Palacký University Olomouc

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The importance of 16S rRNA gene and MALDI-TOF MS analysis in the study of *Leptolyngbya* sensu lato

Bc. Eliška Hajská

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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.

10th May 2021, Olomouc ……

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Abstract:

Cyanobacteria are important primary producers in various aquatic and terrestrial ecosystems. An example of a cyanobacterium occupying both types of environments is *Leptolyngbya* s. l. This taxon is currently considered polyphyletic and requires systematic revision. The Svitava River and stagnant water bodies in its surrounding area were chosen as sampling sites for studying this taxon. Subsequent analyses were enriched with strains from previously sampled terrestrial habitats. Morphology, ecology, 16S rRNA gene, 16S–23S ITS secondary structures, and protein spectra were evaluated in isolated strains. Strains of *Leptolyngbya*, *Nodosilinea*, *Drouetiella*, and *Jaaginema*/*Tildeniella* were analysed using this polyphasic approach. Morphologically similar genera *Anagnostidinema* and *Pseudanabaena* were added to complete the information on the presence of thin filamentous cyanobacteria at the studied sites. Cryptic diversity was revealed in the studied taxa, especially in the genus *Nodosilinea* and *Anagnostidinema*. MALDI-TOF MS was confirmed as a promising type of molecular analysis, potentially useful for future taxonomic works.

Keywords: *Leptolyngbya*, *Nodosilinea*, cyanobacteria, 16S rRNA, MALDI-TOF, taxonomy, cryptic diversity, river

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Abstrakt:

Sinice jsou významní primární producenti v různých vodních i suchozemských ekosystémech. Příkladem sinice obývající oba typy prostředí je *Leptolyngbya* s. l. Tento taxon je v současné době považován za polyfyletický a vyžaduje systematickou revizi. Jako vzorkovací lokality pro studium daného taxonu byla zvolena řeka Svitava a přilehlé stojaté vody. Následné analýzy byly obohaceny o kmeny z dříve vzorkovaných suchozemských habitatů. U izolovaných kmenů byla hodnocena morfologie, ekologie, 16S rRNA gen, 16S–23S ITS sekundární struktury a spektrum proteinů. Pomocí tohoto polyfázického přístupu byly analyzovány kmeny rodu *Leptolyngbya*, *Nodosilinea*, *Drouetiella* a *Jaaginema*/*Tildeniella*. Kmeny morfologicky podobných rodů *Anagnostidinema* a *Pseudanabaena* byly přidány pro doplnění informace o přítomnosti tenkých vláknitých sinic na studovaných lokalitách. U studovaných taxonů, zvláště u rodu *Nodosilinea* a *Anagnostidinema*, byla odhalena kryptická diverzita. MALDI-TOF hmotnostní spektrometrie byla potvrzena jako slibný typ molekulární analýzy, potenciálně využitelný v budoucích taxonomických pracích.

Klíčová slova: *Leptolyngbya*, *Nodosilinea*, sinice, 16S rRNA, ITS, MALDI-TOF, taxonomie, kryptická diverzita, řeka

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1. INTRODUCTION AND OBJECTIVES

Cyanobacteria are microorganisms which share the prokaryotic cell structure with other bacteria and the ability to perform photosynthesis with algae and plants. However, they do not fit precisely onto bacteriology, or botany. Instead, they represent a unique group of organisms which play key roles in various ecosystems and contribute greatly to global biodiversity.

Freshwater cyanobacteria are often associated with stagnant waters where they can produce harmful blooms. It is less well-known they also inhabit running waters, both as plankton and benthos. One example of a benthic riverine cyanobacterium is the taxonomically problematic genus *Leptolyngbya*. It has been observed that molecular diversity within this genus overlaps the morphological diversity. The modern polyphasic approach, integrating different types of data (molecular, morphological, ecological, etc.), represents the best approach for the revision of this polyphyletic taxon. Many taxonomic issues have been resolved by establishing novel, monophyletic taxa. Consequently, it is currently possible to find the leptolyngbyoid morphology in almost 50 genera from four families. The polyphyly, however, persists. For this reason, *Leptolyngbya* s. l. is the subject of investigation within this thesis.

The principal goal of this thesis is to contribute to resolving taxonomic difficulties in *Leptolyngbya* s. l. with the use of a polyphasic approach. For these purposes, the Svitava River and stagnant waters in its surrounding were chosen as model localities. Strains from previously sampled terrestrial habitats were added to complete the information on the morphological and molecular diversity within this taxonomic group. The key questions are:

- whether there is a difference in species composition between lotic, lentic, and terrestrial habitats,
- whether there is a difference in species composition between different reaches of a river,
- whether there is a difference in species composition between directly submerged environment, wet edges, and dry surrounding of the river, and
- whether molecular diversity overlaps the morphological/ecological one.

Finally, an equally important objective of this thesis is to explore the potential of proteomic analysis (MALDI-TOF MS) as a simpler and cheaper alternative to classical DNA analysis.

2. BIODIVERSITY IN RIVERS

2.1. Introduction to fluvial ecosystems

Fluvial ecosystems are essential components of nature. They serve as an environment for a great diversity of organisms. They drain excessive precipitation from the land. They shape the landscape. They connect inland areas with the ocean. As they serve as sources of drinking water for humans and animals and as irrigation for crops, it is not surprising humankind has tended to settle in the proximity of rivers since time immemorial.

In the past, many rivers were negatively affected mostly by pollution and regulation and their ecological value decreased drastically. While there has been an effort to carry out various revitalizations which would restore the natural character of fluvial ecosystems, the problem of pollution has not been sufficiently resolved yet.

Studying rivers (and running waters generally) represents a challenging task because these ecosystems are highly complex and are subject to incessant changes. Moreover, abiotic and biotic factors vary considerably along the stream and differ depending on whether the stream is natural or anthropogenically altered. Yet, the correct understanding of processes in different river reaches is vital if humankind wants to both protect and take advantage of this ecosystem.

Two main approaches to studying the functioning of fluvial ecosystems occurred in the past. The earliest approach subdivided rivers into distinct zones, whereas the later one considered rivers as gradually changing continua (Doretto et al. 2020a). The latter approach predominantly relates to two concepts which influenced the subsequent lotic water studies – the Nutrient Spiraling Concept (Webster et al. 1975, Newbold et al. 1981) and especially the River Continuum Concept (Vannote et al. 1980). Both concepts deal with the longitudinal transport of nutrients along a stream and its relation to biological communities. Nevertheless, the Nutrient Spiraling Concept focuses more on abiotic processes such as retention or reutilization of nutrient particles, while the River Continuum Concept lays bigger stress on interactions between abiotic and biotic components of running waters.

2.2. The River Continuum Concept (Vannote et al. 1980)

The fundamental idea of the RCC is that in flowing waters, there is a longitudinal gradient of abiotic factors which induce biotic responses. Biotic responses predominantly mean changes in proportional representation of functional groups of macroinvertebrates. Functional

groups are groups of organisms which utilize the same type of carbon source. As the availability of different sources chnges within the river, the ratio of functional groups in a community changes as well.

In headwaters, the main carbon source is allochtonous material, for example fallen leaves and wood, so the organic particles are of a bigger size (> 1 mm, CPOM – coarse particulate organic matter). This type of food is preferred by shredders which represent a typical functional group of invertebrates in this reach. As the river widens, the increasing light permeability leads to the transition from heterotrophy to autotrophy. Here, primary producers (e. g. cyanobacteria, algae) form a biomass which serves as a food source for grazers. Lower reaches, on the contrary, are characterized by a switch to heterotrophy again as the light permeability decreases due to turbidity and depth. This part of a river is dependent on the supply of small organic particles (< 1 mm, FPOM – fine particulate organic matter) from the upper reaches and is dominated by collectors. The last group – predators – are present along the whole stream but only in small quantities.

According to this concept, biodiversity reaches its maximum in the middle part of streams and positively correlates with temperature amplitude and primary production (see Fig. 1). The fall of maximum diel temperature amplitude and primary production is caused by a tree canopy and a cold flow of the spring in headstream reaches and due to turbidity and depth in downstream reaches. Consequently, communities must be repeatedly replaced along the stream to maximize the utilization of available energy sources.

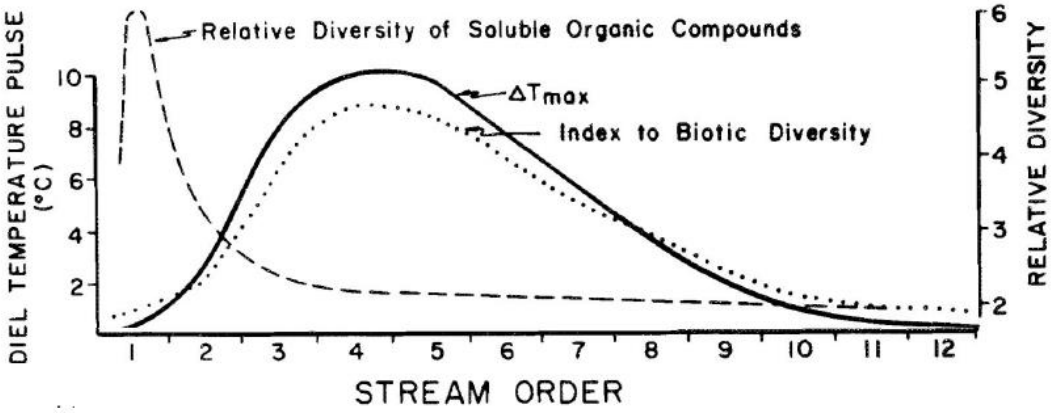


Fig. 1. The relationship between the temperature amplitude and biodiversity along the river continuum (Vannote et al. 1980).

2.3 Modifications of the RCC

Despite its persistent validity, the RCC contains several weaknesses. One discrepancy is that the concept is generalized for various streams but is based on studies of minor temperate running waters (Junk et al. 1989). Moreover, the river continuum was studied only on macroinvertebrates which cannot represent the overall diversity. Further, the concept underestimates factors disrupting the continuous character of running waters. These disruptions may be of a natural (e.g., lakes and tributaries) or anthropogenic (e.g., dams) origin (Doretto et al. 2020). Consequently, the gaps in the RCC induced an effort to formulate new concepts which would take into consideration phenomena neglected by the RCC. Thereafter, the excess of emerging concepts led to an attempt to merge them into one valid concept.

One of the first modifying concepts was the Serial Discontinuity Concept (SDC) (Ward & Stanford 1983) which originated as a reaction to the lack of attention paid to dams in rivers. This concept points out the impact of dams on both the abiotic and biotic components of fluvial ecosystems. As far as the biodiversity is concerned, the effects of impoundments differ substantially between different fluvial reaches. If built in upper reaches, dams prevent the transport of organic matter to lower parts and that limits the biodiversity. In middle parts, the biodiversity below dams is diminished due to the altered thermal regime and increased predictability. Contrariwise, the biodiversity below dams in lower reaches increases, because of enhanced heterogeneity of the environment (Fig. 2).

The original concept, however, perceived lotic waters only as a longitudinal channel, similarly to the RCC, which turned out to be inadequate. Therefore, a four-dimensional model was proposed, which beside the longitudinal dimension considers lateral, vertical, and temporal dimensions (Ward 1989). Following this extension, the Serial Discontinuity Concept was enriched with the influence of floodplains (i.e. lateral dimension) on braided and meandering reaches of rivers (Ward & Stenford 1995). In the new three-reach model, the view on the biodiversity in middle and lower reaches changed considerably. Middle, braided reaches, previously considered highly diverse, were newly described as species-poor because of channel instability. Conversely, in the lower, meandering reaches, the values of biodiversity were estimated to be very high, if the channel communicated with floodplains (Fig. 2).

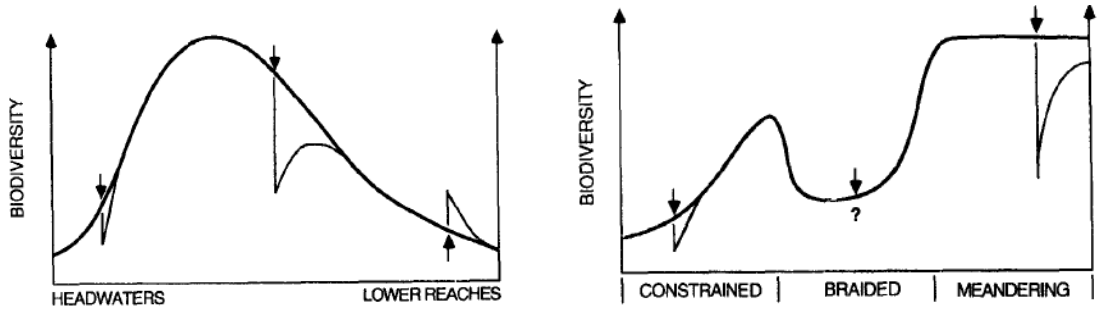


Fig. 2. The downstream changes in biodiversity according to Ward & Stanford (1995). On the left – changes according to the original SDC. On the right – changes according to the extended SDC. Arrows – alterations caused by regulations (dams).

The Flood Pulse Concept (Junk et al. 1989) also emphasizes the significance of floodplains. It says that lower reaches of large, unaltered rivers that flood the surrounding land are independent of the organic matter transported from upper reaches because their lateral supply from flooded land is sufficient in itself. The river-floodplain system consists of three parts – the main channel, permanent lentic habitats, and the aquatic/terrestrial transition zone (floodplain). The importance of the system lies in the offer of various habitats which facilitate high biodiversity. A prerequisite for that is the predictability of flood pulses because irregular pulses prevent organisms from developing adaptations to such extreme changes.

The Patch Dynamics Concept (Townsend 1989) brought a new view on running waters. Its advantage lies in its general applicability to different streams, in contrast with the RCC. The idea of this concept is that each part of a stream is patchy (heterogenous). That means that communities of different segments are influenced by separate species interactions and disturbances. Species interactions involve predominantly competition and predation but both interactions are further modified by disturbances because they can, for example, reduce populations of strong competitors or predators or facilitate the colonization of new species. Thus, frequent disturbances provide a temporal heterogeneity which may result in an increase of biodiversity. The condition for recovering of populations after disturbances is the availability of refuges which protect species from the destructive effects of the disturbance, e.g., spate.

The Riverine Productivity model (Thorp & Delong 1994) enriches the previous concepts with the view on large, constricted rivers, i.e. rivers without access to nutrients from floodplains. The authors state that according to previous concepts, nutrient supply in large rivers depends mainly on headwaters and floodplains. The new model suggests the main

sources of carbon in large, constricted rivers are primary production and organic material from the riparian zone. Hence, the community structure varies among different segments, as it is affected by local conditions.

The River Wave Concept (Humphries et al. 2014) is one of the current concepts which aim to unify the previous ones. The principle of this concept is that river flow varies both in space and time and can be described as a series of waves. These waves can be characterized by their shape, amplitude, wavelength, and frequency, and are influenced by geomorphology, climate, regulation, and other factors. From the source to the mouth, the wavelength of wave increases, while the amplitude decreases. Waves consist of three major parts and each of them indicates which carbon source predominates at a given point and which of the existing concepts describes it. Specifically, the troughs of waves symbolize low or no flow and therefore the carbon sources can be only local; this corresponds with the Riverine Productivity Model. Ascending and descending parts of waves mean the flows are rising or falling. Longitudinal transport from upstream areas is the predominant carbon source here and this part of a wave relates to the RCC. Crests, i.e. tops of waves, represent the highest (flood) flow where floodplains are the main carbon source. This follows up on the Flood Pulse Concept. According to this concept, high species diversity is a result of interactions between waves and geomorphologic objects providing a wide range of habitats, e. g. floodplains, river islands, confluences etc.

