

School of Doctoral Studies in Biological Sciences

University of South Bohemia in České Budějovice
Faculty of Science



***Phylogeny, Phytogeography, and Taxonomy
of Polar Oscillatoriales***

Ph.D. Thesis

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■ Annotation

Morphological and phylogenetic diversity of 143 strains belonging to Oscillatoriales with focus on traditional genera *Phormidium* sensu lato and *Microcoleus* were studied. The 88 strains of *Ph. autumnale*, *Ph. setchelianum*, *Ph. subfuscum*, *Ph. favosum* etc., and *M. vaginatus* confirmed the generic identity with typical *Microcoleus* Desmazières ex Gomont. The necessary nomenclatoric transfers were realized defining the revised genus *Microcoleus*. Based on phylogeny and morphology the taxonomic revision of the Antarctic species *Ph. murrayi* (*Lyngbya murrayi* West & West) was implemented and the genus *Wilmottia* was established. The phylogenetic evaluation of morpho-species included in *Phormidium* group I (*Ph. lloydianum* and *Ph. acuminatum* Gomont) preceded the definition of species *Oxynema thaianum* spec. nova. The biogeography of Antarctic and Arctic strains of *M. vaginatus* (*Ph. autumnale*) based on 16S rDNA and ITS (internal transcribed spacer of the 16S rDNA -23S rDNA ribosomal operon) sequences and strain's morphology was evaluated. The comparison of polar and non polar strains indicated that the Antarctic populations of *M. vaginatus* remained isolated from time of the isolation of the Antarctica from the Gondwana before ~31–45 Ma, whereas the transport of populations within Arctic is relatively frequent even at the present time. It was shown that the polar strains of *M. vaginatus* from the north and south polar areas were not identical.

■ Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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■ List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

- I. **Strunecký, O.**, Elster, J. & Komárek, J. 2010. Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? *Polar Biology* **33**:1419-28(*Strunecký et al., 2010*) (IF = 1.445).
O. Strunecký carried out the morphological evaluation of the strains with cell-size measurements, the DNA extraction, PCR and the sequencing of the strains. He performed the statistical and phylogenetic analyses of the data, interpreted the results and wrote the manuscript. (Strunecký et al., 2010)
- II. **Strunecký, O.**, Elster, J. & Komárek, J. 2011. Taxonomic revision of the fresh-water cyanobacterium „*Phormidium*“ *murrayi* = *Wilmottia murrayi*. *Fottea* **11**:57–71. (IF = 0.978).
O. Strunecký carried out the morphological evaluation of the strains with cell-size measurements, the DNA extraction, PCR and the sequencing of the strains. He performed the statistical and phylogenetic analyses of the data, interpreted the results, wrote incompletely the manuscript and revised it.
- III. Chatchawan, T., Komárek, J., **Strunecký, O.** & Šmarda, J. 2011. Phylogenetic position and ecology of the halophilic cyanobacterium *Oxynema lloydianum* (Gomont) comb. nova, separated from the traditional genus *Phormidium*. *Cryptogamie Algologie* (*accepted*). (IF = 0.25).
O. Strunecký made the preliminary phylogenetic analysis of known strains to specify the phylogenetic position of morpho-types of the strains belonging to Phormidium group I within Oscillatoriales. He carried out DNA extraction, PCR and the sequencing of the strain. He performed phylogenetic analyses of the data, interpreted the results, wrote relevant parts of the manuscript and revised it.
- IV. **Strunecký, O.**, Komárek, J. & Elster, J. 2012. Biogeography of *Phormidium autumnale* (Oscillatoriales, Cyanobacteria) in western and central parts of Spitsbergen. *Polish Polar Research* after revision. (IF = 0.867).
O. Strunecký isolated and purified the majority of strains, carried out the morphological evaluation of the strains with cell-size measurements, the DNA extraction, PCR and the sequencing of the strains. He performed the phylogenetic analyses of the data, interpreted the results and wrote the manuscript.
- V. **Strunecký, O.**, Elster, J. & Komárek, J.. Molecular clock provided evidence for survival of cyanobacteria (Oscillatoriales, *Phormidium autumnale*) in Antarctica from Paleozoic times. *FEMS* submitted.
O. Strunecký designed the study, made the phylogenetic and morphological analysis, calculated the molecular clocks, interpreted the results and wrote the manuscript.
- VI. **Strunecký, O.**, Komárek, J., Johansen, J., Lukesova, A. & Elster, J. manuscript. Molecular and morphological criteria for revision of the genera *Microcoleus* Desmazières ex Gomont and *Phormidium* Kützing ex Gomont (Cyanobacteria), based on strains of *Microcoleus vaginatus* and *Phormidium autumnale*. - manuscript
O. Strunecký collected, isolated and purified part of the strains, carried out the morphological evaluation of the strains with cell-size measurements, the DNA extraction, PCR and the sequencing of the strains. He performed the phylogenetic analyses of the data, interpreted the results and wrote the manuscript.

