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Leptolyngbya sensu lato as a source of cyanobacterial diversity

BACHELOR THESIS

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DECLARATION

I declare that I created this bachelor thesis independently under the supervision of doc. RNDr. Petr Hašler, Ph.D., with the use of cited literature only.

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Abstract:

Leptolyngbya is a genus of filamentous cyanobacteria, currently belonging to the family Leptolyngbyaceae. Due to its extremely small dimensions, this genus is very difficult to study morphologically. In the first part of this thesis, a review focusing on the basic characteristics of cyanobacteria, their taxonomy and the current knowledge about the studied genus *Leptolyngbya* and its relatives was carried out. In the experimental part, the study of the morphology and ecology of leptolyngbyoid species was combined with the protein/peptide analysis called MALDI-TOF mass spectrometry. From 61 collected samples of filamentous cyanobacteria, 24 contained *Leptolyngbya*. Three other related genera (*Nodosilinea*, *Oculatella* and cf. *Stenomitos*) were identified. MALDI-TOF MS confirmed the similar protein/peptide composition among selected *Leptolyngbya* strains. Data obtained from the protein/peptide analysis did not match the morphological data precisely which could indicate that the morphological diversity does not cover the real species diversity within the *Leptolyngbya* genus. Further research is planned to be carried out within the diploma thesis.

Keywords: Cyanobacteria, *Leptolyngbya*, soil, subaerophytic species, MALDI-TOF

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Abstrakt:

Leptolyngbya je rod vláknitých sinic, který v současné době patří do čeledi Leptolyngbyaceae. Kvůli velmi malým rozměrům je obtížné tento rod morfologicky studovat. První část práce je literární rešerše zaměřující se na základní charakteristiku sinic, jejich taxonomii a současnou znalost studovaného rodu *Leptolyngbya* a jemu příbuzných rodů. V praktické části bylo zkombinováno studium morfologie a ekologie leptolyngbyoidních druhů s proteinovou/peptidovou analýzou, označovanou jako MALDI-TOF hmotnostní spektrometrie. Ze 61 vzorků vláknitých sinic 24 obsahovalo rod *Leptolyngbya*. Dále byly identifikovány tři příbuzné rody – *Nodosilinea, Oculatella* a cf. *Stenomitos*. MALDI-TOF analýza potvrdila podobnost v proteinovém/peptidovém složení mezi vybranými kmeny rodu *Leptolyngbya*. Tato data se přesně neshodovala s daty morfologickými, což může naznačovat, že morfologická diverzita zcela nepokrývá skutečnou druhovou diverzitu rodu *Leptolyngbya*. Další výzkum je plánován v rámci navazující diplomové práce.

Klíčová slova: sinice, *Leptolyngbya*, půda, subaerofytické druhy, MALDI-TOF

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1. AIMS OF THE THESIS

The bachelor thesis focuses on three main aims:

1. The review of the morphological variability and diversity of filamentous cyanobacteria of the genus *Leptolyngbya* and of its derived or morphologically similar genera

2. Collecting floristic data, their isolation and morphological assessment

3. MALDI-TOF analysis of selected isolated strains and assessing the usefulness of this method for biotyping of the genus *Leptolyngbya*

The bachelor thesis should form a basis for further research within a diploma thesis.

2. CYANOBACTERIA: DEFINITION AND CHARACTERISTICS

2.1 Cell structure

Cyanobacteria (also Cyanophyta or blue-green algae) are gram-negative bacteria with the ability to carry out photosynthesis of plant type. The cell organization of these ancient organisms is called thallus and can be formed by single cells or filaments, often aggregating in colonies. Cyanobacteria can be distinguished from eukaryotes by the absence of many cell structures, especially nucleus, mitochondria, plastids, endoplasmic reticulum and Golgi apparatus. Cytoskeleton and structures enabling movement are missing, too. The genetic information is stored in a circular molecule of DNA, and together with ribosomes and other structures is freely located in the protoplasm (Kalina & Váňa 2005).

Cyanobacterial cells are separated from the surrounding environment by a multilayered cell envelope which consists of a cytoplasmic membrane, a two-layered cell wall and an external mucilaginous layer. The load-bearing part of the cyanobacterial cell wall is peptidoglycan murein, while the outer layer of the cell wall is mainly composed of lipopolysaccharides (van den Hoek et al. 1995, Hoiczyk & Hansel 2000). A structured external layer is called a sheath; if it is unstructured, it is called a slime (Drews & Weckesser 1982). This layer is predominantly formed of polysaccharides and can contain higher amounts of yellow, red or blue pigments in many colonial and filamentous cyanobacteria (Castenholz 2001). One example of such a pigment is the yellow-brown scytonemin that protects the cells from UV radiation in periods of metabolic inactivity (Castenholz & Garcia-Pichel 2012).

Two types of photosynthetic pigments are present in cyanobacteria – chlorophylls and phycobiliproteins. Chlorophylls are located in thylakoid membranes, either as a single chlorophyll-a or as a combination of chlorophylls a+b, a+c or a+d (Kalina & Váňa 2005). Chlorophyll-a is usually the key reaction center pigment which also participates in light harvesting (Castenholz 2001). There are some exceptions, e.g. the main light-harvesting pigment of *Acaryochloris marina* is chlorophyll-d (Kühl et al. 2005). Phycobiliproteins are located on the outer surface of the thylakoid membrane, organized in supramolecular complexes called phycobilisomes (Sekar & Chandramohan 2008). These complexes play a significant role in the initial parts of photosynthesis as they serve as light harvesting antennae (Liu et al. 2005, Sekar & Chandramohan 2008). Phycobilisomes consist of a triangular core made up of three double discs of allophycocyanin and six rows of discs created by phycocyanin and phycoerythrin attached to the core (van den Hoek et al. 1995). In some cyanobacterial

genera, usually the heterocystous ones, phycoerythrin can be replaced by phycoerythrocyanin (Tooley & Glazer 2002). Light energy is absorbed by phycoerythrin or phycoerythrocyanin, then transferred to phycocyanin, then to allophycocyanin and finally to photosystem II and partially to photosystem I reaction centers (Sekar & Chandramohan 2008).

Phycobiliproteins are also responsible for the color of cyanobacteria. Allophycocyanin is responsible for the bluish green color, phycocyanin for blue, phycoerythrin red and phycoerythrocyanin orange (Grossman et al. 1993, Sekar & Chandramohan 2008). The ratio between phycocyanin and phycoerythrin determines the color of cells and is given by the prevalent wavelengths of light in the environment (van den Hoek et al. 1995, Grossman et al. 1993). This phenomenon is called chromatic adaptation (van den Hoek et al. 1995).

The main storage polysaccharide of cyanobacteria is cyanobacterial starch forming small granules between thylakoids (van den Hoek et al. 1995). Reserves of nitrogen are stored in cyanophycin granules that consist of amino acids arginine and asparagin (Castenholz 2001). Cyanobacteria also store polyphosphate granules (volutin) containing condensed orthophosphates as their phosphorus storage compound (Kalina & Váňa 2005). The enzyme RUBISCO is stored in carboxysomes (Castenholz 2001).

There are several specific structures typical for cyanobacteria. Aerotopes (formerly gas vacuoles) are aggregated gas vesicles which can be described as gas-filled, cylindrically shaped rigid structures with conical end caps (Walsby 1994, Wacklin et al. 2009). These structures are typical for planktonic, water-blooming species and their role is to enable cyanobacteria to migrate vertically in the water column by modulating the relative gas vesicle content (Walsby 1994). In the case of nitrogen depletion in habitats, filamentous cyanobacteria can form specialized cells called heterocytes (Castenholz 2001). The surface of these cells is formed by a thick cell wall, often with a prominent mucilaginous sheath. The cell content is colorless. The first product of the nitrogen fixation in heterocytes is ammonia, then glutamine is created, and in this form it is transported to surrounding vegetative cells (Kalina 1994). Under unfavorable conditions, i.e. inadequate light or heat conditions, lack of nutrients (especially phosphorus) or reduced oxygen availability, cyanobacteria form akinetes – spore-like, thick-walled, non-motile resting cells differentiated from vegetative cells (Kaplan-Levy et al. 2010). These cells are rich in nutrients, e.g. cyanophycin, cyanobacterial starch, lipid and carotenoid pigments (Castenholz 2001). After overcoming unfavorable conditions, new filaments may germinate. The process starts with an increased cell division under the akinete's envelope which

leads to its tearing. A new germling can subsequently emerge from the akinete's envelope (Kaplan-Levy et al. 2010).

2.2 Reproduction

Cyanobacteria reproduce primarily asexually by binary fission. If the fission occurs in a single plane and new cells are completely separated, the resultant populations are unicellular. If the fission occurs in more planes and sheath or gel holds the cells together, colonies may be formed. If the fission occurs in one plane without the separation of new cells, it results in a chain of cells – trichomes (Castenholz 2001).

Sometimes the frequency of cell divisions is too high and dwarf cells (nanocytes) can be developed as a consequence of the lack of time for the growth of daughter cells before the next division (van den Hoek et al. 1995). Some genera of cyanobacteria produce exospores, i.e. little globular spores which are released from the apical part of the cell wall of polarized, club-shaped cells (Kalina & Váňa 2005). Baeocytes form by multiple cell division within one mother cell without being liberated (Casamatta & Hašler 2016). Multicellular filamentous cyanobacteria reproduce by fragments of their filaments which are often motile and are called hormogonia (van den Hoek et al. 1995, Castenholz 2001). Several studies revealed that cyanobacteria can also reproduce "sexually" by recombination, e.g. both intragenic and intergenic recombination was observed in a microcystin synthetase (*mcy*) gene cluster in *Microcystis* species (Tanabe et al. 2004).

The type of reproduction is often characteristic for different cyanobacterial groups. For example, in Bergey's Manual of Systematic Bacteriology, taxa are grouped into subsections depending on whether they are unicellular or filamentous, whether the fission of cells is binary or multiple, and whether true branching is present or not (Castenholz 2001).

2.3 Fossil records and geological age of cyanobacteria

After discovering fossils in Apex Chert of the Warrawoona Group in Western Australia, these structures were considered the oldest findings of microbial communities on Earth, with an age of 3.5 Ga. Structures found in Apex Chert were in many cases morphologically very similar to modern oscillatorian cyanobacteria (Schopf 2000). However, some authors have contested the correct interpretation of these fossils. For example, Brasier et al. (2002) consider structures found in Apex Chert to be secondary artefacts formed from amorphous graphite. The authors point out the presence of branching filaments that are not typical for Oscillatoriales and that

appear in the fossil record much later. After questioning the Apex Chert findings, the microfossils from 3.4 Ga Strelley Pool Formation in Australia seem to be the oldest, indisputable record of life (Betts et al. 2018).

In contrast with the oldest fossils, cyanobacteria younger than 2 Ga are better conserved and thus easier to describe (Schopf 2000, Knoll 2008). The best conserved fossils belong to cyanobacteria forming extracellular sheaths as they are more likely to be preserved than cytoplasm and cell walls (Knoll 2008). The first findings of potential akinetes come from 2.1 Ga cherts in Gabon. The early occurrence of these cells in the fossil record is explained by their higher resistance to postmortem decay than in the case of vegetative cells (Tomitani et al. 2006). The findings of heterocytes are not common in the fossil record because they do not preserve well (Knoll 2008), but it is probable that they developed as an adaptation to highly aerobic conditions (which inhibit nitrogenase used for nitrogen fixation) when the concentration of oxygen in the atmosphere increased rapidly 2.45–2.30 Ga ago. The date of the first cell differentiation of cyanobacteria is therefore established between 2.45 and 2.1 Ga (Tomitani et al. 2006).

Cyanobacteria can be generally conserved in different types of geological material. Some cyanobacteria are preserved pressed along bedding planes in shales, while others can be found in limestones, dolostones, phosphorite, pyrite or silica. Cyanobacterial fossils are also conserved in stromatolites, structures formed by microbial communities interacting with sediments. The oldest well-described stromatolites are 3 Ga old, although it is not clear if cyanobacteria played some role in forming them (Knoll 2008).

2.4 The importance of cyanobacteria

Cyanobacteria are traditionally connected with the development of life on Earth as they are considered the producers of an oxygen atmosphere. The creation of photosystems that derive electrons from water and produce oxygen as a byproduct (Knoll 2008) led to the origin of new, more complex taxa possessing aerobic respiration (Buick 2008). As primary producers and N-fixers, they participate in the carbon and nitrogen cycles (Knoll 2008). Due to their ability to colonize new habitats, cyanobacteria are often pioneer organisms facilitating the life of other organisms (Fott 1967).

The importance of cyanobacteria is also noticeable in symbiotic interactions where the origin of chloroplasts by endosymbiosis is evolutionarily the most essential one

(Kalina & Váňa 2005). This relationship is a result of approximately one billion years of coevolution between the eukaryotic host and its cyanobacterial endosymbiont. The consequence of the origin of endosymbiosis was a great amplification of primary production on Earth (Gould et al. 2008). Apart from that, many other symbiotic interactions between cyanobacteria and eukaryotic organisms have been described. Cyanobacteria that participate in symbiotic relationships usually share similar features, e.g. they are filamentous, form heterocysts and reproduce by hormogonia. Heterocysts are important for fixing nitrogen (which is the benefit provided by cyanobacterium), while hormogonia play a role in infecting the host organism, as they are (in contrast with adult filaments) motile (Adams 2000). A typical example of symbiosis between cyanobacterium and plant is the symbiosis with the water fern *Azolla*. In this relationship, the cyanobiont spends its whole life cycle in the leaves of *Azolla* where it fixes nitrogen and receives fixed carbon from *Azolla* in return (Carrapiço 2016). Despite the long-lasting knowledge of this symbiosis, there is still a debate over whether the cyanobiont belongs to the genus *Anabaena*, *Nostoc* or *Trichormus* (e.g. Carrapiço 2016, Kumar et al. 2019 etc.). Another well-known example is the association of cyanobacteria (or green alga) with fungi which forms the thallus of lichens. In this association, *Nostoc* is the most common genus (Rikkinen et al. 2002). Further cyanobacterial symbiotic interactions include, for example, association with diatoms, mosses, cycads, flowering plants, marine sponges and other eukaryotes (Adams 2000).

Cyanobacteria are also useful for humans. Probably the best-known example is the commercial use of the genus *Arthrospira* (syn. *Spirulina*) as a food supplement rich in proteins, essential fatty acids, vitamins and minerals (Belay 2002). Some cyanobacterial species are indicators of water quality which can be used in the biological analysis of water (Fott 1967). Recently, the use of cyanobacteria as a biofuel has been debated because of their fast growth and easy cultivation (Nozzi et al. 2013).

2.5 The ecology of cyanobacteria

Even though cyanobacteria can be found in different types of environment, they generally prefer places with enough humidity. These habitats do not include only water biotopes – cyanobacteria are also abundant in terrestrial habitats, e.g. soil or rocks.

Terrestrial cyanobacteria occupying rocky substrates can be basically divided into three categories – epilithic (i.e. growing on the substrate surface), hypolithic (i.e. growing under small stones) and endolithic (i.e. growing inside the upper layer of rocks) (Czerwik-Marcinkowska

& Massalski 2018). On cliffs, cyanobacteria sometimes create colorful vertical streaks at places where water flows down (Pentecost & Whitton 2000). Cyanobacteria can also colonize caves, with Chroococcales (represented e.g. by *Gloeocapsa* or *Aphanocapsa*) usually being the most abundant order (Czerwik-Marcinkowska & Massalski 2018). Some species are able to grow through mollusk shells, e.g. *Cyanosaccus atticus*, *Hyella caespitosa* or *Plectonema terebrans* (Pantazidou et al. 2006). Many genera have been reported growing on plants where they often contribute to symbiotic interactions. For example, *Nostoc*, *Anabaena* or *Calothrix* grow on leaves of duckweeds *Lemna* or *Spirodela* where they fix nitrogen (Duong & Tiedje 1985). Examples of genera that can grow on tree barks (epixylically) include *Nostoc* or *Scytonema* (Neustupa & Škaloud 2008). Cyanobacteria also play an important role in the soil as they enhance physical and chemical conditions of soil, stimulate plant growth by producing phytohormones (e.g. *Anabaena, Calothrix, Nostoc*), protect plants from pathogens (*Calothrix elenkenii* or *Fischerella muscicola*), and remove heavy metals from soil (many *Nostoc* species) etc. (Singh et al. 2016).

Water species live either attached to the substrate (benthos) or they float in the water column (plankton). Benthic species grow on substrates such as plants (epiphyton), rocks (epilithon), mud (epipelon), sand (epipsammon) and animals (epizoon) (Poulíčková et al. 2015). Planktonic species living near the water surface with well-developed aerotopes often produce water blooms in fresh waters rich in nitrogen and phosphorus. Cyanobacterial blooms occur predominantly during summer and autumn months in temperate zones, while in the tropics, they can appear practically throughout the whole year. The most frequent bloom-forming cyanobacterial genera are *Anabaena, Oscillatoria, Aphanizomenon* or *Microcystis* (Oliver & Ganf 2000). The increased biomass of cyanobacteria affects other organisms by the production of toxins. Toxins can be classified according to their effect on the human body into five groups – hepatotoxins, neurotoxins, cytotoxins, dermatoxins and irritant toxins (Mankiewicz et al. 2003) or by their chemical structure into cyclic peptides (e.g. microcystins) and alkaloids (e.g. anatoxins) (van Apeldoorn et al. 2007). Cyanobacteria also reduce the amount of oxygen in water during rotting processes caused by biomass decay. Water bloom causes health problems to humans, especially at swimming-pools and dams used as a source of drinking water (Kalina & Váňa 2005).

Cyanobacteria are often generalists tolerating a wide range of environmental conditions, including extreme ones in which even eukaryotic algae often cannot grow (Castenholz 2001).

These habitats can be characterized by the high or low temperature of the water or soil surface, high salt concentrations or extreme values of pH.