The Metacommunity Concept (Leibold et al. 2004) does not belong to concepts formulated primarily for lotic waters and therefore does not follow the RCC. Lately, however, it has formed a basis for many fluvial studies. The concept says communities exist at least at two levels – local (i.e. local communities) and regional (i.e. metacommunities – sets of local communities). While local communities are influenced by species interactions, metacommunities are influenced by dispersal among local communities. The main difference between the RCC (and subsequent works) and this concept is that the RCC emphasizes the influence of environmental heterogeneity on community structure, while the Metacommunity Concept highlights the importance of dispersal (Doretto et al. 2020a). Brown & Swan (2010) employed the concept to study metacommunities in rivers and proposed that processes which determine the structure of communities differ depending on whether the communities are located in a headstream or in a mainstem section. While headstream communities are more isolated and therefore governed by local (environmental) forces, communities in higher order streams depend both on local and regional (dispersal-based) processes. The study was later

extended and called the Network Position Hypothesis (Schmera et al. 2018). The disadvantage of the hypothesis is that it cannot be applied to all taxonomic groups – the only group with convincing results were macroinvertebrates for whom the original hypothesis was postulated. Thus, despite its potential, the concept is not currently applicable (Schmera et al. 2018).

The concepts above, however, do not deal with biodiversity issues only. On the contrary, some of them mention it only marginally, and therefore, subsequent works followed to deduce factors which enhance/reduce species richness. Many authors (e.g., Ward et al. 1999) adopted the intermediate disturbance theory (Connell 1978) and intermediate productivity hypothesis (Grime 1973), which together constitute the dynamic equilibrium model (Huston 1979). The model presumes that the species diversity is highest when the level of disturbance is intermediate. Biodiversity also depends on resources (and thus productivity), because to achieve high biodiversity when there is a surplus of resource, higher levels of disturbance are required (and conversely). Ward et al. (1999) extended the model by adding the impact of different levels of connectivity on biodiversity in river-floodplain systems. Specifically, an intermediate level of connectivity determines the highest biodiversity, as low connectivity results in reduced flow of energy, material and organisms, and increased connectivity in reduced habitat diversity. In this study, the authors highlighted the importance of ecotones (floodplains in this case) for species diversity, as they connect river channels with the surrounding environment. It was observed that ecotones generally support higher species diversity. Hyporheic zones, i. e. places where surface and ground water meet, are another proof of their importance because they constitute an environment where a part of the life cycles of riverine organisms takes place. In addition, they serve as refuges for organisms during unfavorable conditions, and they provide nutrients to riparian flora (Ward & Stenford 1988, 1993).

It follows from the above that species richness depends on a combination of more factors. Most authors highlight heterogeneity – either spatial or temporal – as the major biodiversity promoting factor (Vannote et al. 1980, Junk et al. 1989, Townsend 1989, Humphries et al. 2014 etc.). Palmer et al. (2010), however, questioned the role of heterogeneity as a primary factor enhancing high biodiversity and suggested this issue is more complex, depending on other factors such as food resources, regional species pools, or water quality. Water quality is especially essential, considering the amount of works studying the influence of anthropogenic activity on riverine biota (see further). Another factor –

connectivity – is necessary for species diversity because it enables dispersal (Ward & Stenford 1988, 1993, 1995, Ward et al. 1999, Leibold et al. 2004, Schmera et al. 2018 etc.).

2.4. Anthropogenic influence on riverine biodiversity

As described above, three main conditions seem to be key for maintaining high species diversity in rivers – environmental heterogeneity, connectivity, and the good condition of the water. In anthropogenically modified waters, the importance of all these factors can be evidenced by the reduction of species diversity due to the loss of heterogeneity, connectivity disruption, and pollution. Moreover, the anthropogenic disruption of communities facilitates the spread of invasive species (Rulík et al. 2020).

2.4.1. The loss of heterogeneity

Heterogeneity is mostly associated with habitat diversity, which is achieved by the presence of different substrates, fallen wood and leaves, or water macrophytes in running waters. However, the heterogeneity can also be represented by the range of physical or chemical conditions, e.g., differences in temperature, pH, flow rate, depth, shading, and many others. A great part of heterogeneity is generated by the character of bed and banks.

In the past, many rivers were channelized with the major aim of securing a flood control. River regulations often shortened river lengths and impaired the natural heterogenous character of bottoms and banks. This had a devastating impact on riverine organisms. For instance, Horsák et al. (2009) compared natural, regulated, and previously regulated segments of a river and found that the more modified the river segment, the lower the species richness of macroinvertebrates and the more dominant one functional group – gathering collectors.

Loss of heterogeneity can also result from riparian deforestation. Riparian vegetation is essential because it provides rivers with organic material, captures pollutants and excessive amounts of nutrients from agriculture, ensures shading and generally contributes to bank heterogeneity. Therefore, the loss of vegetation affects the riverine biodiversity very negatively, too. It has been proven, for example, that riparian deforestation reduces bed roughness, availability of organic material, narrows stream channels due to overgrowing riparian grasses, and causes lower nitrogen retention, hence its higher accumulation in the downstream transport (Sweeney et al. 2004).

2.4.2. Connectivity disruption

Anthropogenic activity disrupts not only the longitudinal river continuum, but also the exchange between river and land, i.e. lateral connectivity, and the exchange between river and hyporheic zone, i.e. vertical connectivity (Wohl 2017). Except for spatial disruptions, rivers can become intermittent also in time (Xu 2004, Doretto et al. 2020b).

In the downstream direction, manmade obstacles include mainly transversal objects, such as dams and weirs, which alter hydrological conditions in rivers and constitute migratory barriers. In the Czech Republic, for example, 9 605 migratory barriers were monitored on the stream length of 11 458 km in recent years (AOPK 2020).

The loss of lateral connectivity usually relates to river regulation which disconnects floodplains from their rivers. Because floodplains highly depend on disturbance regimes, their disruption leads to drastic habitat and species decline (Ward et al. 1999). These negative effects are also amplified by land use change, damming and other human interventions (Hein et al. 2016). Altogether these factors contribute to continuing global floodplain decrease and consequently, these ecosystems are currently considered one of the most endangered in the world (Tockner et al. 2010, Hein et al. 2016).

Anthropogenic activity also negatively affects the vertical dimension of rivers. This can be evidenced on disrupted connection between groundwater and surface, on aggravated groundwater or surface water quality, and on change in species composition in the hyporheic zone (Boulton 2007).

Temporal disconnectivity results from flow cessation during periods of drought. Rivers which are subject to regular drying are located mainly in arid and semiarid areas and are called seasonal rivers. Recently, however, the number of these ephemeral rivers has increased (Xu 2004, Doretto et al. 2020b). Xu (2004) proposed a new term – anthropogenic seasonal river (ASR) – which describes a river which used to be perennial but became seasonal due to anthropogenic activity, such as water offtake for agricultural or industrial purposes. The number of seasonal rivers has also increased with continuing global change. Regardless of the cause, the peril of unexpected droughts is that lotic communities are not adapted to them and their recovery after re-inundation seems to depend on the passive drift of organisms from the upper reaches (Doretto et al. 2020b).

2.4.3. Pollution

Rapid industrial and agricultural development together with population growth during the 20th and 21st century worsened the surface water quality very distinctly. Major types of pollution in rivers include eutrophication, acidification, organic pollution, heavy metal pollution, and special attention has been recently given to plastic pollution.

Eutrophication

Although eutrophication mostly relates to lentic waters, it can also represent a serious risk for rivers, especially those with low flow during warm periods (Jarvie et al 2006). The major trigger for eutrophication is a superabundance of nitrogen and especially phosphorus which promotes an excessive growth of primary producers – cyanobacteria, algae and macrophytes. Undesirable amounts of these nutrients in lotic waters originate mostly from field runoff, sewage/industrial effluents, and dams (Jarvie et al. 2006, Glibert 2017). Recently, higher risk of eutrophication has been also associated with climate change (O'Neil et al. 2012, Glibert 2017). Excessive biomass of photoautotrophs threatens species richness as it causes water transparency reduction, dissolved oxygen deficiency, and (if cyanobacteria present) intoxication of riverine biota (Glibert 2017).

Acidification

Massive industrial development in the 20th century caused a rapid increase of sulphate and nitrate emissions which resulted in extensive acidification of European and North American surface waters. That led to species diversity reduction in affected waters. Since the 1980s, emission control programs have been introduced and sulphate and nitrate emissions decreased significantly (Garmo et al. 2020). Although the effect of this improvement on biota is often delayed (Svobodová et al. 2012), in the Czech Republic, many affected streams have already returned to their pre-acidification state (Rulík et al. 2020).

Organic pollution

Certain amounts of organic compounds are a natural component of running waters and these usually have a sufficient self-cleaning capacity. Processing organic compounds comprises degradation by detritivores, bacteria, and fungi, and dilution by runoff. In contrast, much heavier organic pollution is of anthropogenic, specifically urban, agricultural, and industrial origin (Wen et al. 2017). Large amounts of anthropogenic organic pollutants pose a danger of oxygen depletion and whole riverine ecosystem disruption due to intensive bacterial

metabolic activity; moreover, wastewaters pose a risk of spread of dangerous pathogens (Wen et al. 2017).

The extent of organic pollution differs among different parts of the world, depending on numerous factors, such as wastewater treatment standard and intensity of farming and industry (Malaj et al. 2014). In the Czech Republic, organic pollution significantly decreased in the last 30 years (Rulík et al. 2020). However, there are groups of organic pollutants which tend to persist in river sediments, and hence require special attention. Persistent organic compounds (POPs) are extremely dangerous as they are hardly degradable, tend to accumulate in food chains and are toxic to riverine biota (Jones & de Voogt 1999). Commonly monitored POPs in lotic waters are predominantly aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs, PCDFs), polybrominated diphenyl ethers (PBDE), and organochlorine pesticides (OCP) (Kanzari et al. 2014, Kukučka et al. 2015). Recently, pharmaceuticals (e.g., antibiotics) have become an additional subject of investigation in lotic waters not only because of their harmful effects on biota (Ginebreda et al. 2010), but also because of the increasing risk of bacterial resistance development (Xu et al. 2015).

The impact of organic pollution on biodiversity depends on the number, types, and concentrations of pollutants, but in general, their presence leads to species richness losses (Malaj et al. 2014). This occurs not only due to the direct toxicity of these pollutants, but also because they have carcinogenic effects, they impair reproduction, and weaken immunity (reviewed e.g., by Jones & de Voogt 1999 or Vilela et al. 2018).

Heavy metal pollution

Also heavy metals occur in water naturally to some extent, but much more danger lies in metals coming from anthropogenic activity, such as metallurgy, coal combustion, transport, and agriculture. Many of these metals are associated with toxic, carcinogenic, and teratogenic effects, although these effects depend on the form in which they occur. The most dangerous heavy metals include cadmium, arsenic, chromium, mercury, and lead. The highest concentrations are accumulated in sediments wherefrom they can be remobilized back to the water column. A particular danger is represented by mercury which tends to biomagnify within food chains which means that the highest concentrations get to apex predators. (Rulík et al. 2014)

Plastic pollution

The influence of plastics on water ecosystems is currently a subject of extensive research, although most studies focus on their negative impact on marine organisms. These studies show that plastic pollution affects various taxonomic groups and mostly damages their digestion (and thus growth) and reproduction (Cole et al. 2015, Sussarellu et al. 2016, Wang et al. 2019). As regards rivers, it is known that they behave as transport channels delivering plastic particles to the ocean and that plastics present in them occur in undesirable amounts in different parts of the world (Yonkos et al. 2014, Mani et al. 2015, Lahens et al. 2018). However, studies focusing on interactions between plastics and riverine organisms are difficult to find. Microplastics have been observed in the digestive systems of e.g., macroinvertebrates (Windsor et al. 2019) and fish (Sanchez et al. 2014), but the consequences of their presence on individuals, populations, or overall biodiversity remain unclear.

2.5. Cyanobacteria in rivers

Freshwater cyanobacteria are typically associated with harmful blooms in stagnant waters, whereas their presence in running waters is often neglected. These photosynthetic prokaryotes are an integral part of riverine communities, however, as they provide oxygen, nitrogen, food, and refuge to other riverine organisms. Though possessing microscopic dimensions, cyanobacteria are capable of aggregation in macroscopic mats or blooms, and therefore can affect riverine ecosystems considerably.

Like macroinvertebrates, cyanobacteria respond to changes in abiotic conditions along the river continuum. For instance, first-order streams often host species which prefer rocky substrates and tolerate desiccation, while higher-order streams are inhabited by planktic species or benthic species adapted to muddy or sandy substrates (Casamatta & Hašler 2016).

In contrast with motile water eukaryotes, cyanobacteria possess very limited ability to disperse actively. Exceptions include e.g., *Geitlerinema* which possesses highly motile trichomes or *Microcystis* which moves in the water column thanks to gas vacuoles (Casamatta & Hašler 2016). However, the main mechanisms of transport are passive – by water current, air, animals, and human (Kristiansen 1996, Vis 2016). The regrowth of residual populations is another important mechanism ensuring the long-term presence of the given cyanobacterial taxa at a certain locality (McAllister et al. 2016).

There are lots of cyanobacterial taxa of different orders known from rivers, including unicellular, colonial, and filamentous species (see Casamatta & Hašler 2016). Moreover, a considerable part of this biodiversity may remain hidden as it can be detectable at a molecular level only; such species are called cryptic (Casamatta et al. 2003). The taxonomic composition changes throughout the year (Sabater et al. 2003) and among different lotic ecosystems, depending on various factors, e.g., climate, altitude, light and nutrient availability, discharge, type of substrate, presence of grazers, or anthropogenic influence (Casamatta & Hašler 2016).

Anthropogenic activities affect riverine cyanobacteria to a particularly large extent. Excessive amounts of nutrients, artificial objects slowing down water flow, and increased temperature due to global warming are major drivers of harmful cyanobacterial blooms in rivers (Park et al. 2021). Negative effects of bloom-forming cyanobacteria typically include oxygen depletion during their decay, out-competing other species, and toxic metabolites release (Pearl & Otten 2013). Cyanobacterial toxins, however, are not only a matter of planktic cyanobacteria. Recently, high toxin production has also been attributed to benthic cyanobacterial mats (Sabater et al. 2003, Wood et al. 2020). The specific of toxic benthic cyanobacteria is that they occur also in oligotrophic waters (Echenique-Subiabre et al. 2018). The most common toxins are anatoxin and microcystin, further nodularin and cylindrospermopsin (Wood et al. 2020).

Benthic cyanobacteria

A great diversity of potential habitats on river bottoms facilitates a great diversity of benthic cyanobacteria (Wood et al. 2020). The most common riverine benthic cyanobacteria include:

a) coccoid genera, e.g., *Aphanocapsa*, *Chamaesiphon*, *Pleurocapsa*, or *Chroococcus*,

b) nonheterocystous filamentous genera, e.g., *Phormidium*, *Leptolyngbya*, or *Pseudanabaena*, and

c) heterocystous filamentous genera, e.g., *Nostoc*, *Tolypothrix*, or *Calothrix*

(Mohamed et al. 2006, Loza et al. 2013, Casamatta & Hašler 2016). Many studies show *Phormidium* (*Microcoleus*) to be the dominant genus in riverine biofilms (Heath et al. 2010, McAllister et al. 2016, etc.).

The development of benthic cyanobacterial mats has a cyclic nature. A typical cycle of cyanobacterial mat (=accrual cycle) consists of three phases: a) colonization and attachment, b) growth, and c) detachment (Wood et al. 2015a, McAllister et al. 2016).

The cycle begins either by colonization, or by regrowth of relic populations (McAllister et al. 2016). This process is probably facilitated by the presence of organic compounds and bacteria on a substrate (Wood et al. 2015a). The initial development of cyanobacterial mats often occurs in reaches with higher flow velocities which not only do not pose a danger for coherent mucilaginous mats but are also highly beneficial as they bring essential nutrients to them (Biggs et al. 1998).