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Phylogeny, Phytogeography, and Taxonomy of Polar Oscillatoriales

Introduction

Cyanobacteria, the main prokaryotic microorganisms with the phototrophic metabolism play significant roles in both natural and man-made habitats of the biosphere. Their importance in the evolution of life on Earth results from their long history since their origin in early Precambrian (Whitton & Potts, 2000); their life strategy and overall adaptability enabled their successful survival up to the present without any loss of vitality. Thanks to their capability to perform oxygenic photosynthesis, they were directly involved in the origins of the Earth's oxygen-rich atmosphere (Bekker *et al.*, 2004, Tomitani *et al.*, 2006). The members of this phylum exhibit enormous diversity of morphotypes, adaptations, life strategies, and metabolic skills (Whitton & Potts, 2000). Contemporary cyanobacteria have retained key roles as primary producers in a number of environments such as oceans (Honda *et al.*, 1999, Scanlan *et al.*, 2009), freshwater bodies (Lyra *et al.*, 2005, Lyra *et al.*, 2001), wetlands (Rejmánková *et al.*, 2004) and extreme habitats, including hot springs (Miller *et al.*, 2007), cold and hot deserts, polar regions, large range of tropical biotopes, hypersaline and mineral localities (Whitton & Potts, 2000, Strunecký *et al.*, 2010, Chatchawan *et al.*, 2011). The biota of polar areas is predominated by microorganisms and cyanobacteria represent the main primary producers (Casamatta *et al.*, 2005, Elster & Benson, 2004, Komárek *et al.*, 2008, Priscu *et al.*, 1998, Strunecký *et al.*, 2012, Šabacká & Elster, 2006, Vézina & Vincent, 1997). Filamentous types of cyanobacteria belonging to the order Oscillatoriales form the dominant component of polar aquatic and terrestrial ecosystems (Comte *et al.*, 2007, Elster, 2002).

The history of the cyanobacterial determination is much longer than the nomenclatural history of other prokaryotes. Some scientific names of cyanobacteria actually initiate in Linnaeus 1753 (Oren, 2011). During 19th century, description of many cyanobacterial genera were added by botanists such as Roth, Bory, Corda, Hansgirg, M. Kützing, Lyngbye, Agardh etc. Many other botanists described great number of new species on the basis of morphological features only until now when the sequencing of 16S rDNA became fundamental standard. Vast numbers of newly described species created by various taxonomists led to reevaluations and taxonomic revisions of species and genera. The most recent one on Oscillatoriales was compiled by Komárek and Anagnostidis (2005).

Throughout the history of cyanobacterial determination, species belonging to the genus *Phormidium* underwent several major taxonomic revisions, where beside the shape of the cells and filaments, type of end cells and the presence of sheaths were considered as major taxonomic features. At the establishment of the genus *Phormidium* (Gomont, 1892), 29 species were described. In Geitler's monograph (1932) 85 species were included, in the list of *Phormidium*-species by Drouet (1968) more than 260 taxonomic names was presented. Komárek & Anagnostidis (2005) described validly 163 species with many species mostly belonging to the genera *Oscillatoria* and *Lyngbya* relocated to the genus *Phormidium*. The 16S rDNA-based molecular taxonomy that first appeared in the 80th of the 20th century have changed the view of cyanobacterial taxonomy. As shown by recent molecular analyses (Taton *et al.*, 2006, and others., Palinska & Marquardt, 2008, Strunecký *et al.*, 2010, Strunecký *et al.*, 2011 and others) the genus *Phormidium* Kützing ex Gomont 1892 is polyphyletic.