Cyanobacteria occurring in deserts have to deal not only with high temperatures, but also with dessication. Examples of cyanobacteria that developed adaptations to such conditions belong both to filamentous (e.g. *Schizothrix*) and coccoid (e.g. *Synechococcus*) types of thallus (Wynn-Williams 2000). On the other hand, there are several cryophilic species among cyanobacteria which are assigned, for example, to the genera *Leptolyngbya* (Komárek 2007) or *Chroococcidiopsis* (Das & Singh 2017). In the case of *Chroococcidiopsis*, it was discovered that a single species of this genus is able to occupy both hot and cold deserts (Nienow & Friedmann 1993 cited by Wynn-Williams 2000). Species tolerating high salinities are e.g. *Microcoleus chthonoplastes* or *Aphanothece halophytica* (Oren 2000). There are not many cyanobacteria adapted to an extremely low pH, but exceptions do exist, e.g. *Aphanocapsa* sp., *Chroococcus prescotii* or *Oscillatoria* sp. which were detected in Canadian acidic lakes (Kwiatkowski & Roff 1976). Cyanobacteria tolerating a higher pH are more common, e.g. *Plectonema nostocorum* was found growing even at pH 13 (Seckbach & Oren 2007). The success in surviving such conditions can often be explained by the ability to form akinetes and heterocytes, the efficient absorption of photons in cases of reduced light availability, the efficient use of carbon dioxide at low concentrations or by the ability to use hydrogen carbonate ions at a high pH (Castenholz 2001).

3. THE TAXONOMY OF CYANOBACTERIA

As prokaryotic, mostly asexually reproducing organisms with the ability to adapt to diverse environmental conditions, cyanobacteria are very difficult to classify (Komárek 2016, Palinska & Surosz 2014). During the previous century and this one, the cyanobacterial taxonomy has undergone a great development, dealing with numerous obstacles that in many cases have not been fully eliminated yet.

One of the biggest current complications is the conflict between two approaches used for studying cyanobacteria – the botanical and bacteriological one – which is noticeable especially in nomenclature. While botanists (phycologists) consider cyanobacteria photosynthetic organisms with similar ecological niches to eukaryotic algae, the bacteriological approach emphasizes the prokaryotic nature of cyanobacteria shared with other bacteria. Consequently, the cyanobacterial nomenclature is ruled by two codes, botanical and bacteriological, which work on different bases but are both valid (Palinska & Surosz 2014).

The discovery of the prokaryotic nature of cyanobacteria resulted in several other difficulties in taxonomy. For example, taxonomists struggle with searching for a suitable species concept. This issue is described more in detail in the subchapter 3.3 Taxonomic concepts.

3.1 The historical view

The original view on taxonomy of cyanobacteria was based on their morphological features. The introduction of modern methods enabling taxonomists to study organisms in more detail (electron microscopy, molecular analysis etc.) has gradually led to the development of taxonomy that reflects phylogenetic relationships among cyanobacterial taxa (Komárek et al. 2014, Komárek 2016). The goal of the present-day taxonomy is to combine modern laboratory methods with the study of cyanobacterial morphology and ecology which is called a polyphasic approach (Komárek 2016).

3.1.1 Botanical approach

Traditional approach (“Geitler’s system”)

The traditional botanical approach classifies cyanobacteria by their morphological features and their nomenclature is ruled by the Botanical Code (Wilmotte 1994). The origin of traditional cyanobacterial taxonomy dates to the second half of the nineteenth century when the first

taxonomic monographs were published (e.g. Nägeli 1849, then Rabenhorst 1865, Thuret 1875, Bornet & Flahault 1886-1888, Hansgirg 1888, Gomont 1892a,b) (Anagnostidis & Komárek 1985, Palinska & Surosz 2014). The essential work forming the basis of the traditional botanical approach is the work of Lothar Geitler. Geitler's system, based on morphological features and species ecology, was modified many times during the twentieth century by its own author (Geitler 1925, 1932, 1942), but also by other taxonomists.

The first version of Geitler's system (1925) included seven orders of cyanobacteria – Chroococcales, Entophysalidales, Pleurocapsales, Dermocapsales, Siphononematales, Nostocales a Stigonematales. In its later modification (1932) the number of orders was reduced when Geitler adopted Frémy's system including only Chroococcales, Chamaesiphonales and Hormogonales. The final version of Geitler's system (1942) included four orders – Chroococcales, Dermocarpales, Pleurocapsales and Hormogonales, and this system was used and modified by other authors, e.g. Elenkin (1936–1949), Desikachary (1959), Fritsch (1959), Starmach (1966), Kondrateva (1968) or Bourrelly (1970) (Palinska & Surosz 2014, Anagnostidis & Komárek 1985).

Ecophenes approach ("Drouet's system")

At the beginning of the second half of the twentieth century, Francis Drouet proposed a new system in which he reduced drastically the number of cyanobacterial taxa (Drouet & Daily 1956, Drouet 1968, 1973, 1978, 1981 cited by Anagnostidis & Komárek 1985). The reason for such a reduction was that cyanobacteria possess a huge morphological variability under different environmental conditions. Drouet considered the number of described species only ecophenes within one species (Palinska & Surosz 2014). However, classical taxonomists contested this approach and later DNA-DNA hybridizations proved that there was a genotypic difference between taxa placed in a single species by Drouet (Wilmotte 1994). Therefore, this system has not been accepted by phycologists (Anagnostidis & Komárek 1985).

3.1.2 Bacteriological approach ("Stanier's system")

The development of electron microscopy and biochemical analysis led to the discovery of the cyanobacterial prokaryotic nature. Based on this discovery, Stanier et al. (1978) suggested that cyanobacteria should be treated as other bacteria and their taxonomy should be ruled by the Bacteriological Code (Wilmotte 1994). According to this approach, cultured cyanobacterial strains are the basic unit of the cyanobacterial taxonomy (Anagnostidis & Komárek 1985).

Thus, species is only a "consensual construct" created by comparison of similar strains in this approach. Because many bacteriologists avoid phenotypic species description, cultured cyanobacteria are usually assigned the name of genus with a strain code (Palinska & Surosz 2014). The bacteriological taxonomy of cyanobacteria was created by Rippka et al. (1979) and it consists of five sections corresponding with orders of other classifications (Wilmotte 1994). This system formed a basis for Bergey's Manual of Systematic Bacteriology where cyanobacteria are divided into five subsections: I (=Chroococcales), II (=Pleurocapsales), III (=Oscillatoriales), IV (=Nostocales) and V (=Stigonematales) (Castenholz 2001).

The cultivation of cyanobacterial strains turned out to be useful in cyanobacterial taxonomy and became an important part of laboratory practice. However, Palinska & Surozs (2014) pointed out that treating cultured cyanobacterial strains alone underestimates the cyanobacterial diversity in natural conditions.

3.2 Modern polyphasic approach

The introduction of microbiological methods leading to the possibility of 16S rRNA genes sequencing or DNA-DNA hybridization with related organisms permitted the evaluation of differences between cyanobacterial taxa at the molecular level. Even though DNA sequence should determine phylogeny and phylogeny should determine taxonomy (Wayne et al. 1987), molecular methods alone should not be used for the evaluation of phylogenetic relationships because the lengthy way of cyanobacterial cultivation at laboratory conditions can cause numerous morphological and physiological changes, as well as changes in genotype and also the loss of adaptations to environmental conditions (Komárek 2016). A solution to this problem of the discrepancies between morphological/physiological/ecological and molecular features was proposed by Colwell (1970) who introduced a new, polyphasic approach that puts the knowledge obtained by the different methods together. This approach is established on genetic evaluation which is combined with other data from morphological, ecophysiological and ecological analysis (Komárek 2016). It can be useful for revising traditionally described taxa (i.e. based on phenotypic traits) as well as for describing newly discovered taxa, with a goal to reflect phylogenetic relationships in both cases (Komárek et al. 2014).

The polyphasic approach is currently preferred both by bacterial taxonomists (Murray 1990) and traditional taxonomists (Anagnostidis & Komárek 1985, Komárek 2016). Anagnostidis & Komárek utilized this approach while creating the Modern approach to the classification system of cyanophytes (published in 1985), where botanical and bacteriological

approaches are combined. Their broadly recognized system includes four orders – Chroococcales, Oscillatoriales, Nostocales and Stigonematales. One of the newly proposed systems is that of Komárek et al. (2014) consisting of eight orders – Gloeobacterales, Synechococcales, Oscillatoriales, Chroococcales, Pleurocapsales, Spirulinales, Chroococcidiopsidales and Nostocales.

Nevertheless, the modern polyphasic approach still contains several weaknesses. Examples include cryptotaxa, morphologically identical species that differ cytologically or ecologically, or morphotaxa and ecotaxa, morphologically and ecologically separable taxa that are uniform at the molecular level. Moreover, the nomenclature has not been resolved yet, as botanical and bacteriological nomenclatural rules have not been unified (Komárek 2016).

Despite its unresolved obstacles, the polyphasic approach is currently the most desirable one in modern cyanobacterial taxonomy. To improve the current state of taxonomy of cyanobacteria, revisions of genera based on the polyphasic approach are required. Such revisions should include the description of morphological traits observed with light and electron microscopy, habitat characterizations and the molecular analysis of 16S rRNA genes and other markers (Dvořák et al. 2015b).

3.3 Taxonomic concepts

3.3.1 Generic concept

Two tendencies have occurred in cyanobacterial taxonomy when delimitating cyanobacterial genera. The first (usually preferred) one splits taxa into several smaller genera in an attempt to keep monophylly in classification. Here, every group of species with one or more unique feature can be considered a separate genus (Anagnostidis & Komárek 1985). The second approach, by contrast, merges many species into one larger group (genus), but with the risk of creating polyphyletic taxa. This second approach is typically connected with Drouet's system (Komárek 2016).

According to Komárek (2010), genera should be characterized by molecular separation with about 95% or less genetic similarity between 16S rRNA gene sequences compared. This criterion should be combined with at least one autapomorphic cytomorphological feature. In practice, the application of this rule led to splitting some existing genera into more generic units.

There are many features that can characterize cyanobacterial genera. During the history of cyanobacterial taxonomy, different approaches gave priority to different features. The modern polyphasic approach stresses using both molecular and phenotypic features. However, research has proved that some features are more appropriate for defining genera than others. For example, the presence of sheaths, cell size or branching as classification criteria is often not compatible with molecular results (Komárek 2010). On the other hand, some phenotypic features turned out to be very valuable in classification, e.g. cell wall perforations (Palinska & Krumbein 2000) or thylakoids (Komárek 2016).

3.3.2 Species concept

Among taxonomists, the species is considered the basic unit of biological diversity (Wilmotte 1994, Palinska & Surosz 2014). There have been many definitions created for the delineation of species, but most of them are not suitable for cyanobacteria. For example, the broadly accepted Mayr's biological species concept, defining species as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups" (Mayr 1942) is valid only for sexually reproducing eukaryotes. Thus, since cyanobacteria are treated as prokaryotes that reproduce mostly asexually, this concept cannot be used (Johansen & Casamatta 2005, Palinska & Surosz 2014). The problem in bacteriology is that no consensus for defining bacterial species was achieved (Palinska & Surosz 2014). There have been many suggested definitions of prokaryotic species; one of the most widely accepted is a phylo-phenetic species concept defining species as "a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics and is diagnosable by a discriminative phenotypic property" (Roselló-Mora & Amann 2001). Nevertheless, cyanobacteria are a specific group of prokaryotes with several traits shared with eukaryotic algae, so designing a species concept applicable to cyanobacteria is complicated.

The currently predominantly used polyphasic approach combines genomic and phenotypic properties to characterize prokaryotic species (Rosselló-Mora & Amann 2001, Stackebrandt et al. 2002, Palinska & Surosz 2014). The genomic characterization is mainly based on measuring the genetic similarity among isolates. Specifically, it is assessed by the degree to which their genomes hybridize under standard conditions (DNA-DNA hybridization), often in combination with 16S rRNA gene sequence similarity (Palinska & Surosz 2014). DNA-DNA hybridization as a criterion for the definition of bacterial species was proposed by Wayne

et al. (1987) who required 70% and greater DNA-DNA relatedness and 5 °C or less ΔT_m for the same species (T_m = thermal denaturation midpoint, i.e. the temperature at which 50 % of the DNA strands are already denatured; Roselló-Mora & Amann 2001). Different thresholds of percentage similarity of 16S rRNA gene sequences were estimated for differentiating two species, usually between 97 to 99 % (Stackebrandt & Goebel 1994, Kim et al. 2014 etc.), but regardless of the estimated threshold, many authors labeled using 16S rRNA sequences for species delineation as inadequate (Fox et al. 1992, Roselló-Mora & Amann 2001, Komárek 2010 etc.). Johansen & Casamatta (2005), Palinska & Surosz (2014) and some others do not recommend the use of similarity-based species concepts at all. Instead, Johansen & Casamatta (2005) require monophyletic nature of species (recognizable through autapomorphies), the possibility of applying the species concept to both strains in unialgal cultures and natural populations and the possibility of recognizing the ecologically and evolutionarily significant biodiversity of cyanobacteria through the concept. Therefore, they suggest the adoption of the phylogenetic (=monophyletic) species concept sensu Mishler & Theriot (2000) that defines a species as the smallest monophyletic taxonomic group. On that basis, they characterize cyanobacterial species as "monophyletic clusters of strains or natural populations that are diagnosable by some unique combination of traits, those traits being any combination of morphological, biochemical, molecular, or other characteristics". Komárek (2016) finds the species concept a rather conventional measure, as it should have different criteria in different genera. From his point of view, the most appropriate species definition is "a group of populations (+strains), which belong to one and the same genotype (genus), which are characterized by a stabilized phenotype with characteristic features (definable and recognizable with distinct limits of variation) and by the same ecological demands; they should occur repeatedly (in time) in various localities with the same ecological conditions".

3.3.3 Ecotype concept

As bacterial systematics has not found a way of defining a species yet, it was suggested that bacterial taxonomy could be established on different, smaller taxonomic units – ecotypes. Ecotypes are defined as "populations of organisms occupying the same ecological niche, whose divergence is purged recurrently by natural selection". Ecotypes correspond with species of non-bacteriological concepts and therefore species in the bacteriological understanding corresponds rather with a non-bacteriological genus (Cohan 2002). This statement can be proved at the molecular level. If 16S rRNA is used for species demarcation and 1% divergence between strains indicates they are different species, several ecotypes can be hidden in this 1 %.

Consequently, this ecological diversity would not be visible within the current systematics of bacteria (Koeppel et al. 2008). The use of this concept is accepted also in the cyanobacterial taxonomy. For example, Johansen & Casamatta (2005) recommend the use of the ecotype concept in cases where the monophyletic species concept cannot be applied.

3.4 Nomenclature

As noted above, the nomenclature of cyanobacteria is currently ruled by two codes – botanical and bacteriological. Probably the most visible consequence of this situation is the number of names that can be possibly used for this group of ancient organisms. In the botanical view they are seen as Cyanophyta, i.e. microscopical plants, or blue-green algae, according to their similarity to algae. After classifying them as prokaryotic bacteria, the name cyanobacteria has become commonly used. Nowadays, all these names are valid. However, the coexistence of two nomenclatorial codes still causes many problems. For example, cyanobacterial names published under botanical code have no standing under the bacterial nomenclature if they are not described under the bacteriological code, too (Palinska & Surosz 2014).

As no compromise between botanical and bacteriological sides has been proposed, many authors decided to combine instructions from both codes to describe new taxa. Consequently, newly described taxa seem not to follow any of the codes precisely. To resolve such problems, nomenclaturists proposed new suggestions for nomenclatorial rules. One such idea was to create a unified system for all groups of organisms. Another suggestion, to establish special rules for cyanobacteria that would respect both codes, could be more practical. In response to the second idea, a nomenclatural guide for cyanobacteria was designed and published, but it was rejected by both communities (Komárek 2010).

Among bacteriologists, the idea of eliminating names of cyanobacterial taxa and replacing them with numbers and other types of coding emerged. This new system would be accepted only if a) it was as convenient as the current traditional system, b) it was applicable to cyanobacteria from both cultures and natural habitats and c) it was useful for all users of this system (especially ecologists, hydrobiologists and applied scientists). Currently, no such system that could conveniently replace binomial nomenclature is available (Komárek 2010).

4. LEPTOLYNGBYA SENSU LATO

4.1 The position of *Leptolyngbya* sensu lato in a taxonomic system

Leptolyngbya as a separate genus was first described by Anagnostidis and Komárek (1988) who classified the genus in the order Oscillatoriales, fam. Pseudanabaenaceae, subfam. Leptolyngbyoideae. Until that time, *Leptolyngbya* species were known under names of other genera, mostly *Lyngbya*, *Phormidium*, *Plectonema* or *Oscillatoria*. For example, the type genus *Leptolyngbya boryana* was originally named *Plectonema boryanum* Gomont 1899 (Anagnostidis & Komárek 1988). Nevertheless, the similarity between *Lyngbya*, *Phormidium* and *Plectonema* species was also observed before describing *Leptolyngbya* as a genus nova. Rippka et al. (1979) merged these genera into the "LPP group B", named by their first letters, and placed in section III of his system. In Bergey's Manual of Systematic Bacteriology (Castenholz 2001), based on the system created by Rippka et al. (1979), the genus *Leptolyngbya* is classified in subsection III (=Oscillatoriales).