The subsequent successful growth of mats depends on favorable conditions in a river. For instance, the highest biomass of *Phormidium*-dominated biofilms was found in riffle areas on cobbles and boulders in periods of increased temperatures (Echenique-Subiabre et al. 2018). As far as nutrients are concerned, McAllister et al. (2016) generalize that proliferations of *Phormidium*-dominated biofilms occur mainly if a sufficient amount of dissolved inorganic nitrogen is present, while concentrations of phosphorus can be limited. A possible explanation for the toleration of lower concentrations of phosphorus is that cyanobacterial mats have their separate biochemistry and can utilize phosphorus from sediments which get caught in them (Wood et al. 2015b). Echenique-Subiabre et al. (2018) observed that the appearance of *Phormidium*-dominated biofilms also reflects light conditions – biofilms in shallow parts of a river are thicker, while those in deeper parts are thin with a higher percentage of coverage. Grazers are another controlling factor of the growth of cyanobacterial mats (Scott & Marcarelli 2012). It has been proposed that the pressure of grazers could be responsible for the toxin production by benthic cyanobacteria (McAllister et al. 2016).

The final phase of the accrual cycle is the mat detachment. Its causes vary. External factors include shear stress and substrate disturbance during higher flow velocities (McAllister et al. 2016). Another mechanism is the detachment of mats due to buoyancy caused by bubbles of oxygen from photosynthesis which get trapped among filaments (Quiblier et al. 2013). Detached mats can be washed up on the river shores where they represent a potential health risk for both animals and humans if containing toxic species (Wood et al. 2015a).

Recognizing the real diversity of riverine benthic cyanobacteria represents a challenging task, because many of these taxa are known to be polyphyletic. Examples include

Phormidium/*Microcoleus* (Casamatta et al. 2005, Hašler et al. 2012), *Geitlerinema/Anagnostidinema* (Casamatta et al. 2005, Hašler et al. 2012, Johansen et al. 2017), or *Leptolyngbya* (Casamatta et al. 2005, Mai et al. 2018, Cordeiro et al. 2020). This is a consequence of a limited number of morphological features, a known cryptic diversity, and the lack of studies focusing on these taxa. Therefore, future revisions are necessary (Casamatta et al. 2005, Hašler et al. 2012, Johansen et al. 2017).

Planktic cyanobacteria

Cyanobacterial plankton is most abundant in reaches which resemble stagnant waters. Such conditions are attained in higher-order streams with very slow flow (Casamatta & Hašler 2016) or in dam reservoirs which release higher amounts of planktic cyanobacteria in the downstream direction (Hašler et al. 2007, Grabowska & Mazur-Marzec 2011).

The abundance of planktic cyanobacteria varies during a year and depends on physical variables such as temperature, conductivity, or discharge (Hašler et al. 2007). It also reflects availability of nutrients (both N and P) and the top-down control of organisms feeding on phytoplankton (Minaudo et al. 2021). Various anthropogenic activities contribute to changes in planktic communities. Consequently, species composition and richness in natural and influenced streams can differ substantially (Hašler et al. 2007).

A characteristic feature of many planktic cyanobacteria is their ability to form toxic blooms which impair water quality and limit species richness. Bloom forming genera reported from rivers include e.g., *Microcystis*, *Anabaena*, *Planktothrix*, and *Aphanizomenon* (Hindák et al. 2006, Hašler et al. 2007). A problematic species capable of bloom formation in rivers is also *Cylindrospermopsis raciborskii* which is widely distributed in tropical areas, but recently it has invaded many temperate freshwater ecosystems (Dvořák & Hašler 2007). Other frequently observed genera in rivers are e.g., *Merismopedia*, *Snowella*, *Aphanocapsa*, *Pseudanabaena*, or *Oscillatoria* (Hindák et al. 2006, Hašler et al. 2007).

The peak abundance of toxic planktic cyanobacteria positively correlates with water temperature (Read et al. 2014). This represents a serious future threat in view of onward climate change which (together with eutrophication) will probably lead to more frequent proliferations of toxic planktonic cyanobacteria in rivers (Pearl & Otten 2013). Thus, it is essential to monitor the state of cyanobacterial proliferations and to find ways to control them not only in lentic water bodies, but also in running waters. Reducing light availability, using chemical agents, bacteria or viruses, or manipulation with flow are possible ways of dealing

with harmful cyanobacterial blooms (Mitrovic et al. 2011, Pearl & Otten 2013). The most effective way, however, is nutrient limitation (Pearl & Otten 2013, Minaudo et al. 2021). A potential disadvantage of this attitude is that the absence of phytoplankton due to lack of phosphorus could facilitate the excessive growth of benthic toxic cyanobacteria which do not require high concentrations of P in water for their growth (Minaudo et al. 2021). For this reason, new strategies for an effective fight against harmful cyanobacteria will probably be necessary in the future.

3. LEPTOLYNGBYA SENSU LATO

3.1. Taxonomy

Leptolyngbya is a common, yet frequently overlooked genus of filamentous non-heterocystous cyanobacteria. The genus was established by Anagnostidis and Komárek (1988), although its species were known sooner, e.g., under the names of *Lyngbya*, *Phormidium*, or *Plectonema* (Anagnostidis & Komárek 1988).

The position of *Leptolyngbya* in a taxonomic system has changed since the establishment of this genus (Fig. 3). Before the description of *Leptolyngbya* as a separate genus, fine species of *Lyngbya*, *Phormidium* or *Plectonema* were clustered in the "LPP group B" based on their morphological similarity (Rippka et al. 1979). When *Leptolyngbya* gen. nov. was established in 1988, it was classified in the order Oscillatoriales, fam. Pseudanabaenaceae, subfam. Leptolyngbyoideae. Komárek et al. (2014) transferred the genus into the order Synechococcales and described a new family – Leptolyngbyaceae. Within the most recent revision, the Leptolyngbyaceae family was divided into four families – Leptolyngbyaceae, Oculatellaceae, Prochlorotrichaceae, and Trichocoleaceae (Mai et al. 2018). Despite its inconspicuous appearance, *Leptolyngbya* s. l. is the most species-rich group in the Synechococcales order (see Tables 1−4).

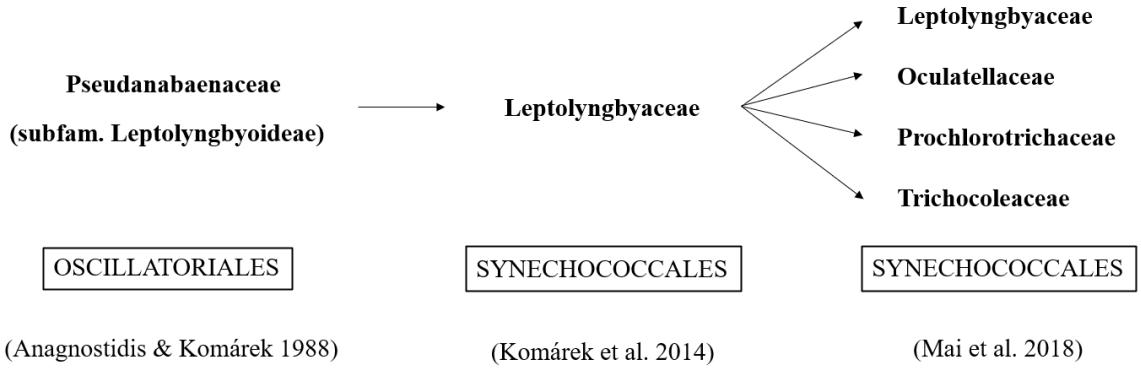


Fig. 3. Scheme of historical development of classification of *Leptolyngbya* s. l.

The genus *Leptolyngbya* has been known to be polyphyletic since its description (Komárek & Anagnostidis 1988, Casamatta et al. 2005, Mai et al. 2018, Cordeiro et al. 2020). The polyphasic approach, i.e. approach merging molecular data with morphology/ecology/ultrastructure, is currently recommended for resolving taxonomic issues in cyanobacteria, including the polyphyly of *Leptolyngbya* (Komárek 2016).

The number of genera within the group *Leptolyngbya* s. l. has recently increased considerably. This has been happening for two major reasons. First, several genera have been split out of *Leptolyngbya* s. s. based on the increasing availability of molecular data. This is the case of *Nodosilinea* (Perkerson et al. 2011), *Cartusia* (Mai et al. 2020), and several other genera. Secondly, many genera have been described from previously understudied environments. For instance, *Neosynechococcus* was isolated from a peatbog (Dvořák et al. 2014), *Timaviella* from caves (Sciuto et al. 2017), and *Aegeococcus*, *Cyanolege*, *Metis*, *Rhodoploca*, and *Thalassoporum* from sponges (Konstantinou et al. 2021).

Leptolyngbya s. l. currently includes 49 genera from four families which are listed in Tables 1−4. The tables were created based on records in AlgaeBase (Guiry & Guiry 2021), Web of Science, and Google Scholar.

Table 1. Genera of the Leptolyngbyaceae family.

Genus	Authors of description	Publication	No. of sp.
Aegeococcus	Konstantinou & Gkelis	Konstantinou et al. 2021	2
Albertania	Zammit et al.	Zammit et al. 2018	2
Alkalinema	Vaz et al.	Vaz et al. 2015	1
Arthronema	Komárek & Lukavský	Komárek & Lukavský 1988	2
Chamaethrix	Dvořák et al.	Dvořák et al. 2017	1
Chroakolemma	Becerra-Absalón & Johansen	Becerra-Absalón et al. 2018	3
Cymatolege	Konstantinou & Gkelis	Konstantinou et al. 2021	2
Euryhalinema	Chakraborty & Mukherjee	Chakraborty et al. 2019	1
Kovacikia	Miscoe, Pietrasiak & Johansen	Miscoe et al. 2016	1
Leibleinia	(Gomont) Hoffmann	Hoffmann 1985	15
Leptodesmis	Raabová, Kovacik & Strunecký	Raabová et al. 2019	2
Leptoelongatus	Chakraborty & Mukherjee	Chakraborty et al. 2019	1
Leptolyngbya	Anagnostidis & Komárek	Anagnostidis & Komárek 1988	136
Leptothoe	Konstantinou & Gkelis	Konstantinou et al. 2021	3
Limnolyngbya	Li & Li	Li & Li 2016	1
Metis	Konstantinou & Gkelis	Konstantinou et al. 2021	1
Monilinema	Malone et al.	Malone et al. 2020	1
Myxacorys	Pietrasiak & Johansen	Pietrasiak et al. 2019	3
Neosynechococcus	Dvořák, Hindák, Hašler, Hindáková	Dvořák et al. 2014	1
Onodrimia	Jahodářová, Dvořák, Hašler	Jahodářová et al. 2017a	1
Pantanalinema	Vaz et al.	Vaz et al. 2015	1
Phormidesmis	Turicchia et al.	Turicchia et al. 2009	5
Pinocchia	Dvořák, Jahodářová & Hašler	Dvořák et al. 2015	1
Planktolyngbya	Anagnostidis & Komárek	Anagnostidis & Komárek 1988	15
Plectolyngbya	Taton et al.	Taton et al. 2011	1
Rhodoploca	Konstantinou & Gkelis	Konstantinou et al. 2021	1
Romeria	(Raciborski) Koczwara	Geitler 1932	18
Scytolyngbya	Song & Li	Song et al. 2015	1
Stenomitos	Miscoe & Johansen	Miscoe et al. 2016	5
Tapinothrix	Sauvageau	Sauvageau 1892	21
Thalassoporum	Konstantinou & Gkelis	Konstantinou et al. 2021	1

Table 2. Genera of the Oculatellaceae family.

Genus	Authors of description	Publication	No. of sp.
Cartusia	Mai, Johansen & Pietrasiak	Mai et al. 2018	1
Drouetiella	Mai, Johansen & Pietrasiak	Mai et al. 2018	3
Elainella	Jahodářová, Dvořák & Hašler	Jahodářová et al. 2017b	1
Kaiparowitsia	Mai, Johansen & Bohunická	Mai et al. 2018	1
Komarkovaea	Mai, Johansen & Pietrasiak	Mai et al. 2018	1
Oculatella	Zammit et al.	Zammit et al. 2012	13
Pegethrix	Mai, Johansen & Bohunická	Mai et al. 2018	4
Shackletoniella	Strunecký, Raabová & Bernardová	Strunecký et al. 2020	1
Thermolyngbya	Sciuto & Moro	Sciuto & Moro 2016	2
Tildeniella	Mai, Johansen & Pietrasiak	Mai et al. 2018	3
Timaviella	Sciuto & Moro	Sciuto et al. 2017	5
Trichotorquatus	Pietrasiak & Johansen	Pietrasiak et al. 2021	4

Table 3. Genera of the Prochlorotrichaceae family.

Genus	Authors of description	Publication	No. of sp.
Haloleptolyngbya	Dadheech et al.	Dadheech et al. 2012	2
Halomicronema	Abed et al.	Abed et al. 2002	2
Lagosinema	Akagha & Johansen	Akagha et al. 2019	1
Nodosilinea	Perkerson & Casamatta	Perkerson et al. 2011	10
Prochlorothrix	Burger-Wiesma et al.	Burger-Wiesma et al. 1989	2

Table 4. Genera of the Trichocoleaceae family.

Genus	Authors of description	Publication	No. of sp.
Trichocoleus	Anagnostidis	Anagnostidis 2001	19

3.2. Markers used in the taxonomy of *Leptolyngbya* s. l.

3.2.1. Morphology

Studying morphological differences is the traditional way of distinguishing between cyanobacterial taxa. *Leptolyngbya* can be characterized by forming long wavy or almost straight filaments which can be rarely pseudobranched, with or without hyaline sheaths. Trichomes are 0,5−3.5 μm wide, immotile or with indistinct trembling, producing motile or immotile hormogonia. Cells are cylindrical, isodiametric, longer or shorter than wide, with thylakoids arranged peripherally. Reproduction occurs as trichome fragmentation, with or without the participation of necridic cells (Komárek & Anagnostidis 2005).

Typical morphological features that differ among leptolyngbyoid species are constrictions at the crosswalls, the presence and the width of sheaths, the width of trichomes, and color (Komárek & Anagnostidis 2005). The length of cells and the presence of necridic cells are important for distinguishing between subgenera *Leptolyngbya* (isodiametric cells, necridic cells present) and *Protolyngbya* (cells longer than wide, necridic cells absent) (Komárek & Anagnostidis 2005). Several genera outside *Leptolyngbya* s. s. possess their own autapomorphic morphological features. *Nodosilinea*, for instance, forms nodules along the filament (Perkerson et al. 2011), *Onodrimia* forms tree-like tufts of hormogonia that attach to other filaments (Jahodářová et al. 2017a), and apical cells of *Oculatella* possess a colorful spot at their tips (Zammit et al. 2012).

However, the morphology of *Leptolyngbya* is very primitive and the number of species substantially exceeds the number of possible phenotypes. Such species which cannot be distinguished based on morphology are called cryptic (Casamatta et al. 2003). Cryptic diversity is also known from higher taxonomic levels. Many genera are morphologically indistinguishable since they were described mainly based on molecular analyses. Even features such as nodules previously described as distinctive for *Nodosilinea* do not currently relate to this genus only – after the revision of the Leptolyngbyaceae family (Mai et al. 2018), a very similar feature was observed also in a newly described *Pegethrix*.

With the ongoing development of molecular methods, morphology alone is not sufficient for precise species determination or for descriptions of new taxa. Thus, information about morphology should be always combined with other types of data (Komárek et al. 2016).

3.2.2. Ecology

Leptolyngbya s. l. occurs both in aquatic and terrestrial environments, from polar to tropical regions (Komárek 2007, Jahodářová et al. 2017a). Despite the cosmopolitan distribution of this genus, many species have specific ecological demands, and therefore ecological data can provide valuable information (Komárek & Anagnostidis 2005). For this reason, the basic identification key by Komárek & Anagnostidis (2005) divides the genus into ecological groups in the first step.