Whereas the genus *Phormidium* is polyphyletic with many morphotypes, genotypes and ecotypes, this study was focused on genetic diversity of *Phormidium* group VII (according to Komárek & Anagnostidis 2005). This group is characterized by trichomes shortly attenuated towards ends and with apical cells with calyptra. The cells are moreless isodiametric. *Phormidium autumnale* [Agardh] Trevisan ex Gomont belongs to core species of this group; representatives of this type are common worldwide in various habitats. Due to the high degree

of both environmentally and culture cultivation induced morphological variability, the sequencing is essential for the proper taxonomic evaluation of this species. Phylogenetic analysis suggests that *Phormidium autumnale* is very close to *Microcoleus vaginatus* (the type species of the genus *Microcoleus* Desmazières ex Gomont 1892) and falls into the same phylogenetic group (Boyer *et al.*, 2002, Siegesmund *et al.*, 2008, Strunecký *et al.*, 2010, Strunecký *et al.*, 2012). Other species of Phormidium group VII were supposed to be found all over the world in various biotopes including marine.

However, the comparison with the type species of the genus *Phormidium* is crucial for the taxonomic evaluation of other genera. The Geitler's (1932) description of *Phormidium* type species *Ph. lucidum* followed original description of Kützing (1843) and Gomont (1892), based on Agardh's (1827) „*Oscillatoria lucida*“, described from atmophytic mat collected on the walls of thermal spring in Carlsbad (Karlovy Vary, Czech Republic). Komárek & Anagnostidis (2005) emphasized the thermal biotope as one of critical features of *Ph. lucidum*, which belongs according to their classification to the group VIII. The distinctive feature in morphology of *Ph. lucidum* consist of short cells with 1/2 -1/4 width/length ratio and width of trichomes 6-8 µm. The genetic and ecological characterization of this cluster (based mainly on *Phormidium irriguum*, related to *Phormidium lucidum*) was presented by Sciuto & al. (in press).

Aims

This thesis was originally focused on taxonomic evaluation of populations belonging to the genus *Phormidium* from polar regions, where it forms one of the dominant cyanobacterial species. However, the wider consortium of strains was obtained from different parts of the world and the morphology of strains was compared with ultrastructure and phylogeny of 16S rDNA and ITS, where possible.

The main aims of this study were:

1. Discriminate the strains from polar regions belonging supposedly to *Phormidium* group VII (Komárek & Anagnostidis, 2005) by molecular methods
2. Find out the morphological and ecological properties of strains belonging to *Ph. autumnale*/*M. vaginatus* group
3. Biogeography of cyanobacteria
4. Taxonomic revision of species belonging to *Phormidium* sensu lato

Results

This thesis consists of six papers – two papers already published (paper I and II), one paper accepted for publication (III), one paper after revision (paper IV), one submitted (V) and one manuscript prior to submitting (paper VI).

Paper I

Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? *Polar Biology* 33: 1419-1428

A total of 26 *Phormidium* strains were studied using a polyphasic approach, 18 from Arctic (Svalbard, Ellesmere Island and Scandinavian Arctic—Abisko) and Antarctic (Antarctic Peninsula—King George and James Ross Island) regions, and 8 from temperate regions. A phylogeographical comparison between polar (Arctic and Antarctic) and non-polar strains of

the cyanobacterial genus *Phormidium*, was conducted. Within the *Phormidium autumnale* cluster, genetic similarity of 16S rDNA was more related to geographical proximity of strain origin than to morphological similarity. It was seen that in the cluster belonging to *Phormidium autumnale* the geographical limitation played a prominent role. However, the cyanobacterial strains found in Europe suggested that the distribution areas of some morpho and genotypes of clusters overlap. We gathered a first firm evidence that the *Ph. autumnale* cluster was evidently a very characteristic type and represented an isolated clade within the traditional genus *Phormidium*. According to morphological features and the structure of trichomes, it is most similar and thus probably belongs to the genus *Microcoleus*.