Leptolyngbya is a highly problematic genus because of its polyphyletic character, known from the very beginning of its establishment (Anagnostidis & Komárek 1988). Thus, achieving monophyly in *Leptolyngbya* and related taxa through revisions is the current aim of taxonomists dealing with this issue (Albertano & Kováčik 1994, Dvořák et al. 2017, Mai et al. 2018 etc.). This effort is carried out mainly by dividing formerly described *Leptolyngbya* genus into new and independent taxa. However, revisions have not only led to describing new genera – the classification of *Leptolyngbya* was also modified at higher taxonomic levels. According to the new classification proposal by Komárek et al. (2014), *Leptolyngbya* and its relatives were moved from Oscillatoriales into Synechococcales, fam. Leptolyngbyaceae stat. novo. In the newest revision of Synechococcales (Mai et al. 2018), Leptolyngbyaceae were even split into more families – Leptolyngbyaceae, Prochlorotrichaceae, Oculatellaceae and Trichocoleaceae. These substantial changes are a matter of the last twenty years. During that period, many new genera were described and classified in Leptolyngbyaceae and it seems that many other new descriptions will follow. Therefore, it is possible that the increasing number of sources giving the information about phylogenetic relationships within this family (order) will lead to other changes in the taxonomic system.

4.2 *Leptolyngbya* and its relatives

When Anagnostidis and Komárek (1988) recognized Leptolyngbyoidae as a subfam. nova, they included three genera in this subfamily – *Leptolyngbya*, *Planktolyngbya* and *Leibleinia*. *Planktolyngbya* was described as a planktonic genus, *Leptolyngbya* and *Leibleinia* as genera creeping on substrate (in the case of *Leibleinia* usually on plants) (Komárek & Anagnostidis 2005).

As mentioned above, the revisions of genera at the beginning of this century caused changes in the classification system of cyanobacteria. Some genera were split into smaller ones, while some genera were combined with respect to their morphological and molecular relationships. A typical example of the first case is *Oculatella* (Zammit et al. 2012) which was split off the *Leptolyngbya* genus and according to the current revision, it belongs to the independent family Oculatellaceae (Mai et al. 2018). The latter situation can be demonstrated by *Phormidesmis molle,* which was initially classified as *Phormidium molle,* but molecular analysis proved its independence and close relationship with leptolyngbyoid cyanobacteria (Turicchia et al. 2009). Except for revised genera, the current family Leptolyngbyaceae contains many entirely newly described ones, e.g. *Scytolyngbya* (Song et al. 2015), *Onodrimia* (Jahodářová et al. 2017b) and many others.

According to Komárek et al. (2014), the family Leptolyngbyaceae includes the following genera: *Leptolyngbya*, *Haloleptolyngbya*, *Halomicronema*, *Leibleinia*, *Neosynechococcus*, *Nodosilinea*, *Oculatella*, *Planktolyngbya*, *Plectolyngbya*, *Phormidesmis*, *Prochlorothrix* and *Trichocoleus*.

According to Mai et al. (2018), Leptolyngbyaceae include: *Alkalinema*, *Arthronema*, *Chamaethrix*, *Kovacikia*, *Leptolyngbya*, *Limnolyngbya*, *Myxacorys*, *Neosynechococcus*, *Onodrimia*, *Pantanalinema*, *Phormidesmis*, *Pinocchia*, *Plectolyngbya*, *Planktolyngbya*, *Romeria*, *Scytolyngbya*, *Stenomitos* and *Tapinothrix*. Oculatellaceae consist of *Cartusia*, *Elainella*, *Drouetilla*, *Komarkovaea*, *Tildeniella*, *Kaiparowitsia*, *Oculatella*, *Pegethrix*, *Thermoleptolygbya*, *Timaviella* and *Trichotorquatus*. Prochlorotrichaceae contain *Haloleptolyngbya*, *Halomicronema*, *Nodosilinea* and *Prochlorothrix*. Trichocoleaceae contain a single genus *Trichocoleus*.

The genus *Leptolyngbya* will be described in detail in subchapter 4.3. Other genera of Leptolyngbyaceae, Oculatellaceae, Prochlorotrichaceae and Trichocoleaceae sensu

Mai et al. (2018) + newly described *Chroakolemma*, *Marileptolyngbya* and *Salileptolyngbya* (not included in Mai et al. 2018) will be described in subchapter 4.4.

4.3 *Leptolyngbya* Anagnostidis & Komárek 1988

4.3.1 Morphology

Filaments are long, thin, solitary or more often in clusters, floating or ± attached to the substrate, finely waved or almost straight, usually not attenuated towards the ends (Komárek & Anagnostidis 2005). Hyaline sheaths are firm and thin, their presence depends on environmental conditions (Komárek & Anagnostidis 2005). Trichomes are 0.5 to 3 μm wide, without conspicuous motility, sometimes with indistinct trembling (Komárek & Anagnostidis 2005, Castenholz 2001). Cells can be cylindrical, isodiametric or shorter than wide (Komárek & Anagnostidis 2005). True branching does not occur in this genus, false branching is possible and depends on the sheath strength (Anagnostidis & Komárek 1988, Castenholz 2001, Komárek & Anagnostidis 2005). The cell content is usually homogenous, often with recognizable chromato- and centroplasma (Komárek & Anagnostidis 2005). Thylakoids are arranged peripherally in number three to seven, gas vesicles are missing, chromatic adaptation is possible (Anagnostidis & Komárek 1988, Albertano & Kováčik 1994, Komárek & Anagnostidis 2005). Pores in the cell wall are present (Albertano & Kováčik 1994). Constrictions at the cross-walls are usually present, but sometimes almost indistinct (Anagnostidis & Komárek 1988, Castenholz 2001).

Leptolyngbya reproduces by trichome disintegration (Komárek & Anagnostidis 2005). Hormogonia breaking up from the apical parts of trichomes are motile or immotile (Castenholz 2001). Necridic cells may be present or absent (Komárek & Anagnostidis 2005). Divided cells reach their full size before the next division (Anagnostidis & Komárek 1988).

4.3.2 Ecology

*Leptolyngby*a is a common cyanobacterial genus with a cosmopolitan distribution. It is known from various habitats, including some extreme ones. Its species colonize both aquatic and terrestrial ecosystems: water habitats include fresh waters, seas and mineral and thermal springs, whereas on land, *Leptolyngbya* grows on soil, rocks, walls or tree barks (Komárek & Anagnostidis 2005).

In Central Europe, *Leptolyngbya* predominantly occupies stagnant and flowing waters, e.g. *L. perforans, L. ochracea, L. fontana, L. subtilis, L. tenerrima, L. boryana*, etc., wet rocks, walls or greenhouses, e.g. *L. gloeophila, L. compacta, L. henningsii* or *L. subtilissima*. Some species can be found in soils, e.g. *L. foveolarum, L. notata* or *L. hansgirgiana*. Some are present in thermal springs, e.g. *L. gelatinosa* or *L. thermobia*, or in salty inland waters, e.g. *L. halophila* (Komárek & Anagnostidis 2005).

Some *Leptolyngbya* species are able to withstand extremely cold conditions and therefore can be found in polar regions. Many of those species have been described in Antarctica. Examples include *L. antarctica*, which colonizes bottoms of constantly frozen lakes and is probably an endemic species to this continent, *L. erebi*, which occurs in small stagnant waters, wet soils or cryoconits on glaciers, and *L. nigrescens*, which prefers wet rocks (Komárek 2007). *Leptolyngbya* has also been discovered in mats formed on ice shelves in Arctic regions (Vincent et al. 2004).

In contrast, some species are adapted to the life in deserts where they are forced to deal with extreme temperatures, often in combination with high irradiation and extreme drought. For instance, *L. ohadii* is a desiccation-tolerant species (Raanan et al. 2016) whose populations increase with decreasing rainfalls in the Negev desert, Israel (Hagemann et al. 2017).

High temperatures are typical also for thermal springs where many species of *Leptolyngbya* have been described, too. According to Sciuto & Moro (2016), *Leptolyngbya* can be considered one of the most abundant cyanobacterial genera in thermal environments. Many species have been described from Yellowstone National Park, either from geysers, where e.g. *L. geysericola* resists temperatures 59–84 °C, or from hot springs, where e.g. *L. cartilaginea*, *L. rubra*, *L. subterranea* or *L. yellowstonensis* occur (Copeland 1936, Komárek & Anagnostidis 2005). *Leptolyngbya* sp. present in thermal springs in Tunisia was proved to have a beneficial, antioxidant effects on human health, as it contains higher amounts of phenols, flavonoids and vitamin C (Trabelsi et al. 2016). Other thermophilic species have been described from thermal springs in Kamchatka, Indonesia, equatorial Africa, Puerto Rico (Anagnostidis & Komárek 2005), Patagonia (Mackenzie et al. 2013), Himalayas (Singh et al. 2018), Australia (McGregor & Rasmussen 2008) and other places.

Some marine *Leptolyngbya* species have a significant influence on marine ecosystems. In the Caribbean and Philippines, *Leptolyngbya* sp. producing microcystin was confirmed to be one of contributors to the black band disease which negatively affects corals by lysing

their tissue (Myers et al. 2007, Richardson et al. 2007). *Leptolyngbya* was also found growing in association with marine sponges (Pagliara & Caroppo 2011, Konstantinou et al. 2016). The strains isolated from these sponges were also tested on their cytotoxic and antimitotic effects which were in both cases confirmed (Pagliara & Caroppo 2011).

Soil species are abundant world-wide in different habitats where they can bring various benefits. In areas with inhospitable conditions, they may serve as pioneer organisms (Lin & Wu 2014, Roncero-Ramos 2019). Also, *Leptolyngbya* plays a significant role in agriculture, e.g. in rice fields, as it is known for increasing the concentrations of nitrogen in soils by fixing atmospheric nitrogen which leads to enhancing the conditions for the plants' growth. Moreover, the production of phytohormone auxin by *Leptolyngbya* causes the increased growth of adventitious roots of cultivated plants (Ahmed et al. 2014).

All this information proves that the genus *Leptolyngbya* is a widespread, species-rich taxon with great ecological diversity. The ability of *Leptolyngbya* species to endure such extremes as frost, heat, drought or excessive or insufficient irradiation makes them successful colonizers of almost all possible habitats on Earth.

4.3.3 Determination of *Leptolyngbya* species

The small proportions and the simple morphology of this genus make species determination difficult (Komárek & Anagnostidis 2005, Johansen & Casamatta 2005, Zhou et al. 2018 etc.). In contrast with morphology, the specific ecological demands of *Leptolyngbya* species can simplify their complicated determination (Komárek & Anagnostidis 2005). These two characteristics form the basis for the determination of *Leptolyngbya* species in Süsswasserflora von Mitteleuropa (Komárek & Anagnostidis 2005). Here, *Leptolyngbya* is divided into two subgenera – *Leptolyngbya* and *Protolyngbya* which differ by cells proportions and the mechanism of trichome disintegration. Specifically, the cells of *Leptolyngbya* are ± isodiametric and the disintegration of trichomes is accompanied by the participation of necridic cells. By contrast, the cells of *Protolyngbya* are distinctly longer than wide and the necridic cells are not present. Both subgenera are further divided into six ecological groups:

Leptolyngbya: I – freshwater species living in stagnant and flowing waters, II – species occurring in mineral and thermal springs, III – endogloeic species, living in the mucilage of other cyanobacteria and algae, IV – soil species, V – subaerophytic species, mostly from wet rocks and walls, VI – marine and halophilic species

Protolyngbya: VII – freshwater species living in stagnant and flowing waters, VIII – species from mineral and thermal springs, IX – endogloeic species, living in the mucilage of other cyanobacteria and algae, X – soil species, XI – subaerophytic species, mostly from wet rocks and walls, XII – marine and halophilic species

However, the morphological and ecological characteristics alone are not a sufficient source of information for determination of *Leptolyngbya* species because of the frequent presence of cryptic species within cyanobacterial genera, including *Leptolyngbya* (Li & Li 2016, Jahodářová et al. 2017b). According to the current conception of cyanobacterial taxonomy, the molecular evaluation should be incorporated when describing and determining species, too.

4.4 Genera closely related to *Leptolyngbya*

In the following list of genera, the source of all information about each genus is identical to the source given after the name of the genus, except for cases when a different source is mentioned in the text. Genera within families are sorted alphabetically.

4.4.1 Leptolyngbyaceae

Alkalinema Vaz et al. 2015

Alkalinema was discovered in saline-alkaline lakes in the Brazilian Pantanal wetland. This cyanobacterium, with trichomes often interwoven in a specific formation, possesses a huge pH tolerance (4–11). One species, *A. pantanalense*, has been described within the genus so far.

Identification methods: the study of morphology, 16S rRNA gene, 16S–23S rRNA ITS sequence and secondary structures, growth responses to culture pH.

Morphology: trichomes immotile, often arranged in interwoven mats, without sheaths, but surrounded by a diffluent mucilage; cells ± isodiametric or longer than wide, 1.5–4.1 μm long and 1.1–2.2 μm wide, with a homogenous, reddish to brownish content; apical cells narrowed or rounded-conical; reproduction via hormogonia.

Type species: *Alkalinema pantanalense* Vaz et al. 2015

Arthronema Komárek & Lukavský 1988

Arthronema africanum, originally described as *Pseudanabaena africanum*, was discovered in the oasis Waw en-Namus in Central Sahara in Libya (Schwabe & Simonsen 1961). Komárek & Lukavský (1988) isolated the same species that was found in southern Kuwait, in the depth of 1–2 cm under the wet sand covered with crystalline salts at the bottom of dry lake, and classified it as a new taxon – *Arthronema africanum*. C-phycocyanin isolated from this species was tested for its antitumor activity on Graffi tumor in hamsters and based on the positive results, its possible use in pharmacology and medicine in the future was proposed (Gardeva et al. 2014).

Identification methods: morphological and ecophysiological analysis.

Morphology: trichomes thin, blue-green, of unequal width from 0.8 to 5 μm in different parts of trichomes and in trichomes from different conditions; sheaths not developed; cells ± isodiametric with slight constrictions at the cross-walls; special swollen cells present probably

as an adaptation to haline conditions; thylakoids peripherally in number of 2–6; apical cells widely rounded and flattened, sometimes slightly narrowed or widened; the cell division shifted to one cell end; short, few-celled fragments separated from trichomes common in cultures; both trichomes and hormogonia immotile.

Type species: *Arthronema africanum* (Schwabe & Simonsen) Komárek & Lukavský 1988

Chamaethrix Dvořák et al. 2017

Chamaethrix was isolated from a soil crust in Everglades National Park in Florida, USA. The genus can be distinguished from *Leptolyngbya* both by morphological (e.g. more trichomes in a common sheath) and molecular features.

Identification methods: morphological and molecular analysis (16S rRNA gene and 16S-23S rRNA ITS secondary structures).

Morphology: filaments solitary or in mats, straight or undulate, occasionally with false branching; sheaths colorless or rarely colored brownish to black, distinct, firm, thin or thick, sometimes containing two trichomes; cells isodiametric or longer than wide, constrictions at the cross-walls slight or missing; apical cells rounded to conical; reproduction by hormogonia, usually with the help of necridic cells.

Type species: *Chamaethrix vaginata* Dvořák et al. 2017

Chroakolemma Becerra-Absalón & Johansen in Becerra-Absalón et al. 2018

Chroakolemma was distinguished as a separate genus after confirming its independence on genetically similar *Scytolyngbya* and morphologically similar *Chamaethrix*. The genus is specific by creating blackish sheaths. *Chroakolemma* was isolated from two localities in Central Mexico where it was found in semi-desert soil crusts. Three species have been described within the genus, *Ch. opaca*, *Ch. pellucida* and *Ch. edaphica*.

Identification methods: the study of morphology and ecology, the molecular analysis (16S rRNA gene and 16S–23S ITS secondary structures).

Morphology: filaments solitary or intricated, entangled with other filamentous cyanobacteria, filaments cultured on agar forming thin and compact biofilms, sometimes coiled, occasionally false-branched; sheaths firm, thin and colorless, becoming thick and colored with age, opened at the end, with one trichome per sheath; trichomes isopolar, fine, cylindrical, sometimes tapering at the ends, straight or coiled, constricted at the cross-walls; cells pale blue-green,

isodiametric or longer than wide, without aerotopes, with rare granules; the cell content homogenous; apical cells conical or rounded; reproduction via hormogonia, with the help of necridic cells.

Type species: *Chroakolemma opaca* Becerra-Absalón & Johansen 2018

Kovacikia Miscoe, Pietrasiak & Johansen in Miscoe et al. 2016

Kovacikia was found in Waikapala'e Cave on Kauai (Hawai), associated with moss growing on the cave walls. Only one species has been described within the genus so far.

Identification methods: the study of morphology and ecology, phylogenetic analysis of the 16S rRNA gene.

Morphology: filaments unbranched, often containing one or more coiled trichomes; sheaths colorless, often absent; trichomes constricted at the cross-walls; cells longer than wide, with parietal thylakoids; without necridic cells; terminal cells mostly rounded.

Type species: *Kovacikia muscicola* Miscoe, Pietrasiak & Johansen in Miscoe et al. 2016

Limnolyngbya Li & Li 2016

The type species *Limnolyngbya circumcreta* was initially described as *Lyngbya circumcreta* West 1907 and later as *Planktolyngbya circumcreta* Anagnostidis & Komárek 1988 (Anagnostidis & Komárek 1988). The studied samples of *Limnolyngbya* were collected in lakes in Eastern China, but the genus is also distributed in Northern Europe where it inhabits ponds, lakes and reservoirs.

Identification methods: the study of morphology and ultrastructure (light and transmission electron microscopy), molecular analysis of the 16S rRNA gene and 16S–23S rRNA ITS secondary structures.

Morphology: filaments solitary, freely floating, regularly or irregularly spirally to narrowly screw-like coiled, trichomes pale blue-green or greyish, with no or slight constrictions at the cross-walls, sheaths firm, thin and colorless; cells quadrate or slightly cylindrical with max. width 2.15 μm; thylakoids parietal, with four to eight in parallel; apical cells rounded.

Type species: *Limnolyngbya circumcreta* (West) Li & Li 2016

Marileptolyngbya Zhou & Ling in Zhou et al. 2018

The samples of *Marileptolyngbya* were obtained from the tropical seagrass *Thalassia hemperichii* in Xincun Bay, China. The genus currently contains only one species *Marileptolyngbya sina*.