3.2.2.1. Freshwater species in central Europe

Leptolyngbya s. s.

Freshwater species occupy bottoms of lotic or lentic habitats as endolithon, epilithon, epipsammon, epiphyton, or metaphyton (Komárek & Anagnostidis 2005, Kaštovský et al. 2010). The presence of particular species often reflects conditions in the aquatic ecosystem, e.g., trophy, pollution, or temperature (Komárek & Anagnostidis 2005). Common species of the subgenus *Leptolyngbya* in central Europe include *L. boryana*, *L. foveolarum*, *L. tenerrima*, and *L. subtilissima*; the subgenus *Protolyngbya* is most frequently represented by *L. angustissima*, *L. valderiana*, and *L. tenuis* (Komárek & Anagnostidis 2005, Kaštovský et al. 2010).

Type species *L. boryana* is a typical freshwater cyanobacterium, occurring predominantly as metaphyton among algae and water plants (Komárek & Anagnostidis 2005). The species tolerates pollution and high trophy (Loza et al. 2013). It was shown to be resistant to arsenic, thus has a potential to be utilized in bioremediation in aquatic ecosystems (Zhu et al. 2020).

L. foveolarum is a euryvalent species which inhabits submerged habitats as well as moist soils or margins of mineral and thermal springs. Like *L. boryana*, it is resistant to water pollution (Komárek & Anagnostidis 2005).

L. tenerrima grows in metaphyton, frequently in waters with higher concentrations of organic matter and nutrients (Komárek & Anagnostidis 2005).

L. subtilissima is a subaerophytic species, but it is also known from stony littoral zones of rivers, lakes and ponds (Komárek & Anagnostidis 2005).

L. angustissima thrives both in stagnant and flowing waters, as well as in moist soils and on walls and rocks in various regions from the tropics to Antarctica

(Komárek & Anagnostidis 2005). This species was suggested as an indicator of oligotrophic waters with low conductivity, low inorganic nitrogen, and high dissolved oxygen (García & Aboal 2014).

L. valderiana is a cosmopolitan species growing in stagnant and flowing waters, sometimes also in brackish waters and sea (Komárek & Anagnostidis 2005).

Leptolyngbya tenuis can be found in moist soils or in shallow stagnant waters (Komárek & Anagnostidis 2005). The species was also reported from oligotrophic running waters (Loza et al. 2013).

Leptolyngbya s. l.

Leibleinia epiphytica is the most common species of the genus *Leibleinia* (Kaštovský et al. 2010). It is a freshwater epiphyte growing on *Cladophora*, *Oedogonium*, and other algae or cyanobacteria (Komárek & Anagnostidis 2005). The whole genus *Leibleinia* is problematic because there is a lack of morphological features and molecular data are missing (Komárek & Anagnostidis 2005, Casamatta & Hašler 2016).

Phormidesmis molle (originally *Phormidium molle*) is a cosmopolitan, euryvalent species occurring as epi/metaphyton in mesotrophic to eutrophic stagnant waters or soils (Turicchia et al. 2009, Kaštovský et al. 2010). *Phormidesmis communis* was recently described as a freshwater species from Polar regions but is expected to have a cosmopolitan distribution (Raabová et al. 2019).

The most frequently encountered species of *Planktolyngbya*, *P. limnetica*, occurs in meso/eu/hypertrofic stagnant waters freely as a plankton or entangled among other algae (Komárek & Anagnostidis 2005, Kaštovský et al. 2010). *P. contorta* is a rarer representative of this genus (Kaštovský et al. 2010)

Short trichomes of *Romeria* species can be found in freshwater environments as epiphyton, metaphyton, or plankton (Komárek & Anagnostidis 2005). Although the genus is species-rich, all species seem to be rare in central Europe (Kaštovský et al. 2010).

Oculatella is predominantly a terrestrial genus inhabiting soils (Osorio-Santos et al. 2014, Becerra-Absalón et al. 2020, Jung et al. 2020). However, *O. hafneriensis* was described from the bottom of a lake in Austria (Osorio-Santos et al. 2014), thus it could possibly occur in the Czech Republic as well.

Drouetiella lurida is a freshwater species with a worldwide distribution (Komárek & Anagnostidis 2005, Mai et al. 2018). *D. lurida* (originally *Leptolyngbya lurida*) is known mainly from stagnant waters (Komárek & Anagnostidis 2005). It has a specific purple/brown color in actively growing populations (Mai et al. 2018).

Tapinothrix is a heteropolar, species-rich, mostly epilithic genus. *T. janthina* and *T. crustacea* occur commonly in running waters, but *T. janthina* prefers silicate substrates, while *T. crustacea* thrives on calcium carbonate substrates (Komárek & Anagnostidis 2005). *T. stagnalis* is an epiphytic species occupying eutrophic stagnant waters (Komárek & Anagnostidis 2005, Kaštovský et al. 2010).

Prochlorothrix is a green cyanobacterium containing chlorophylls a+b, and thus resembles green algae more than cyanobacteria (Burger-Wiesma et al. 1989). However, the genus belongs to the group of *Leptolyngbya* s. l. as it used to be a part of the Leptolyngbyaceae family (Komárek et al. 2014) and the current family Prochlorotrichaceae contains typical leptolyngbyoid taxa such as *Nodosilinea* (Mai et al. 2018). *Prochlorothrix hollandica* is a rather rare planktonic species discovered in a shallow eutrophic lake (Burger-Wiesma et al. 1989, Pinevich et al. 2012).

3.2.2.2. Soil species in central Europe

Leptolyngbya s. s.

Typically soil species of the subgenus *Leptolyngbya* are not reported from central Europe. The only recorded species from this subgenus is *L. foveolarum* which is still considered rather a freshwater species (Komárek & Anagnostidis 2005).

Subgenus *Protolyngbya* is richer in edaphic species. *L. nostocorum* is probably the most common and grows in various soils, moss pools, marshes, jars of water, and in the mucilage of other cyanobacteria or algae (Komárek & Anagnostidis 2005, Kaštovský et al. 2010). *L. voronichiniana* is a soil species growing also as an epiphyte on colonies of *Nostoc commune*; *L. hansgirgiana* commonly occurs on wet soils, especially in the proximity of tree bases; *L. tenuis* and *L. notata* are known both from edaphic and freshwater environments (Komárek & Anagnostidis 2005).

Leptolyngbya s. l.

Chroakolemma edaphica (formerly *Leptolyngbya edaphica*) is a soil/subaerophytic species of newly established genus *Chroakolemma* (Komárek & Anagnostidis 2005, Becerra-Absalón et al. 2018). Other species, *C. opaca* a *C. pellucida* were described from semi-desert soil crusts (Becerra-Absalón et al. 2018).

Oculatella is a genus originally described from hypogea as *Lyngbya* sp. (Albertano & Grilli-Caiola 1988), later as *Leptolyngbya* "Albertano/Kováčik-red" (Komárek & Anagnostidis 2005), and finally as an independent genus (Zammit et al. 2012). Recently, several species were described from arid and semi-arid soils (Osorio-Santos et al. 2014, Becerra-Absalón et al. 2020) and one species was isolated from arctic soil crust (Jung et al. 2020). *Oculatella* sp. was also reported from wet soil in the Czech Republic (Hajská 2019).

Genus *Nodosilinea* contains four soil species – *N. conica*, isolated from an American desert (Perkerson et al. 2011), *N. ramsarensis*, described from soil in the proximity of a thermal spring in Iran (Heidari et al. 2018), and *N. signiensis*, forming mats on soils in Antarctica (Radzi et al. 2019). The fourth species, *N. epilithica*, was originally isolated from a house wall (Perkerson et al. 2011) but was sampled from soils too (Temraleeva 2018). As regards temperate zones, an unspecified *Nodosilinea* species was isolated from a dry puddle in the Czech Republic (Hajská 2019).

Pseudophormidium hollerbachianum (formerly *L. hollerbachiana*) is a wide-spread soil species of *Pseudophormidium*, currently belonging to the *Microcoleaceae* family. Pietrasiak et al. (2019), however, analysed strains from American deserts which were formerly assigned to *P. hollerbachianum* and described two species of a novel genus *Myxacorys*, belonging to the Leptolyngbyaceae family based on molecular analyses. Evidence for the polyphyly of the genus *Pseudophormidium* can be found also in Osorio-Santos et al. (2014). *P. hollerbachianum*, well-known also from central European soils (Komárek & Anagnostidis 2005, Kaštovský et al. 2010), could therefore belong to the group of *Leptolyngbya* s. l.

Phormidesmis molle is a freshwater species occurring also in soils (Turicchia et al. 2009, Kaštovský et al. 2010). Other soil species of the genus were observed in Polar regions (Raabová et al. 2019).

Soil species are also possible to find in other genera, such as *Chamaethrix* (Dvořák et al. 2017), *Kaiparowitsia* (Mai et al. 2018), *Trichocoleus* (Mühlsteinová et al. 2014), *Trichotorquatus* (Pietrasiak et al. 2021), and others. Nevertheless, habitats/regions where they were sampled differ considerably from typical temperate conditions of the central Europe, thus the likelihood of their occurrence in the Czech Republic is not certain.

3.2.3. Molecular markers

Genetic markers

Molecular sequencing is the basic method used in taxonomic works which are based on the currently recommended polyphasic approach (Komárek 2016). In modern cyanobacterial taxonomy, 16S rRNA gene and 16S−23S ITS region are the most widely used DNA markers (Vaz et al. 2015, Dvořák et al. 2017, Sciuto et al. 2017, Mai et al. 2018 etc.).

16S rRNA gene codes the small subunit of (cyano)bacterial ribosomes. This part of DNA is a useful phylogenetic tool as it is present in all bacteria, it contains both conservative and variable regions, and it is large enough to provide statistically relevant information (Patel 2001). The gene was shown to be appropriate for distinguishing taxa at generic level, but its use for discerning taxa at lower/higher levels has been questioned (Fox et al. 1992, Mareš 2018).

The second broadly used genetic marker is 16S−23S ITS region. The abbreviation ITS stands for internal transcribed spacer which is located between genes for small (16S) and large (23S) ribosomal subunits. This region is suitable for species delimitation, but it should not be used as a single marker (Mareš 2018). ITS sequences can be utilized for constructing phylogenetic trees and for predicting RNA secondary structures (Sciuto et al. 2017).

An alternative option is to obtain sequences from more loci and to utilize them for multilocus phylogenetic tree constructions. Such robust data are useful e.g., for revisions of taxa above the genus level (Komárek et al. 2014, Mareš 2018).

Protein markers

Molecular data do not necessarily need to be of a genomic origin. The use of peptide/protein mass spectra is a recently developing method applicable for elaborate taxonomic studies, as well as for other biological studies (Singhal et al. 2015, Šebela et al. 2018). It was shown that peptide/protein composition (displayed as a peptide/protein mass spectrum) is species-specific

and trees constructed based on this type of data are comparable to 16S rRNA phylogenetic trees (Šebela et al. 2018).

The technique used for obtaining peptide/protein spectra is called MALDI-TOF MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The potential of MALDI-TOF MS lies in its rapidness, cheapness, simplicity, and high sensitivity in comparison with genetic markers (Singhal et al. 2015). These benefits are achieved largely because of the possibility of using intact cells (Singhal et al. 2015, Šebela et al. 2018).

The number of studies utilizing the method of MALDI-TOF MS is currently limited (Imanishi et al. 2016, Sun et al. 2016, Šebela et al. 2018). However, these studies provided promising results which prove the potential of this method to be utilized in a variety of biological studies, including the taxonomic ones (Šebela et al. 2018).

4. METHODS

4.1. Sampling

Samples for subsequent analyses were obtained from the Svitava River and stagnant water bodies in its surrounding area.

Svitava River

The Svitava River is a 97-km-long river which flows in eastern Bohemia (the Czech Republic) (EDPP 2021). It springs in a coniferous forest near the town of Svitavy, flows in a southern direction, and merges with the Svratka River in the city of Brno. Six municipalities with the population above 3,000 inhabitants (Svitavy, Letovice, Rájec-Jestřebí, Blansko, Adamov, and Brno) are situated on this river (ČSÚ 2021).

Water quality is measured regularly in specific profiles of the river. According to the latest data, the Svitava River belongs to streams with the most unsatisfactory water quality in the Morava River drainage basin; in several profiles (Letovice, Blansko), more than one indicator of water pollution was found during the latest measurements (Procházková et al. 2020).

Samples were collected in July 2019. Before sampling, the river was divided into imaginary segments of the length ± 10 km. In each segment, one point was chosen as a sampling site (Fig. 5). At each of these sites, three samples were obtained – one from the river bottom, one from the boundary between water surface and bank, and one from the soil surface on the bank (Fig. 4). Physical parameters (pH, temperature, and conductivity of the water) were measured during the sampling. In addition, pH and conductivity of soil from the river surrounding were measured after the transfer of samples to the phycological laboratory at Palacký University in Olomouc.

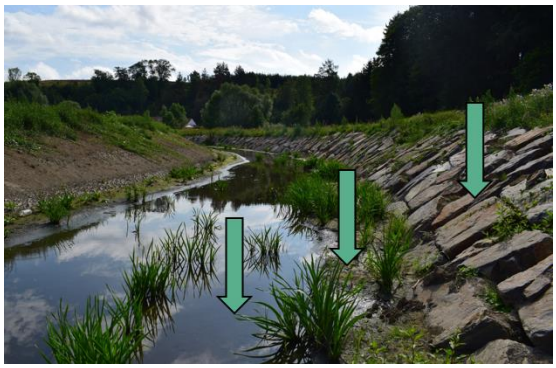


Fig. 4. Sampling site with labelled points of sample collection.

