium* cf. *subfuscum* I-Roc, but the green isolate did not grow in culture. Similar cases have been documented in past, but little is known about the causes of this phenomenon (KOMÁREK 2006). It is interesting that the *Phormidium* cf. *amoenum* colouration was a kind of greyish blue-green, an intermediate mixture of blue-green and grey of *Phormdium autumnale* CB. No other colour was ever observed in *Phormidium* cf. *amoenum* strains. This particular colour combination is most likely a mere coincidence and there is no explanation for the existence of either one or more coloured variants in a single population at the moment. However, this phenomenon has most likely something to do with adaptation. Either it could reflect the existence of some constraint imposed by the environnment (e.g. the particular colouration of *Phormidium* cf. *amoenum* may offer some considerable advantage compared to other possible colourations for some reason, and therefore it outcompeted them) or it could be a fortuitous result of a particular population history (differently coloured mutants just did or did not appear).

3.1.7. A note on the inherited and environmentally induced variability of morphological characters

Problem with the use of morphological characters for taxonomic purposes dwells, among others, in the difficulty to decide whether these traits are inherited or environmentally induced (WILMOTTE & GOLUBIĆ 1991). It is not known how many and which genes are reponsible for particular traits or if those traits have some adaptive meaning. Apical cell shape and cell width were shown to be quite stable, as already mentioned. Can trichome width be somehow related to the thylakoid arrangement, as cyanobacteria with wider trichomes usually have radial thylakoid pattern? Perhaps yes, but the stability of apical cell shape is quite amazing. It is conceivable that the genes coding for the apical cell shape are only slightly influenced by environmental conditions for some reason, but why this would be so remains unknown. Many more studies on the relation between genotypic and morphological traits will have to be undertaken in order to discover the genetic basis of individual traits, to estimate the range of environmentally induced variability and to establish the importance of various morphological characters for taxonomic purposes on a more profound level.

3.2. Thylakoid arrangement

Two major thylakoid patterns were observed in the studied strains (Fig. 1-8). *Geitlerinema* cf. *lemmermannii* P-G, P-SA, *Geitlerinema* cf. *pseudacutissimum* KLABOUCHOVA 1987/1, *Geitlerinema* cf.*acuminatum* HINDAK 1967/39, *Getlerinema* sp. GROWTHER/1459-6 and *Leptolyngbya* cf. *boryana* P-BJ1 have parietally (concentrically) arranged thylakoids while the rest of the strains have more or less radial thylakoid pattern. Some differences were observed between the strains with the same thylakoid arrangement. *Geitlerinema* cf. *lemmermannii* P-G and P-SA have concentric thylakoids which usually constitute a trianguloid fringe around the interior of the cell. A few thylakoids arranged across the cell were seen in *Geitlerinema* cf.*acuminatum* HINDAK 1967/39 and *Getlerinema* sp. GROWTHER/1459-6 on several occasions in addition to the parietally arranged ones. Whether this is a mere chance or it has some taxonomic meaning is not clear. Radial thylakoids are rather variable in appearance, but this can be due to the life cycle stage of the strains in the moment of specimen preparation or to the specimen preparation itself.

3.3. Phylogenetic analysis

3.3.1. Characterization of *rrn* operons

The number of ribosomal operons of each strain is listed in Table 1. Minimum of two ribosomal operons was found in:

- *Geitlerinema* cf. *lemmermannii* P-G (approx. 99.1% sequence identity, 99.4% seq. id. excluding indels)
- *Phormidium animale* HINDAK 1963/108 (approx. 88 % seq. id., 97% seq. id. without indels)
- *Phormidium* cf. *formosum* P-FW (85,7% seq. id., 99.6% seq. id.without indels)
- *Phormidium autumnale* CB-V (98,9% seq. id., 99,4% seq. id. without indels).

Indications that *Geitlerinema* cf. *lemmermannii* P-SA, *Phormidium autumnale* CB-G and *Phormidium sp* B-Tom may too have more than one *rrn* operon exist. This would not be surprising especially in the case of the former two strains, whose close relatives were shown to have two *rrn* operons. However, potential second operons share more than 99.5% sequence similarity with the rest of the clones, which means that the observed differences can be due to the erroneous nucleotide incorporation by Taq polymerase. The same holds for *Phormidium autumnale* CB-V, in which the possibility of a third operon exists. On the other hand, sequences of the three *Phormidium* cf.*amoenum* strains are virtually identical although they belong to isolates from distant localities and 3-4 clones were sequenced for each strain. *Getlerinema* sp. GROWTHER/1459-6 is a special case. Two operons were identified with very low sequence similarity (73% seq. id., 84% seq. id. without indels). One of them is very similar to that of *Phormidium animale* HINDAK 1963/108 and the other one is more similar to various *Phormidium autumnale* sequences. Only latter sequence was used in phylogenetic analysis. There is, of course, a possibility of contamination, but it is pretty low. In addition, this strain with parietal thylakoids falls into the radial thylakoid group, no matter which one of these two sequences is used. Moreover, it is morphologically almost identical with *Phormidium animale* HINDAK 1963/108. Whether these peculiarities are due to the long time the strain has been in culture (more than 30 years) or it was already isolated as such is a question which can hardly be resolved.

3.3.1.1. 16S-23S ITS region characterization

The length of the 16S-23S ITS region and the presence or absence of tRNA genes in it are listed in Table 1.

16S-23S ITS region varies in length substanitally, from 369 bp in *Phormidium* cf. *formosum* P-FW to 678 bp in *Getlerinema* sp. GROWTHER/1459-6. 16S-23S ITS region of 16 strains contains both tRNA genes. However, *Phormidium* cf. *formosum* P-FW and *Getlerinema* sp. GROWTHER/1459-6 have also the second operon with no tRNA genes. *Phormidium* cf. *okenii* Led-Z, cf. *formosum* P-O, *Phormidium* cf. *irriguum* KOVACIK 1987/5 and one operon of *animale* HINDAK 1963/108 also have no tRNA genes. In all other strains only $tRNA^{Ile}$ gene was detected. Many 16S-23S ITS with none or only $tRNA^{Ile}$ gene were found in comparison with previous studies, where most ITS had both tRNA genes (TATON et al. 2006b, MARQUARDT & PALINSKA, 2006). However, ITS sequences are few and some other studies indicate that the arrangement including only $tRNA^{Ile}$ is also quite common (BOYER et al. 2001, BOYER et al. 2002).

3.3.2. Molecular variability between different strains from same locality

Different strains isolated from the same locality have virtually identical *rrn* operons, with the exception of the *Phormidium autumnale* CB strains (95% seq. id., 97% excluding indels). *Phormidium autumnale* CB strains are, as already mentioned, each of different colour. This is interesting from the ecotype theory of bacterial speciation point of view (COHAN 2001). The theory says that inidivdual clones in bacterial populations (ecotypes) are free to diverge as they do not reproduce sexually. Once in a while, however, an "adaptive mutant" occurs and it outcompetes the rest of the clones, which purges the population of nearly all its diversity at all loci. So, the permanent coexistence of two or more strains of the same ecotype is impossible. However, a novel trait can be acquired by lateral transfer or mutation by one of the ecotype strains, which enables e.g. utilization of some new resource. So, when the adaptive mutant appears, it outcompetes all other strains except that one, which can now diverge permanently and give rise to a new ecotype. *Phormidium autumnale* CB strains may represent two different ecotypes, as there is considerable sequence divergence between them. It is not too convincing, however, that the different colouration would be a trait "powerful" enough to trigger the speciation event. Perhaps it is more likely, that the two strains were isolated during the "divergence period" of the population and so got stabilized as they had been released from competition pressure by the isolation event. On the other hand, striking resemblance of *Phormidium* cf.*amoenum* strains from different localities on both molecular and morphological level could be explained by the fact that both German and Italian strains arose recently from a common ancestor. However, it is still amazing, having in mind just described theory, that they are so alike. It is therefore more likely that some unknown selection pressure influences the strains. Both samples were collected in coniferous forests. Nevertheless, mountain spruce forest of the temperate zone and Mediterranean stone pine forest do not seem to have much in common. Perhaps some property of the litter of those coniferous trees imposes selection pressure on the cyanobacterial population, but this should be confirmed by further research.
These are only hypotheses and surely many others are also conceivable.

3.3.3. Problems concerning cyanobacterial sequences in public databases

Very little is known about morphology, ecology or often even of the geographical origin of the vast amount of strains whose sequences are deposited in public databases (e.g. WILMOTTE & HERDMAN 2001). I have even found a sequence, denoted as "*Oscillatoria salina* clone T7" under the accession number DQ080032, which is by no means of cyanobacterial origin. Moreover, it is not even a small ribosomal subunit RNA gene, but shares most sequence identity with some *Vibrio* proteins. Such sequences can cause a great deal of confusion in the phylogenetic analysis. Incorrect taxonomic designation of sequences in public databases has been a big problem for almost 20 years (WILMOTTE & GOLUBIĆ 1991, CASAMATTA et al. 2003, WILLAME et al. 2006). Unfortunately, some researches "determine" their cyanobacterial strains on the basis of comparison of their sequences to the public databases. If the most similar sequence is e.g. "*Oscillatoria*", they use the same name for their sequence and the problem misidentification gets even worse (e.g. JING et al. 2005). In addition, many authors refer phylogenetic relationship among those sequences (CASAMATTA et al. 2003). A good example is again *Phormidium*, often claimed to be polyphyletic on the basis of the phylogenetic analysis of sequences belonging to morphotypes which respond to the

description of other validly defined genera (e.g. TENEVA et al. 2005, MARQUARDT & PALINSKA 2007).

3.3.4. The phylogenetic tree

The alignment of 16S rDNA sequences is 1202 bp long and comprises 139 sequences.
Maximum parsimony and MrBayes trees are not shown, because they are largely the same as the ML tree (see below). The branching order of the large clusters is somewhat different in the MP tree, but as the bootstrap supports are negligible these slight incongruences were not taken into account. It is important to notice, that only 386 out of 1202 characters were parsimony informative and both consistency index (CI=cca 0,2) and homoplasy index (HI=cca 0,8) indicate a great portion of parallel evolution in the dataset. However, smaller clusters encountered also in the ML tree are well supported. The same holds for MrBayes tree, except for the fact that there is significant basal polytomy, so absolutely nothing can be said about the branching order of these supported clusters.
The Maximum Likelihood tree with ML and MP bootstrap values as well as with Bayesian posterior probabilities is shown in Figure 9. Four major clusters can be identified in this tree. However, the bootstrap supports are extremely low and these clusters cannot be considered credible. The unability to identify relations among cyanobacteria on larger evolutionary scale was noticed already by GIOVANONNI et al. 1988. He and later researchers (HONDA et al. 1999, WILMOTTE & HERDMAN 2001) believe that this is probably due to the rapid diversification of cyanobacteria immediately after the acquisition of oxygenic photosynthesis. This implies that the branching order of the main cyanobacterial lineages as well as their factual form may remain unrevealed forever, at least in 16S rDNA inferred phylogeny. On the other hand, some smaller clusters are well statistically supported.

3.3.4.1. Clusters containing sequences obtained in this study

Well supported clusters containing sequences obtained in this study are represented in Figure 10.
Clusters A, "*Phormidium* 2", C and G contain sequences of strains which have radial thylakoid arrangement. Ultrastructure is not known for many members of these groups, but the same thylakoid pattern would be probably found in the most. However, there is already one known exception to the rule - *Getlerinema* sp. GROWTHER/1459-6, which has concentric thylakoids. Peculiarities concerning this strain are described above. Nevertheless, if the sequence really belongs to this strain, then the reversion to the parietal thylakoid arrangement must have occurred. Clusters D, E and F contain the author´s sequences belonging to the strains with concentric thylakoid arrangement.