Paper II

Taxonomic revision of the freshwater cyanobacterium „*Phormidium*“ *murrayi* = *Wilmottia murrayi*. *Fottea* 11: 57–71.

We evaluated 23 morphologically similar strains or natural populations resembling *Ph. murrayi* from the Antarctica and few other geographical regions; 6 of them were studied by us by molecular methods. The clade formed by those strains belonged to a group of strains identified traditionally as *Phormidium murrayi*, described from the Antarctica originally in 1911 as *Lyngbya murrayi* by W. et G.S.West. Morphological characteristics and 16S rDNA similarity corresponding with typical *Ph. murrayi* were shared mostly by the specimens from the Antarctica. Molecular analyses confirmed that *Ph. murrayi* represented a special taxonomic group (on the generic level) inhabiting commonly shallow freshwater classified as a special genus in cyanobacterial taxonomy (*Wilmottia* gen. nov.) according to the recent “polyphasic approach”. The type species of *Wilmottia*, *W. murrayi*, was determined up to now to be characteristic for the Antarctica. We suggested that other phylogenetically and morphologically similar types from other regions represent possibly different taxa (species) of the same generic unit

Paper III

***Oxynema*, a new genus separated from the genus *Phormidium* (Cyanophyta). *Cryptogamie Algologie* (accepted).**

We have studied another group of species (mainly the strains from salterns in Thailand), which was classified earlier in the genus *Phormidium*, but it represented an isolated cluster according to 16S rRNA gene sequencing and was characterized by specific uniform and morphological features. Because this whole group represents a phylogenetically and morphologically distinctly separated cluster, we described it as a special taxon *Oxynema* genus novum, in agreement with Botanical Nomenclatoric Code. All of the genetically most related clusters had genetic similarity less than 93% and differed by distinct autapomorphic features. The filaments of members of *Oxynema* are cylindrical, narrowed and bent at the ends, commonly attenuated to a terminal elongated, ± sharply pointed cells without calyptra. Thylakoids in cells are distinctly radially arranged, similarly as in the genera *Microcoleus* and “*Phormidium autumnale*”-type. The ecology of all members, which belong potentially to these types is also similar: all species from this cluster were recorded from halophilic habitats, less frequently from thermal springs and soil biotopes with higher salt contents.

Paper IV

Biogeography of *Phormidium autumnale* (Oscillatoriales, Cyanobacteria) in western and central parts of Spitsbergen. *Polish Polar Research*, after revision

We cultivated 25 strains of *Ph. autumnale* from Svalbard and compared them with available strains from surrounding regions. The comparison of strains, based on of 16S rDNA and 16S-

23S rDNA intergenic spacer sequences, revealed identity of strains from Ellesmere Island, Canadian Arctic and Abisko, Sweden with strains from Spitsbergen (Svalbard). The rate of colonization of *Ph. autumnale* from lotic habitats is relatively high and we suggest geese as a main transmission vector from surrounding lands. Strains of *Ph. autumnale* were positioned in the phylogenetic tree according to their occurrence in similar biotopes. An apparent grouping factor was the duration of availability of water in lakes and long-lasting streams in contrast to rapid and repeated desiccation in soil and on wetted rock in the spray zone of waterfalls. Strains that grow in very cold waters just above the melting point of snow or ice formed another phylogenetic cluster. Strains investigated in this study showed morphological similarity in shape of trichomes and calyptras of majority of studied specimens. Cell diameter except for terminal cells ranged from 3 to 10 µm for all studied strains. Comparison of 16S rDNA sequences of the genus *Ph. autumnale* with the previously published definition of the species *Microcoleus vaginatus* revealed the identity of these two species.

Paper V

Molecular clock provided evidence for survival of cyanobacteria (Oscillatoriales, *Phormidium autumnale*) in Antarctica from Paleozoic times *FEMS Microbial Ecology* submitted.