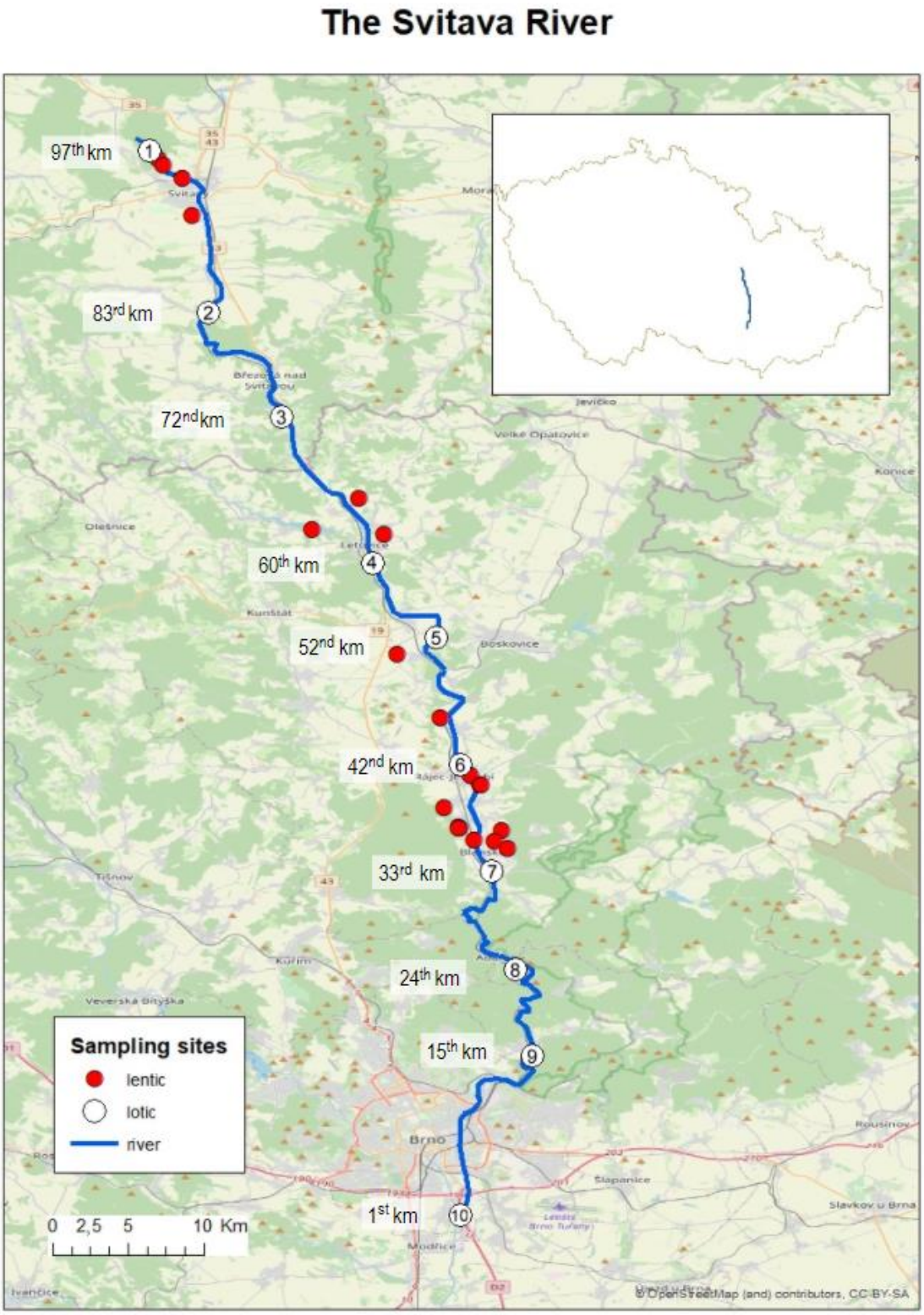


Fig. 5. The map of the Czech Republic with labeled Svitava River. 1 – Svitavy, 2 – Hradec nad Svitavou, 3 – Moravská Chrastová, 4 – Letovice, 5 – Mladkov, 6 – Rájec-Jestřebí, 7 – Blansko, 8 – Adamov, 9 – Bílovice nad Svitavou, 10 – Brno. Created in the ArcGIS 10.4 (ESRI 2016).

Fig. 6. Sampling sites along the Svitava River. 1 – Svitavy, 2 – Hradec nad Svitavou, 3 – Moravská Chrastová, 4 – Letovice, 5 – Mladkov, 6 – Rájec-Jestřebí, 7 – Blansko, 8 – Adamov, 9 – Bílovice nad Svitavou, 10 – Brno.

Lentic sampling sites

Stagnant water bodies chosen as sampling sites were situated in the proximity of the Svitava River. Samples were obtained in June 2020 from a variety of benthic substrates – stones, dead leaves and branches, mud, or sand. Four sampling sites were quarries, one was a large water reservoir, but most sites were constituted by ponds. Like in the Svitava River, physical parameters (pH, temperature, and conductivity) were measured at each site. The location of these sampling sites is depicted in appendices 1−4.

Terrestrial samples

To assess morphological and molecular differences between populations from freshwater and terrestrial habitats, strains from the bachelor thesis were added to the analyses. These samples were obtained from meadows, gardens, edges of puddles, and other terrestrial habitats. Strains from Ploština, Vlachovice, and Vysoké Pole were collected by Bc. Adéla Smolíková.

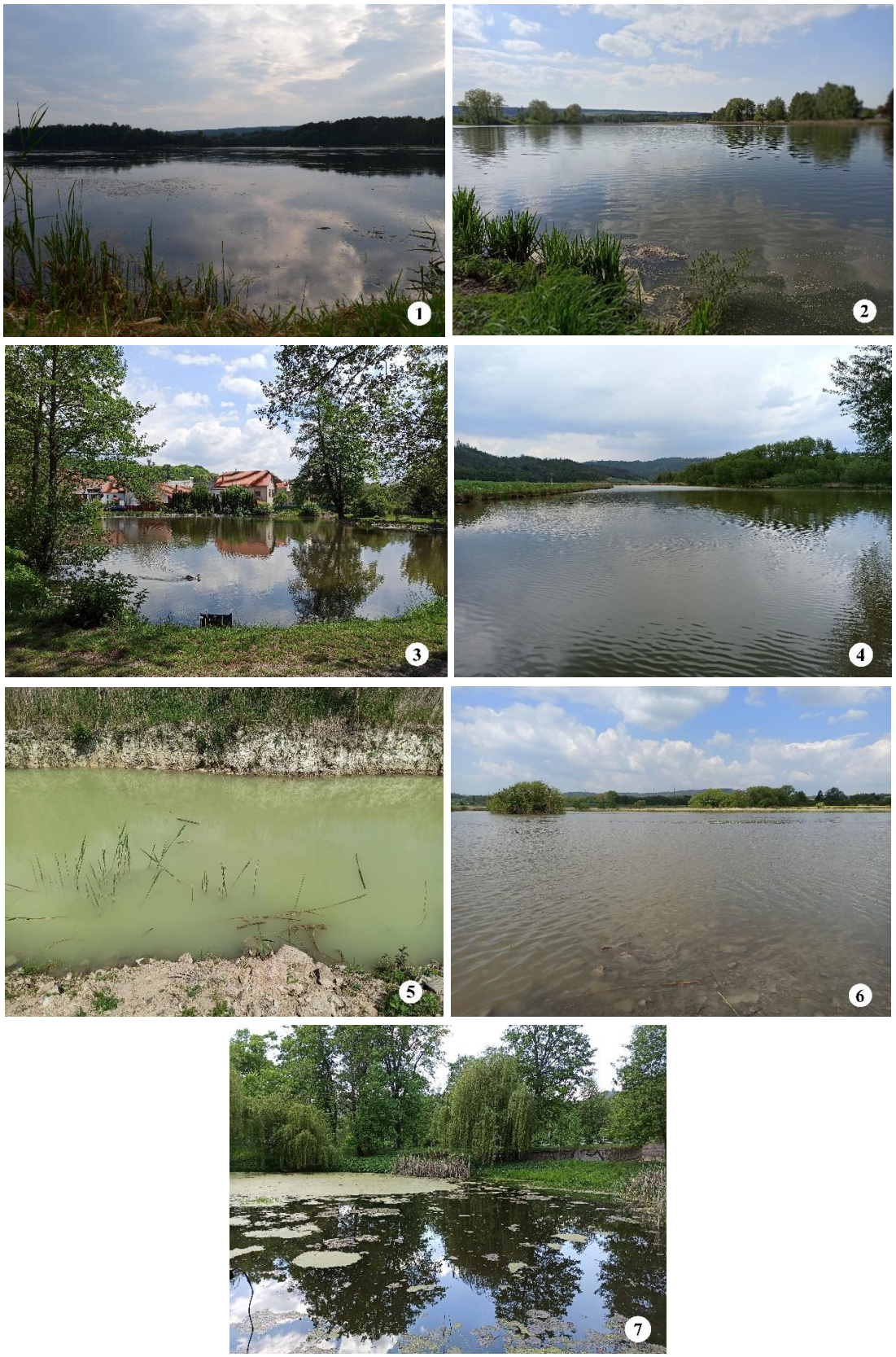


Fig. 7. Stagnant water bodies. 1 – Rosnička Pond, 2 – Svitavský Pond, 3 – Klimšák Pond, 4 – Klemovák Pond, 5 – Dolní Lhota Quarry, 6 – Skalice Pond, 7 – Blansko Pond.

4.2. Cultivation

Zehnder (Z) medium (Staub 1961) was used for the cultivation of cyanobacteria. Pure strains were obtained by continuous transfer of cyanobacterial biomass to new test tubes with fresh Z medium. Purification through drops of sterile water (described in Hajská 2019) was performed to secure clonal growth from one filament. All treatment with cultures was performed in a horizontal box (AURA HZ 48) to prevent them from being contaminated.

4.3. Analyses

4.3.1. Morphological analysis

The morphology of strains was continuously studied using a light microscope (Zeiss Primo Star, objective 40× and immerse objective 100×) with an attached camera (AxioCam Erc5s, 5 MPx). Photographs of strains were captured and edited using AxioVision Rel. 4.8.1. Further editing was done in Zoner Photo Studio 14. Identification key by Komárek and Anagnostidis (2005) was used for species determination.

4.3.2. 16S rRNA gene and 16S−23S ITS region analysis

PCR amplification and sequencing

Cyanobacterial DNA was isolated with the use of the CTAB method (Doyle J. 1991). 16S rRNA gene and 16S−23S ITS region were amplified using the PCR primers P2 (forward; 5‘–GGGGAATTTTCCGCAATGGG–3‘) and P1 (reverse; 5‘–CTCTGTGTGCCTAGGTATCC–3‘) (Boyer et al. 2002). The reaction solution was prepared for the volume 40 μl/sample (Master-mix with the polymerase Emerald 20 μl, P1 1 μl, P2 1 μl, sterile water 17 μl, and 1 μl of template DNA). The PCR reaction was performed under the following conditions: initial denaturation (10 s, 98 °C), 25 cycles of denaturation (10 s, 98 °C), annealing (30 s, 57 °C), and extension (1 min 40 s, 72 °C), and the final extension (10 min., 72 °C).

PCR products were analyzed using gel electrophoresis and purified using the commercial kit E. Z. N. A. Cycle Pure Kit (Omega Bio-Tek, Georgia, USA) following the manufacturer’s manual.

PCR products were commercially sequenced by Sanger sequencing (Macrogen Europe B. V., Amsterdam, Netherlands) using two additional primers: P5 (forward, 5‘–TGTACACACCGCCCGTC–3‘) and P8 (reverse, 5’– AAGGAGGTGATCCAGCCACA–3‘)

(Boyer et al. 2001, 2002). Sequences were then assembled and proofread using the Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA).

Phylogenetic analysis

Two datasets of 16S rRNA gene sequences were used for phylogenetic trees constructions. The first one contained only sequences from isolated strains (a total of 18 sequences) and was used for the comparison with results from the MALDI-TOF MS analysis. The second dataset contained the same sequences plus similar sequences which were obtained using BLAST from the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (a total of 46 sequences). Multiple sequence alignment was performed in MEGA X, ver. 10.1.5 (Kumar et al. 2018) using the Muscle algorithm (Edgar 2004). The alignment was trimmed using the GBlocks, version 0.91b (Castresana 2000). Aligned sequences from the first dataset consisted of 1,066 positions, sequences from the second one of 942 positions.

Maximum likelihood and maximum parsimony analyses were performed in MEGA X with 500 Bootstrap replications. The most appropriate model for ML analysis was evaluated using the Jmodel test (Posada 2008). Based on Akaike Information Criterion, the GTR+G+I model (Nei & Kumar 2000) was selected for the analysis.

The Bayesian inference was performed in MrBayes 3.2.7 (Ronquist & Huelsenbeck 2003) with four runs (each with 3 heated and 1 cold chains) for 5 000 000 generations. The analysis was performed using the GTR model. The sampling frequency was each 1000^{th} generation. Twenty-five percent of trees were discarded as burn-in.

The table with p-distances was generated in MEGA X. The prediction of 16S−23S ITS secondary structures was performed using the Mfold web server (Zuker 2003).

4.3.3. MALDI-TOF MS analysis

MALDI-TOF MS analysis was performed on sixteen strains. During a preparation for analysis, small amounts of cyanobacterial biomass were inserted into pits of a MALDI-TOF plate. One μl of matrix [sinapic acid (SA, 15 $mg.ml^{-1}$) and ferulic acid (FA, 5 $mg.ml^{-1}$) dissolved in acetonitrile (ACN):trifluoro acetic acid (TFA, 2 %) = 7:3] was added to each pit where this mixture dried and crystallized (Šebela et al. 2018). Peptides/proteins were then separated and detected in a spectrometer (Microflex LRF, Brucker Daltonics Inc.). The spectral data were checked and processed using flexAnalysis 3.4 and MALDI Biotyper 3.1 (Brucker Daltonics Inc.).

Hierarchical clustering of selected spectra was made using RStudio interface with MALDIquant and pvclust packages (Gibb & Strimmer 2012, Suzuki & Shimodaira 2006). Spectra with available 16S rRNA gene sequences were selected for clustering. Transformation and calibration of spectra was performed prior to their alignment. Complete clustering and correlation distance methods were used for a tree construction. Spectral clustering were transformed into nexus file using MALDIrppa package (Palarea-Albaladejo et al. 2018) and a direct comparison between 16S rDNA data and MALDI-TOF MS spectra was made using the SplitsTree4 (Huson & Bryant 2006).

5. RESULTS

5.1. Sampling sites and environmental variables

River

Three samples from permanently submerged environments contained leptolyngbyoid taxa. These samples were obtained at localities 4 (Letovice), 7 (Blansko) and 9 (Bílovice nad Svitavou). Water pH in these three sampling sites varied from 7.6 to 7.9; the range of conductivity was 379−531 μS.cm^{-1} (see Table 5). Samples from these localities were usually dominated by *Phormidium*/*Microcoleus* species, while leptolyngbyoid species were represented only by a few individual filaments hidden among other taxa.

Samples taken on the boundary between water surface and bank contained leptolyngbyoid taxa also in three cases. They were collected at localities 5 (Mladkov), 6 (Rájec-Jestřebí), and 7 (Blansko). Like in strictly aquatic samples, leptolyngbyoid species from the edge of the river represented only a minor part of the cyanobacterial community.

Four samples obtained from soils near the river contained leptolyngbyoid taxa. These samples were collected at localities 2 (Hradec nad Svitavou), 4 (Letovice), 6 (Rájec-Jestřebí), and 9 (Bílovice nad Svitavou). At these sampling sites, cyanobacteria mostly did not form any visible mats and the soil appeared to be bare. There was not a great diversity of cyanobacterial/algal taxa in soil samples, thus the isolation of leptolyngbyoid species for subsequent analyses did not pose such a difficulty as in the samples from an aquatic environment. The range of soil pH at localities where *Leptolyngbya* s. l. was present was 6.3−8.7; the conductivity was 79–899 μS.cm^{-1} (Table 5).

Stagnant waters

Altogether 35 samples were taken from stagnant water bodies (Table 6). Almost all habitats were submerged; only several samples were collected from the boundary between water surface and bank. Leptolyngbyoid taxa were found in five samples. The pH range in these sampling sites was narrow, 8.0–8.4, and the conductivity was 356 to 807 μS.cm^{-1}. Three samples were epipelic, two epilithic. Several samples of *Leptolyngbya* s. l. were also obtained from edges of puddles.

Terrestrial habitats

Terrestrial samples outside the river/pond reach were obtained mostly from meadows and gardens. *Leptolyngbya* s. l. was abundant in these samples. As these samples were collected during my bachelor studies when measurements of physical parameters were not performed, values of pH and conductivity are not available.

Table 5. Sampling sites along the Svitava River.

Locality	River km	Soil		Water				GPS (N)	GPS (E)
		pH	Conductivity [µS.cm^{-1}]	pH	Conductivity [µS.cm^{-1}]	t [°C]	Substrate		
Svitavy – spring	97	5.9	10	6.7	121	18.5	epipelon	49°46'55"	16°26'45"
Hradec nad Svitavou	83	6.3	899	7.4	863	23.5	epipsammon	49°41'06"	16°28'51"
Moravská Chrastová	72	6.8	198	7.8	445	12.9	epilithon	49°37'23"	16°31'27"
Letovice	60	7.4	79	7.9	379	13.8	epipsammon, epilithon	49°32'09"	16°34'43"
Mladkov	52	6.9	238	7.9	463	17.4	epipsammon, epilithon	49°29'29"	16°37'00"
Rájec-Jestřebí	42	7.2	55	7.8	462	18.8	epipelon, epilithon	49°24'57"	16°37'52"
Blansko	33	7.2	50	7.6	531	20.4	epipelon	49°21'08"	16°38'58"
Adamov	24	6.9	133	7.9	470	19.4	epipsammon, epilithon, epiphyton	49°17'36"	16°39'50"
Bílovice nad Svitavou	15	8.7	430	7.7	462	21.1	epilithon	49°14'32"	16°40'26"
Brno	1	7.6	152	7.6	479	22.4	epipsammon, epilithon	49°08'50"	16°37'51"

Table 6. Sampling sites – stagnant waters.