Identification methods: the study of morphology and ultrastructure (light microscopy, scanning and transmission electron microscopy), ecology and molecular analysis based on 16S rRNA gene and 16S–23S ITS secondary structures.

Morphology: thallus blue-green; filaments straight, curved or entangled, not branched; sheaths thin and colorless; trichomes uniseriate, cylindrical; cells longer than wide, cylindrical, with distinct constrictions at the cross-walls, not attenuated towards the ends; apical cells rounded.

Type species: *Marileptolyngbya sina* Zhou & Ling in Zhou et al. 2018

Myxacorys Pietrasiak et al. 2015 provis. in Komárek et al 2014

"Myxacorys" is a clade of soil species whose description is currently not available in any literature (Mai et al. 2018). The name of this genus is mentioned only in several taxonomic papers (e.g. Komárek et al. 2014, Komárek 2016) as a part of Leptolyngbyaceae family.

Neosynechococcus Dvořák, Hindák, Hašler & Hindáková in Dvořák et al. 2014

The genus was described based on a strain isolated from the peat bog Klin in Slovakia. The habitats of *Neosynechococcus* include hyaline cells of peat moss (*Sphagnum*), cyanobacterial sheaths, dead cells of desmids and dead crustaceans and it also appears alone in detritus. The genus was named after *Synechococcus* because of its similar morphology and was placed among Synechococcacae. According to Mai et al (2018), *Neosynechococcus* belongs to the Leptolyngbyaceae family.

Identification methods: the study of morphology, ecology and ultrastructure, analysis of 16S rRNA genes, 16S–23S ITS and *rbcL* loci.

Morphology: cells solitary or in irregular clusters, occasionally forming pseudofilaments; cells oval or cylindrical with a homogenous content and parietal thylakoids, blue-green colored, reproducing by binary fission.

Type species: *Neosynechococcus sphagnicola* Dvořák, Hindák, Hašler & Hindáková in Dvořák et al. 2014

Onodrimia Jahodářová, Dvořák & Hašler in Jahodářová et al. 2017b

Strains were isolated from the bark of tree branches submersed in a hot-water spring in a rainforest in West Java. Despite its similarities to *Leptolyngbya*, *Onodrimia* differs with the tree-like structures formed by hormogonia and hormocytes during reproduction.

Identification methods: morphological and physiological analysis, the study of ecology, phylogenic analysis of 16S rRNA and predicting 16S–23S ITS secondary structures.

Morphology: filaments in mats or occasionally creeping, straight to bent, sometimes coiled or entangled together, with colorless sheaths, roundly closed at the ends or opened after hormogonia release, exceeding trichome or with trichome protruding from sheath; frequently with false branching; immotile trichomes narrowed towards the ends; cells usually rectangular, isodiametric to longer than wide, with visible parietal chromatoplasma and inner pale centroplasma; end cells rounded or conical, without calyptra; reproduction by hormogonia or hormocytes with the help of necridic cells; both hormogonia and hormocytes form groups of tree-like tufts that attach to other filaments via sheath.

Type species: *Onodrimia javanensis* Jahodářová, Dvořák & Hašler in Jahodářová et al. 2017b

Pantanalinema Vaz et al. 2015

The strains of *Pantanalinema* were isolated from saline-alkaline lakes in the Brazilian Pantanal wetland. The genus contains only one described species, *Pantanalinema rosaneae*.

Identification methods: the study of morphology, 16S rRNA gene, 16S–23S rRNA ITS sequence and secondary structures, growth responses to culture pH.

Morphology: filaments entangled and flexuous, forming olive green colonies; sheaths hyaline, firm, attached to the trichome, always present; trichomes with a slight gliding motility; cells isodiametric or wider than long, 1.2–3.1 μm long and 1.5–3.1 μm wide, slightly constricted at the cross-walls, with a brownish-green or olive green homogenous content; apical cells cylindrical with a rounded to slightly conical apex; the formation of hormogonia by cell disintegration (false necridic cells) or trichome fragmentation.

Type species: *Pantanalinema rosaneae* Vaz et al. 2015

Phormidesmis Turicchia et al. 2009

The type species of this genus, *Phormidesmis molle*, was originally described as *Phormidium molle* Gomont 1892. After molecular analysis had proved that the species is too genetically distant from other *Phormidium* genera, a new genus was established. *Phormidesmis* can be distinguished from other genera by the shape of cells (barrel-shaped to spherical) and the absence of differentiated apical cells. The samples of *Phormidesmis* were collected in the Belizean marshes, but the former *Phormidium molle* is known as a cosmopolitan species, occurring especially in tropical areas.

Identification methods: the study of morphology, molecular evaluation (16S rRNA).

Morphology: filaments grouped in mats or clusters, rarely solitary, straight or slightly coiled, with facultative sheaths; sheaths firm, thin, colorless or slightly yellowish-brownish; trichomes constricted at the cross-walls, not attenuated towards the ends; cells isodiametric or slightly longer or shorter than wide, occasionally with visible chromatoplasma, apical cells rounded, without typical calyptra.

Type species: *Phormidesmis molle* (Gomont) Turicchia et al. 2009

Pinocchia Dvořák, Jahodářová & Hašler in Dvořák et al. 2015a

The strains of *Pinocchia* were isolated from samples collected from plankton and periphyton of the lake Hồ Đâu Co, province Đồng Nai in Vietnam. This monospecific genus could be considered a cryptogenus within the genus *Pseudanabaena* if based solely on morphology. However, the phylogenetic analysis altogether with geographical data support the hypothesis of the independence of *Pinocchia* as a separate genus.

Identification methods: the study of morphology and ecology, molecular analysis (16S rRNA, 16S–23S ITS secondary structures).

Morphology: filaments solitary or in mats; sheaths thin, colorless, facultative; trichomes straight or bent, constricted at the cross-walls, motile, 2 to 34 cells; cells with distinct centro– and chromatoplasma; cell length variable within the filament, cells connected with hyaline bridges; cell content homogenous or with small granules; apical cells often elongated and differentiated; reproduction by hormogonia without the help of necridic cells.

Type species: *Pinocchia polymorpha* Dvořák, Jahodářová & Hašler in Dvořák et al. 2015a

Plectolyngbya Taton et al. 2011

Plectolyngbya was isolated from the coastal lakes of the Larsemann Hills region in Antarctica. The genus also occurs in other Antarctic lakes where it grows under specific conditions, e.g. in areas where the average temperature is below 3 °C during the summer season and where drying and freezing occurs periodically for more than 8 months in a year. The genus combines some features typical for other related genera, e.g. the morphology of trichomes corresponds to *Leptolyngbya*, the type of false branching is the same as in case of *Pseudophormidium* and the occasional multiple arrangement of trichomes in the sheaths is a feature shared with *Schizothrix*.

Identification methods: the study of morphology, ultrastructure and 16S rRNA gene sequences.

Morphology: filaments solitary, in clusters or forming mats, 0.8–4 μm wide; sheaths thin with false branching of both tolypotrichoid and scytonematoid types; trichomes cylindrical, not or slightly attenuated towards the ends, not or slightly constricted at the cross-walls; cells isodiametric or slightly shorter or longer than wide, with thin cross-walls; thylakoids parietal with facultative circular formations; reproduction by fragmentation of trichomes or hormocytes.

Type species: *Plectolyngbya hodgsonii* Taton et al. 2011

Planktolyngbya Anagnostidis & Komárek 1988

Planktolyngbya as a separate genus was first proposed by Anagnostidis & Komárek (1988). Until that time, the *Planktolyngbya* species were known under the names of other genera. The type species basionym *Lyngbya limnetica* Lemmermann 1898 has been renamed many times, e.g. to *Oscillatoria splendida var. limnetica* (Lemmermann) Playfair 1938 or to *Planktolyngbya subtilis* (West) Anagnostidis & Komárek 1988 when describing *Planktolyngbya* as a genus nova. The correct name of the type species is *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg 1992. The genus contains several species inhabiting freshwater planktonic environments (Komárek & Anagnostidis 2005).

Identification methods: the study of morphology and ecology.

Morphology: filaments straight or ± spirally coiled, solitary, free−floating, not attenuated towards the ends; sheaths thin, firm, colorless, rarely with false branching; trichomes immobile; cells cylindric, up to 3 μm wide, with parietally arranged thylakoids; necridic cells absent.

Type species: *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg 1992

Romeria (Raciborski) Koczwara in Geitler 1932

Romeria species are distributed mostly in fresh waters (springs, streams, rivers, ponds, lakes), but e.g. *Romeria cryophila* occupies a specific habitat – the snow fields of the High Tatras (Komárek & Anagnostidis 2005).

Identification methods: the study of morphology and ecology.

Morphology: trichomes solitary or in clusters, without sheaths, but with a colorless mucilaginous envelope, containing one or rarely more trichomes; trichomes mostly short, fine, irregular and fragile, often curved or screw-like coiled, with 1–8 helices, 0.6–3 μm wide, usually constricted at the cross-walls; cells cylindrical to long-cylindrical or barrel-shaped, always longer than wide; apical cells rounded, capable of dividing; thylakoids arranged parietally; reproduction by trichome fragmentation into small hormocytes in solitary cells (Komárek & Anagnostidis 2005).

Type species: *Romeria leopoliensis* (Raciborski) Koczwara in Geitler 1932

Salileptolyngbya Zhou in Zhou et al. 2018

Salileptolyngbya was obtained from the South China Sea, specifically from planktonic organisms that were trawled up from a depth of 200 m. One species was described within the genus.

Identification methods: the study of morphology and ultrastructure (light microscopy, scanning and transmission electron microscopy), ecology; molecular analysis based on 16S rRNA gene and 16S–23S ITS secondary structures.

Morphology: thallus blue-green; filaments straight or curved to floating mats, not branched; sheaths thick, multilayered; cells cylindrical and elongated, with distinct constrictions at the cross-walls; apical cells rounded; reproduction via hormogonia.

Type species: *Salileptolyngbya diazotrophicum* Zhou in Zhou et al. 2018

Scytolyngbya Song & Li in Song et al. 2015

Scytolyngbya was first isolated from wet stones in a freshwater well with lowered light conditions in central China (Xishui county, Hubei Province). The genus can be morphologically distinguished from other leptolyngbyoid genera by repeated false branching and thick sheaths.

Identification methods: the study of morphology and molecular analysis based on 16S rRNA sequences and 16S-23S rRNA ITS secondary structures.

Morphology: thallus pale bluish-green to yellow-brown; filaments bent, entangled, nonmotile, with repeated false branching; branches mostly narrower than the main filaments; sheaths originally thin, colorless, later yellow-brown, widened and firm; trichomes thin, monoseriate, single within filaments, cylindrical along the whole length; cells longer than wide, cylindrical, non-granular, distinctly constricted at the cross-walls, not attenuated towards the ends; apical cells rounded.

Type species: *Scytolyngbya timoleontis* Song & Li in Song et al. 2015

Stenomitos Miscoe & Johansen in Miscoe et al. 2016

The type species *Stenomitos rutilans* was isolated from Waikapala'e Cave in Hawai, associated with moss growing on the cave walls. Two other species were assigned to this genus – *S. frigidus* comb. nov. (basionym *Phormidium frigidum* Fritsch 1912) and *S. tremulus* comb. nov. (basionym *Pseudanabaena tremula* Casamatta et al. 2005). The genus *Stenomitos* is closely related to *Neosynechococcus* Dvořák et al. 2014 and the question of describing *Stenomitos* species as a part of *Neosynechococcus* arose. However, these two genera are morphologically two completely different microorganisms, so merging them to the same genus would be highly disputable.

Identification methods: morphology and ecology, phylogenetic analysis of the 16S rRNA gene.

Morphology: filaments without false branching, less than 2.5 μm wide, with thin sheaths; trichomes short, untapered, without necridia; cells longer than wide, with parietally arranged thylakoids; end cells cylindrical, rounded, similar to intercalary cells.

Type species: *Stenomitos rutilans* Miscoe & Johansen in Miscoe et al. 2016

Tapinothrix Savageau 1892

Tapinothrix is a taxonomically complicated genus. At the beginning of the second half of the 20th century, it was transferred to *Homoeothrix* (Komárek & Anagnostidis 2005), but *Tapinothrix*, together with several other genera, as a part of *Homoeothrix* made the whole genus polyphyletic (Johansen et al. 2011). To improve the taxonomic situation of both genera, Bohunická & Johansen removed many *Homoeothrix* species to the *Tapinothrix* genus (Bohunická et al. 2011). *Tapinothrix* mostly occurs in fresh and marine waters, but it has also

been discovered on terrestrial substrates, e.g. moistened rocks (Komárek & Anagnostidis 2005) or sandstone seep walls (Bohunická et al. 2011).

Identification methods: the study of morphology and ecology.

Morphology: filaments simple, not or rarely laterally branched, heteropolar, erect, solitary or in small fascicles, attached by one, basal end to the substrate, occasionally radially-oriented with bases in the center of the colony; sheaths thin or rarely slightly widened, firm, hyaline, yellowish colored; trichomes cylindrical, thin, straight or coiled, mostly to 3 μm wide, constricted or not constricted at the cross-walls, narrowed to the ends, sometimes elongated in thin, hyaline hair with elongated cells; reproduction by hormogonia which are released from the upper part of trichomes after the separation of the terminal hair (Komárek & Anagnostidis 2005).

Type species: *Tapinothrix bornetii* Sauvageau 1892

4.4.2 Oculatellaceae

Cartusia Mai, Johansen & Pietrasiak in Mai et al. 2018

Cartusia is a subaerophytic species found in the ruins of the Cartusian Monastery in the Slovak Paradise National Park. The type species *Cartusia fontana* was originally described as *Lyngbya fontana* Hansgirg 1892 and later as *Leptolyngbya fontana* (Hansgirg) Komárek in Anagnostidis 2001. Samples of former *Leptolyngbya fontana* were previously collected in the mountainous regions of the Czech Republic. As the samples from Slovak Paradise and Czech mountains matched morphologically and ecologically, they were merged into one species. Genus *Cartusia* is also morphologically similar to *Drouetiella fasciculata* and some *Pegethrix* species. Based on molecular analysis, there is a high similarity in 16S rRNA gene sequences between *Cartusia fontana* and *"Marsacia ferruginosa"* nom. nud. (97.2 %) and *Elainella saxicola* (97 %). However, *"M. ferruginosa"* differs in ITS sequence and *E. saxicola* in morphology (with false branching and the presence of a single trichome per sheath).

Identification methods: the study of morphology and ecology, the molecular analysis of 16S rRNA gene sequences and 16S–23S ITS secondary structures.

Morphology: filaments straight or flexuous, at times with more than one trichome in a common sheath, sometimes forming fascicles of trichomes, without false branching; sheaths firm, thin, colorless; trichomes not tapering, not or only slightly constricted at the cross-walls, up to 3.5 μm wide; cells mostly shorter than wide or isodiametric.

Type species: *Cartusia fontana* (Hansgirg) Mai, Johansen & Pietrasiak in Mai et al. 2018

Drouetiella Mai, Johansen & Pietrasiak in Mai et al. 2018

The type species *Drouetiella lurida* was initially described as *Phormidium luridum* Gomont 1892 and later as *Leptolyngbya lurida* Anagnostidis & Komárek 1988. According to Anagnostidis & Komárek (2005), it is a freshwater species with a worldwide distribution. Three other species have been described within this genus – *D. hepatica* Mai, Johansen & Bohunická 2018, *D. fasciculata* Mai, Johansen & Pietrasiak 2018 and *Drouetiella* sp. ANT.LH52.2. *D. hepatica* was found in a subaerial limestone in Sucha Bela gorge in the Slovak Paradise National Park, *D. fasciculata* on a large seep wall and waterfall in Navajo Sandstone in Utah and *Drouetiella* sp. ANT.LH52.2. was isolated from Antarctic environment and currently lacks morphological description.

Identification methods: the study of morphology, molecular analysis of 16S rRNA and 16S–23S ITS secondary structures.

Morphology: filaments mostly solitary, sometimes consolidated into fascicles, with infrequent single false branching; sheaths clear, thin, firm, occasionally widened; trichomes untapered, straight, flexuous or spirally coiled, but not in nodules, slightly constricted at the cross-walls; cells mostly longer than wide, becoming ± isodiametric in dividing trichomes, rarely with a central granule in the cytoplasm; thylakoids arranged parietally; apical cells cylindrical, untapered, rounded, without calyptra; reproduction via trichome fragmentation, necridia absent.

Type species: *Drouetiella lurida* (Gomont) Mai, Johansen & Pietrasiak in Mai et al. 2018

Elainella Jahodářová, Dvořák & Hašler in Jahodářová et al. 2017a

Elainella is a cyanobacterial genus morphologically similar to *Plectonema* or *Pseudophormidium*. The strains of *Elainella* were isolated from ephemeral waterbodies in the forest, Cat Tien National Park, province Đồng Nai, Vietnam and from granite and sand from Pongour waterfall 875 m above sea level, province Lâm Đồng, Vietnam.

Identification methods: the study of morphology and ecology, genomic sequencing (focusing especially on differences in the average nucleotide identity, 16S rRNA genes, genome size and composition).

Morphology: colonies macroscopic, dark green, in fascicles or tufts; filaments yellow-green, green, grey-green, straight, curved, undulate, often with loops; sheaths colorless, thin and distinct, variable in length; sheaths exceeding trichomes or trichomes protruding from sheaths; trichomes cylindrical, not attenuated towards the ends, not or slightly constricted at the cross-walls, immotile; false branching present, usually both of chiasmatic type formed after breakage of trichome loop formation, and Y type formed during the simultaneous growth of hormogonia; cells isodiametric or longer than wide, 1.7–2.6 μm wide and 1.3–3.8 μm long; with distinguishable peripheral chromatoplasma and central pale nucleoplasma, often with granules, without aerotopes; apical cells rounded, without calyptra; reproduction by necridic cells, via trichome breakage and subsequent disintegration, releasing hormogonia.