In this study we used 15 previously analyzed strains of *Ph. autumnale* from the Antarctic. We tested the hypothesis of their survival through the glaciations using relaxed and strict molecular clock methods in the analysis of a 16S rDNA within the strains collected in vicinity of maritime Antarctica, mainly at James Ross Island. We estimated that the biogeographic history of Antarctic cyanobacteria belonging to *Ph. autumnale* lineages has ancient origins. The oldest exceeded the break-up of Gondwana and originated somewhere at the supercontinent between 442 Ma and 297 Ma. Enhanced speciation rate was found around the time of the opening of the Drake Passage (~31–45 Ma) with start of glaciations (~43 Ma). The high morphological diversification of *Ph. autumnale* suggested the co-evolution of lineages and formation of complex associations with different morphologies. Our results supported the hypothesis that long-term survival took place in glacial refuges, resulting in a specific endemic cyanobacterial Antarctic flora.

Paper VI

Molecular and morphological criteria for revision of the genera *Microcoleus* Desmazières ex Gomont and *Phormidium* Kützing ex Gomont (Cyanobacteria), based on strains of *Microcoleus vaginatus* and *Phormidium autumnale*. manuscript

This paper extends the conclusions from previous papers that traditional taxonomy of cyanobacteria depends mostly upon morphological characteristics and must be reviewed by means of modern, combined bacteriological as well as botanical methods. Our article follows the revision of the cluster of typical *Microcoleus* based on the type species *M. vaginatus* and presents its close relation to a part of the traditional genus *Phormidium* (sine typo, based on the cluster of *Phormidium autumnale* –type). Analyses based on 16S rDNA gene sequences and ITS of 88 analysed strains proved the generic identity of this cluster with typical *Microcoleus*. The necessary nomenclatoric transfers were realized in the article. Whole group *Phormidium* VII was nomenclatorically transferred into the genus *Microcoleus* and the family of Microcoleaceae Strunecký, Johansen et Komárek, familia nova [syn. (p.p. sine typo): subfam. Microcoleoideae Hansgirg, 1892] was introduced.

General discussion, conclusions and perspectives

The submitted thesis deals with taxonomically very problematic genus *Phormidium*. The most complex comparison of different morpho and genospecies in a new concept of the genus *Microcoleus* is described in **paper VI**. There is in fact only one comparable published work on one genus in taxonomy of Oscillatoriales, the widespread marine mat-forming species *Microcoleus chthonoplastes* (with 21 isolated strains) that had been transferred to the genus *Coleofasciculus* (Casamatta et al., 2005). The morphological comparison of the 88 cultivated strains based on phylogeny of 16S rDNA together with ITS, and their morphological comparison with previously described species sensu Komárek and Anagnostidis (2005) showed that the group "Phormidium VII" does not correspond with the type species of *Phormidium* (*Ph. lucidum*, member of the group VIII (Komárek & Anagnostidis, 2005). It means that the group "Phormidium VII" has to be nomenclaturally transferred into the genus *Microcoleus*. Moreover, there are no 16S rDNA sequences of marine origin similar to newly formed *M. vaginatus/Ph. autumnale* clade in the Genbank. Whereas the "Phormidium VII" group include a lot of species of marine or brackish origin, it is very likely that those species will have to be excluded from the genus *Microcoleus*. However, the species found in both mineral-waters and seaside environment of comparable morphology with newly formed genus *Microcoleus* but without capability to form calypthras - group "Phormidium II" often determined as *Ph. animale* (*Oscillatoria animalis* Agardh 1827) form the most proximate cluster (unpublished results). It was also shown that some features used for taxonomic determination must be revised. The thickness of sheaths is one dilemma; whereas the majority of studied strains had adequate sheaths, some of the strains genetically belonging to *Microcoleus* produced thick layered sheaths that were previously associated with the genus *Porphyrosiphon* Kützing ex Gomont, 1892. The variety of the end cell is also remarkable. In vast number of the strains, namely the strains from Svalbard, only a very small portion of trichomes is attenuated to the end with small rounded cell that is metabolically dead (Tashyreva, pers. comm.). Some strains did not produce the calypthra in culture either, hence it is most probable that some important species of "Phormidium group V", such as *Ph. puteale*, *Ph. tergestinum*, or *Ph. retzii* that does not form calypthras at all, also belong to the genus *Microcoleus*.