3.3.4.1.1. Cluster A

Cluster A is further divided in "*Phormidium* 1" and "*Oscillatoria* 1" clusters.

- "*Phormidium* 1" cluster consists of two well supported subclusters.
  - "*Phormidium* 1.1" is dominated by the author´s sequences of the "*Phormidium autumnale*" group, but also contains sequences of *Microcoleus vaginatus* PCC 9802

and "*Oscillatoria*" (probably *Phormidium*) sp. PCC 7112. The two lone sequences in "*Phormidium* 1" cluster belong to *Phormidium autumnale* CB-G and CB-V strains. All 16S rDNA sequences of "*Phormidium* 1.1" cluster and those of *Phormidium autumnale* CB-G and CB-V have a 11-bp insert in variable loop six (V6) on the 16S rRNA gene (bp 423–433), which has previously been reported for desert *Microcoleus vaginatus* strains (BOYER et al. 2002) as well as for related Antarctic (NADEAU et al. 2001, CASAMATTA et al. 2003, TATON et al. 2006a) and *Phormidium* sp. NIVA-CYA 203 (RUDI et al. 1997). Thus, this group of taxa has obviously cosmopolitan distribution. Moreover, all strains falling into this group have rather uniform basic morphological characters - they are at least 5 μm wide and all have calyptra. Congruence between basic morphological and molecular characters as well as high sequence similarity inspite of the cosmopolitan distribution indicate that either this is a relatively young cyanobacterial group with considerable dispersal potential or that it is quite conserved on the DNA level, so it managed to remain almost unchanged during long period of time.

▪ "*Phormidium* 1.2" contains 16S rDNA sequences of *Tychonema bourrelyii* and polar *Phormidium autumnale* and *Microcoleus antarcticus* strains in addition to the author´s *Phormdium* cf. *subfuscum* I-Roc strain.

Two more sequences are situated on the basis of this cluster - *Getlerinema* sp. GROWTHER/1459-6 and *Phormidium* cf. *tergestinum* Drak. Neither morphological nor ultrastructural similarities exist between those two strains and they differ from the rest of the cluster. The two sequences are quite divergent, so it is possible that they are first two sequenced representatives of a potential "*Phormidium* 1" sister cluster.

It is interesting to notice that, inspite of high overall sequence similarity of sequences, it is possible to distinguish between the strains of the author´s "*Phormidium autumnale*" group isolated from different locations. It is probably due to the fact that each of the three groups of strains (CB, BW - I-Sab and Kvet) is adapted to somewhat different habitat type. All other related sequences belong to the strains isolated from distant geographical regions, which is the additional source of potential variation. I do not believe that such location-specific grouping would occur if the sequences would originate from strains isolated e.g. from ecologically similar puddles around Czech Republic or perhaps Europe.

With the exception of *Tychonema bourrelyi* and probably *Phormidium* sp. NIVA-CYA 203 (the sequence is not used in this analysis) all strains of this cluster are isolated either from soil or from some type of benthic habitat (puddles, seepages, lakes, streams). On the other hand they are adapted to very different environmental conditions - cold, high light intensity, drought etc. None of the strains is marine. As far as known, all representatives of this cluster have radial thylakoid arrangement, same cell division type and all have calyptra. Thus, the basic cell and trichome morphology support the uniformity of the strains on molecular level. It seems that the low amount of variation on 16S rDNA level is not an obstacle for those strains to adapt to various environmental conditions, or to vary in morphological traits such as slime production and the cell content appearance, which are considered sufficient to assign these strains to different species of even genera.

- "*Oscillatoria* 1" cluster can be divided in the "*Oscillatoria sancta*" and well defined marine *Trichodesmium* genus (BEN-PORATH et al. 1993, ABED et al. 2006) with the uncertain position of *Hydrocoleum* sp. The first one contains the author´s sequences of *Oscillatoria sancta* 1970/SAG 74.79 and *Oscillatoria* cf. *curviceps* Fkv-3 and Fkv-4 and *Oscillatoria sancta* PCC 7515. *"Trichodesmium"* cluster is marine, while "*Oscillatoria sancta*" seems to be freshwater/terrestrial. That is why it is most likely that *Hydrocoleum* sp. belongs to the former cluster and the uncertainty of its position is probably caused by the short length of its 16S rDNA sequence.

3.3.4.1.2. "Phormidium 2" cluster

"*Phormidium* 2" cluster contains closely related taxa of the genus *Phormidium*, as far as known with radial thylakoid pattern and with more or less conical-narrowed apical cells without calyptra. It is interesting that, although the two *Phormidium* cf. *formosum* strains are very similar morphologically, they do not group together in the phylogenetic tree. *Phormidium* cf. *formosum* P-O seems to be closely related to the African hot spring *Phormidium* cf. *terebriformis* KR2003/25 strain. Also this *Phormidium* cluster contains strains from distant geographic regions. Strains are closely related on molecular level, but as far as known they occur in dissimilar habitats such as hot springs or temperate fish pond.

3.3.4.1.3. Cluster C

Cluster C contains two minor subclusters, one of which is further divided into two groups. Some morphological data are available for *Lyngbya* cf. *confervoides* VP0401 in addition to those for *Phormidium* cf. *aerugineo-coeruleum.* There are no data for the Thai *Planktothricoides raciborskii* OR1-1 strain, but some are available for closely related *Planktothricoides raciborskii* INBaOR. It is interesting that all strains in this cluster were isolated from the habitats with higher conductivity. *Lyngbya* cf. *confervoides* VP0401 and *Geitlerinema* sp. PCC 7105 are marine, "*Phormidium*" sp. UTCC 487 was isolated from potassium mine drainage and "*Phormidium*" sp. ETS-05 is from thermal mud. The original habitat of *Planktothricoides raciborskii* OR1-1 is not known, but the already mentioned *Planktothricoides raciborskii* INBaOR comes from a very polluted lake Inbanuma (Japan), so some similar habitat may be postulated for *P. raciborskii* OR1-1. *Phormidium* cf. *aerugineo-coeruleum* was found in a tropical freshwater aquarium. There were many fish in that aquarium and their faeces could have caused a higher electrolyte concentration (this is, however, not confirmed). So, although that the strains of this cluster seem to be quite diverse on molecular level compared to previous clusters, it seems that they share some basic ecophysiological (probably inherited) trait that enables the survival in habitats with higher ion concentration. The image, though, might change as more representatives of this group are sequenced.

3.3.4.1.4. Cluster G

Cluster G contains only two sequences - those of *Phormidium* cf. *irriguum* KOVACIK 1987/5 and *Phormidium autumnale* UTEX 1580. Unfortunately, no morphological data are available for the latter strain, so it cannot be compared with cluster A *Phormidium autumnale* strains. The thylakoid pattern is known in *Phormidium* cf. *irriguum* KOVACIK 1987/5 and it is radial. These two strains and the clusters containing *Microcoleus sociatus* MPI 96MS.KID, *Phormidium ambiguum* M-71 and *Oscillatoria princeps* NIVA-CYA 150 are always found in the vicinity of heterocytous cyanobacteria in the tree. Nothing can be said about the exact branching order and relations between these strains, as the bootstrap

supports are very low and the exact grouping of the strains changes with the method and the particular parameters used in the phyologenetic analysis. Nevertheless, they are never found in the distant branches of the tree.

Clusters D, E and F contain sequences of the strains with the concentric thylakoid arrangement.

3.3.4.1.4. Cluster D

Geitlerinema cf. *pseudacutissimum* KLABOUCHOVA 1987/1 and *Getilerinema carotinosum* AICB 37 (cluster D) share 96% sequence identity (97% without indels) and the assignment of those strains to different species seems to be justified. Otherwise they are very similar morphologically and both have a bit elongated apical cell. They are closely related to unicellular "*Microcystis* and co." cluster (again poorly supported by bootstrap values).

3.3.4.1.4. Cluster E

Cluster E ecompasses two sequences of *Geitlerinema* cf. *lemmermannii* (P-G and P- SA) and a strain identified as "*Limnothrix redekei*" from lake Kastoria, Greece (GKELIS et al. 2005). All tree strains are morphologically very similar according to the available photographs. The misidentification of "*Limnothrix redekei*" probably occurred because all three strains have polar granules, somewhat resembling gas vesicles. These three strains are most closely related to the unicellular *Cyanobium gracile* PCC 6307 and *Synechococcus elongatus* PCC 6301 strains. It is interesting that all mentioned strains are isolated from freshwater biotops (perhaps terrestrial, but by no means saline), while all nearby branches contain primarily halophilic (halotolerant?) strains.

3.3.4.1.4. Cluster F

Cluster F contains polar *Leptolyngbya antarctica* ANT.LH18.1, thermophile "*Oscillatoria*" sp. J-24-Osc, *Leptolyngbya* sp. CENA 112 from a Brazilian facultative waste stabilization pond, Leptolyngbya sp. "VRUC 135 Albertano 1987/1" from Roman hypogea (BELLEZZA et al. 2003) and *Leptolyngbya* cf. *boryana* P-BJ1 of unknown origin. It is morphologically and ecologically diverse group according to the available data.

3.3.4.2. Other well supported clusters

Other well supported clusters are shown in Figure 11.

3.3.4.2.1. "Arthrospira" cluster

Arthrospira is morphologically and genetically well defined and coherent genus (NELISSEN et al. 1994, MANEN & FALQUET 2002, ZHANG et al. 2005). It is closely related to *Phormidium* cf. *terebriformis* AB2002/07, a strain from alkaline-saline lake Nakuru (Kenya), with somewhat wavy, but by no means regularly coled trichomes. A sister taxon of this group is a salt marsh strain *Lyngbya aestuarii* PCC 7419. Also this group seems to be at least halotolerant.

3.3.4.2.2. "Marine Lyngbya" cluster

"Marine Lyngbya" cluster encompasses 16S rDNA sequences of two *Lyngbya* strains. Their relation to *Symploca* sp. VP377 is not supported by bootstrap values in ML and MP trees. However, the posterior probability value for this branch is high (0.94) in the MrBayes phylogenetic tree. All tree strains come from coral reef ecosystems of Micronesia.

3.3.4.2.3.. "LPP 1" cluster

"LPP 1" cluster contains, among others, sequences of marine "LPP-group B" MBIC 10597 and "*Phormidium*" sp. MBIC 10025 strains from Japan. The rest of the strains are isolated from various habitats in Antarctica. All these strains are constricted at the cross-walls and, as far as known, not more than 3µm wide (no morphological data are available for "*LPP-group*" QSSC8cya strain).

3.3.4.2.4. "LPP 2" cluster

"LPP 2" cluster is comprised of brackish, certainly misidentified "*Schizothrix calcicola*" CIBNOR and marine "*LPP-group B*" MBIC 10087, "*Phormidium*" sp. MBIC 10003 and *Leptolyngbya* sp. PCC 7375 strains. This group is morphologically similar to the previous one. Its relatedness to the well defined halophilic *Halomicronema* genus (ABED et al. 2002) is poorly supported by bootstrap values.

3.3.4.2.5. "Leptolyngbya" and "Pseudanabaenaceae" clusters

The rest of the clusters, except for the well defined *Planktothrix* group (Suda et al. 2002), are composed of various strains with thin filaments. However, behind the simple morphology a great amount of molecular diversity is hiding and little congruence between morphological, molecular and ecological data exists (KAŠTOVSKÝ, per. comm. ex JOHANSEN et al., in prep.). Therefore, these clusters will not be discussed in detail.