Type species: *Elainella saxicola* Jahodářová, Dvořák & Hašler in Jahodářová et al. 2017a

Kaiparowitsia Mai, Johansen & Bohunická in Mai et al. 2018

Kaiparowitsia is a newly discovered genus containing one species, *Kaiparowitsia implicata*. The strains were isolated from a small horizontal seep wall in sandstone of the Kaiparowits Plateau formation in the Grand Staircase-Escalante National Monument in Kane County, Utah, USA. *Kaiparowitsia* is morphologically similar to *Tildeniella nuda,* but it differs in forming *Arthronema*-like outgrowths.

Identification methods: the study of morphology and molecular analysis (16S rRNA genes and 16S–23S rRNA ITS secondary structures).

Morphology: filaments flexuous, entangled, sometimes fasciculated, with one to several trichomes in a common sheath, unbranched; sheaths thin, colorless; trichomes bent, flexuous, entangled, occasionally forming nodules, less than 2 μm wide; cells cylindrical, longer than wide, sometimes with outgrowths; apical cells rounded; hormogonia and necridia absent.

Type species: *Kaiparowitsia implicata* Mai, Johansen & Bohunická in Mai et al. 2018

Komarkovaea Mai, Johansen & Pietrasiak in Mai et al. 2018

Komarkovaea is currently a monospecific genus found in a waterfall in El Yunque National Forest in Puerto Rico. Its morphology is comparable to some *Leptolyngbya* species, but its trichomes are considerably wider.

Identification methods: the study of morphology and molecular analysis (16S rRNA gene and 16S–23S rRNA ITS sequencing).

Morphology: filaments simple, without branching, with differences in width between post-hormogonial and mature filaments; sheaths firm, thin and colorless; trichomes constricted at the cross-walls, rarely tapering; cells isodiametric, shorter or longer than wide, with thylakoids arranged parietally.

Type species: *Komarkovaea angustata* Mai, Johansen & Pietrasiak in Mai et al. 2018

Oculatella Zammit et al. 2012

Oculatella is the type genus of the Oculatellaceae family. The genus was first isolated from calcareous surfaces of hypogea and catacombs in Rome (Italy) and Malta where it grew under specific conditions, such as high humidity or reduced light availability. *Oculatella* is easily distinguishable from other genera by the presence of a colored spot at the tip of apical cells and

the shape of cells which are distinctly longer than wide (Zamit et al. 2012). Several other species have been described from various habitats, e.g. arid or semi-arid soils, a desert waterfall, a temperate lake and a Hawaiian sea cave (Osorio-Santos et al. 2014).

Identification methods: the study of morphology (light and electron microscopy) and ecology, pigment analysis (confocal laser scanning microscopy and spectral analysis – CLSM-SA), molecular analysis (16S rRNA gene and 16–23S ITS sequencing and secondary structure predicting).

Morphology: filaments forming reddish, sometimes greenish compact subaerophytic biofilms on calcareous substrates; filaments fine, coiled in clusters, wavy, mostly without false branching; sheaths colorless, thin, firm, usually attached to a trichome or slightly distant, occasionally open at the ends; trichomes fine, 1–3 μm wide, cylindrical, slightly constricted at the cross-walls, not attenuated towards the ends; cells cylindrical, longer than wide, with a homogenous content, rarely with granules; apical cells conical-rounded, with an orange spot at the tip; cells dividing by symmetrical crosswise binary fission; reproduction by hormogonia produced by fragmentation of trichomes, mostly without the formation of necridic cells.

Type species: *Oculatella subterranea* Zammit et al. 2012

Pegethrix Mai, Johansen & Bohunická in Mai et al. 2018

Five species of *Pegethrix* were identified when describing the new genus. *P. bostrychoides* and *P. olivacea* were isolated from a sandstone seep wall in Strait Cliffs Formation, Utah, USA. Similarly, the samples *P. convoluta* and *P. indistincta* came from a large seep wall and waterfall in Navajo Sandstone in Utah, USA. *Pegethrix* sp. ANT.LH70.1 and ANT.LMA.1 were collected in a water body of Larsemann Hills in Antarctica.

Identification methods: the study of morphology and ecology, the molecular analysis (16S rRNA gene and 16S–23S rRNA ITS).

Morphology: filaments mostly solitary, occasionally with more trichomes within one sheath, or with loose nodule formation, with infrequent single or double false branching; sheaths clear, thin and firm to soft and widened, but never diffluent; trichomes straight, flexuous, or entangled within a sheath into a loose nodule, sometimes spirally coiled, slightly constricted at the cross-walls; slow gliding motility in trichomes without sheaths, not tapered; cells mostly shorter than wide, sometimes with granules in cytoplasm, thylakoids arranged parietally; apical cells

rounded, without calyptra; involution cells with axillary bud-like structures rare; reproduction by trichome fragmentation via disintegration at necridia, sometimes necridia absent.

Type species: *Pegethrix bostrychoides* Mai, Johansen & Bohunická in Mai et al. 2018

Thermoleptolyngbya Sciuto & Moro 2016

Thermoleptolyngbya is a cyanobacterial genus worldwide distributed occupying thermal (and often also alkaline) environments. The genus mostly corresponds to the previously described group VIII of *Protolyngbya* (Komárek & Anagnostidis 2005), specifically to the second subgroup including species with trichomes narrower than 2 μm. Two species were described within the genus by Sciuto & Moro (2016), *T. albertanoae* and *T. oregonensis*. More species are expected to be recognized.

Identification methods: the study of morphology, ultrastructure and ecology, molecular and phylogenetic analysis of 16S rRNA gene and 16S–23S ITS region.

Morphology: filaments in blue-green mats on substrates and large floating membranaceous aggregates in liquid cultures; trichomes flexuous, isopolar, unbranched; sheaths thin, colorless, surrounding only one trichome; cells longer than wide, usually 1.2–6.5 μm long and 0.8-2 μm wide; thylakoids arranged parietally; reproduction by trichome fragmentation into short hormogonia, necridic cells absent; rich in allophycocyanin and C-phycocyanin.

Type species: *Thermoleptolyngbya albertanoae* Sciuto & Moro 2016

Tildeniella Mai, Johansen & Pietrasiak in Mai et al. 2018

The genus currently contains two species – *T. torsiva* and *T. nuda*. *T. torsiva* was isolated from the Prielom Hornadu gorge in the Slovak Paradise National Park, but findings come also from Germany. *T. nuda* was discovered on a wet stone wall in Stansstaad, Switzerland.

Identification methods: the study of morphology, molecular and phylogenetic analysis (16S rRNA genes and 16S–23S rRNA ITS secondary structures).

Morphology: filaments straight, flexuous or spirally coiled, with or without sheath; sheaths thin, firm, colorless if present; trichomes untapered, not or slightly constricted at the sometimes almost invisible cross-walls, less than 3 μm wide; cells longer than wide, apical cells rounded; without necridia and hormogonia.

Type species: *Tildeniella torsiva* Mai, Johansen & Pietrasiak in Mai et al. 2018

Timaviella Sciuto & Moro in Sciuto et al. 2017

The strains of *Timaviella* were isolated and described as *T. circinata* and *T. karstica* from the Giant Cave in the Italian Alps where they grew under typical cave conditions, characterized by high humidity and constant temperature (11°C). The light was available through artificial lighting, as the cave was accessible to tourists. Several other species were described, e.g. *T. obliquedivisa*, *T. radians* and *Timaviella* sp. WMT-WP7-NPA (Mai et al. 2018). Furthermore, *T. edaphica* comb. nov. (syn. *Plectonema edaphicum* or *Leptolyngbya edaphica*) was assigned to this genus by Vinogradova & Mikhaulyuk (2018).

Identification methods: the study of morphology, ultrastructure and ecology; phycobiliprotein analysis; the phylogenetic reconstruction based on the 16S rRNA gene and 16S–23S ITS region; the prediction of 16S–23S ITS secondary structure.

Morphology: filaments long, flexuous and curved, red-brown; trichomes often false-branched, slightly constricted at the cross-walls, sheathed; cells isodiametric or longer than wide, 1.1–4.4 μm long, 1.2–2.1 μm wide; thylakoids arranged parietally; apical cells rounded or tapered; reproduction by fragmentation of trichomes into short hormogonia, necridic cells absent.

Type species: *Timaviella circinata* Sciuto & Moro in Sciuto et al. 2017

Trichotorquatus Petrasiak & Johansen 2015 provis. in Komárek et al. 2014

"Trichotorquatus" is a species lacking any proper description (Mai et al. 2018). The name of this genus is mentioned only by Komárek et al. (2014) as a part of the Leptolyngbyaceae family and in Mai et al. (2018) as a part of the Oculatellaceae family.

4.4.3 Prochlorotrichaceae

Haloleptolyngbya Dadheech et al. 2012

Haloleptolyngbya alcalis was discovered in the saline-alkaline Lake Nakuru in Kenya where it serves as an alternative food source for Lesser Flamingos at periods when the populations of the main primary producer, *Arthrospira fusiformis*, fluctuate and the amounts of their biomass are reduced (Dadheech et al. 2012). Another species, *Haloleptolyngbya elongata*, was isolated from the saline-alkaline crater lake Dziani Dzaha (dominated also by *Arthrospira fusiformis)* on Mayotte Island in Indian Ocean (Cellamare et al. 2018).

Identification methods: the study of morphology and ultrastructure (light microscopy, scanning electron microscopy and transmission electron microscopy), the study of ecology, the molecular analysis of 16S rRNA gene, 16S–23S ITS (incl. determining secondary structures) and beta and alpha subunits including the intergenic spacer (*cpc*BA–IGS) of phycocyanin operon.

Morphology: thallus thin, pale to bright-blue; filaments solitary or forming dense floating mats, tychoplanktonic or attached to the substrate; filaments long, straight or wavy, 1.2–1.9 μm wide; trichomes constricted at the cross-walls; sheaths firm, colorless; cells cylindrical, elongated or isodiametric, 1.2–2.1 μm long, 1.2–1.9 μm wide, without aerotopes; the cell content heterogenous with pale centroplasma and dense chromatoplasma; apical cells rounded without calyptra; reproduction by fragmentation of trichomes.

Type species: *Haloleptolyngbya alcalis* Dadheech et al. 2012

Halomicronema Abed et al. 2002

The strains of the type species *Halomicronema excentricum* were isolated from hypersaline artificial ponds in Eilat, Israel. Except of this species, two other species have been described. *H. metazoicum* was discovered living in association with a marine sponge *Petrosia ficiformis* (Caroppo et al. 2012) and occurring on leaves of *Posidonia oceanica* in Mediterranean Sea (Ruocco et al. 2018). *H. hongdechloris* was isolated from stromatolites in Shark Bay in Western Australia (Chen et al. 2012).

Identification methods: the study of morphology and ultrastructure (light and transmission electron microscopy), physiology and ecology, molecular analysis based on 16S rRNA gene sequencing, the study of chemotaxonomic markers – carotenoids and mycosporine-like amino acids.

Morphology: filaments very thin, possessing slow gliding motility, with thin, colorless, diffluent sheaths; trichomes narrow, ± 1 μm wide, without constrictions at the cross-walls; cells longer than wide, with the length of 2–8 μm, with thylakoids often asymmetrically distributed; apical cells rounded; gas vesicles present.

Type species: *Halomicronema excentricum* Abed et al. 2002

Nodosilinea Perkerson & Casamatta in Perkerson et al. 2011

Nodosilinea is a cyanobacterial genus differing from other leptolyngbyoid species by forming nodules under conditions with reduced light availability. The type species *N. nodulosa* was originally identified as *Leptolyngbya nodulosa* and was first isolated from the South China Sea (Li & Brand 2007). Several other species were described within the genus – *N. epilithica* Perkerson & Casamatta 2011, *N. bijugata* (Kong.) Perkerson & Kováčik 2011 and *N. conica* Perkerson & Johansen 2011 (Perkerson et al. 2011). Two other species, *N. radiophila* Heidari & Hauer 2018 and *N. ramsarensis* Heidari & Hauer 2018 were described within the genus (Heidari et al. 2018). *Nodosilinea* species occupy both water and terrestrial biotopes – e.g. *N. nodulosa* can be found in marine habitats, *N. epilithica* was isolated from a house wall and *N. conica* is a desert species (Perkerson et al. 2011). *N. radiophila* was isolated from benthic mat in a thermal spring and *N. ramsarensis* from a soil near the thermal spring in Iran (Heidari et al. 2018).

Identification methods: the study of morphology and ultrastructure (light and transmission electron microscopy), molecular analysis (16S rRNA gene sequencing and the determination of 16S–23S ITS secondary structures).

Morphology: filaments usually consisting from a single trichome, but occasionally becoming multiseriate, with nodules formed under reduced light conditions, with sheaths mostly present; sheaths thin, soft and colorless; trichomes immotile, slightly to distinctly constricted at the cross-walls; cells ± isodiametric or longer than wide, without aerotopes, with thylakoids arranged peripherally.

Type species: *Nodosilinea nodulosa* (Li & Brand) Perkerson & Casamatta in Perkerson et al. 2011

Prochlorothrix Burger-Wiesma et al. 1989

Samples of *Prochlorothrix* were obtained from a mixed water column of a shallow, highly eutrophic lake (Lake Loosdrecht, Netherlands).

Identification methods: the study of morphology and ultrastructure (light and transmission electron microscopy), the analysis of the G+C content of the DNA.

Morphology: trichomes without well-defined sheaths, nonmotile, of a variable length, with constrictions at the cross-walls; cells cylindrical, dividing by binary fission in a single plane; without differentiated apical cells; reproduction by trichome fragmentation.

Type species: *Prochlorothrix hollandica* Burger-Wiesma et al. 1989

4.4.4 Trichocoleaceae

Trichocoleus Anagnostidis 2001

Trichocoleus is a filamentous cyanobacterial genus distinguishable from other leptolyngbyoid genera mainly by the presence of multiple trichomes in a common sheath (Mühlsteinová et al. 2014). The type species *T. delicatulus* was originally identified as *Microcoleus delicatulus* West & West 1896. Similarly, most of the species described within this genus were formerly assigned as *Microcoleus* species as well (Anagnostidis 2001). *Trichocoleus* species occupy a diverse scale of environments, e.g. fresh and marine waters, swamps, thermal springs, rocks (Komárek & Anagnostidis 2005), wet rock seeps (Johansen et al. 2008), soils (Komárek & Anagnostidis 2005, Dulić et al. 2017), including desert soils (Mühlsteinová et al. 2014) etc.

Identification methods: the study of morphology, ecology and molecular features (not specified).

Morphology: filaments solitary or rarely densely aggregated forming mats, containing a few or numerous trichomes; sheaths ± cylindric or rarely attenuated towards the ends, diffluent, colorless; trichomes 0.5–3 μm wide; cells cylindric, always longer than wide, with ± homogenous content; apical cells conic, apiculate or rounded, without calyptra.

Type species: *Trichocoleus delicatulus* (West & West) Anagnostidis 2001

5. METHODS

5.1 The sampling

5.1.1 The sampling strategy

Samples were collected in the period from October 2017 to November 2018, mostly in Bohemia (the region of Pardubice and Vysočina) and Moravia (the region of Olomouc and Zlín). One sample originates from Slovakia. The habitats were in all cases terrestrial, although some of them were located near stagnant or flowing water. Both natural and urban environments were used for sampling. Soil species were isolated mostly from the soil of fields, meadows, forest pathways, parks, gardens and greenhouses, further from dry puddles and shores of rivers and ponds. Several samples were collected from stony substrates, e.g. from stones on the shores of rivers and ponds, from rocks and walls. The presence of a putative cyanobacterial population was detected visually, as they formed typical blue-green or green mats on substrates they inhabited. The samples were usually collected in periods after rain, mostly in spring and fall, but also in summer. No samples were collected in the period from December to March.

Samples were scraped from substrate with a scalpel, placed into small plastic bags or plastic test tubes and transported to the phycological laboratory at the Palacký University, Department of Botany, where the species richness was studied, predominantly with the use of light microscopy.

A total of 61 samples of filamentous cyanobacteria were collected. Samples transported to the laboratory were checked with the use of light microscope. The samples containing *Leptolyngbya* or filamentous cyanobacteria similar to *Leptolyngbya* were then cultivated in liquid Z medium and on agar plates.

5.1.2 Description of sampling sites

5.1.2.1 Nezdín

Samples were collected from soil on the edge of a dirt road and from soil near a forest spring called Nezdínská studánka.

5.1.2.2 Lisovská skála

Samples were scraped from a pathway in a forest near a place called Lisovská skála.

5.1.2.3 Svitavy

Samples collected in Svitavy come both from stony substrates and soil. Stony substrates included stones on the shore of the Svitava river, the shore of the Rosnička pond, the shore of a pond in the Park of Jan Palach and a smaller rocky formation in a forest. One sample was taken from soil, specifically from the wet edge of puddles on a pathway near a sandstone quarry behind the town.

5.1.2.4 Ústí and Orlicí

The sample was taken from the edge of a pathway in a park called Wolkerovo údolí.

5.1.2.5 Olomouc

Samples from Olomouc were taken from several habitats. Two samples originate from a park called Bezručovy sady – from a huge rock and from the shore of a stream called Mlýnský potok. One sample was collected from soil next to a puddle near the building of Moravská vysoká škola Olomouc (Moravian Business College Olomouc). Another one was scraped from a dry puddle next to the Kaufland supermarket.

5.1.2.6 Grygov

Samples were scraped from the edge of a pathway near a forest called Les království, further from the edges of a puddle in front of the forest and also from a pathway in the forest.

5.1.2.7 Poděbrady and Horka and Moravou

The sample from Poděbrady was collected from wet soil on a pathway on a meadow, whereas the sample from Horka nad Moravou was collected from wet soil on a shore of a pond.