Because of the growing knowledge of unclear taxonomical status of various species in Oscillatoriales (e. g. Taton et al., 2006, Taton et al., 2003) and polyphyletic origin of some genera (Engene et al., 2010, etc., Bruno et al., 2009, Lokmer, 2007) based on phylogenetic comparison of 16S rDNA, the taxonomic revision of various taxonomic units should be prepared. Based on discussions in taxonomic community (Komárek, pers. comm.), the level of genera seems to be most appropriate according to relatively high stability of 16S rDNA. This was conducted in **papers II and III**, where in close association with traditional cyanobacterial taxonomy based on morphological features, two groups of cultivated, morphologically and genetically well-defined strains were transferred into new genera. Moreover, a taxonomic revisions of other groups of collected and sequenced strains obtained for the taxonomic revisions of "Phormidium VII" will follow beyond the scope of this thesis.

The high level of conservation at 16S rDNA among cyanobacteria limits its use for determination at the intrageneric level (Rosselló-Mora & Amann, 2001, Boyer et al., 2002) and highly limits this marker in biogeographic studies (**paper V**). Therefore, the ITS is frequently used to enhance marker variability within populations of one species (Iteman et al., 2002). Until now, there was little accurate evidence of either cosmopolitan or endemic distribution of cyanobacterial species, mainly because of the absence of molecular analyses (Seckbach, 2007) or the use of small 16S rRNA gene sequences (Garcia-Pichel et al., 1996), which could lead to inaccurate or biased interpretations (Comte et al., 2007). A few biogeographical studies based on ITS of freshwater or soil Chroococacean cyanobacteria were done in previous years. ITS analysis of van Gremberghe et al. (2011) suggests the cosmopolitan distribution of *Microcystis aruginosa*, whereas Bahl et al. (2011) proposes that long term acclimatization of

Chroococcidiopsis sp. based on Bayesian relaxed-clock of the 16S -23S ITS rDNA to hot and cold deserts started already in Proterozoic.

Papers I, IV and V reflect those growing tendencies to compare species and genospecies in cyanobacteria. The label "genospecies" was introduced in **paper VI** and reflects the strains that retain very high similarity in 16S rDNA (and/or ITS or other parts of the genome), whereas the strains possess relatively highly diverse morphology. It was suggested, mainly in **paper V**, that long time isolation of Antarctic strains led to evolution of various morpho and ecospecies that originated from common ancestor specialized to different biotopes, and their morphology and supposedly physiology changed. The **paper IV** deals with biogeography pattern of *Ph. autumnale* group from north polar areas and shows that the biogeography patterns are formed mainly on small range scale and transport of natural populations can occur relatively often. Moreover for clarification of biogeography in the open system of temporal and tropic biomes much bigger collection of strains must be obtained.

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The Ph.D. thesis is based on following manuscripts:

Paper I

Strunecký O, Elster J & Komárek J (2010) Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? *Polar Biology* 33: 1419-1428.

The manuscript has been published

Abstract: We present a phytogeographical comparison between polar (Arctic and Antarctic) and non-polar strains of the cyanobacterial genus *Phormidium*, which plays a key role in Arctic and Antarctic ecosystems as primary producer. A total of 26 *Phormidium* strains were studied using a polyphasic approach, 18 from Arctic (Svalbard, Ellesmere Island and Scandinavian Arctic—Abisko) and Antarctic (Antarctic Peninsula—King George and James Ross Island) regions, and 8 from temperate sites (mostly situated in Central Europe). A phylogenetic tree was constructed and compared with similar 16S rRNA sequences retrieved from Genbank. Within the *Phormidium autumnale* cluster, genetic similarity of 16S rDNA was more related to geographical proximity of strain origin than to morphological similarity. No genetic identity of *Phormidium* strains from north and south polar regions was found. The cluster *Phormidium autumnale* apparently belongs to generic entities in which geographical limitation plays a prominent role. However, the cyanobacterial strains found in Europe suggest that the distribution areas of some *Phormidium* cyanobacteria overlap. The *Phormidium autumnale* cluster is evidently a very characteristic type and represents an isolated clade within the traditional genus *Phormidium*. According to morphological features and the structure of trichomes, it is most similar and thus probably belongs to the genus *Microcoleus*.