Locality	Sample origin	pH	t [°C]	Conductivity [µS.cm^{-1}]	GPS (N)	GPS (E)
Retention Basin (Svitavy)	pit filled with water epipelon	7.3	23.8	62	49°46'35"	16°27'03"
Rosnička Pond (Svitavy)	metaphyton	8.4	25.2	362	49°46'23"	16°27'13"
Svitavský Pond (Svitavy)	epilithon metaphyton	8.1	18.2	421	49°45'54"	16°27'53"
Lánský Pond (Svitavy)	epiphyton epipelon	8.2	17.3	382	49°44'36"	16°28'15"
Pond in Hradec nad Svitavou	epiphyton metaphyton epipelon	7.5	18.7	563	49°41'07"	16°28'53"
Slatinka Pond	epilithon	7.9	10.8	514	49°34'29"	16°34'12"
Letovice Reservoir	metaphyton epilithon	8.1	14.4	369	49°33'20"	16°32'33"
Letovický Pond	epipelon epilithon	8.1	15.7	581	49°33'10"	16°35'07"
Skalice Pond	epiphyton (branches)	8.0	15.1	807	49°28'54"	16°35'36"
Klemovák Pond	epipelon epilithon	8.1	17.4	431	49°26'37"	16°37'09"
Klimšák Pond	epiphyton epilithon	8.1	17.0	503	49°24'33"	16°38'12"

Table 6 cont.

Locality	Sample origin	pH	t [°C]	Conductivity [µS.cm^{-1}]	GPS (N)	GPS (E)
Sládkovy Rybníčky A	epilithon	7.9	62.2	1,039	49°24'13"	16°38'32"
Sládkovy Rybníčky B	epipsammon	8.2	63.0	868	49°24'14"	16°38'35"
Spešov Quarry	metaphyton (moss) epipelon	8.1	61.9	321	49°23'25"	16°37'15"
Dolní Lhota – Quarry A	epipelon	8.2	65.0	388	49°22'42"	16°37'48"
Dolní Lhota – Quarry B	epilithon epipelon	8.1	62.6	356	49°22'42"	16°37'46"
Dolní Lhota – Quarry C	epilithon	8.0	67.6	381	49°22'42"	16°37'45"
Blansko Pond	epiphyton epilithon	7.8	57.9	520	49°22'16"	16°38'19"
Zborovec Pond	epiphyton (branch, cattail) metaphyton (moss) epilithon	7.8	57.4	604	49°22'35"	16°39'19"
Sloupečník Pond	epilithon	7.8	61.7	433	49°22'13"	16°39'02"
Palava Pond	epipelon	8.1	64.2	422	49°21'57"	16°39'32"

5.2. Morphological analysis

Species of *Leptolyngbya*, *Leibleinia*, *Nodosilinea*, *Jaaginema*, and *Drouetiella* were observed in collected samples. Morphologically similar *Anagnostidinema* and *Pseudanabaena* species were added to complete the information on the presence of thin filamentous cyanobacteria at the studied sites.

5.2.1. River

Morphological features of strains isolated from the Svitava River are summarized in Table 7.

***Leptolyngbya* sp. E6**

Morphology: Filaments rather short (max. length 125 μm), slightly undulated, isopolar, rarely pseudobranched; sheath thin and colorless; trichomes blue green to green, constricted at the cross walls, ± 1.5 μm wide; cells isodiametric; apical cell rounded; reproduction by motile hormogonia (trembling); necridic cells not observed (Fig. 8).

Habitat: Soil near the river (pH 6.3, conductivity 899 $\mu S.cm^{-1}$).

Locality: 2 (Hradec nad Svitavou).

Fig. 8. *Leptolyngbya* sp. E6 (Hradec nad Svitavou). Scale bar 5 μm.

***Leptolyngbya* sp. s. l. E3**

Morphology: Filaments mostly short (max. length 225 μm), straight or slightly undulated, isopolar, not pseudobranched; sheath very thin, almost inconspicuous; trichomes blue green to green, constricted at the cross walls, 2.0−2.3 μm wide; cells isodiametric or slightly shorter/longer than wide; apical cell conical, mostly distinctly elongated; hormogonia slightly motile (trembling), mostly very short; necridic cells not observed. (Fig. 9).

Habitat: Soil near the river (pH 7.4, conductivity 79 $\mu S.cm^{-1}$).

Locality: 4 (Letovice).

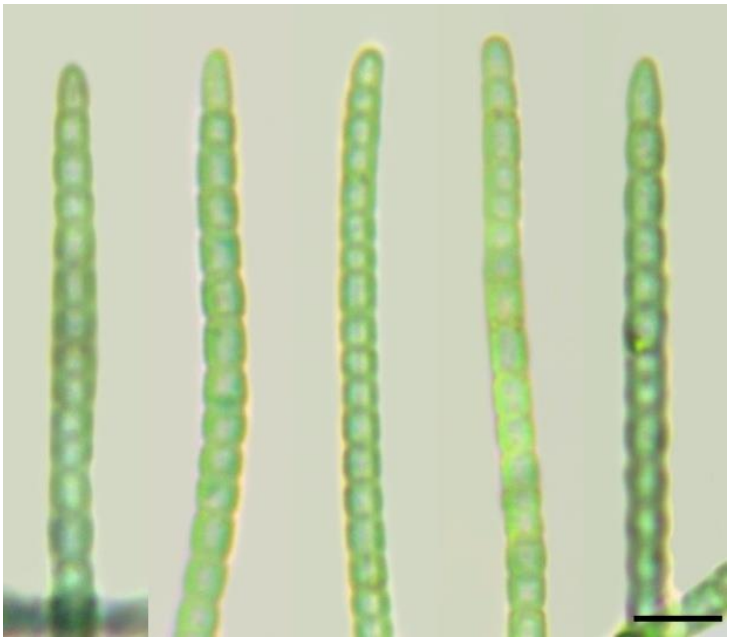


Fig. 9. *Leptolyngbya* sp. s. l. E3 (Letovice). Scale bar 5 μm.

***Leptolyngbya foveolarum* E31**

Morphology: Filaments long, slightly undulated, isopolar; sheath thin and colorless; trichomes blue green to green, long, constricted at the cross walls, 2.0 μm wide; cells isodiametric; apical cells rounded; hormogonia and necridic cells present (Fig. 10).

Habitat: Boundary between aquatic and terrestrial environment (water pH 7.9, conductivity 463 $\mu S.cm^{-1}$).

Locality: 5 (Mladkov).

***Pseudanabaena catenata* E30**

Morphology: Filaments rather short (max. length 175 μm), undulated, with thin, colorless sheath; trichomes olive green/reddish, very distinctly constricted at the cross walls, 1.9 μm wide; cells mostly slightly longer than wide or isodiametric, rarely shorter than wide; apical cell rounded to obtuse conical; hormogonia motile; necridic cells absent (Fig. 11).

Habitat: Soil near the river (pH 6.9, conductivity 238 $\mu S.cm^{-1}$).

Locality: 5 (Mladkov).

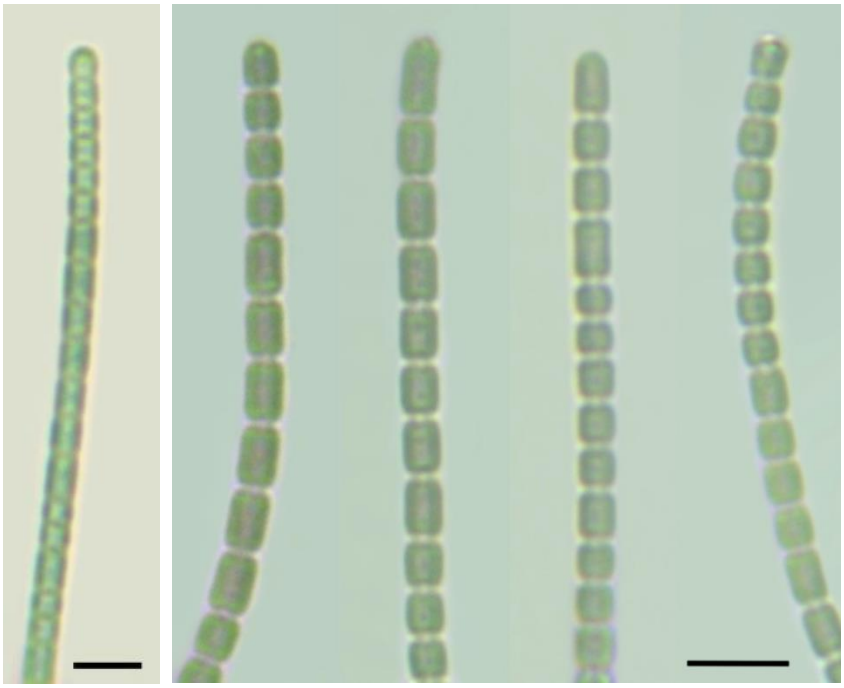


Fig. 10. *Leptolyngbya foveolarum* E31 (Mladkov). Scale bar 5 μm.

Fig. 11. *Pseudanabaena catenata* E30 (Mladkov). Scale bar 5 μm.

***Drouetiella lurida* E7**

Morphology: Filaments usually 625−750 μm long, straight or undulated, isopolar; sheath thin and colorless; false branching rarely observed; trichomes olive-green/reddish/brownish; rather indistinctly constricted at the cross walls, ± 1.7 μm wide; cells isodiametric or slightly shorter/longer than wide, sometimes containing a granule; apical cell rounded; hormogonia immotile, without necridic cells (Fig. 12).

Habitat: Boundary between aquatic and terrestrial environment (water pH 7.8, conductivity 462 $\mu S.cm^{-1}$).

Locality: 6 (Rájec-Jestřebí).

Fig. 12. *Drouetiella lurida* E7 (Rájec-Jestřebí). Arrows – granules. Scale bar 5 μm.

***Leptolyngbya* sp./*Nodosilinea epilithica* E5**

Morphology: Filaments long (max. length 1250 μm), slightly undulated, isopolar; sheath thin and colorless; false branching present; trichomes green to blue green, distinctly constricted at the cross walls, ± 1.7 μm wide; cells isodiametric or slightly longer than wide; apical cell rounded; hormogonia immotile, necridic cells present (Fig. 13).

Habitat: Soil near the river (pH 7.2, conductivity 55 $\mu S.cm^{-1}$).

Locality: 6 (Rájec-Jestřebí).

Note: *Nodosilinea epilithica* based on 16S rRNA and ITS 16S−23S secondary structures analyses, but characteristic nodules were not observed under laboratory conditions.

***Leibleinia* sp. E32**

Morphology: Filaments long, undulated, sheath distinct, but colorless; trichomes green, not or only very slightly constricted at the cross walls, 1.1−1.3 μm wide; cells longer than wide; apical cell obtuse conical; hormogonia present, necridic cells not observed (Fig. 14).

Habitat: Soil near the river (pH 7.2, conductivity 55 $\mu S.cm^{-1}$), growing spirally as an epiphyte on *Microcoleus* sp.

Locality: 6 (Rájec-Jestřebí).

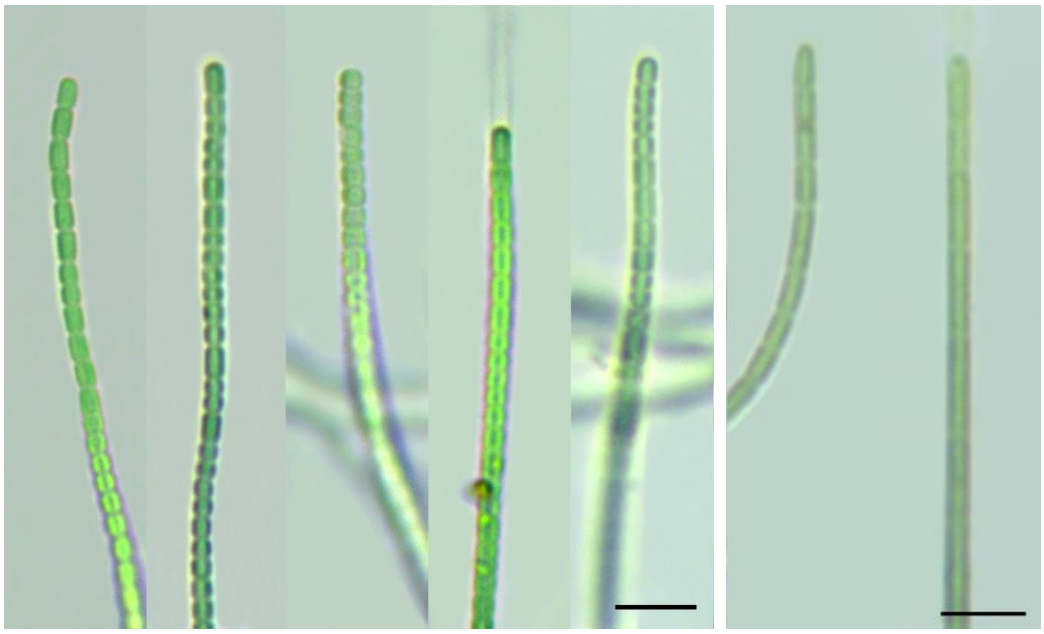


Fig. 13. *Leptolyngbya* sp./*Nodosilinea epilithica* E5 (Rájec-Jestřebí). Scale bar 5 μm.

Fig. 14. *Leibleinia* sp. E32 (Rájec-Jestřebí). Scale bar 5 μm.

***Leptolyngbya* cf. *margaritata* E33**

Morphology: Filaments long, undulated, isopolar; sheath distinct, thick and colorless, up to 3.4 μm wide; trichomes blue green to green, constricted at the cross walls, 1.7 μm wide; cells isodiametric or slightly longer than wide; apical cell rounded; hormogonia present, necridic cells not observed (Fig. 15).

Habitat: Mud with half-decayed leaves on the river bottom (pH 7.6, conductivity 531 $\mu S.cm^{-1}$).

Locality: 7 (Blansko).

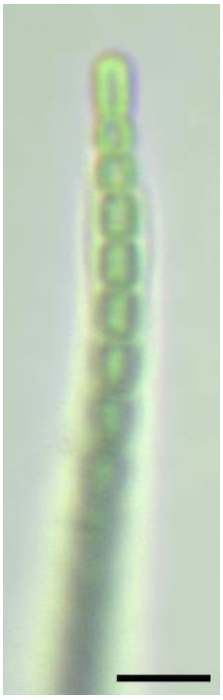


Fig. 15. *Leptolyngbya* cf. *margaritata* E33 (Blansko). Scale bar 5 μm.

***Leptolyngbya* sp./*Nodosilinea epilithica* E2**

Morphology: Filaments long (>1000 μm), undulated, isopolar; sheath thin, almost indistinct; false branching not observed; trichomes blue green, constricted at the cross walls, 1.3 μm wide; cells ± isodiametric; apical cell rounded to obtuse conical; hormogonia immotile; necridic cells present (Fig. 16).

Habitat: Soil near the river (pH 8.7, conductivity 430 $\mu S.cm^{-1}$).

Note: *Nodosilinea epilithica* based on 16S rRNA and ITS 16S−23S secondary structures analyses, but characteristic nodules were not observed under laboratory conditions.

Locality: 9 (Bílovice nad Svitavou).