5.1.2.8 Ploština and Vysoké Pole

Samples from Ploština were collected from a bare soil on a meadow and from forest soil near a stream. Samples from Vysoké Pole come from a dry puddle on a meadow, from soil on a mowed meadow, from forest soil near a stream and from soil in a greenhouse. These samples were collected by Adéla Smolíková.

5.1.2.9 Sivá Brada (SK)

The sample was obtained from the edges of a mineral spring.

Sampling sites are displayed in Fig. 1.

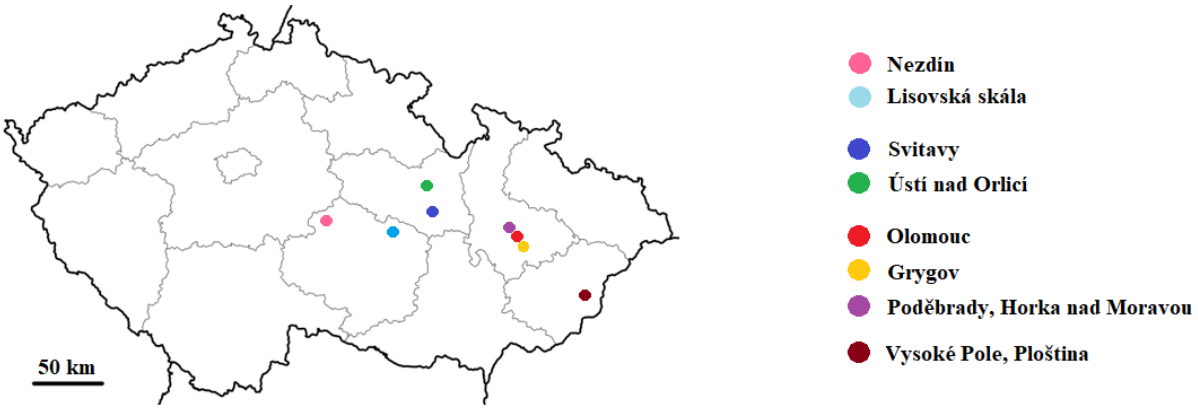

Fig. 1. The map of the Czech Republic with labeled sampling sites (template used from https://wiki.rvp.cz/Kabinet/Mapy/Mapa_%C4%8CR).

5.2 The cultivation

5.2.1 The preparation of Z medium and agar

To prepare 1 liter of Z medium, a large conical glass flask was used. The flask was filled with 0.5 liter of deionized water and then other components were added (for stock solutions and doses see Table 1). The remaining volume was refilled with distilled water. The mixed liquid was equally divided into 0.5 glass bottles which were sterilized (150 kPa, 121 °C, 30 min) and then stored in a laboratory refrigerator (4 °C). Agar plates were prepared from 1.5% agar using 90 mm Petri dishes.

Table 1: List of macro- and microelements used for the preparation of Z medium stock solution and added volumes.

Component	Stock	Volume used
$NaNO_3$	9.34 g/200 ml	10 ml
$Ca(NO_3)_2.4H_2O$	1.18 g/200 ml	10 ml
K_2HPO_4	0.62 g/200 ml	10 ml
$MgSO_4.7H_2O$	0.50 g/200 ml	10 ml
Na_2CO_3	0.42 g/200 ml	10 ml
Fe/EDTA solution	0.138 g $FeCl_3.6H_2O$ in 5ml 0.1M HCl 0.186 g EDTA-Na_2 in 5ml 0.1M HCl	10 ml
Gaffron's solution		80 µl

5.2.2. The preparation of cyanobacterial cultures

All treatment with samples was done in a flowbox (horizontal box AURA HZ 48) to prevent them from being contaminated. The samples collected in their natural habitats were transferred to Petri dishes containing Z medium, using sterilized (i.e. those which were previously held in a flame) inoculating loops. Samples in Petri dishes were cultivated at room temperature.

5.2.3 Obtaining pure cultures

After a few weeks of cultivation, the samples were transferred to Z medium in plastic test tubes or to agar plates. Samples in plastic test tubes were placed in a cultivation room (22 °C, 16/8 h light/dark regime), while samples on agar plates were transferred to a cultivation box to prevent the agar from fast desiccation (22 °C, 16/8 h light/dark regime). The grown cyanobacterial biomass was then repeatedly checked for contaminations of other species and purified with the aim of obtaining monospecific strains.

To isolate pure strains, several special methods were used. For cultures with bacterial contamination, an antibiotic mixture was applied. The composition of the mixture sensu Andersen (2005) is given in Table 2. For 10 ml of sample, the amount 0,1 ml of antibiotic mixture was applied for 36 hours.

Table 2: The composition of antibiotic mixture applied on cyanobacterial cultures for 36 hours.

Component	Concentration
Penicilin G	100 mg/10 ml
Gentamycin	25 mg/10 ml
Streptomycin	25 mg/10 ml

The second method used for obtaining pure strains was transferring single filaments to drops of sterile water with the use of glass micropipettes and light microscope. The making of micropipettes with an extremely small diameter consisted of two basic steps: first of holding the central part of a glass Pasteur pipette in a flame and second of pulling the ends of the glass fiber in opposite directions. Achieving the preferred diameter and length of a micropipette was regulated by the speed of pulling the ends of the glass fiber and then by pinching the fiber with tweezers to remove redundant parts of the micropipettes. Micropipettes prepared this way were used for transferring single filaments on a microscope slide from one drop to another. The correct transfer was continuously checked through a microscope (magnification 100×).

After several transfers, the filament was placed in a plastic test tube with Z medium which was then cultivated in a cultivation box under the same conditions as described above.

5.3 Determination and documentation

During the preparation of pure strains, the filaments were continuously studied and documented. A light microscope (Zeiss Primo Star, objective 40× and immerse objective 100×) connected with camera (AxioCam ERc5s, 5 MPx) was used. Photographs were captured and edited in program AxioVision Rel. 4.8.1. Further editing of photographs was done in Zoner Photo Studio 14. Determination key by Komárek and Anagnostidis (2005) was used for species determination.

5.4 MALDI-TOF analysis

The technique used is called Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The advantage of this method is low cost, rapidness, simplicity and high sensitivity (Singhal et al. 2015). It works as follows: the analyzed sample and matrix (usually a weak acid) co-crystalize on a plate which is placed in a mass spectrometer (Lay 2000, Graham et al. 2007). These two substances are then exposed to a laser beam which results in their evaporation (Graham et al. 2007). Here, the molecules of the matrix transfer the laser energy to molecules of the analyzed sample during the process of ionization and prevent the proteins from being disintegrated. These ionized molecules of analyte in a gas phase are then detected by a time-of-flight mass spectrometer where ions with a smaller mass have higher velocity than larger ones when moving through the evacuated tube in the spectrometer (Graham et al. 2007). Ions with different mass-to-charge ratios (m/z) are separated from each other and the time needed for getting to the end of the tube varies (Singhal et al. 2015). Different mass-to-charge ratios form spectrums (peptide mass fingerprint – PMF) that are characteristic for different taxa and can be utilized for the identification of samples studied (Lay 2000, Singhal et al. 2015, Schubert & Kostrzewa 2017).

Seven leptolyngbyoid strains were analyzed using MALDI-TOF MS analysis. The samples were obtained from a rocky formation in a forest in Svitavy (Bc6), wet soil on the shore of a pond in Horka and Moravou (Bc8), a mowed meadow in Vysoké Pole (Bc9), soil near a sandstone quarry in Svitavy (Bc10), the edge of a dirt road in Nezdín (Bc12), wet soil next to a stream in a park in Olomouc (Bc13) and from soil in a greenhouse in Vysoké Pole (Bc15).

Small amounts of cyanobacterial filaments with 6.5 µl of matrix were put into sterile Eppendorf tubes. The matrix consisted of sinapic acid (SA), ferulic acid (FA) and trifluoro acetic acid (TFA) as a solvent. With a micropipette, 1 µl of the mixture was inserted into a pit in a plate where it dried and crystallized. Proteins of analyte were then separated and detected in a spectrometer (Microflex LRF, program Biotyper, Brucker Daltonics Inc.).

The spectral data were processed using language R with the package MALDIquant (Gibb & Strimmer 2012).

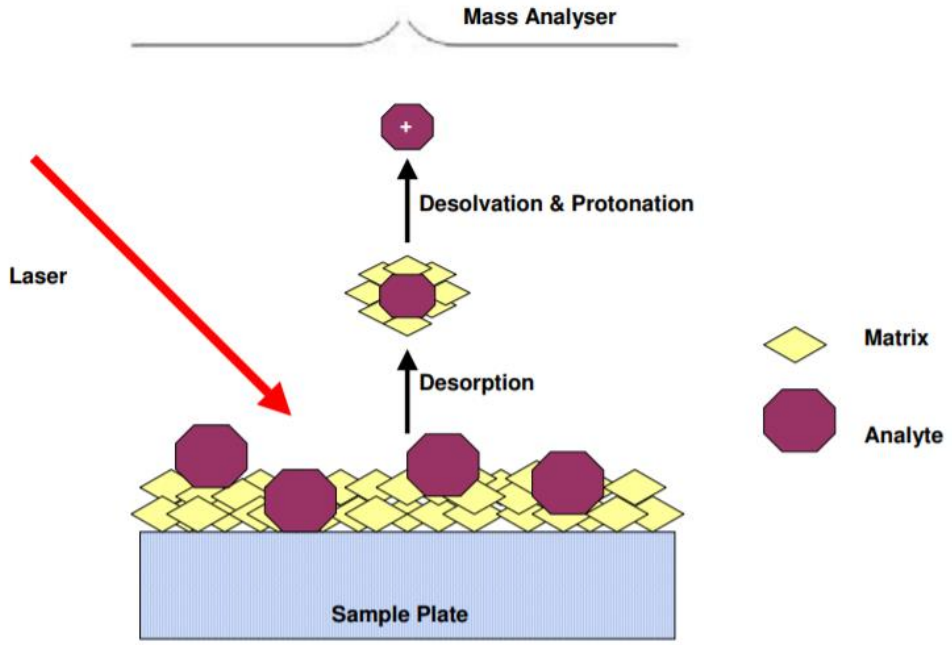

Fig. 2 The scheme of the process of MALDI-TOF (taken from Graham et al. 2007).

6. RESULTS

From 61 collected samples of filamentous cyanobacteria, 24 samples contained the genus *Leptolyngbya*, two samples *Nodosilinea*, two samples *Oculatella* and one sample cf. *Stenomitos*. The species composition of samples after the transfer to the laboratory was very heterogenous. Apart from leptolyngbyoid species, the presence of other cyanobacteria and algae was frequent. Common cyanobacterial genera found together with leptolyngbyoid species were e.g. *Phormidium* or *Geitlerinema*. Algae were mostly represented by diatoms and other coccoid species, but filamentous species (e.g. *Cladophora glomerata*) were also often observed. Soil samples sometimes contained organisms from the phylum Nematoda. The effort to remove all non-leptolyngbyoid species from the cultures during the cultivation resulted in obtaining seven pure strains which were used for the MALDI-TOF protein/peptide analysis.

6.1 Morphological analysis

6.1.1 *Leptolyngbya*

Twenty-four monospecific cultures of *Leptolyngbya* were obtained from natural samples. Seventeen of them contained *Leptolyngbya* sensu stricto, seven of them belonged to the subgenus *Protolyngbya*.

Based on the habitat where the samples were collected, 14 isolates from 24 were identified as members of the group IV (soil species with isodiametric cell proportions). Ten of them were determined as *Leptolyngbya foveolarum* (Fig. 3a–h). Filaments were straight or curved, sometimes arranged parallelly, with filaments width ranging from 1.0 to 1.6 μm. The sheath was thin, usually hardly visible. Trichomes were constricted at the cross-walls and not attenuated towards the ends. The cells were isodiametric or slightly shorter or longer than wide, with homogenous content. Apical cells were rounded, occasionally slightly elongated. Reproduction via hormogonia was observed in the majority of cases. Necridic cells were usually absent. The rest of isolates differed from *L. foveolarum* in morphological features, e.g. with cells distinctly shorter than wide (Fig. 3i), with trichomes extremely constricted at the cross-walls (Fig. 3j) or conversely without any constrictions at the cross-walls. These four isolates were not determined at the species level.

One species was assigned to group II, as it was found forming large mats on wet edges of a mineral spring (Fig. 3l). The filaments were straight or slightly curved, ± 1.9 μm wide, with constrictions at the cross-walls. It was not possible to determine the species.

Two strains with isodiametric cells were isolated from a rocky substrate and therefore were assigned to group V. Both of them were identified as *Leptolyngbya compacta* (Fig. 3m). Filaments were ± 1.5 μm wide. Trichomes were constricted at the cross-walls. The reproduction by hormogonia was without the help of necridic cells.

The subgenus *Protolyngbya* was found in seven cultures. Six of them were assigned to group X (soil species) and one to group XI (subaerophytic species). Three isolates of group X were identified as *Leptolyngbya voronichiana* (Fig. 3n, 3o). The filaments of these strains were 1.0–1.1 μm wide, with trichomes slightly, and usually almost indistinctly constricted at the cross-walls. Cells were cylindrical, 1.4–2.4 μm long. Hormogonia nor necridic cells were observed. The remaining isolates from group X were determined only as *Leptolyngbya* sp. Single strain of the group XI had filaments of an average width of 2.2 μm. Trichomes possessed distinct sheaths and were not constricted at the cross-walls. Cells were ± 1.5× longer than wide. Necridic cells were observed.

6.1.2 *Nodosilinea*

Nodosilinea was found in two cultures (Fig. 4). The genus was identified based on nodules formed within filaments and on visible necridic cells. Apart from these two features, the filaments shared identical morphology to *Leptolyngbya* sp. (the width of filaments, cell proportions, constrictions at the cross-walls etc.).

6.1.3 *Stenomitos*

Stenomitos was observed in one sample (Fig. 5a, 5b). The filaments of this genus were colored green-purple, with distinct sheaths and occasional pseudobranching. The width of filaments was ± 2 μm. Trichomes were constricted at the cross-walls, cells were isodiametric, slightly shorter or longer than wide. Necridic cells were present in this strain.

6.1.4 *Oculatella*

Oculatella was found in two samples (Fig. 5c, 5d). The genus was identified based on dark granules in the apical cells. The width of filaments ranged from 1.1 to 1.6 μm. The sheath was very thin, hardly visible. Constrictions at the cross-walls were slight. Cells were cylindrical, longer than wide, with homogenous content. The reproduction was via hormogonia, without the help of necridic cells.

A summary of the whole morphological analysis is given in Tables 3 and 4.

Table 3: List of species of *Leptolyngbya* with the characteristics of their morphology and occurrence.

Group	Species	Constrictions	Width of filaments [μm]	Sheath	Necridic cells	Hormogonia	Cell proportions	Habitat	Locality	Date of collection
II	*Leptolyngbya* sp.	+	1.9	+	+	+	± isodiametric or slightly s/w*	wet soil near a mineral stream	Sivá Brada	16.05.2018
IV	*L. foveolarum*	+	1.5	+	–	+	isodiametric or slightly s/w	edge of a path in front of a forest	Grygov	14.10.2018
IV	*L. foveolarum*	+	1.2	+	–	+	isodiametric or slightly l/w**	pathway in a forest	Grygov	14.10.2018
IV	*L. foveolarum*	+	1.5	+	+	+	isodiametric	dry puddle next to a supermarket	Olomouc	23.09.2018
IV	*L. foveolarum*	+	1.5	+	+	–	isodiametric or s/w	soil near a forest stream	Ploština	18.10.2018
IV	*L. foveolarum*	+	1	+	–	+	isodiametric or slightly s/w	soil near a spring	Nezdín	28.09.2018
IV	*L. foveolarum*	+	1.6	+	–	+	isodiametric	soil next to a puddle	Olomouc	23.07.2018
IV	***L. foveolarum* (Bc10)**	+	1.5	+	–	+	isodiametric	soil near a sandstone quarry	Svitavy	14.10.2017
IV	***L. foveolarum* (Bc15)**	+	1.1	+	–	+	isodiametric or slightly s/w	soil in a greenhouse	Vysoké Pole	16.10.2017
IV	***L. foveolarum* (Bc13)**	+	1.5	+	–	+	± isodiametric	wet soil next to a stream in a park	Olomouc	23.07.2018
IV	***Leptolyngbya* sp. (Bc12)**	+	1.7	+	–	+	mostly s/w (± 1 μm long)	edge of a dirt road	Nezdín	28.09.2018
IV	*Leptolyngbya* sp.	+	1.7	+	–	+	isodiametric, s/w or l/w (1.3–2.5 μm long)	dry puddle on the edge of a forest	Grygov	14.10.2018

Table 3 cont.