3.3.4.3. Position of the rest of the studied strains in the tree

Phormidium sp. B-Tom seems to be most closely related to the Antarctic *Phormidium murrayi* Ant-Ph58 strain. However, the bootstrap support values are low again (only posterior probability value in MrBayes tree exceeds 0.5 limit, but only by one hundredth) and nothing can be said for sure about the relatedness of these strains. Equally unclear is their relation to *Microcoleus chthonoplastes* PCC 7420, uncultured "*Plectonema*" sp. Pc49 and "*Oscillatoria boryana*" BDU92181 (*Phormidium boryanum*) strains, situated in nearby branches of the tree. *Phormidium* sp. B-Tom is a case of a strain with rather wide trichomes and parietal thylakoid pattern. No ultrastructural data on other strains of this group are available.
Geitlerinema cf. *acuminatum* HINDAK 1967/39 is found in the vicinity of the symbionts of marine invertebrates and "*Oscillatoria earlei*" (*Getilerinema earlei*). The bootstrap support is low and 16S rDNA is quite divergent in members of this group. Nevertheless it is interesting that both *Geitlerinema earlei* and *Geitlerinema* cf. *acuminatum* have sharply pointed apical cells.

3.3.4.4. Relation between molecular and ecological data

Many well supported clusters, such as "LPP 1", "LPP 2", "*marine Lyngbya*", cluster C, "*Trichodesmium*" and "*Arthrospira*", contain solely, or at least mainly, isolates from habitats with higher conductivity (marine, mineral and hot springs, extremely polluted lakes). The two "LPP" clusters and *Halomicronema* are ecologically (physiologically?) and morphologically quite homogenous and maybe even related to each other. There is some evidence that morphologically similar strains from similar habitats are closely related (MARQUARDT & PALINSKA 2007). On the other hand, "*Phormidium* 2", "*Pseudanabaenaceae*" and "*Leptolyngbya*" clusters are ecologically extremely heterogenous. It is difficult to say, if the discrimination between e.g. halophilic (halotolerant) strains from different evolutionary groups will ever be possible without DNA sequencing. The possibility of finding e.g. some ultrastructural trait typical for a given group cannot be excluded. Relative stability of ecological traits in some groups and its complete lack in others could have many different causes. It is possible that the same ecological trait has a different molecular background in those groups. So, in some groups it would be rather conserved, while in others it could change freely. The other possibility is that some cyanobacterial lineages in general are able to evolve more rapidly than the others for some unknown reason.

4. Conclusion

The topology of the obtained phylogenetic tree is congruent with the results from other authors (e.g. COMTE et al. 2006, WILLAME et al. 2006).
Genera *Phormidium* and *Oscillatoria* sensu Anagnostidis & Komárek 2005 are polyphyletic. The representatives of *Phormidium* are found in at least four clusters ("*Phormidium* 1", "*Phormidium* 2", cluster C, cluster G) while those of *Oscillatoria* are found in two clusters ("*Oscillatoria* 1" and *Oscillatoria princeps* sequence). It seems that there are also at least two lineages of cyanobacteria with radial thylakoid arrangement. One of them (*Oscillatoria princeps* lineage) is related to heterocytous cyanobacteria and only few sequences of the members of this branch are available. Thus, its internal structure and molecular variability and coherence are unknown at the moment. The second cluster is poorly supported by the bootstrap values as a whole, but five well supported clusters are found within. On the other hand, both main "clusters" (the first one consists of several "double-sequence" clusters) branch off near the root of the phylogenetic tree. So, it cannot be excluded that those clusters represent an ancient cyanobacterial lineage with so divergent sequences and such amount of homoplasy, that it would be impossible to confirm its monophyly, at least on the basis of 16S rDNA. This question could perhaps be solved by use of some other slowly evolving gene. As the bootstrap support for the observed branching pattern is extremely low, this hypothesis does not seem so improbable.
No thin (< 3 μm) strain has radial thylakoid arrangement as far as known. On the contrary, strains wider than cca 6 μm have radial thylakoid arrangement with the exception of *Phormidium* sp. B-Tom. Unfortunately, nothing about the ultrastructure of closely related strains of *Phormidium murrayi* and others is known. The taxa with the intermediate width may have both radial and parietal thylakoids (e.g. *Phormidium animale* vs. *Geitlerinema* sp. Growther). This indicates the need for great caution in taxonomic determination of this group of strains.
16S rDNA reflects neither distinctions in characters such as the form of mucilage nor ecological variability within these groups. Nevertheless, basic morphological and ultrastructural characters are congruent with 16S rDNA based phylogeny in two major *Phormidium* clusters (*"Phormidium* 1*"* and *"Phormidium* 2*"*).

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Appendix

Table 1. Basic characteristics of the strains used in this study, further details are in the text (a[1] - the numbers in the first row are 25% and 75% quantiles, the numbers in the second row are the range and the median)

strain designation	species		originally determined as:	cell width (µm)[1]	cell length/width ratio[1]	apical cell width (µm)[1]	apical cell length/width ratio[1]	habitat	geographical origin	minimal number of *rrn* operons	t-RNA^{Ile}	t-RNA^{Ala}	approx.ITS length (bp)
P-BJ1	*Leptolyngbya* cf. *boryana*		*Oscillatoria* sp.	1,9-2,3 (1,3-3,4; 2,1)	0,7-1,0 (0,4-2,1; 0,85)	1,6-2,1 (1,3-3,2; 1,8)	0,8-1,2 (0,4-2,0; 1,0)	unknown	Pakistan	1	+	+	506
P-G	*Geitlerinema* cf. *lemmermannii*		*Oscillatoria* sp.	1,4-1,7 (1,1-2,1; 1,5)	2,2-3,2 (1,3-4,9; 2,7)	1,4-1,6 (1,2-1,9; 1,5)	2,4-3,1 (1,7-4,4; 2,8)	unknown	Pakistan	2	+	-	485
P-SA	*Geitlerinema* cf. *lemmermannii*		*Oscillatoria* sp.	1,8-2,1 (1,2-2,7; 1,9)	1,6-2,6 (0,6-4,5; 2,0)	1,6-1,9 (1,3-2,3; 1,8)	1,3-2,4 (0,2-4,1; 1,8)	unknown	Pakistan	1 (2?)	+	-	485
KLABOUCHOVA 1987/1 (CCALA 142)	*Geitlerinema* cf. *pseudacutissimum*		*Geitlerinema* cf. *splendidum*	1,8-2,1 (1,0-2,8; 1,9)	1,3-1,9 (0,8-6,4; 1,6)	1,5-2,0 (1,1-2,6; 1,8)	1,5-2,4 (0,8-4,2; 1,9)	soil, meadow	Czech Republic, river Luznice valley	1	+	-	510
HINDAK 1967/39 (CCALA 141)	*Geitlerinema* cf.*acuminatum*		*Phormidium* cf. *animale*	3,5-4,1 (2,6-5,0; 3,9)	0,8-1,1 (0,5-1,8; 1,0)	2,9-3,9 (1,5-5,0; 3,3)	1,1-1,9 (0,7-4,3; 1,3)	soil	Romania, town Brasov, Poiana Brasov soil	1	+	+	490
GROWTHER/1459-6 (CCALA 138)	*Getlerinema* sp.		*Phormidium animale*	3,6-4,3 (2,5-5,3; 3,9)	0,8-1,1 (0,4-1,6; 0,9)	2,5-3,3 (1,6-4,6; 3,0)	1,1-1,6 (0,5-2,6; 1,3)	freshwater	United Kingdom, London, University College	2 ?	-	-	409
											+	+	678
HINDAK 1963/108 (CCALA 140)	*Phormidium animale*		*Phormidium animale*	4,1-5,0 (2,4-6,6; 4,5)	0,6-0,8 (0,3-1,4; 0,7)	2,5-3,3 (1,5-5,4; 2,9)	1,1-1,8 (0,5-3,9; 1,4)	soil	Italy, crater of the volcano Vesuv	2	+	-	468
													502
Led-Z	*Phormidium* cf. *okenii*			5,6-6,5 (3,7-7,5; 6,1)	0,5-0,8 (0,3-1,8; 0,6)	3,3-5,1 (2,5-7,5; 3,8)	1,0-2,1 (0,4-3,1; 1,4)	a pond, benthos	Czech Republic, Lednice na Moravě, park	1	-	-	372
P-FW	*Phormidium* cf. *formosum*		*Oscillatoria* sp.	4,0-4,5 (2,2-5,1; 4,2)	0,4-0,7 (0,3-1,3; 0,6)	2,1-3,0 (1,5-5,0; 2,6)	1,2-2,0 (0,4-3,0; 1,5)	unknown	Pakistan	2	+	+	636
											-	-	369
P-O	*Phormidium* cf. *formosum*		*Oscillatoria* sp.	5,0-5,8 (3,9-7,1; 5,3)	0,4-0,6 (0,3-1,0; 0,5)	3,6-4,9 (2,0-7,0; 4,1)	0,7-1,9 (0,2-3,0; 1,5)	unknown	Pakistan	1	-	-	351
Kvet-0	*Phormidium autumnale*	*Phormidium autumnale* group		5,3-5,8 (3,9-6,5; 5,6)	0,4-0,6 (0,2-1,0; 0,5)	3,4-5,3 (2,3-6,2; 4,8)	0,8-1,0 (0,6-1,4; 0,9)	ox-bow, benthos	Czech Republic, Lednice na Moravě, Květné lake (Dyje river)	1	+	+	573
Kvet-1	*Phormidium autumnale*			5,7-6,4 (4,3-7,2; 6,1)	0,3-0,5 (0,2-1,0; 0,4)	3,6-5,5 (2,7-6,2; 4,3)	0,7-0,9 (0,4-1,2; 0,8)	ox-bow, benthos	Czech Republic, Lednice na Moravě, Květné lake (Dyje river)	1	+	+	573
Kvet-2	*Phormidium autumnale*			5,3-5,8 (4,2-7,1; 5,5)	0,4-0,7 (0,2-1,6; 0,6)	3,3-5,1 (2,4-6,5; 3,9)	0,8-1,0 (0,6-1,6; 0,9)	ox-bow, benthos	Czech Republic, Lednice na Moravě, Květné lake (Dyje river)	2	+	+	573
BW-0	*Phormidium* cf. *amoenum*			5,9-6,6 (5,1-8,4; 6,2)	0,3-0,6 (0,1-1,5; 0,4)	4,2-5,4 (2,2-6,6; 5,0)	0,8-1,1 (0,6-1,6; 0,9)	puddle in the spruce forest	Germany, near border cross.point Prášily- Gsenget - Scheureck	1	+	+	563
BW-1	*Phormidium* cf. *amoenum*			6,0-6,6 (4,4-7,3; 6,3)	0,5-0,8 (0,2-1,1; 0,6)	3,3-4,6 (2,2-6,6; 3,9)	1,1-1,4 (0,7-2,0; 1,2)	puddle in the spruce forest	Germany, near border cross.point Prášily- Gsenget - Scheureck	1	+	+	563
I-Sab	*Phormidium* cf. *amoenum*			6,0-6,7 (3,6-7,5; 6,4)	0,6-0,9 (0,3-1,3; 0,8)	2,9-4,3 (2,3-7,0; 3,3)	1,0-1,5 (0,5-2,0; 1,1)	ditch in a stone pine wood	Italy, Sabbaudia town	1	+	+	563
CB-G	*Phormidium autumnale*			6,5-7,4 (5,3-8,3; 6,9)	0,3-0,5 (0,2-0,7; 0,4)	3,1-6,3 (2,0-7,8; 4,7)	0,6-1,0 (0,5-1,7; 0,8)	puddle in the vicinity of the dump yard	Czech Republic, České Budějovice town	1 (2?)	+	+	548
CB-V	*Phormidium autumnale*			6,1-7,3 (4,5-8,2; 6,8)	0,3-0,5 (0,2-0,8; 0,4)	3,2-4,2 (2,2-7,2; 3,6)	0,8-1,1 (0,6-0,6; 1,0)	puddle in the vicinity of a dump yard	Czech Republic, České Budějovice town	2 (3?)	+	+	574
													580
I-Roc	*Phormdium* cf. *subfuscum*			6,9-7,8 (5,5-9,7; 7,4)	0,2-0,4 (0,1-0,8; 0,4)	3,3-5,1 (2,4-7,1; 3,9)	0,6-0,9 (0,3-1,5; 0,7)	small water-fall	Italy, Roccadaspide town surroundings	1	+	+	549