Paper II

Strunecky O, Elster J & Komarek J (2011) Taxonomic revision of the freshwater cyanobacterium "*Phormidium*" *murrayi* = *Wilmottia murrayi*. *Fottea* 11: 57-71.

The manuscript has been published

Abstract: The cyanobacterial genus *Phormidium* is polyphyletic, as follows from recent molecular and phenotypic analyses. Several isolated clusters were found also in Antarctic populations (Taton et al. 2006, Taton et al. 2010, Strunecký et al. 2010b). A few of them have already been described or revised on the generic level (e.g., *Phormidesmis*, *Microcoleus*). One of the separate clusters belongs to a group of strains identified traditionally as *Phormidium murrayi*, described from the Antarctica originally

in 1911 as *Lyngbya murrayi* by W. et G.S.West. We evaluated 23 morphologically similar strains or populations resembling *Ph. murrayi* from the Antarctica and few other geographical regions; 6 of them were studied by us by molecular methods. Morphological characteristics and 16S rDNA similarity corresponding with typical *Ph. murrayi* were shared mostly by the specimens from the Antarctica. Molecular analyses confirmed that *Ph. murrayi* represents a special taxonomic group (on the generic level) inhabiting commonly shallow freshwater classified as a special genus in cyanobacterial taxonomy (*Wilmottia* gen. nov.) according to the recent "polyphasic approach". The type species of *Wilmottia*, *W. murrayi*, was determined up to now to be characteristic for the Antarctica. Other phylogenetically and morphologically similar types from other regions represent possibly different taxa (species) of the same generic unit. The specific status of similar or related strains from other regions should be therefore solved in the future.

Paper III

Chatchawan T, Komárek J, Strunecký O & Šmarda J (2011) *Oxynema*, a new genus separated from the genus *Phormidium* (Cyanophyta). *Cryptogamie Algologie* 33:41-59

The manuscript has been published

Abstract: Taxonomic revision of the traditional polyphyletic cyanobacterial genus *Phormidium* is based on molecular sequencing combined with the definition of distinct, autapomorphic features. Several genera, clearly separated from each other (with genetic similarity lower than 95), were already defined and separated from this widely conceived generic unit (*Phormidesmis*, *Wilmottia* and others). All of these new generic taxa are characterized by morphological markers also. We have studied another group of species (mainly the strains from salterns in Thailand), which was classified earlier in the genus *Phormidium*, but it represents an isolated cluster according to 16S rRNA gene sequencing and is characterized by specific uniform and morphological features. Because this whole group represents a phylogenetically and morphologically distinctly separated cluster (see *Phormidium* group I sensu Komárek & Anagnostidis 2005), we describe it as a special taxon *Oxynema* genus novum, in agreement with the Botanical Nomenclatoric Code (ICBN; McNeill & al. ed., 2007). The genetically most related clusters always have genetic similarity less than 93 and differ by distinct autapomorphic features. The filaments of members of *Oxynema* are cylindrical, narrowed and bent at the ends, commonly attenuated to a terminal elongated, more or less sharply pointed cells without calyptra. Thylakoids in cells are distinctly radially arranged, similarly as in the genera *Microcoleus* and "*Phormidium autumnale*"-type. The ecology of all members, which belong potentially to these types, is also similar: all species from this cluster were recorded from halophilic habitats, less frequently from thermal springs and soil biotopes with higher salt contents.