Group	Species	Constrictions	Width of filaments [μm]	Sheath	Necridic cells	Hormogonia	Cell proportions	Habitat	Locality	Date of collection
IV	*Leptolyngbya* sp.	+	1.1	–	–	–	isodiametric	soil along a pathway in a park	Ústí nad Orlicí	22.09.2018
IV	*Leptolyngbya* sp.	–	1.9	–	–	–	isodiametric	bare wet soil on a meadow	Poděbrady	21.09.2018
IV	*Leptolyngbya* sp.	+	1.2	+	+	–	isodiametric or slightly l/w	dry puddle on a meadow	Vysoké Pole	21.05.2018
V	*L.* cf. *compacta*	+	1.6	+	–	+	isodiametric	stones on the shore of a pond	Svitavy	04.11.2018
V	*L.* cf. *compacta*	+	1.5	+	–	+	isodiametric	rock in a park	Olomouc	23.04.2018
X	*L.* cf. *voronichiniana*	+	1	+	–	–	l/w (± 1.6 μm long)	soil near a spring	Nezdín	28.09.2018
X	*L.* cf. *voronichiniana*	+	1.1	+	–	–	l/w (± 2.4 μm long)	wet soil near a forest stream	Lisovská skála	28.09.2018
X	*L.* cf. *voronichiniana*	+	1.1	+	–	–	l/w (1.4–2.3 μm long)	soil from a meadow	Ploština	18.10.2018
X	*Leptolyngbya* sp.	+	1.4	+	+	–	mostly l/w	soil near a forest stream	Vysoké Pole	05.11.2018
X	***Leptolyngbya* sp. (Bc9)**	+	1.9	+	–	+	l/w (up to 2x longer than wide)	mowed meadow	Vysoké Pole	21.05.2018
X	***Leptolyngbya* sp. (Bc8)**	–	1.5	–	–	–	l/w (up to 2x longer than wide)	wet soil on the shore of a pond	Horka nad Moravou	12.11.2018
XI	***Leptolyngbya* sp. (Bc6)**	–	2.2	+	–	–	l/w (± 1.5x longer than wide)	stones in a forest	Svitavy	11.10.2017

* s/w = shorter than wide; ** l/w = longer than wide; **in bold** cyanobacterial strains used for MALDI-TOF MS

Table 4: List of leptolyngbyoid genera out of *Leptolyngbya* with the characteristics of their morphology and occurrence.

Genus	Constrictions	Width of filaments [µm]	Sheath	Necridic cells	Hormogonia	Cell proportions	Habitat	Locality	Date of collection
Nodosilinea sp.	+	1.3	+	+	+	± isodiametric	stones on the shore of a pond	Svitavy	15.10.2017
Nodosilinea sp.	+	1.3	+	+	+	± isodiametric	dry puddle on a meadow	Vysoké Pole	04.11.2018
Stenomitos (cf.)	+	2	+	+	–	isodiametric or slightly s/w* or l/w**	stones on a river shore	Svitavy	15.10.2017
Oculatella sp.	+	1.1	+	–	+	distinctly l/w	soil along a pathway in a park	Ústí nad Orlicí	22.09.2018
Oculatella sp.	+	1.6	+	–	+	slightly l/w	soil next to a puddle	Olomouc	23.07.2018

* s/w = shorter than wide, ** l/w = longer than wide

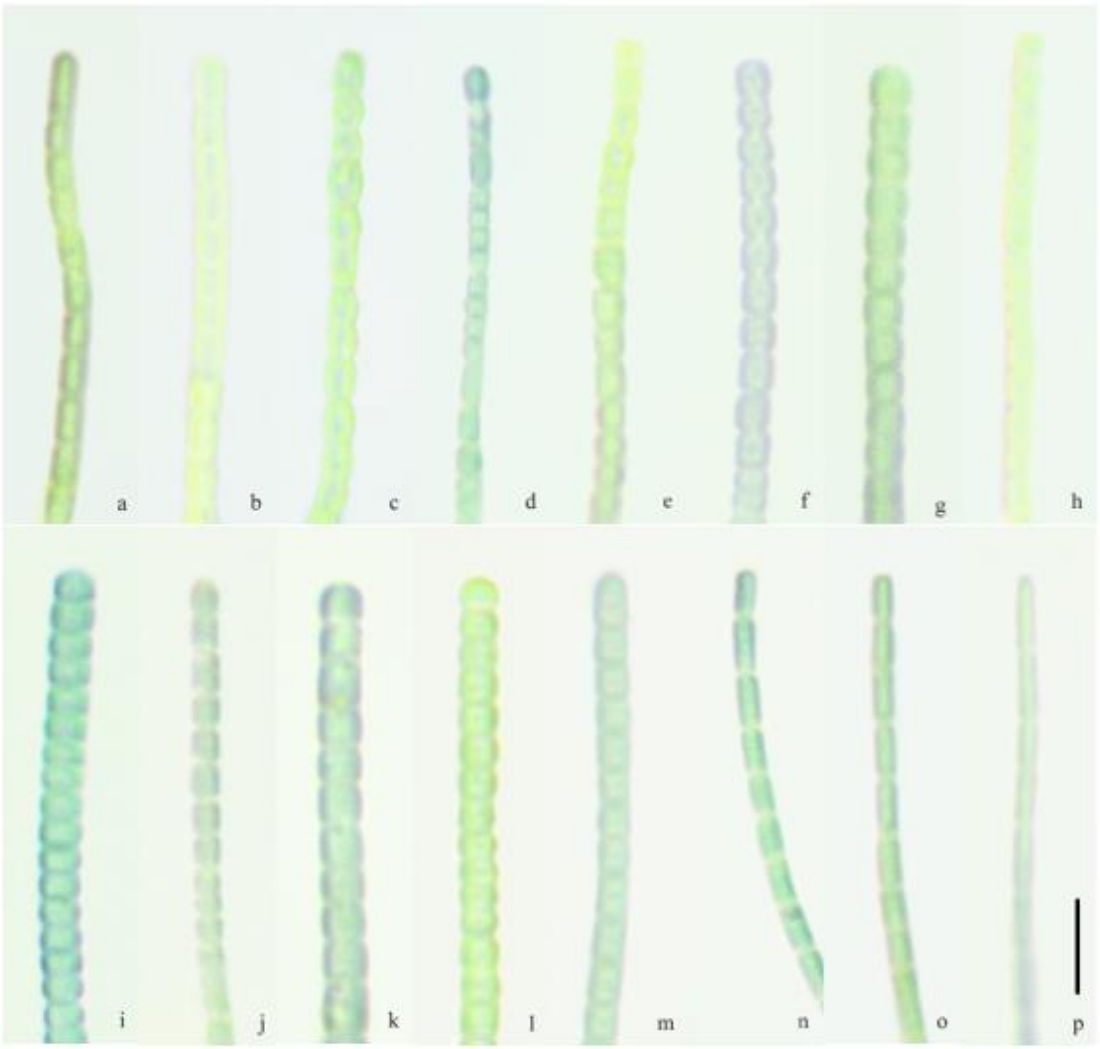

Fig. 3. *Leptolyngbya foveolarum* (group IV) from sampling sites: a – pathway in a forest in Grygov, b – pathway in front of a forest in Grygov, c – dry puddle next to a supermarket in Olomouc, d – soil near a forest spring in Nezdín, e – soil next to a puddle in Olomouc, f – soil near a sandstone quarry in Svitavy, g – soil near a forest stream in Ploština, h – greenhouse in Vysoké Pole; *Leptolyngbya* sp. (group IV): i – dirt road in Nezdín, j – soil along a pathway in a park in Ústí and Orlicí; k – dry puddle on the edge of a forest in Grygov; *Leptolyngbya* sp. (group II): l – wet soil near a mineral stream in Sivá Brada; *Leptolyngbya* cf. *compacta* (group V): m – wet stones on a shore of the Rosnička pond in Svitavy; *Leptolyngbya* cf. *voronichiana* (group X): n – soil near a forest spring in Nezdín, o – wet soil near a forest stream in Lisovská skála, p – soil from a meadow in Ploština. Scale bar 5 μm.

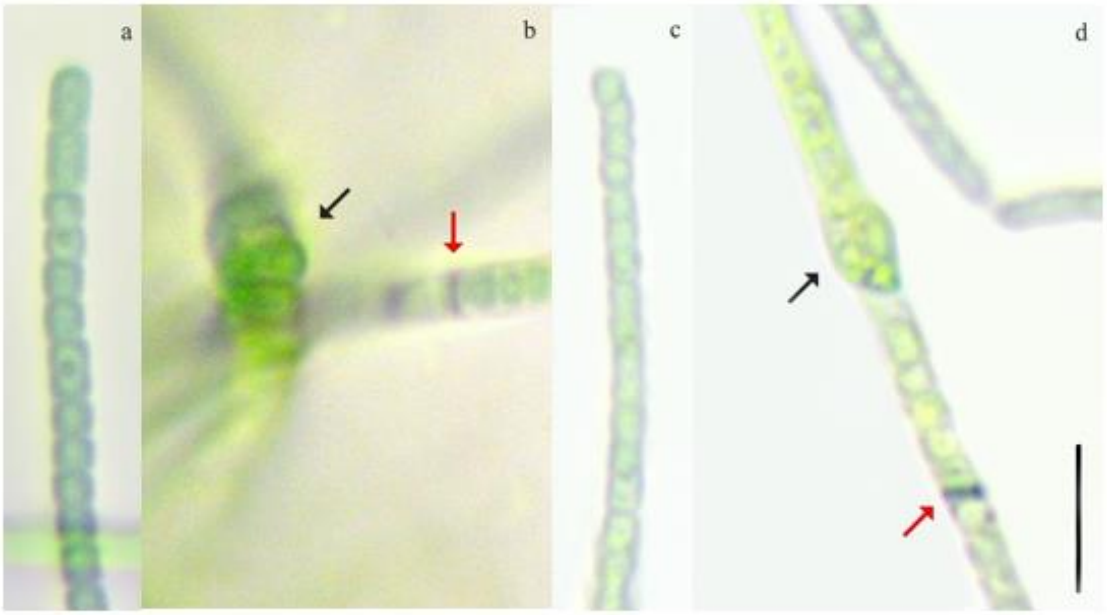

Fig. 4. *Nodosilinea* sp.: a, b – pond in a park in Svitavy; c, d – dry puddle on a meadow in Vysoké Pole (black arrows point to nodules, red arrows point to necridic cells). Scale bar 5 μm.

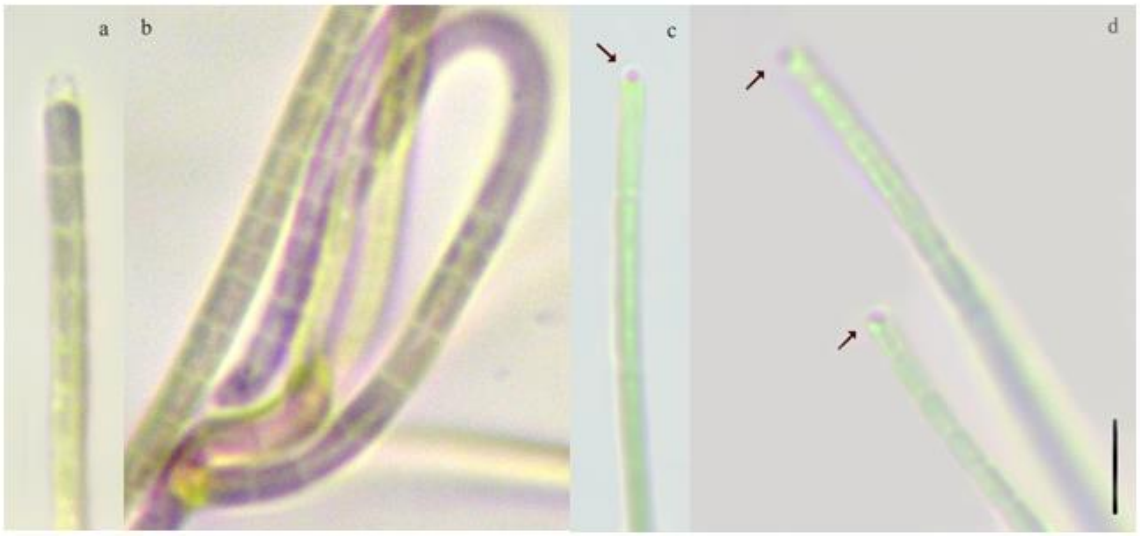

Fig. 5: a, b – *Stenomitos* sp. isolated from stones on the shore of the Svitava river; c, d – *Oculatella* sp. isolated from soil along a pathway in a park in Ústí and Orlicí and from soil next to a dry puddle in Olomouc (black arrows point to the colored tips of filaments – important diagnostic feature for *Oculatella*). Scale bar 5 μm.

6.2 MALDI-TOF MS

Seven pure strains were analyzed with MALDI-TOF MS. The branches of the strains analyzed in the clustering tree were hierarchically clustered according to similarities in their protein/peptide composition. Strains of *Chroococcus* and *Chroococcidiopsis* species were utilized as outgroups. Numbers in red represent Approximately Unbiased (AU) p-values, numbers in green Bootstrap Probability (BP) values. Strongly supported data are those with AU p-value higher than 95 %. Strains analyzed formed two main clusters (Fig. 6). The first one consisted of two strains which were joined to the cluster of compared *Leptolyngbya* spp. with AU p-value 96 %. The remaining strains formed the second, independent cluster, connected with the rest of *Leptolyngbya* strains with AU p-value 96 %.

The first cluster contained strains Bc13 and Bc15. These strains were paired with AU p-value 88 %.

The second cluster contained the rest of isolates – Bc10, Bc6, Bc8, Bc12 and Bc9. Bc10+Bc6 were paired with AU p-value 97 % and Bc12+Bc9 with AU p-value 89 %. Bc12+Bc9 formed a cluster with Bc8 with AU p-values 93 %. This cluster was paired with Bc10+Bc6 with AU p-value 96 %.

These results indicate that the strains of the first cluster (Bc13 and Bc15) are probably members of *Leptolyngbya* sensu stricto. The strains from the second cluster could belong to related genera, e.g. *Oculatella*, *Schizothrix*, *Pseudophormidium* etc.

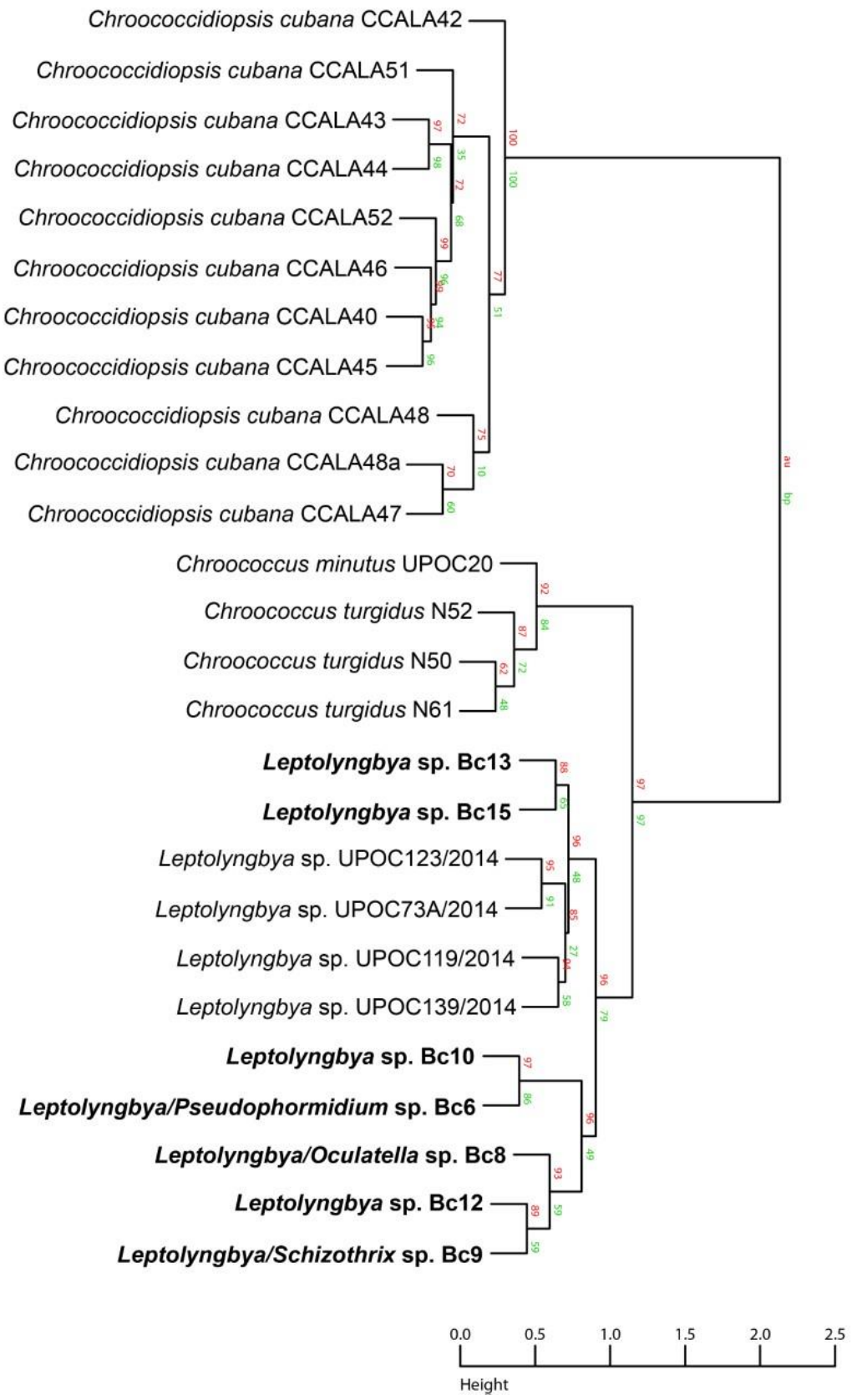

Fig. 6. Clustering tree based on MALDIquant analysis with highlighted branches of studied strains. Numbers in red represent Approximately Unbiased (AU) p-values, numbers in green Bootstrap Probability (BP) values.

7. DISCUSSION

The results from morphological analysis proved that leptolyngbyoid species are commonly abundant both in soils and on stony substrates. However, samples of *Leptolyngbya* collected from soils predominated over samples from stones, because stony substrates were mostly occupied by coccoid algae instead of leptolyngbyoid cyanobacteria.

The determination of species turned out to be complicated for several reasons. First, Komárek and Anagnostidis (2005) offer only one species, *Leptolyngbya foveolarum*, for group IV (soil species with isodiametric cells) in their determination key. Within this thesis, most of *Leptolyngbya* samples were assigned to this group. Although these samples were mostly morphologically very similar, some strains differed distinctly (in the width/length ratio or the extent of constrictions at the cross-walls). Thus, some of the species assigned to group IV were not determined at the species level. A similar situation occurred in group II in which only two species were described by Komárek and Anagnostidis (2005), but none of them matched the description of the studied strain. Kaštovský et al. (2018) state that *L. foveolarum* can also grow on the edges of mineral and thermal springs, and because the studied strain was morphologically very similar to other strains of this species, it could possibly be assigned to this one. Similarly, species from group IV that cannot be assigned to *L. foveolarum* may belong to species described within different ecological groups.