Fkv-3, Fkv4	***Oscillatoria* cf. *curviceps***		**13,2-15,1 (11,8-16,6; 14,4)**	**0,2-0,4 (0,1-0,6; 0,3)**	**11,8-13,6 (9,0-15,5; 12,8)**	**0,4-0,6 (0,2-0,8; 0,5)**	**flowerpot in a tropical greenhouse**	**Czech Republic, Prague, Botanical garden**	**1**	+	+	**457**
KOCH 1970/SAG 74.79 (CCALA 135)	***Oscillatoria sancta***	***Oscillatoria sancta***	**14,6-17,0 (9,0-18,8; 15,7)**	**0,2-0,4 (0,1-0,5; 0,3)**	**12,6-14,7 (5,5-16,8 (13,6)**	**0,3-0,5 (0,2-0,6; 0,4)**	**greenhouse**	**Germany, University of Gottingen, Botanical Garden**	**1**	+	+	**458**
KOVACIK 1987/5 (CCALA 759)	***Phormidium* cf. *irriguum***	***Oscillatoria limosa***	**6,7-7,7 (5,5-9,2; 7,1)**	**0,3-0,5 (0,1-0,8; 0,4)**	**6,5-7,7 (5,7-8,4; 7,1)**	**0,4-0,6 (0,3-2,6; 0,5)**	**littoral of a sand pit lake**	**Slovakia, Bratislava, Strkovec lake**	**1**	-	-	**427**
Drak	***Phormidium* cf. *tergestinum***		**7,1-7,8 (5,8-8,8; 7,4)**	**0,3-0,5 (0,2-0,7; 0,4)**	**6,7-7,1 (5,8-8,7; 7,1)**	**0,5-0,7 (0,4-1,0; 0,6)**	**flowing water**	**Czech Republic, Dračice brook**	**1**	+	+	**520**
B-Tom	***Phormidium* sp.**		**5,5-6,3 (4,4-7,2; 5,9)**	**0,6-0,8 (0,3-1,7; 0,7)**	**4,8-5,8 (3,2-7,2; 5,4)**	**0,8-1,2 (0,5-1,8; 1,0)**	**wet rock near seashore**	**Brasil, Tominhos**	**1 (2?)**	+	-	**475**
R-aq	***Phormidium* cf. *aerugineo-coeruleum***		**5,5-6,8 (4,7-8,2; 6,0)**	**0,4-0,6 (0,2-1,0; 0,5)**	**3,3-5,4 (1,8-6,7; 4,1)**	**0,7-1,0 (0,6-2,2; 0,8)**	**freshwater (brackish?) aquarium**	**(Czech Republic)**	**1**	+	-	**500**

Table 2. PCR and Cycle sequencing programmes used in this study

PCR	temperature	time	cycles
initial denaturation	94˚C	5 min	
denaturation	94˚C	45 s	10
annealing	57˚C	45 s	
extension	72˚C	2 min	
denaturation	94˚C	45 s	26
annealing	54˚C	45 s	
extension	72˚C	2 min	
final extension	72˚C	7 min	
sequencing	temperature	time	cycles
initial denaturation	94˚C	1 min	
denaturation	94˚C	30 s	30
annealing	50˚C	45 s	
extension	60˚C	4 min	

Table 3. Primers used in this study; CYA359F and Primer 18 were used for PCR, ther rest were used for sequencing

primer	5‘-3‘ sequence	target sequence	author
CYA359F	GGG GAA TTT TCC GCA ATG GG	359-378 of 16S rDNA	NUBEL et al. 1997
Primer 18	CTC TGT GTG CCT AGG TAT CC	36-45 of 23S rDNA	WILMOTTE 1994
Primer 14	TGT ACA CAC CGG CCC GTC	1334-1350 of 16S rDNA	WILMOTTE 1993
Primer 14 reverse complement	GAC GGG CCG GTG TGT ACA	1350-1334 of 16S rDNA	WILMOTTE 1993
CYA781F	AAT GGG ATT AGA TAC CCC AGT AGT C	approx. bp 800 of 16S rDNA	NUBEL et al. 1997
WAW1486R	AAG GAG GTG ATC CAG CCA CA	unspecified, 16S-23S ITS region	WILMOTTE 1993
M13R	CAG GAA ACA GCT ATG A	both primers target sequences in TOPO TA CLONING® KIT vector	
M13F	GTA AAA CGA CGG CCA GT		

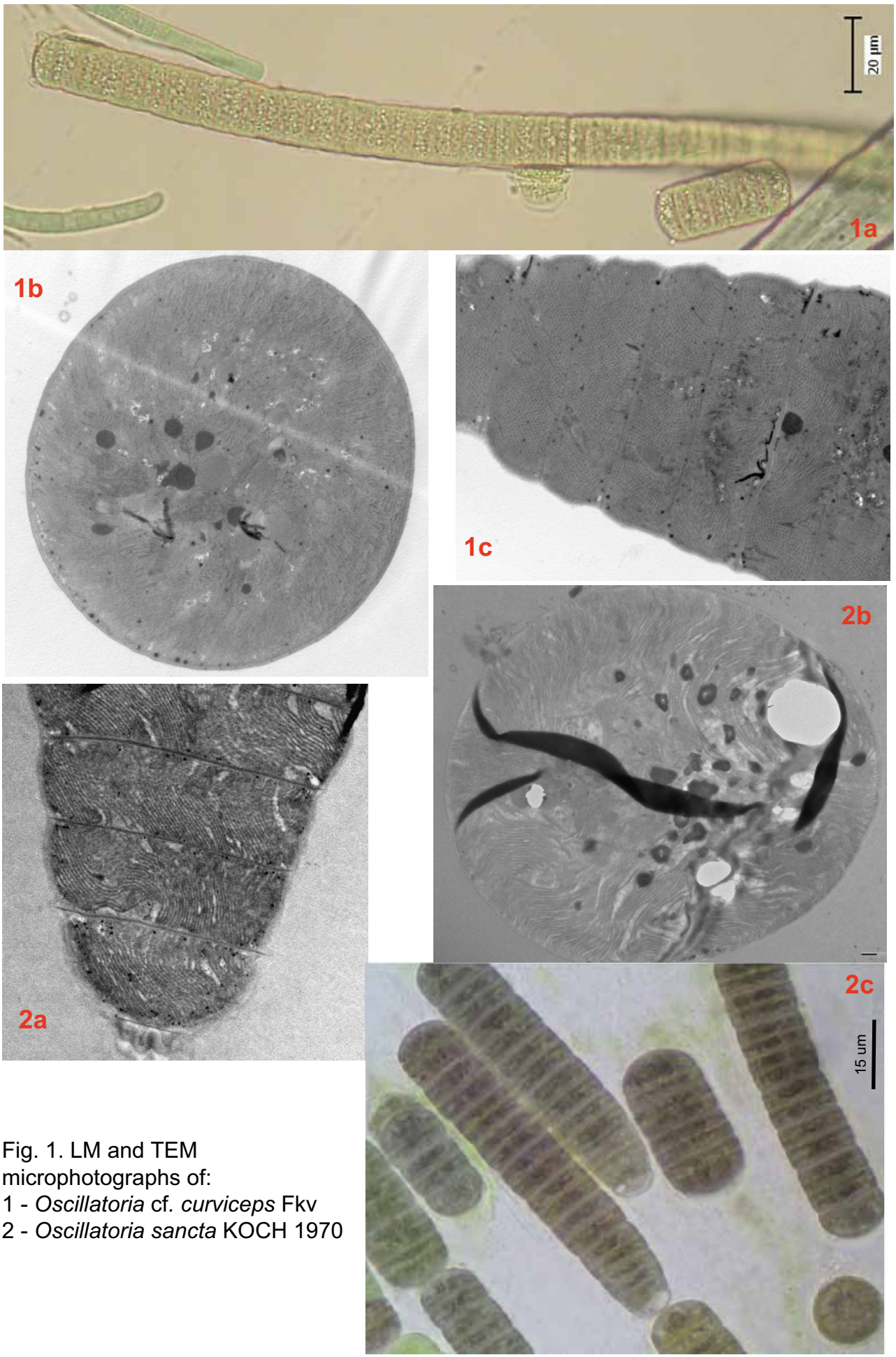


Fig. 1. LM and TEM microphotographs of:
1 - *Oscillatoria* cf. *curviceps* Fkv
2 - *Oscillatoria sancta* KOCH 1970

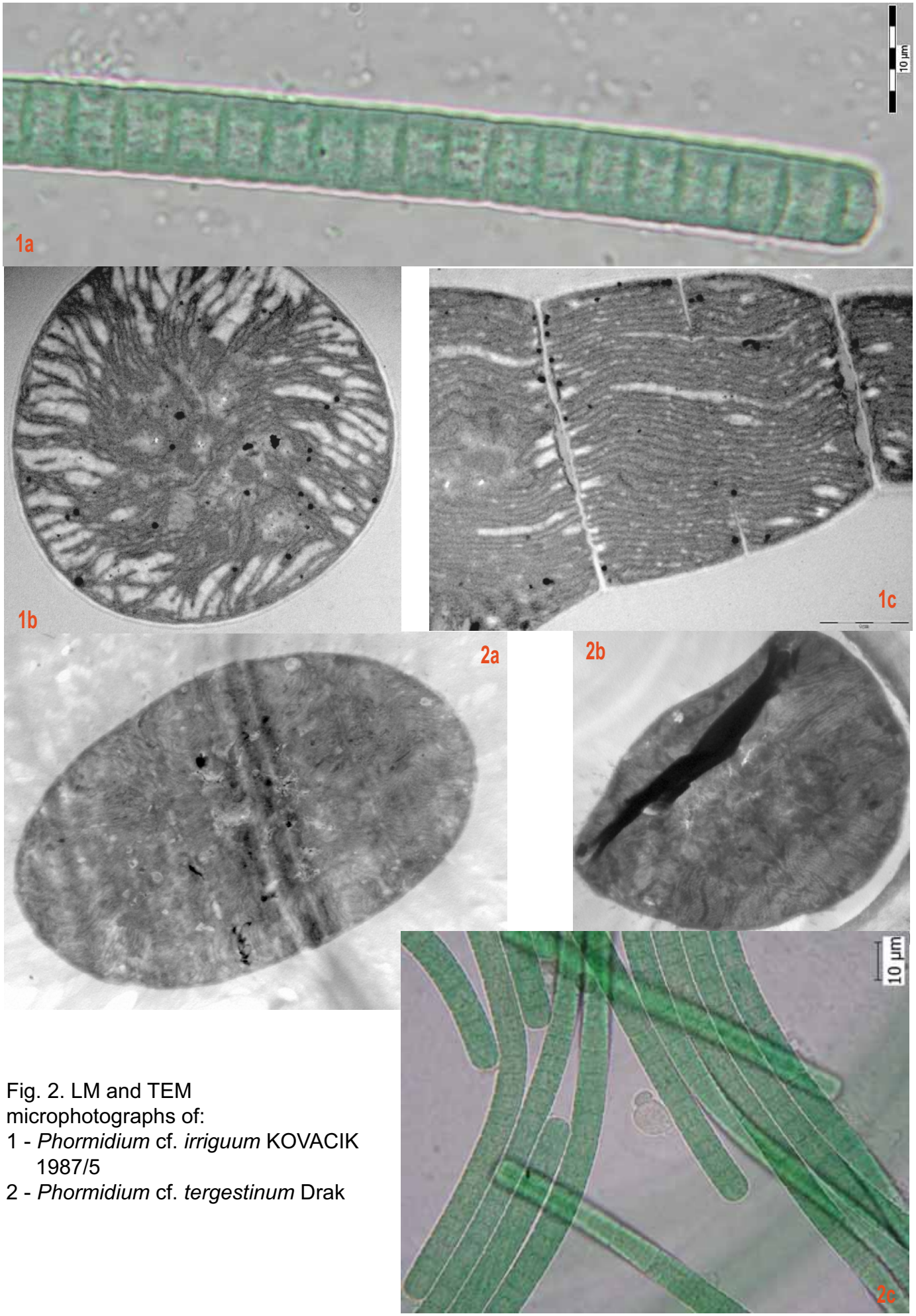


Fig. 2. LM and TEM microphotographs of:
1 - *Phormidium* cf. *irriguum* KOVACIK 1987/5
2 - *Phormidium* cf. *tergestinum* Drak