Paper IV

Strunecký O, Komárek J & Elster J (2012) Biogeography of *Phormidium autumnale* (Oscillatoriales, Cyanobacteria) in western and central parts of Spitsbergen. *Polish Polar Research*

The manuscript has been submitted after revision

Abstract: Filamentous types from the order Oscillatoriales, particularly the species *Phormidium autumnale* Kützing ex Gomont 1892, have widely diverse morphotypes which dominate in Arctic aquatic microbial mats and wet soils. We cultivated 25 strains of *Ph. autumnale* from Svalbard and compared them with available strains from surrounding regions. The comparison of strains, based on 16S rDNA and 16S-23S rDNA intergenic spacer sequences, revealed the similarity of strains from Ellesmere Island, the Canadian Arctic and Abisko, Sweden with strains from Svalbard. The rate of colonization of *Ph. autumnale* from aquatic habitats is relatively high and we suggest geese as a main transmission vector from surrounding lands. Strains of *Ph. autumnale* were positioned in the phylogenetic tree according to their occurrence in similar habitats. An apparent clustering factor is the duration of availability of water in lakes and long-lasting streams in contrast to rapid and repeated desiccation in soil and on wetted rock in the spray zone of waterfalls. Strains that grow in very cold waters just above the melting point of snow or ice form a distinct genetic group. The strains investigated in this study show morphological similarity in the shape of the trichomes of the studied specimens. Overall, the cell diameter, except for terminal cells, of our strains varied between 3 and 10 µm. Comparison of 16S rDNA sequences of the genus *Ph. autumnale* with the previously published definition of the species *Microcoleus vaginatus* revealed the identity of these two species.

Paper V

Strunecký O, Elster J & Komárek J (2012) Molecular clock provided evidence for survival of cyanobacteria (Oscillatoriales, *Phormidium autumnale*) in Antarctica from Paleozoic times *FEMS Microbiol Ecol*

The manuscript has been submitted after revision

Abstract: Cyanobacteria are well adapted to freezing and desiccation; they have been proposed as possible survivors of comprehensive Antarctic glaciations. Filamentous types from the order Oscillatoriales, especially the species *Phormidium autumnale* Kützing ex Gomont 1892, have widely diverse morphotypes which dominate in Antarctic aquatic microbial mats, seepages and wet soils. Currently little is known about the dispersion of cyanobacteria in Antarctica and of their population history. We tested the hypothesis that cyanobacteria survived Antarctic glaciations directly on site after the Gondwana-break up by using the rRNA gene relaxed and strict molecular clock in the analysis of the 16S rRNA gene. We estimated that the biogeographic history of Antarctic cyanobacteria belonging to *Ph. autumnale* lineages has ancient origins. The

oldest go further back in time than the break-up of Gondwana and originated somewhere on the supercontinent between 442 Ma and 297 Ma. Enhanced speciation rate was found around the time of the opening of the Drake Passage (~31–45 Ma) with beginning of glaciations (~43 Ma). Our results, based primarily on the strains collected in maritime Antarctica, mostly around James Ross Island, support the hypothesis that long-term survival took place in glacial refuges. The high morphological diversification of *Ph. autumnale* suggested the co-evolution of lineages and formation of complex associations with different morphologies, resulting in a specific endemic Antarctic cyanobacterial flora.

Paper VI

Strunecký O, Komárek J, Johansen J, Lukesova A & Elster J Molecular and morphological criteria for revision of the genera *Microcoleus* Desmazières ex Gomont and *Phormidium* Kützing ex Gomont (Cyanobacteria), based on strains of *Microcoleus vaginatus* and *Phormidium autumnale*. *Journal of Phycology*.

Supposed to be submitted in one month after review of a coauthor

Abstract: The traditional taxonomy of cyanobacteria depends mostly upon morphological characteristics and must be reviewed by means of modern, combined bacteriological as well as botanical methods. Our article follows the revision of the cluster of typical *Microcoleus* based on the type species *M. vaginatus* and presents its close relation to a part of the traditional genus *Phormidium* (sine typo, based on the cluster of *Phormidium autumnale* –type). Analyses based on 16S rRNA gene sequences and ITS of 88 analysed strains prove the generic identity of this cluster with typical *Microcoleus*. The necessary nomenclatoric transfers are realized in the article, in agreement with rules of botanical nomenclature. The main characters, defining the revised genus *Microcoleus* are evaluated and presented.

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