***Leptolyngbya* sp./*Nodosilinea bijugata* E4**

Morphology: Filaments mostly 175−375 μm long, undulated, isopolar; sheath thin and colorless; false branching rare; trichomes pale green, almost indistinctly constricted at the cross walls, ± 1.2 μm wide; cells longer than wide, occasionally containing a granule near the cross walls; apical cell rounded; hormogonia immotile, necridic cells not observed (Fig. 17).

Habitat: Stones in river (pH 7.7, conductivity 462 $\mu S.cm^{-1}$).

Locality: 9 (Bílovice nad Svitavou).

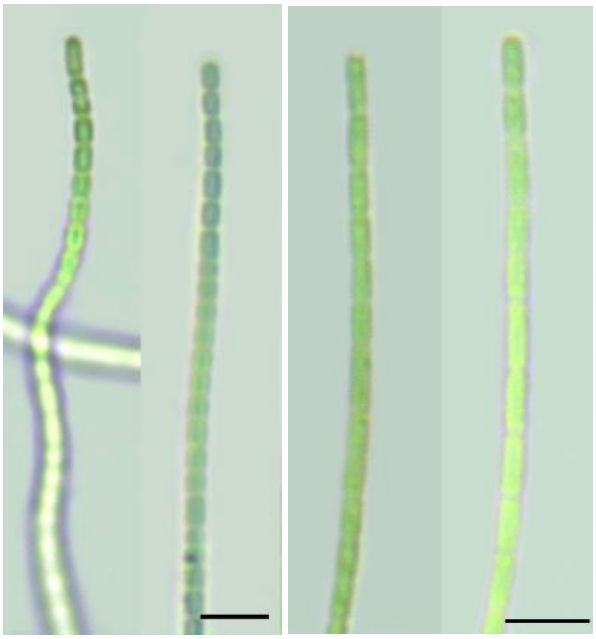


Fig. 16. *Leptolyngbya* sp./*Nodosilinea epilithica* E2 (Bílovice nad Svitavou). Scale bar 5 μm.

Fig. 17. *Leptolyngbya* sp./*Nodosilinea bijugata* E4 (Bílovice nad Svitavou). Scale bar 5 μm.

***Anagnostidinema amphibium* E25**

Morphology: Filaments of various lengths, up to 625 μm long, straight or slightly undulated, isopolar; sheath thin, inconspicuous; trichomes blue green, not constricted at the cross walls, motile (gliding movement), ± 2.0 μm wide; cells mostly longer than wide, rarely isodiametric, containing granules; apical cell rounded; hormogonia present, necridic cells absent (Fig. 18).

Habitat: Boundary between aquatic and terrestrial environment (water pH 7.6, conductivity 479 $\mu S.cm^{-1}$).

Locality: 10 (Brno).

Fig. 18. *Anagnostidinema amphibium* E25 (Brno). Arrow – granule. Scale bar 5 μm.

Table 7. Table of strains isolated from the Svitava River. Sequenced strains are in **bold.**

Strain	Species	Width [µm]	Constrictions	Necridic cells	Hormogonia	Sheath	False branching	Cell dimensions	Apical cell	Environ-ment	Habitat	Locality
E6	*Leptolyngbya* sp.	1.5	+	–	$+_m$	+	+	isodiametric	rounded	terrestrial	soil	2
E3	*Leptolyngbya* sp.	2.0–2.3	+	–	$+_m$	+	–	isodiametric, slightly s/w, or l/w	conical	terrestrial	soil	4
E31	*L. foveolarum*	2.0	+	+	+	+	–	isodiametric	rounded	boundary	soil/river	5
E30	*Pseudanabaena catenata*	1.9	+	–	$+_m$	+	–	isodiametric or l/w	rounded/ conical	terrestrial	soil	5
E7	*Drouetiella lurida*	1.7	±	–	$+_i$	+	+	isodiametric, slightly s/w, or l/w	rounded	boundary	soil/river	6
E5	*Leptolyngbya* sp./ *Nodosilinea epilithica*	1.7	+	+	$+_i$	+	+	isodiametric or slightly l/w	rounded	terrestrial	soil	6
E32	*Leibleinia* sp.	1.1−1.3	±	–	+	+	–	l/w	obtuse conical	terrestrial	soil	6
E33	*L.* cf. *margaritata*	1.7	+	–	+	+	–	isodiametric or slightly l/w	rounded	aquatic	mud with leaves	7
E2	*Leptolyngbya* sp./ *Nodosilinea epilithica*	1.3	+	+	$+_i$	+	–	isodiametric	rounded/ conical	terrestrial	soil	9
E4	*Leptolyngbya* sp./ *Nodosilinea bijugata*	1.2	±	–	$+_i$	+	+	l/w	rounded	aquatic	stones	9
E25	*Anagnostidinema amphibium*	2.0	–	–	+	+	–	l/w	rounded	boundary	soil/river	10

+ present, – absent, $+_m$ present and motile, $+_i$ present and immotile, s/w – shorter than wide, l/w – longer than wide

5.2.2. Ponds

Morphological features of strains isolated from ponds are summarized in Table 8.

***Leptolyngbya boryana* E1**

Morphology: Filaments usually shorter than 200 μm, slightly undulated, sometimes growing radially from one point under laboratory conditions; false branching present; sheath thin and colorless, prominent after hormogonia release; trichomes blue green, at times pale, distinctly constricted at the cross walls, ± 2 μm wide; cells isodiametric; apical cell rounded; hormogonia immotile, necridic cells present (Fig. 19).

Habitat: Boundary between aquatic and terrestrial environment (water pH 8.4, conductivity 362 $\mu S.cm^{-1}$).

Locality: Rosnička Pond.

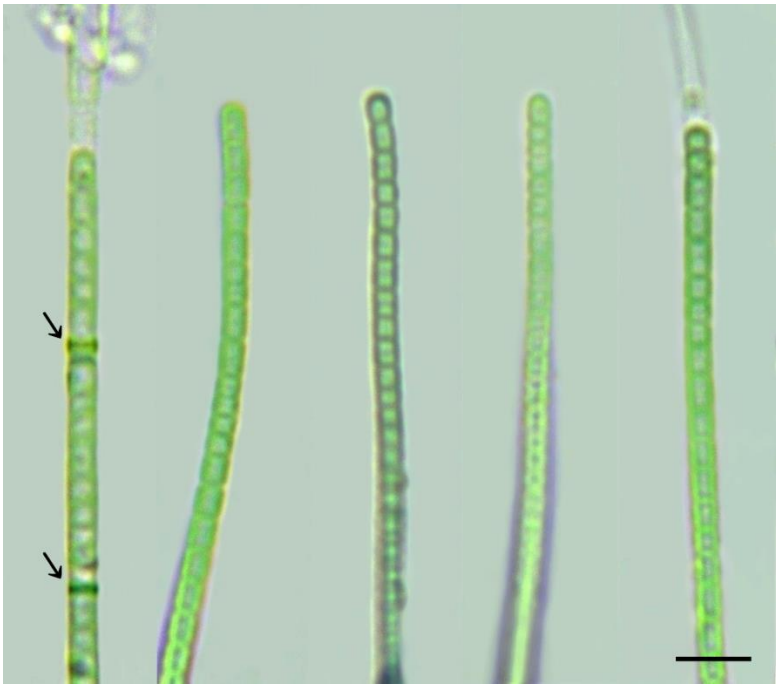


Fig. 19. *Leptolyngbya*/*Leibleinia* sp. E34 (Rosnička). Scale bar 5 μm.

***Leptolyngbya*/*Leibleinia* sp. E34**

Morphology: Filaments very thin, isopolar; sheath inconspicuous and colorless; trichomes blue green, pale, slightly constricted at the cross walls, 1.3 μm wide; cells longer than wide; apical cell rounded; hormogonia present, necridic cells not observed (Fig. 20).

Habitat: Half-submerged stones on the pond shore.

Locality: Rosnička Pond.

***Leptolyngbya cf. foveolarum* E35**

Morphology: Filaments very long (up to 2500 μm), curved, isopolar, without false branching; sheath thin and colorless; trichomes blue green, constricted at the cross walls, 1.5 μm wide; cells isodiametric; apical cell rounded; hormogonia immotile; necridic cells not observed (Fig. 21).

Habitat: Pond epipelon (pH 8.1, conductivity 431 $\mu S.cm^{-1}$).

Locality: Klemovák Pond.

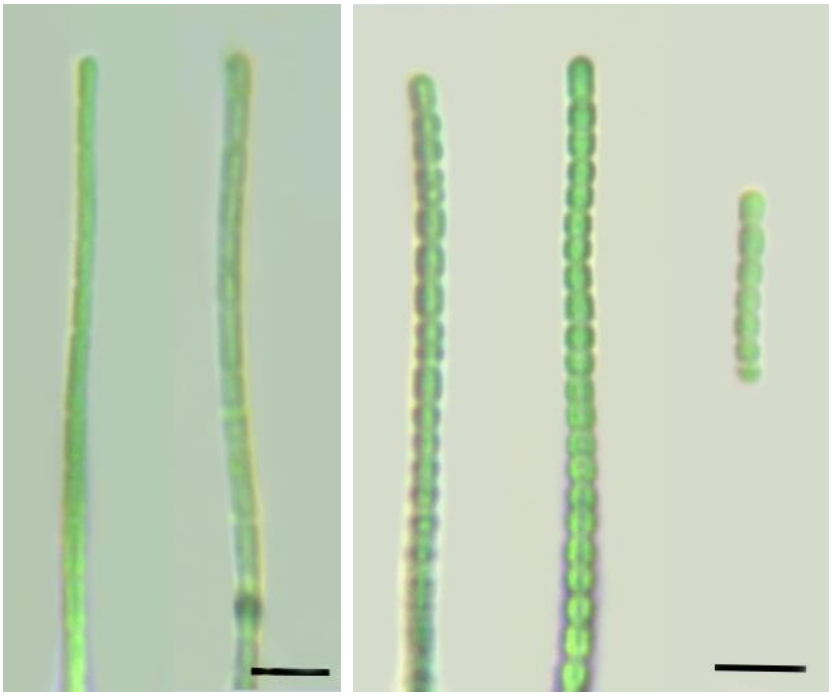


Fig. 20. *Leptolyngbya*/*Leibleinia* sp. E34 (Rosnička). Scale bar 5 μm.

Fig. 21. *Leptolyngbya* cf. *foveolarum* E35 (Klemovák). Scale bar 5 μm.

***Nodosilinea* sp. E36**

Morphology: Filaments long (max. length 900 μm), undulated, sometimes pseudobranched, forming nodules under laboratory conditions; sheath thin and colorless; trichomes blue green, distinctly constricted at the cross walls, 1.9 μm wide; cells isodiametric; apical cell rounded; hormogonia immotile; necridic cells present (Fig. 22).

Habitat: Submerged stones (pH 8.1, conductivity 356 $\mu S.cm^{-1}$).

Locality: Dolní Lhota Quarry.

***Leptolyngbya foveolarum* E37**

Morphology: Filaments ≤ 750 μm, undulated, isopolar, occasionally pseudobranched; sheath thin and colorless; trichomes blue green, distinctly constricted at the cross walls, 1.75 μm wide; cells isodiametric; apical cell rounded; hormogonia immotile; necridic cells present (Fig. 23).

Habitat: Submerged stones (pH 8.0, conductivity 807 $\mu S.cm^{-1}$).

Locality: Skalice Pond.

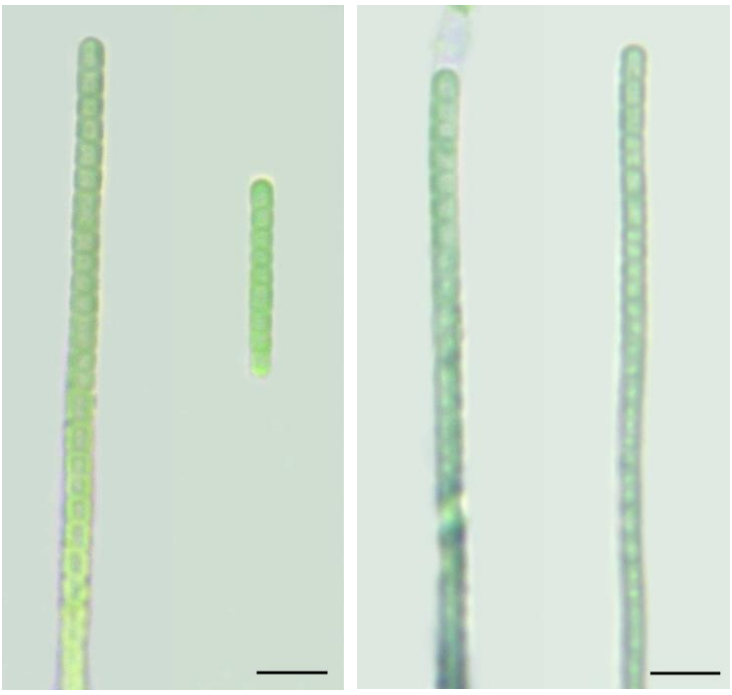


Fig. 22. *Nodosilinea* sp. E36 (Dolní Lhota Quarry). Scale bar 5 μm.

Fig. 23. *Leptolyngbya foveolarum* E37 (Skalice Pond). Scale bar 5 μm.

***Anagnostidinema amphibium* E24**

Morphology: Filaments rather short (max. length 250 μm), straight/slightly undulated; sheath thin, inconspicuous; trichomes blue green, not constricted at the cross walls, motile (gliding movement), ± 1.2 μm wide; cells mostly longer than wide, rarely isodiametric; apical cells rounded; hormogonia motile, necridic cells absent (Fig. 24).

Habitat: Decaying leaves and little branches in the littoral zone of a pond (pH 7.5, conductivity 563 $\mu S.cm^{-1}$).

Locality: Pond in Hradec nad Svitavou.

***Anagnostidinema pseudacutissimum* E38**

Morphology: Filaments rather short (max. length 275 μm), slightly undulated; sheath thin, inconspicuous; trichomes blue green, not constricted at the cross walls, motile (gliding movement), ± 1.3 μm wide, slightly narrowed towards ends; cells mostly longer than wide, rarely isodiametric; apical cell rounded; hormogonia motile, sometimes very short, necridic cells absent (Fig. 25).

Habitat: Submerged stones in a pond (pH 8.1, conductivity 503 $\mu S.cm^{-1}$).

Locality: Klimšák Pond.

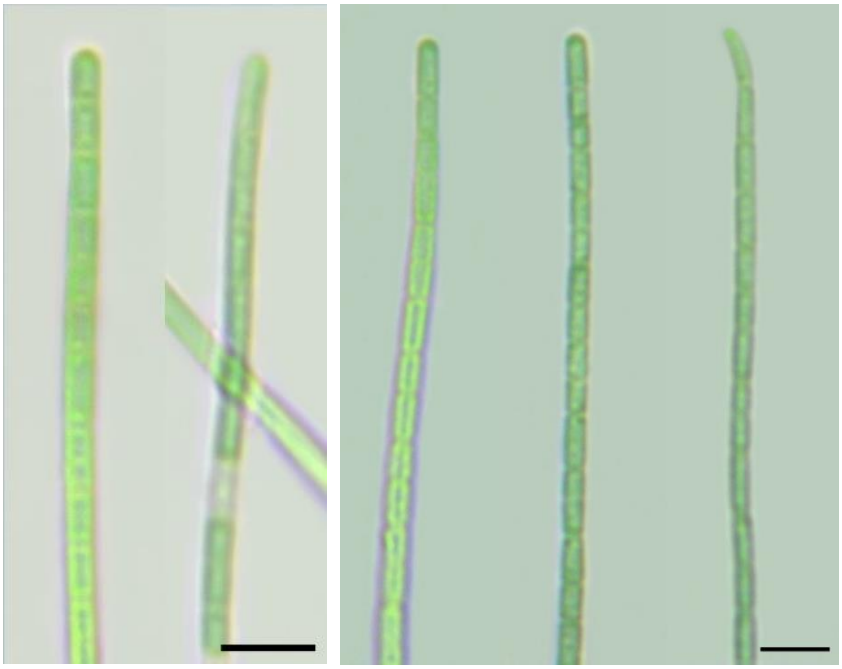


Fig. 24. *Anagnostidinema amphibium* E24 (Hradec nad Svitavou). Scale bar 5 μm.