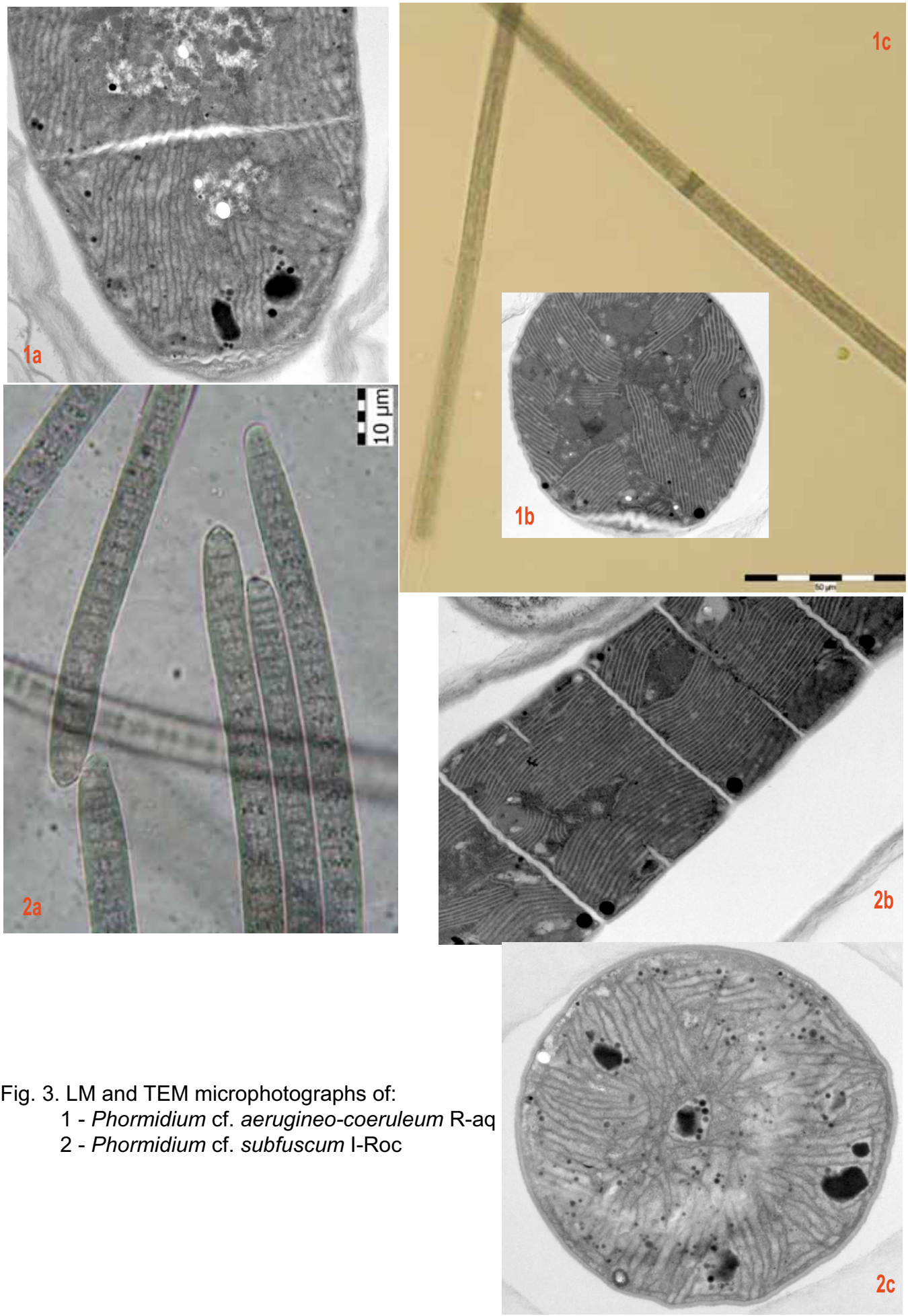


Fig. 3. LM and TEM microphotographs of:
1 - *Phormidium* cf. *aerugineo-coeruleum* R-aq
2 - *Phormidium* cf. *subfuscum* I-Roc

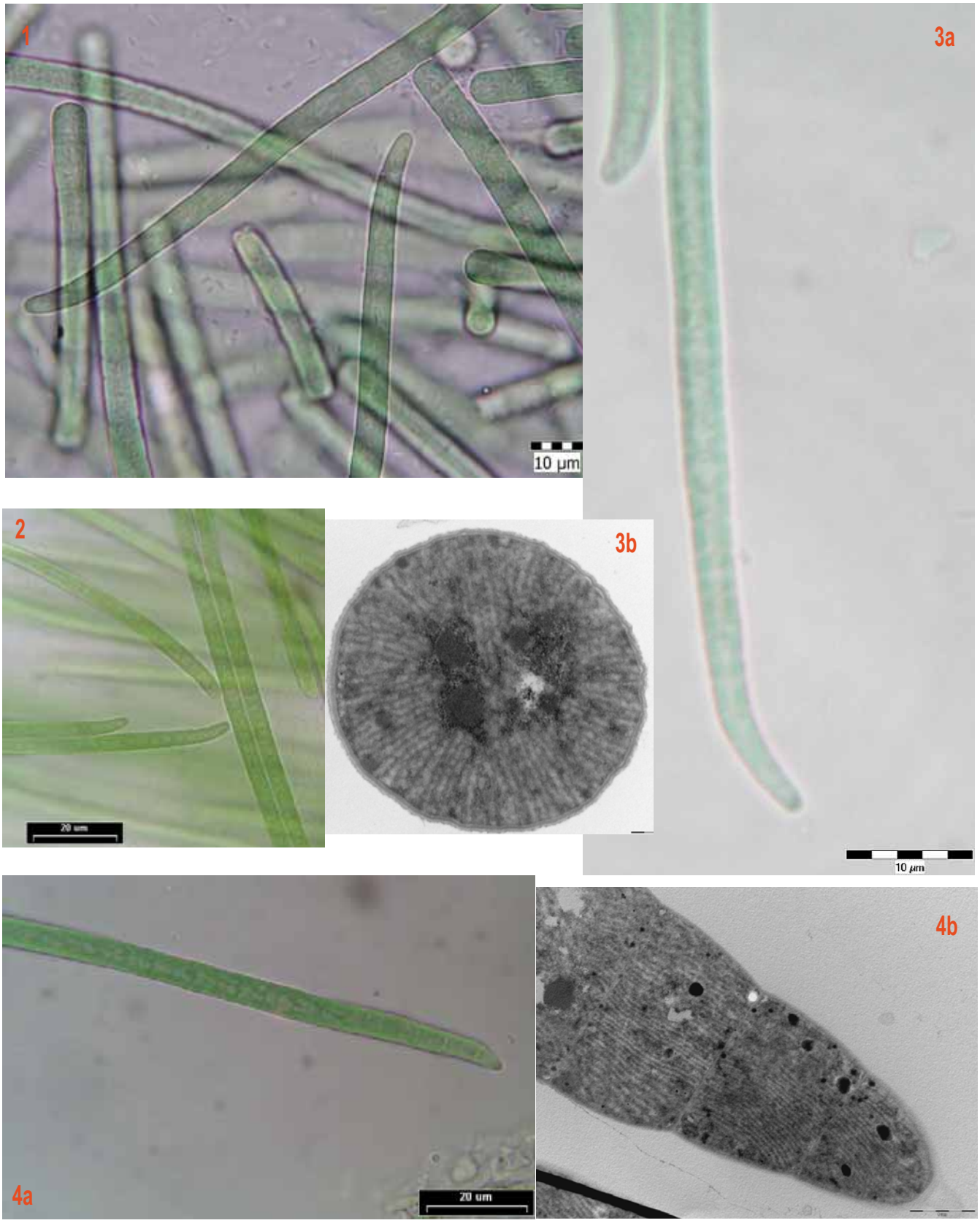


Fig. 4. LM and TEM microphotographs of:
1 - *Phormidium* cf. *okenii* Led-Z
2 - *Phormidium animale* HINDAK 1963/108
3 - *Phormdium* cf. *formosum* P-FW
4 - *Phormdium* cf. *formosum* P-O

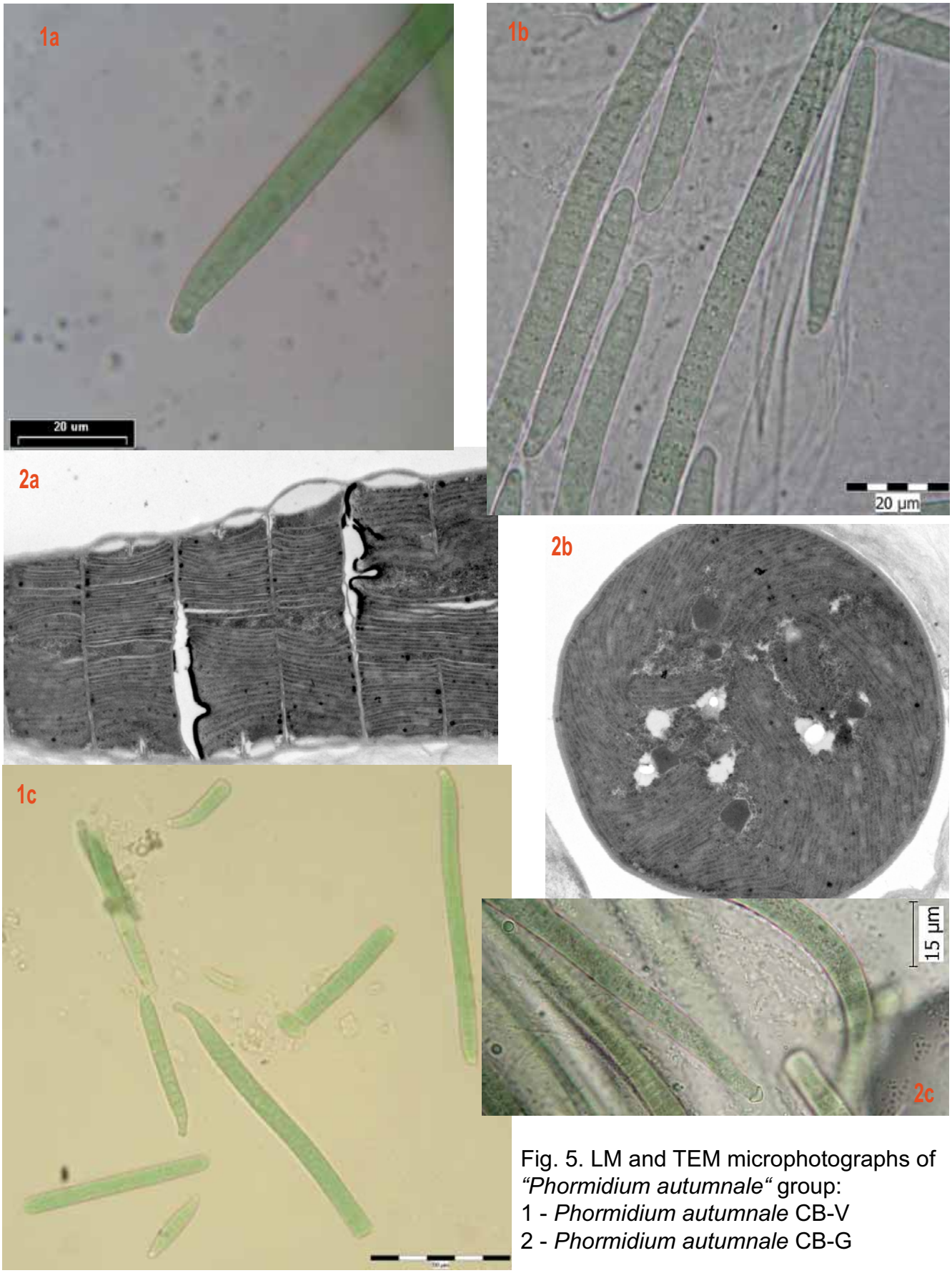


Fig. 5. LM and TEM microphotographs of *"Phormidium autumnale"* group:
1 - *Phormidium autumnale* CB-V
2 - *Phormidium autumnale* CB-G