The second obstacle complicating the determination of some species was the reverse of the previously mentioned one. Some ecological groups sensu Komárek and Anagnostidis (2005) contain too many species with only slight differences in morphology, increasing the risk of a wrong determination. This was the case of *Protolyngbya*. Consequently, some species within this subgenus remain undetermined.

A specific problem arose when determining the strain isolated from stones of the Svitava river shore. This strain was eventually assigned to *Stenomitos* Miscoe & Johansen 2016. Although the studied strain was phenotypically similar to *Stenomitos*, especially in color, there were striking differences indicating that the studied strain could belong to another genus. These dissimilarities included e.g. pseudobranching of filaments or the presence of necridic cells which are features that have not been reported for *Stenomitos*. However, no other suitable genus was suggested as an alternative to *Stenomitos*. A more elaborate study will follow in the diploma thesis.

MALDI-TOF MS analysis provided data which were relatively strongly supported (AU p-values of two main clusters were 96 %). When these data were compared with data obtained by the morphological analysis, some coincided precisely while others were more or less inconsistent. The first situation occurred in strains Bc13 and Bc15 which joined the cluster of compared *Leptolyngbya* spp. Based on morphology, they were assigned to *Leptolyngbya foveolarum*, and the result of MALDI TOF MS was not in conflict with this statement. On the contrary, strains Bc10 and Bc12 were morphologically identical to *L. foveolarum*, too, but their position in the clustering tree was distant from the rest of *Leptolyngbya* sensu stricto spp. Remaining strains of the cluster (Bc6, Bc8 and Bc9) were assigned to the subgenus *Protolyngbya* (groups X and XI) based on their morphology, which was in agreement with their close position in the clustering tree. Nevertheless, the correct determination of these strains remains uncertain, so these strains could also belong to closely related genera, such as *Pseudophormidium*, *Oculatella* or *Schizothrix*.

The discrepancies between morphological data and data obtained by MALDI-TOF MS may be explained in several ways. The first one could be the presence of slight contaminations. Even though there was an effort to obtain absolutely pure strains, some contaminants could have stayed unnoticed and therefore been included in samples analyzed. This could have influenced the results of MALDI-TOF MS. On the other hand, a low density of contaminants should not produce distinct protein signals in the MS spectrum – they should fluctuate only under critical signal/noise (S/N) ratio important for peak detection. Thus, the second possible source of these discrepancies is the presence of cryptic species, a known issue in the taxonomy of leptolyngbyoid cyanobacteria (see e.g. Osorio-Santos et al. 2014, Dvořák et al. 2015a, Li & Li 2016 etc.). That would explain why morphologically identical strains occurred in different parts of a clustering tree. Regardless of these unsolved problems, MALDI-TOF MS itself seems to be an appropriate tool for cyanobacterial classification, as it was confirmed to provide data consistent with data obtained by 16S rRNA sequencing, at least for the genus *Chroococcidiopsis* (Šebela et al. 2018).

However, due to the small number of strains analyzed, it would be premature to try to interpret results of the MALDI-TOF analysis at this moment. Further research is planned to be carried out within the diploma thesis where more strains will be included in the protein/peptide analysis. That could shed light on currently unresolved issues.

8. CONCLUSION

Within this thesis, it was confirmed that leptolyngbyoid species contribute significantly to the cyanobacterial diversity in soils and on stony substrates. That can be evidenced by the number of various habitats where these species were found and by the number of samples in which they were present (almost half of the collected samples). Based on the frequent abundance of leptolyngbyoid species in the samples, they can be considered common taxa in soil and stony habitats.

Morphological analysis revealed that the most frequent leptolyngbyoid genus in the localities studied was *Leptolyngbya* sensu stricto, especially the species *Leptolyngbya foveolarum*. Other genera, such as *Oculatella* or *Nodosilinea*, were rather minor contributors to the cyanobacterial diversity.

The outcome of the MALDI-TOF MS analysis was a clustering tree with relatively strongly supported branches. For this reason, MALDI-TOF MS seems to be a useful tool for assessing the diversity of taxa studied and thus is planned to be more utilized within the diploma thesis, as it was applied only to selected strains in the bachelor thesis.

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10. APPENDICES

List of photographic appendices:

Appendix 1: Wet soil near puddles on a pathway near a sandstone quarry behind Svitavy.

Appendix 2: The wet surrounding of a puddle near the Kaufland supermarket.

Appendix 3: Rocky formation in the coniferous forest behind Svitavy.

Appendix 4: Submerged stones on the shore of a pond in the Park of Jan Palach in Svitavy.

Appendix 5: Wet soil and stones on the edge of the Rosnička pond in Svitavy.

Appendix 6: Work in laboratory – flowbox.

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Appendix 1: Wet soil near puddles on a pathway near a sandstone quarry behind Svitavy.

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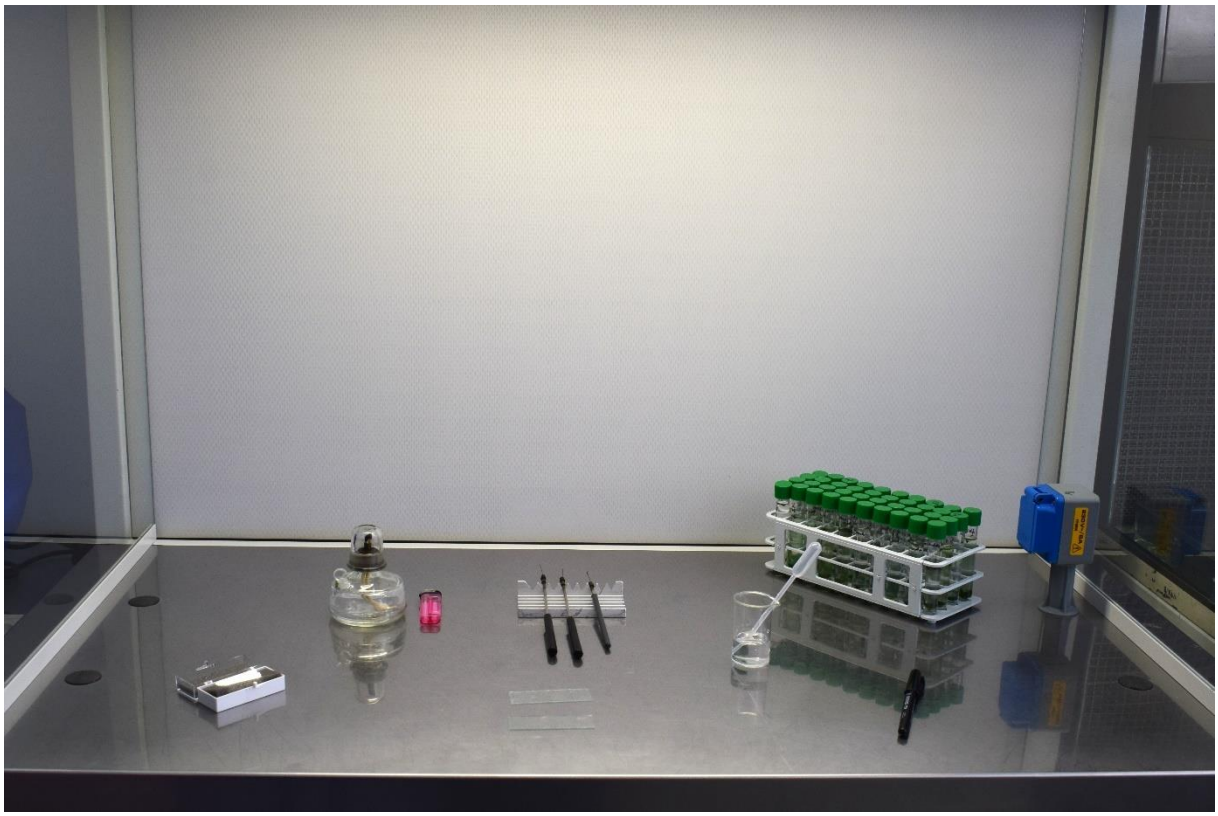
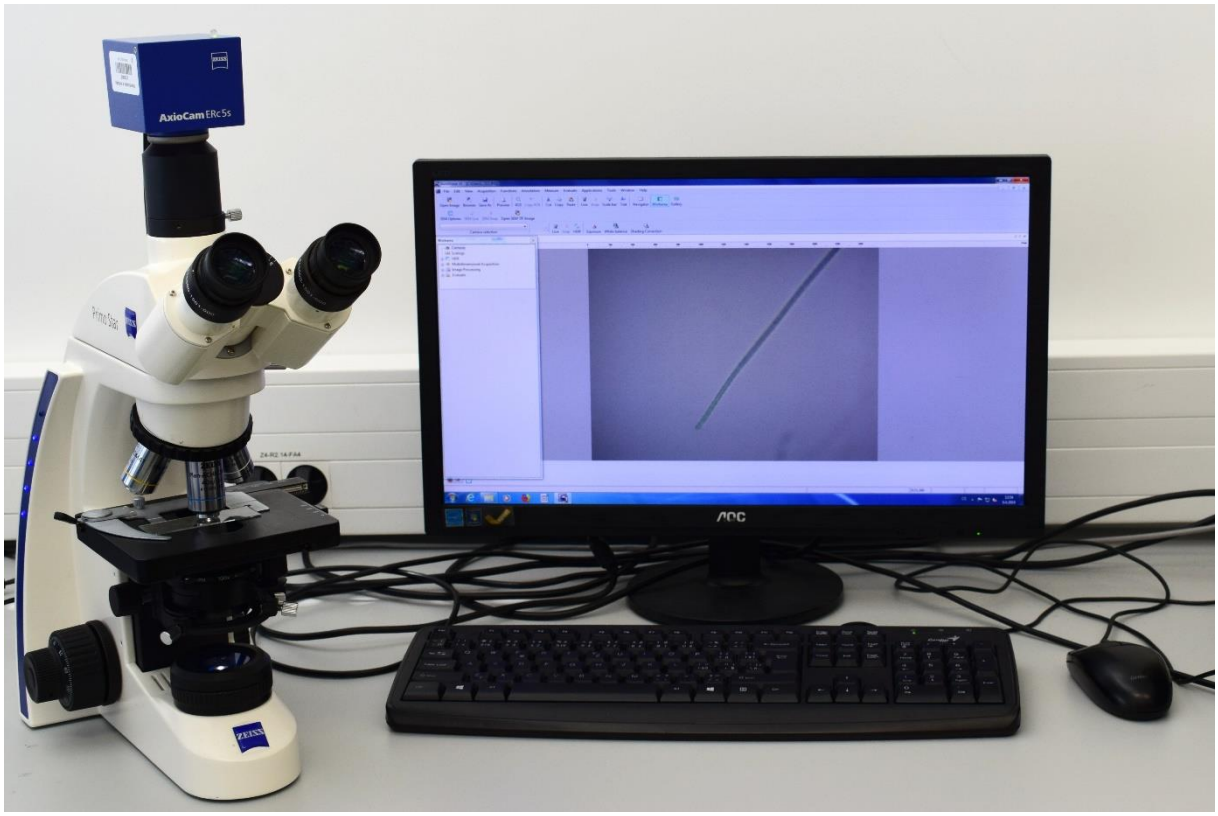
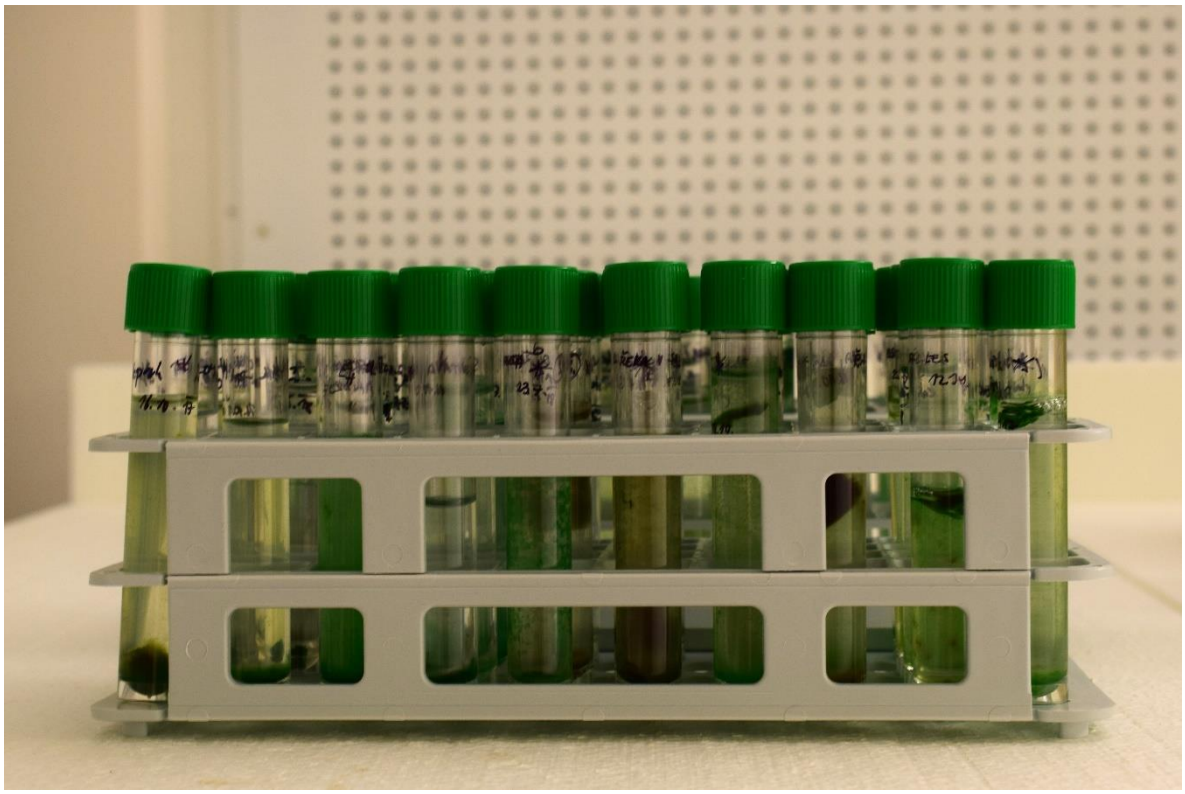


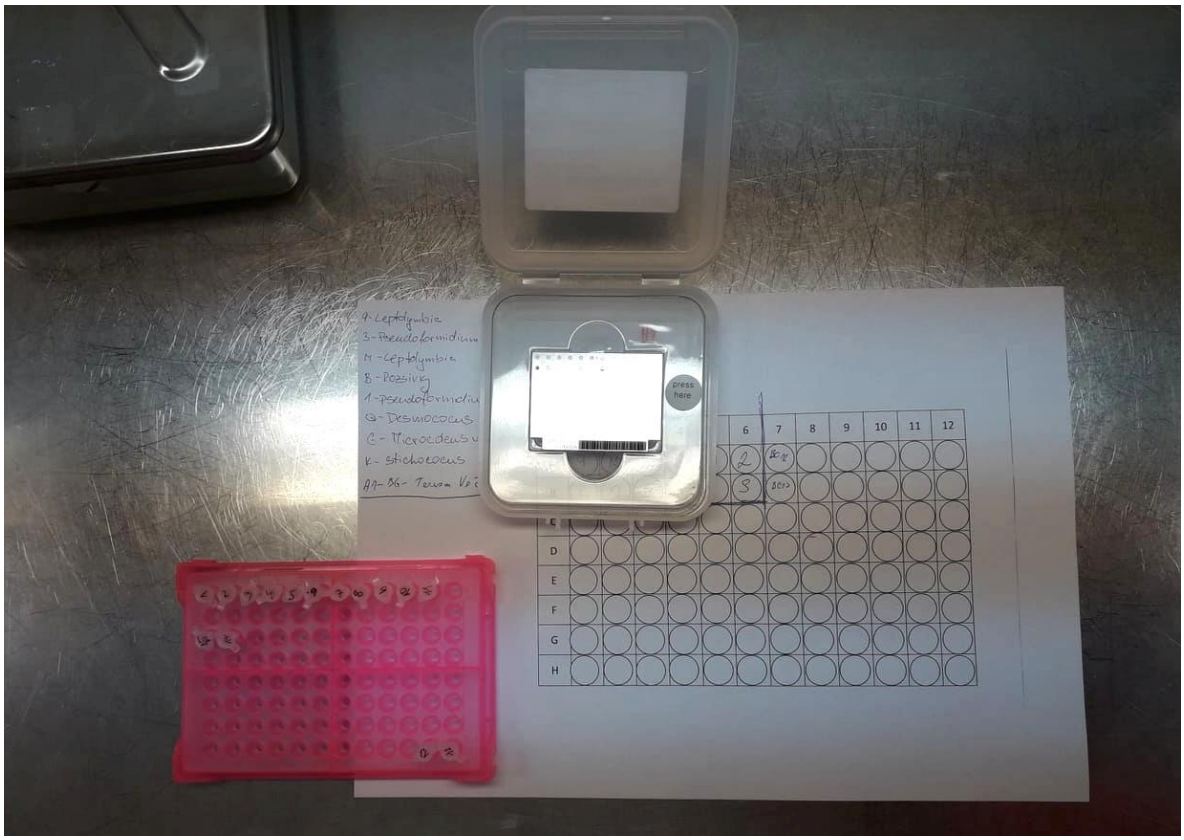
Appendix 6: Work in laboratory – flowbox.



Appendix 7: Work in laboratory – microscope with camera attached to the computer.



Appendix 8: Work in laboratory – strains in Z medium stored in cultivation room.



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