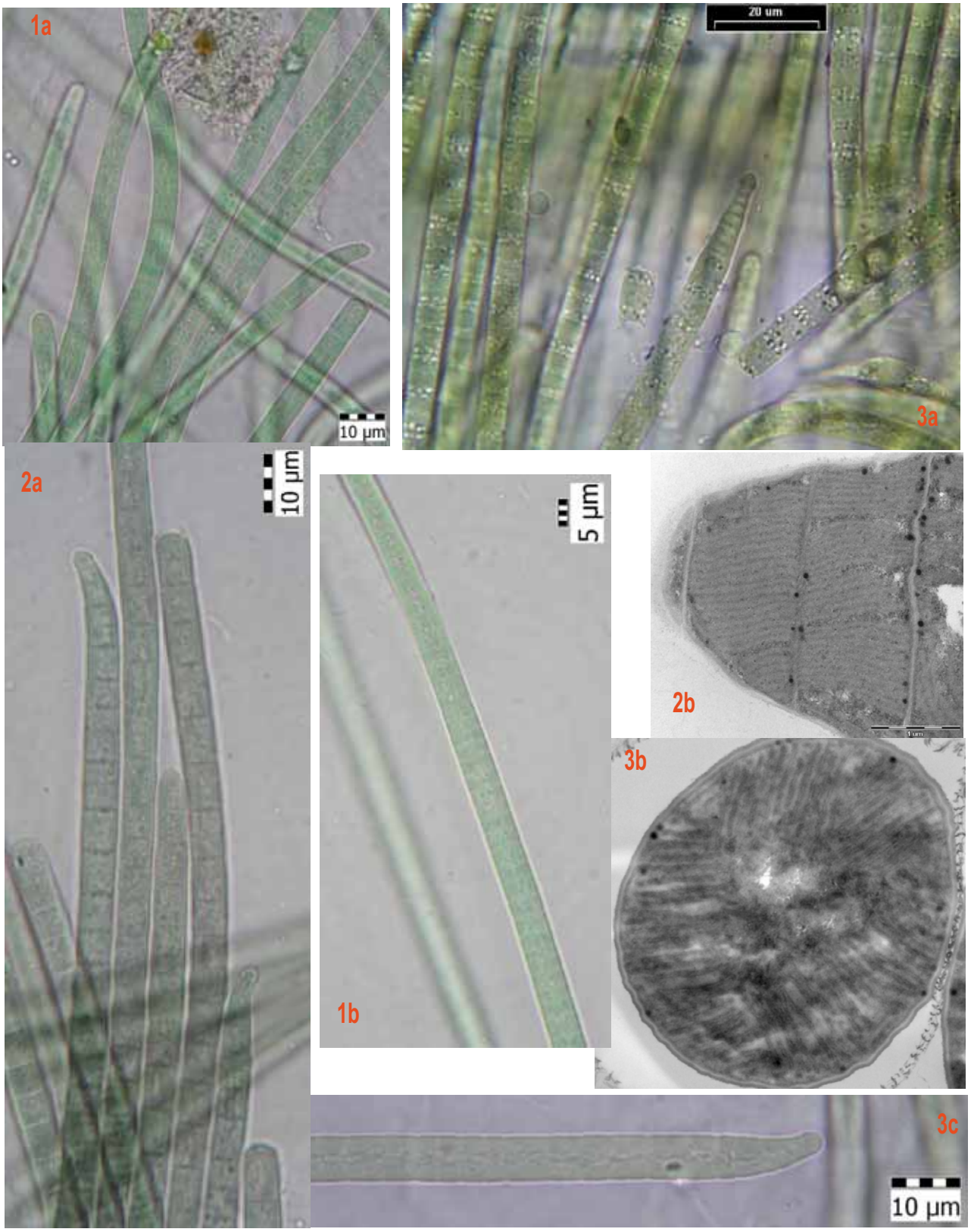


Fig. 6. LM and TEM microphotographs of *"Phormidium autumnale"* group:
1 - *Phormidium autumnale* Kvet-0
2 - *Phormidium* cf. *amoenum* BW-0
3 - *Phormidium* cf. *amoenum* I-Sab

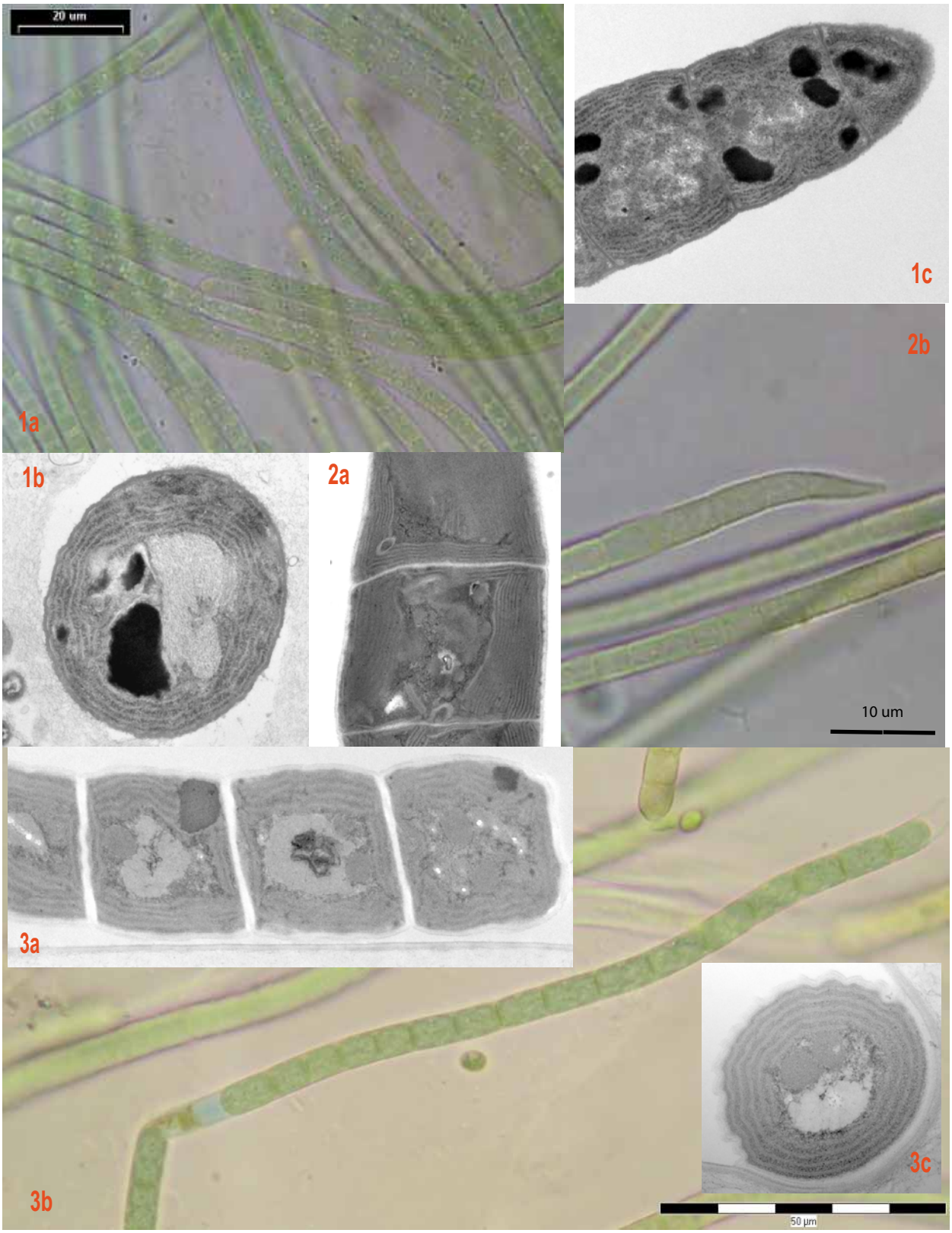


Fig. 7. LM and TEM microphotographs of:
1 - *Geitlerinema* sp. GROWTHER/1459-6
2 - *Geitlerinema* cf. *acuminatum* HINDAK 1967/39
3 - *Phormidium* sp. B-Tom

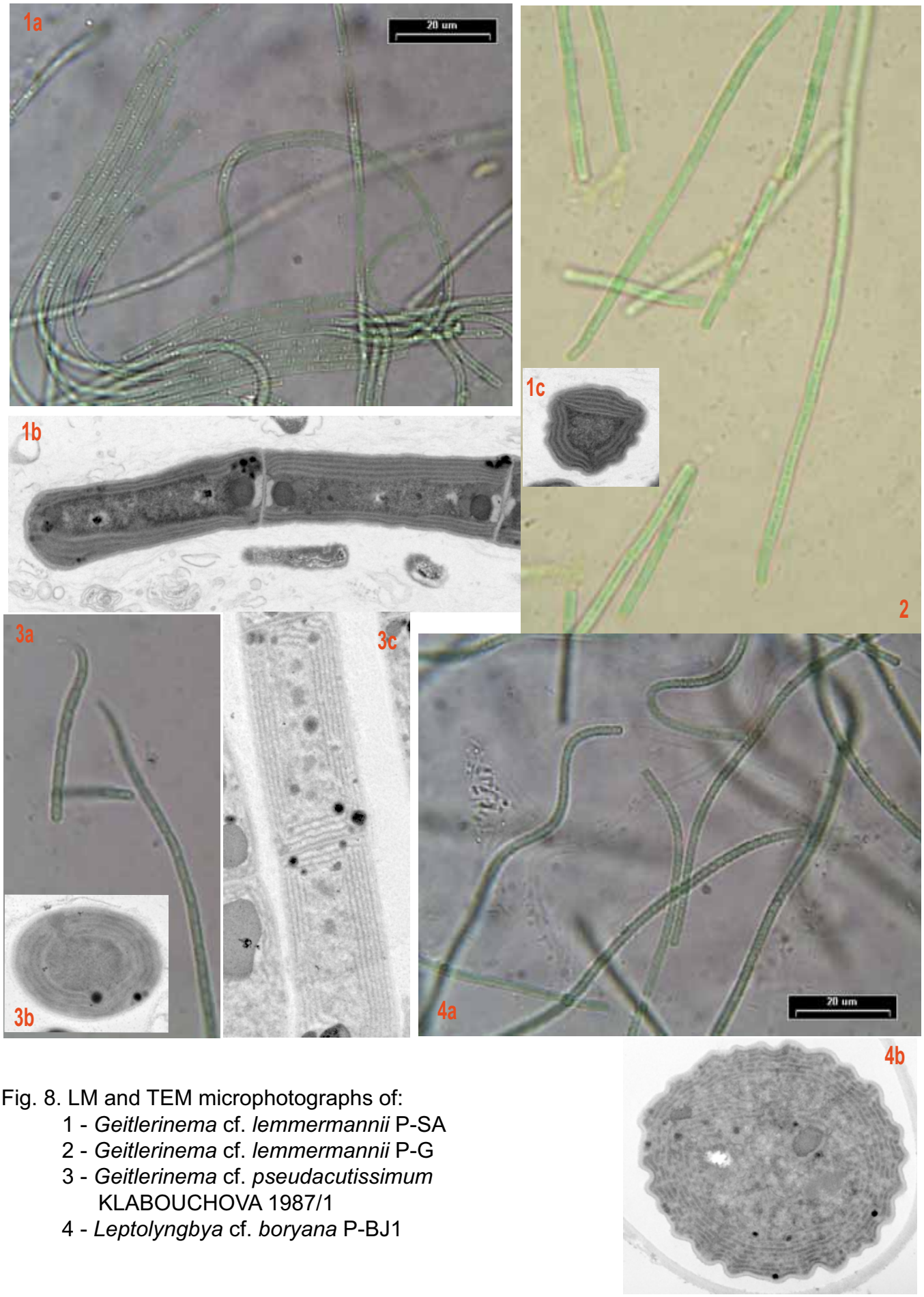
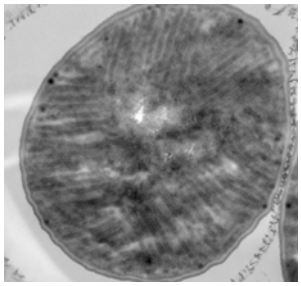
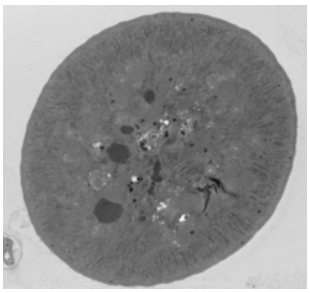
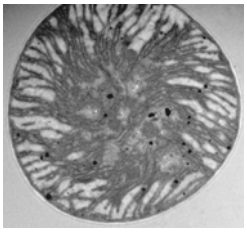


Fig. 8. LM and TEM microphotographs of:
1 - *Geitlerinema* cf. *lemmermannii* P-SA
2 - *Geitlerinema* cf. *lemmermannii* P-G
3 - *Geitlerinema* cf. *pseudacutissimum* KLABOUCHOVA 1987/1
4 - *Leptolyngbya* cf. *boryana* P-BJ1







PhyML v2.4.4
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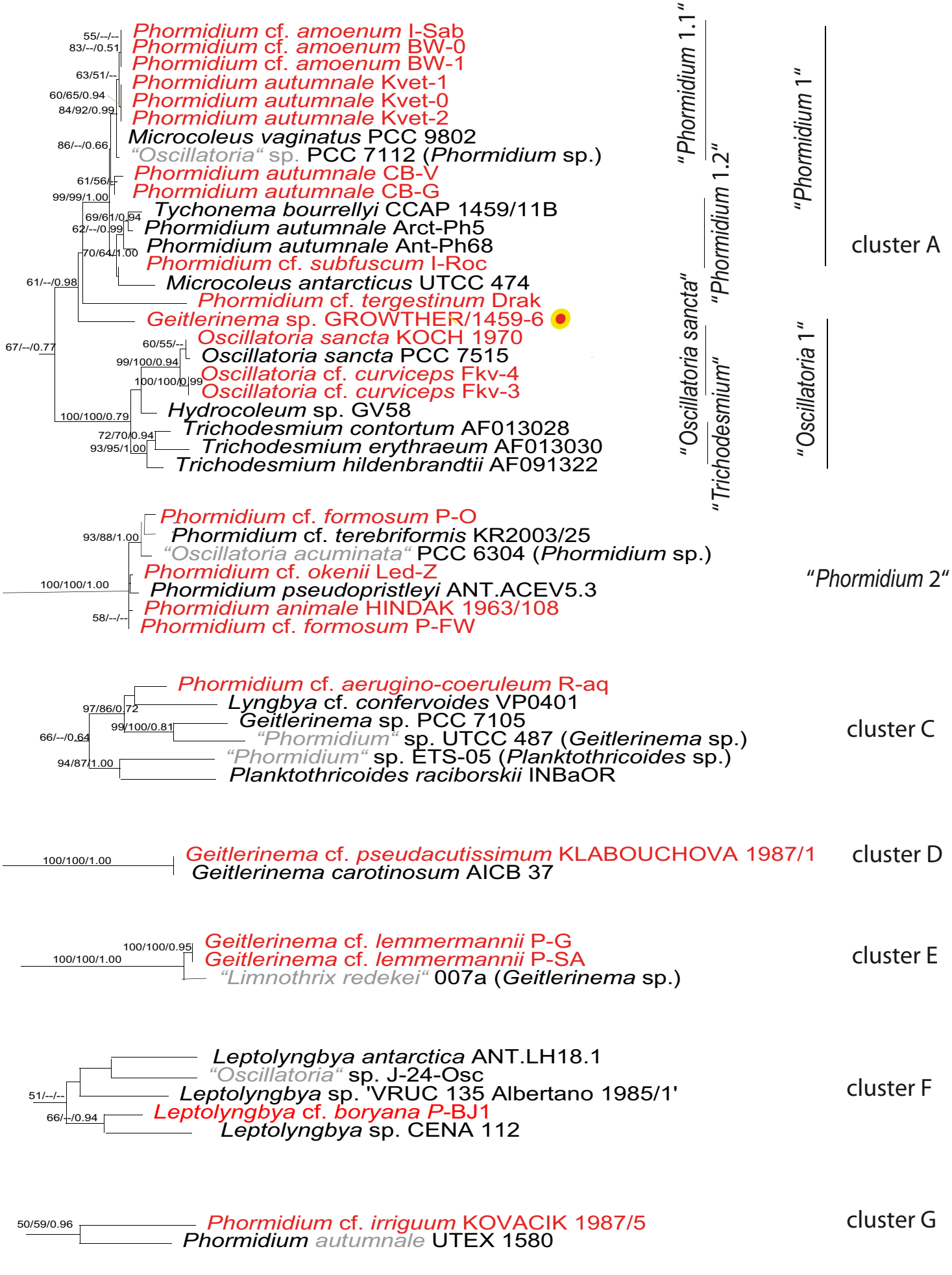
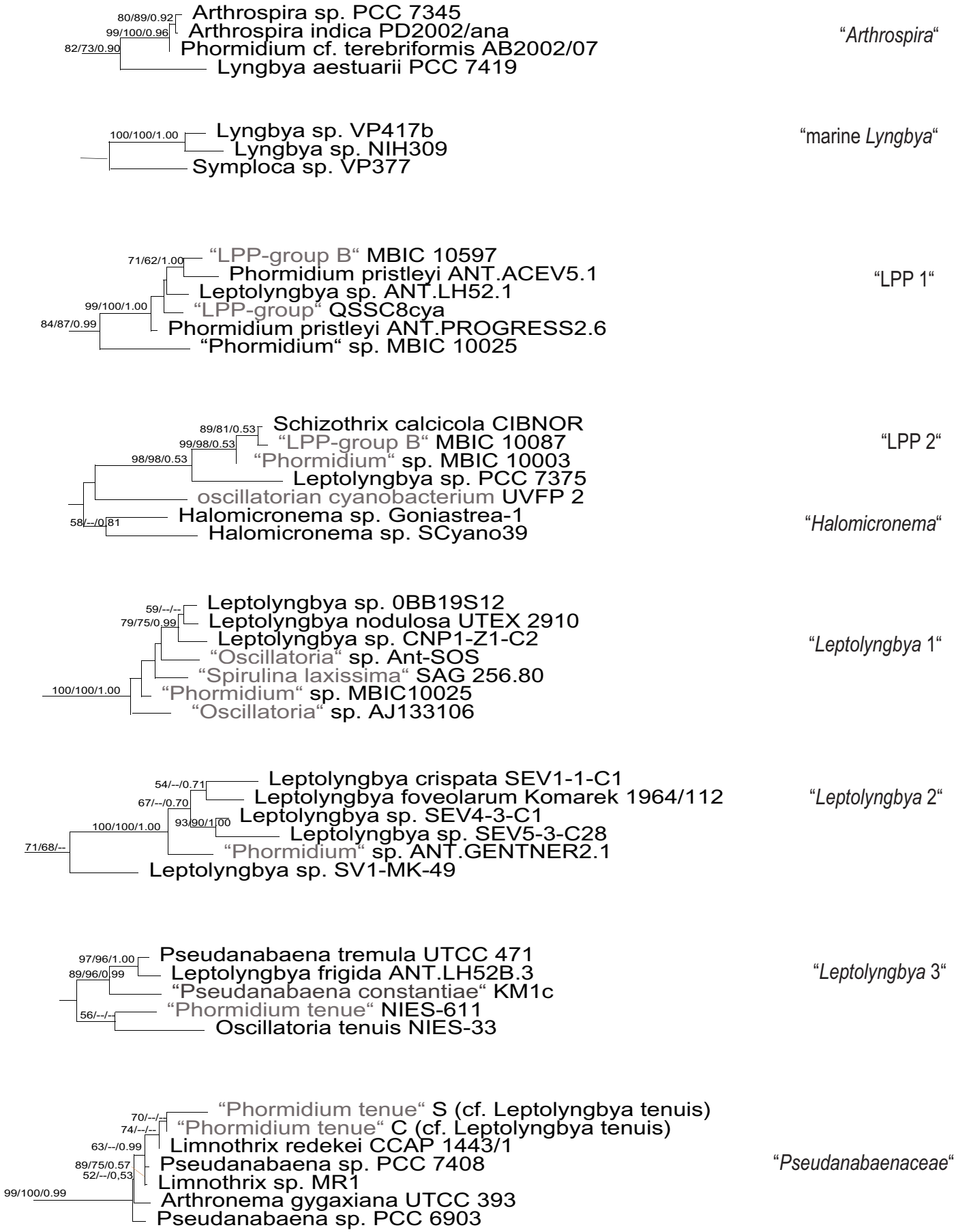
nucleotide substitiution model: **GTR+I+Γ**
number of substitution categories: **6**
proportion of invariant sites: **0.503**
gamma shape parametr: **0.640**

Phormidium cf. *amoenum* I-Sab
Phormidium cf. *amoenum* BW-0
Phormidium cf. *amoenum* BW-1
Phormidium autumnale Kvet-1
Phormidium autumnale Kvet-0
Phormidium autumnale Kvet-2
Microcoleus vaginatus PCC 9802
"*Oscillatoria*" sp. PCC 7112 = *Phormidium* sp.
Phormidium autumnale CB-V
Phormidium autumnale CB-G
Tychonema bourrellyi CCAP 1459/11B
Phormidium autumnale Arct-Ph5
Phormidium autumnale Ant-Ph68
Phormidium cf. *subfuscum* I-Roc
Microcoleus antarcticus UTCC 474
Phormidium cf. *tergestinum* Drak
Geitlerinema sp. GROWTHER/1459-6
Oscillatoria sancta KOCH 1970
Oscillatoria sancta PCC 7515
Oscillatoria cf. *curviceps* Fkv-4
Oscillatoria cf. *curviceps* Fkv-3
Hydrocoleum sp. GV58
Trichodesmium contortum AF013028
Trichodesmium erythraeum AF013030
Trichodesmium hildenbrandtii AF091322
"*Oscillatoria*" sp. CYA 127 = *Planktothrix* sp.
Planktothrix sp. 1LT27S08
Planktothrix pseudagardhii T19-6'-6
Planktothrix mougeotii TK4-5
Phormidium cf. *formosum* P-O
Phormidium cf. *terebriformis* KR2003/25
"*Oscillatoria acuminata*" PCC 6304 = *Phormidium* sp.
Phormidium cf. *okenii* Led-Z
Phormidium pseudopristleyi ANT.ACEV5.3
Phormidium animale HINDAK 1963/108
Phormidium cf. *formosum* P-FW
Arthrospira sp. PCC 7345
Arthrospira indica PD2002/ana
Phormidium cf. *terebriformis* AB2002/07
Lyngbya aestuarii PCC 7419
Phormidium cf. *aerugino-coeruleum* R-aq
Lyngbya cf. *confervoides* VP0401
Geitlerinema sp. PCC 7105
"*Phormidium*" sp. UTCC 487 = *Geitlerinema* sp.
"*Phormidium*" sp. ETS-05 (*Planktothricoides* sp.)
Planktothricoides raciborskii INBaOR
Halospirulina sp.'MPI S3'
Spirulina subsalsa PD2002/gca
"*Synechococcus*" sp. PCC 7202 = *Cyanobacterium stanierii*
Spirulina subsalsa AB2002/06
Halothece sp. MPI 96P605
"*Oscillatoria rosea*" M-220 = *Phormidium roseum*
Synechocystis sp. PCC 6803
Microcystis aeruginosa PCC 7941
Cyanothece sp. PCC 7424
Gloeocapsa sp. PCC 73106
Geitlerinema cf. *pseudacutissimum* KLABOUCHOVA 1987/1
Geitlerinema carotinosum AICB 37
Lyngbya sp. VP417b
Lyngbya sp. NIH309
Symploca sp. VP377
uncultured "*Plectonema*" sp. Pc49
Phormidium sp. B-Tom
Phormidium murrayi Ant-Ph58
Microcoleus chthonoplastes PCC 7420
"*Oscillatoria boryana*" BDU92181 = *Phormidium boryanum*
"*Oscillatoria* cf. *corallinae*" X84812 = *Phormidium corallinae*
"*Oscillatoria* cf. *spongeliae*" 310P1 = cf. *Hormoscilla spongeliae*
"*Oscillatoria earlei*" NTAP016 = *Geitlerinema earlei*
Geitlerinema cf. *acuminatum* HINDAK 1967/39
"LPP-group B" MBIC 10597
Phormidium pristleyi ANT.ACEV5.1
Leptolyngbya sp. ANT.LH52.1
"LPP-group" QSSC8cya
Phormidium pristleyi ANT.PROGRESS2.6
"*Phormidium*" sp. MBIC 10025
Geitlerinema cf. *lemmermannii* P-G
Geitlerinema cf. *lemmermannii* P-SA
"*Limnothrix redekei*" 007a = *Geitlerinema* sp.
"*Synechococcus*" sp. PCC 6307 = *Cyanobium gracile*
Synechococcus elongatus PCC 6301
Schizothrix calcicola CIBNOR
"LPP-group B" MBIC 10087
"*Phormidium*" sp. MBIC 10003
Leptolyngbya sp. PCC 7375
oscillatorian cyanobacterium UVFP 2
Halomicronema sp. Goniastrea-1
Halomicronema sp. SCyano39
"LPP-group" MBIC 10086
Leptolyngbya sp. 0BB19S12
Leptolyngbya nodulosa UTEX 2910
Leptolyngbya sp. CNP1-Z1-C2
"*Oscillatoria*" sp. Ant-SOS
"*Spirulina laxissima*" SAG 256.80
"*Phormidium*" sp. MBIC10025
"*Oscillatoria*" sp. AJ133106
Leptolyngbya crispata SEV1-1-C1
Leptolyngbya foveolarum Komarek 1964/112
Leptolyngbya sp. SEV4-3-C1
Leptolyngbya sp. SEV5-3-C28
"*Phormidium*" sp. ANT.GENTNER2.1
Leptolyngbya sp. SV1-MK-49
uncultured "*Arthrospira*" sp. TRK22
"*Oscillatoria amphigranulata*" 23-3 = *Pseudanabaena amphigranulata*
Phormidium pristleyi ANT.LH66.1
Leptolyngbya sp. SEV5-5-C6
Pseudanabaena tremula UTCC 471
Leptolyngbya frigida ANT.LH52B.3
"*Pseudanabaena constantiae*" KM1c
"*Phormidium tenue*" NIES-611
Oscillatoria tenuis NIES-33
Leptolyngbya antarctica ANT.LH18.1
"*Oscillatoria*" sp. J-24-Osc
Leptolyngbya sp. 'VRUC 135 Albertano 1985/1'
Leptolyngbya cf. *boryana* P-BJ1
Leptolyngbya sp. CENA 112
Leptolyngbya sp. PCC 9221
Leptolyngbya sp. CNP1-Z1-C2 2
Phormidium sp. CCG7
oscillatorian cyanobacterium UVFP 3
Phormidium ambiguum M-71
Microcoleus sociatus MPI 96MS.KID
Oscillatoria princeps NIVA-CYA 150
Phormidium cf. *irriguum* KOVACIK 1987/5
Phormidium autumnale UTEX 1580
Nodularia spumigena PCC 73104
Fischerella muscicola PCC 7414
"*Phormidium tenue*" S = cf. *Leptolyngbya tenuis*
"*Phormidium tenue*" C = cf. *Leptolyngbya tenuis*
Limnothrix redekei CCAP 1443/1
Pseudanabaena sp. PCC 7408
Limnothrix sp. MR1
Arthronema gygaxiana UTCC 393
Pseudanabaena sp. PCC 6903
Pseudanabaena sp. PCC 6802
"*Phormidium mucicola*" M-221 = *Pseudanabaena mucicola*
Pseudanabaena sp. PCC 7367
Gloeobacter violaceus PCC 7421
Lactobacillus casei I-5

0.1


55/--/--
83/--/0.51
Phormidium cf. amoenum I-Sab
Phormidium cf. amoenum BW-0
Phormidium cf. amoenum BW-1
63/51/--
Phormidium autumnale Kvet-1
60/65/0.94
84/92/0.99
Phormidium autumnale Kvet-0
Phormidium autumnale Kvet-2
86/--/0.66
Microcoleus vaginatus PCC 9802
"Oscillatoria" sp. PCC 7112 (Phormidium sp.)
61/56/--
Phormidium autumnale CB-V
99/99/1.00
Phormidium autumnale CB-G
69/61/0.94
Tychonema bourrellyi CCAP 1459/11B
62/--/0.99
Phormidium autumnale Arct-Ph5
Phormidium autumnale Ant-Ph68
70/64/1.00
Phormidium cf. subfuscum I-Roc
61/--/0.98
Microcoleus antarcticus UTCC 474
Phormidium cf. tergestinum Drak
Geitlerinema sp. GROWTHER/1459-6
60/55/--
Oscillatoria sancta KOCH 1970
67/--/0.77
99/100/0.94
Oscillatoria sancta PCC 7515
100/100/0.99
Oscillatoria cf. curviceps Fkv-4
Oscillatoria cf. curviceps Fkv-3
100/100/0.79
Hydrocoleum sp. GV58
72/70/0.94
Trichodesmium contortum AF013028
93/95/1.00
Trichodesmium erythraeum AF013030
Trichodesmium hildenbrandtii AF091322
"Phormidium 1.1"
"Phormidium 1.2"
"Oscillatoria sancta"
"Trichodesmium"
"Phormidium 1"
"Oscillatoria 1"
cluster A
Phormidium cf. formosum P-O
93/88/1.00
Phormidium cf. terebriformis KR2003/25
"Oscillatoria acuminata" PCC 6304 (Phormidium sp.)
Phormidium cf. okenii Led-Z
100/100/1.00
Phormidium pseudopristleyi ANT.ACEV5.3
Phormidium animale HINDAK 1963/108
58/--/--
Phormidium cf. formosum P-FW
"Phormidium 2"
Phormidium cf. aerugino-coeruleum R-aq
97/86/0.72
Lyngbya cf. confervoides VP0401
99/100/0.81
Geitlerinema sp. PCC 7105
66/--/0.64
"Phormidium" sp. UTCC 487 (Geitlerinema sp.)
94/87/1.00
"Phormidium" sp. ETS-05 (Planktothricoides sp.)
Planktothricoides raciborskii INBaOR
cluster C
100/100/1.00
Geitlerinema cf. pseudacutissimum KLABOUCHOVA 1987/1
Geitlerinema carotinosum AICB 37
cluster D
100/100/0.95
100/100/1.00
Geitlerinema cf. lemmermannii P-G
Geitlerinema cf. lemmermannii P-SA
"Limnothrix redekei" 007a (Geitlerinema sp.)
cluster E
Leptolyngbya antarctica ANT.LH18.1
"Oscillatoria" sp. J-24-Osc
51/--/--
Leptolyngbya sp. 'VRUC 135 Albertano 1985/1'
66/--/0.94
Leptolyngbya cf. boryana P-BJ1
Leptolyngbya sp. CENA 112
cluster F
50/59/0.96
Phormidium cf. irriguum KOVACIK 1987/5
Phormidium autumnale UTEX 1580
cluster G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82/73/0.90
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Arthrospira indica PD2002/ana
Phormidium cf. terebriformis AB2002/07
Lyngbya aestuarii PCC 7419
"Arthrospira"
100/100/1.00
Lyngbya sp. VP417b
Lyngbya sp. NIH309
Symploca sp. VP377
"marine Lyngbya"
71/62/1.00
99/100/1.00
84/87/0.99
"LPP-group B" MBIC 10597
Phormidium pristleyi ANT.ACEV5.1
Leptolyngbya sp. ANT.LH52.1
"LPP-group" QSSC8cya
Phormidium pristleyi ANT.PROGRESS2.6
"Phormidium" sp. MBIC 10025
"LPP 1"
89/81/0.53
99/98/0.53
98/98/0.53
58/--/0.81
Schizothrix calcicola CIBNOR
"LPP-group B" MBIC 10087
"Phormidium" sp. MBIC 10003
Leptolyngbya sp. PCC 7375
oscillatorian cyanobacterium UVFP 2
Halomicronema sp. Goniastrea-1
Halomicronema sp. SCyano39
"LPP 2"
"Halomicronema"
59/--/--
79/75/0.99
100/100/1.00
Leptolyngbya sp. 0BB19S12
Leptolyngbya nodulosa UTEX 2910
Leptolyngbya sp. CNP1-Z1-C2
"Oscillatoria" sp. Ant-SOS
"Spirulina laxissima" SAG 256.80
"Phormidium" sp. MBIC10025
"Oscillatoria" sp. AJ133106
"Leptolyngbya 1"
54/--/0.71
67/--/0.70
93/90/100
100/100/1.00
71/68/--
Leptolyngbya crispata SEV1-1-C1
Leptolyngbya foveolarum Komarek 1964/112
Leptolyngbya sp. SEV4-3-C1
Leptolyngbya sp. SEV5-3-C28
"Phormidium" sp. ANT.GENTNER2.1
Leptolyngbya sp. SV1-MK-49
"Leptolyngbya 2"
97/96/1.00
89/96/0.99
56/--/--
Pseudanabaena tremula UTCC 471
Leptolyngbya frigida ANT.LH52B.3
"Pseudanabaena constantiae" KM1c
"Phormidium tenue" NIES-611
Oscillatoria tenuis NIES-33
"Leptolyngbya 3"
70/--/--
74/--/--
63/--/0.99
89/75/0.57
52/--/0,53
99/100/0.99
"Phormidium tenue" S (cf. Leptolyngbya tenuis)
"Phormidium tenue" C (cf. Leptolyngbya tenuis)
Limnothrix redekei CCAP 1443/1
Pseudanabaena sp. PCC 7408
Limnothrix sp. MR1
Arthronema gygaxiana UTCC 393
Pseudanabaena sp. PCC 6903
"Pseudanabaenaceae"