Fig. 25. *Anagnostidinema pseudacutissimum* E38 (Klimšák Pond). Scale bar 5 μm.

***Anagnostidinema carotinosum* E29**

Morphology: Trichomes of various lengths (125−875 μm), undulated, green to blue green, not constricted at the cross walls, 1.6 μm wide, not or slightly attenuated towards the ends; cells longer than wide, containing granules; apical cell conical to obtuse conical; hormogonia motile; necridic cells absent (Fig. 26).

Habitat: Pond wet shore.

Locality: Pond in Horka nad Moravou.

Note: Determination at the species level is based on molecular data (16S rRNA gene).

Fig. 26. *Anagnostidinema carotinosum* E29 (Horka nad Moravou). Arrow – carotenoid granule. Scale bar 5 μm.

Table 8. Table of strains isolated from ponds. Sequenced strains are in **bold**.

Strain	Species	Width [µm]	Constrictions	Necridic cells	Hormogonia	Sheath	False branching	Cell dimensions	Apical cell	Environment	Habitat	Locality
E1	*L. boryana*	2.0	+	+	$+_i$	+	+	isodiametric	rounded	boundary	wet soil	Rosnička
E34	*Leptolyngbya/Leibleinia* sp.	1.3	+	–	+	+	–	l/w	rounded	boundary	wet stones	Rosnička
E35	*L.* cf. *foveolarum*	1.5	+	–	$+_i$	+	–	isodiametric	rounded	aquatic	mud	Klemovák
E36	*Nodosilinea* sp.	1.9	+	+	$+_i$	+	+	isodiametric	rounded	aquatic	stones	Dolní Lhota
E37	*L. foveolarum*	1.75	+	+	$+_i$	+	+	isodiametric	rounded	aquatic	stones	Skalice
E24	*A. amphibium*	1.2	–	–	$+_m$	+	–	l/w or isodiametric	rounded	aquatic	decaying leaves and branches	Hradec nad Svitavou
E38	*A. pseudacutissimum*	1.3	–	–	$+_m$	+	–	l/w or isodiametric	rounded	aquatic	stones	Klimšák
E29	*A. carotinosum*	1.6	–	–	$+_m$	+	–	l/w	conical	boundary	wet soil	Horka nad Moravou

+ present, – absent, $+_m$ present and motile, $+_i$ present and immotile, s/w – shorter than wide, l/w – longer than wide

5.2.3. Puddles

Morphological features of strains isolated from puddles are summarized in Table 9.

***Leptolyngbya boryana* E23**

Morphology: Filaments max. 250 μm, undulated, isopolar, occasionally pseudobranched; sheath thin and colorless; trichomes blue green, sometimes pale, distinctly constricted at the cross walls, ± 2.3 μm wide; cells ± isodiametric; apical cell rounded to obtuse conical; hormogonia immotile; necridic cells present (Fig. 27).

Habitat: Wet puddle edge near a pond.

Locality: Svitavy, in the proximity of the Svitavský Pond.

***Leptolyngbya* cf. *foveolarum* E11**

Morphology: Filaments max. 750 μm long, undulated, isopolar, sometimes pseudobranched; sheath inconspicuous and colorless; trichomes blue green to pale green, constricted at the cross walls, 1.5−1.8 μm; cells isodiametric or shorter than wide; apical cell rounded; hormogonia motile (trembling); necridic cells present (Fig. 28).

Habitat: Wet puddle edge near a stream.

Locality: Olomouc (Bezručovy sady).

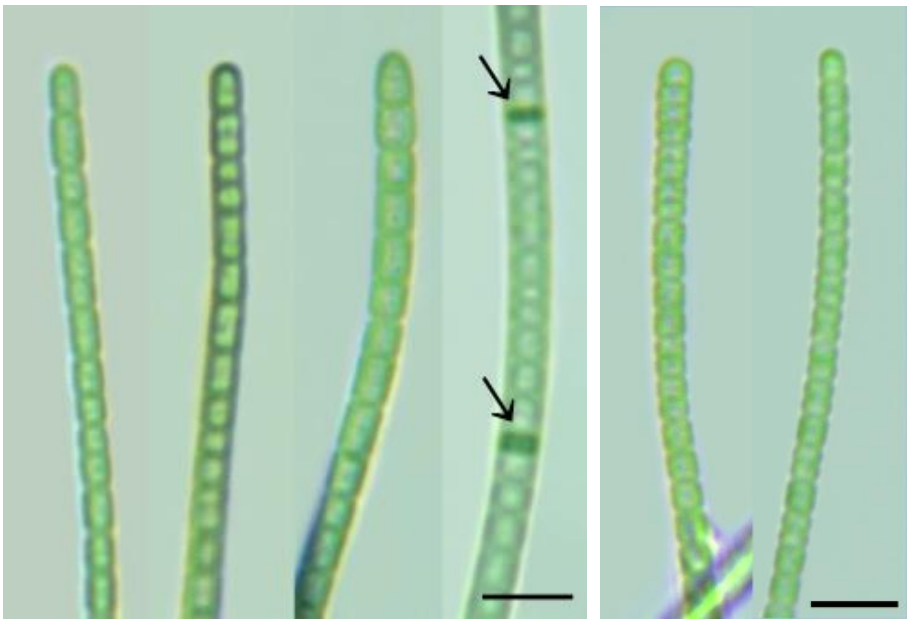


Fig. 27. *Leptolyngbya boryana* E23 (Svitavy). Arrows – necridic cells. Scale bar 5 μm.

Fig. 28. *Leptolyngbya* cf. *foveolarum* E11 (Olomouc). Scale bar 5 μm.

***Leptolyngbya* sp. E12**

Morphology: Filaments usually 375−1000 μm long, slightly undulated, isopolar, sometimes pseudobranched; sheath colorless; trichomes bright blue green, constricted at the cross walls, 2.9 μm wide; cells isodiametric; apical cell rounded; hormogonia motile; necridic cells present (Fig. 29).

Habitat: Wet puddle edge near a forest.

Locality: Grygov.

***Anagnostidinema pseudacutissimum* E26**

Morphology: Trichomes of various lengths (160−500 μm), undulated, blue green to green, not constricted at the cross walls, motile, 1.4 μm; cells longer than wide, with granules; apical cell rounded; hormogonia motile; without necridic cells (Fig. 30).

Habitat: Wet puddle edge in the city center.

Locality: Brno (Olympia park).

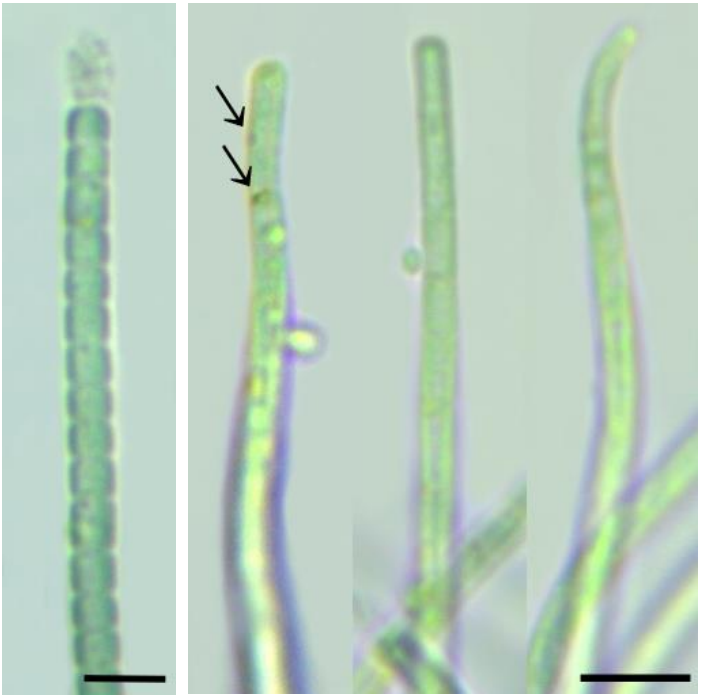


Fig. 29. *Leptolyngbya* sp. E12 (Grygov). Scale bar 5 μm.

Fig. 30. *Anagnostidinema pseudacutissimum* E26 (Brno). Arrows – granules. Scale bar 5 μm.

Table 9. Table of strains isolated from puddles. Sequenced strains are in **bold**.

Strain	Species	Width [µm]	Constrictions	Necridic cells	Hormogonia	Sheath	False branching	Cell dimensions	Apical cell	Locality
E23	*L. boryana*	2.3	+	+	$+_i$	+	+	isodiametric	rounded/conical	Svitavy
E11	*L.* cf. *foveolarum*	1.5–1.8	+	+	$+_m$	+	+	isodiametric or s/w	rounded	Olomouc
E12	*Leptolyngbya* sp.	2.9	+	+	$+_m$	+	+	isodiametric	rounded	Grygov
E26	*A. pseudacutissimum*	1.4	–	–	$+_m$	–	–	l/w	rounded	Brno

+ present, – absent, $+_m$ present, motile, $+_i$ present, immotile, s/w – shorter than wide, l/w – longer than wide

5.2.4. Soil

Morphological features of strains isolated from soils are summarized in Table 10.

***Jaaginema* sp. E10**

Morphology: Filaments long (>750 μm), flexuous, isopolar, always immotile; sheath conspicuous colorless or sometimes dark; trichomes bright blue green, constricted at the cross walls, 1.0−1.2 μm; cells cylindrical, distinctly longer than wide; apical cell rounded; hormogonia immotile; necridic cells absent (Fig. 31).

Habitat: Moist forest soil near a spring.

Locality: Nezdín.

***Drouetiella lurida* E13**

Morphology: Filaments usually 150-300 μm long, straight or undulated, isopolar, sometimes pseudobranched; sheath thin and colorless; trichomes olive-green/reddish/brownish; rather indistinctly constricted at the cross walls, ± 1.5 μm wide; cells isodiametric or slightly longer than wide; apical cell rounded; hormogonia immotile; necridic cells absent (Fig. 32).

Habitat: Meadow soil.

Locality: Ploština.

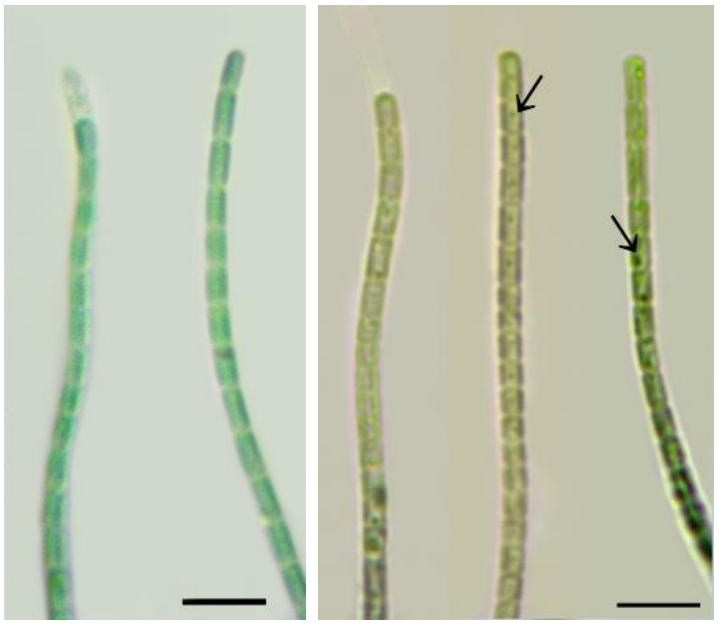


Fig. 31. *Jaaginema* sp. E10 (Nezdín). Scale bar 5 μm.

Fig. 32. *Drouetiella lurida* E13 (Ploština). Arrows – granules. Scale bar 5 μm.

***Nodosilinea epilithica* E15**

Morphology: Filaments long (1000 μm), undulated , isopolar, sometimes pseudobranched; sheath colorless usually inconspicuous, sometimes distinct; filaments forming nodules of various sizes; trichomes blue green, distinctly constricted at the cross walls, ± 1.9 μm wide; cells isodiametric; apical cell rounded; hormogonia immotile; necridic cells present (Fig. 33).

Habitat: Soil under trees.

Locality: Olomouc (Svatý Kopeček).

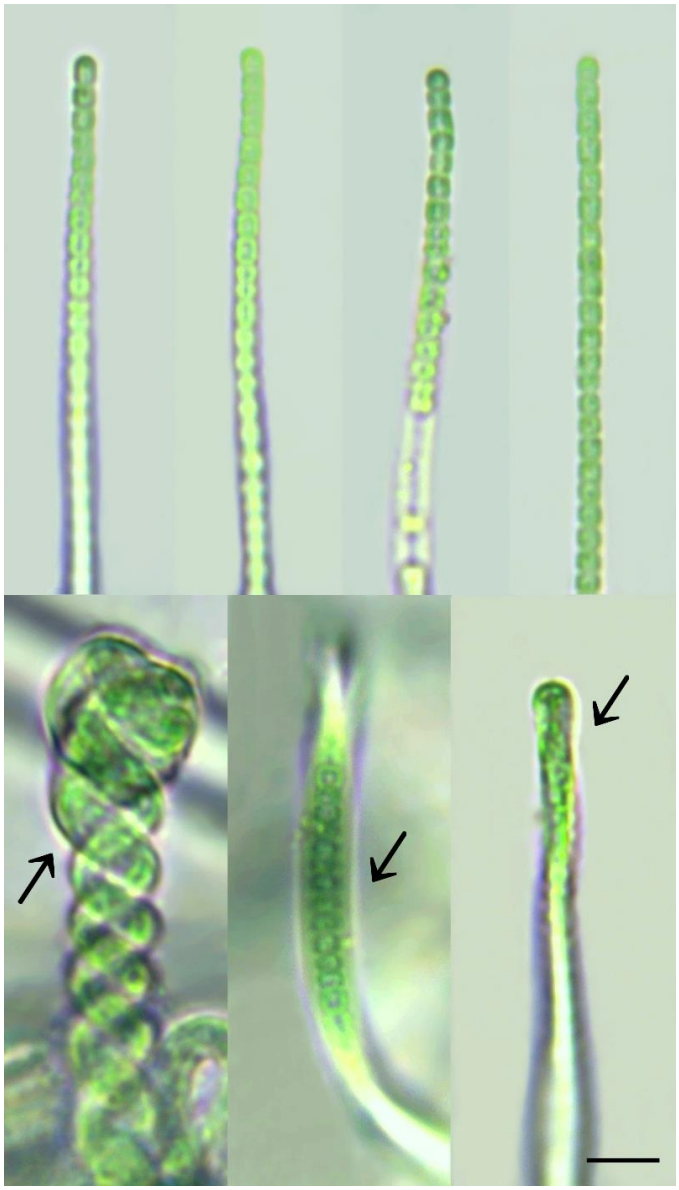


Fig. 33. *Nodosilinea epilithica* E15 (Olomouc). Arrows – nodule formation in different stages of development. Scale bar 5 μm.

***Nodosilinea epilithica* E19**

Morphology: Filaments of various lengths (75−750 μm), undulated, isopolar, rarely pseudobranched, at times forming loose nodules; sheath colorless, usually conspicuous, especially in older filaments; trichomes blue green to green, constricted at the cross walls, ± 1.7 μm wide; cells isodiametric; apical cell rounded; hormogonia motile (trembling); necridic cells present (Fig. 34).

Habitat: Meadow soil.

Locality: Vlachovice.

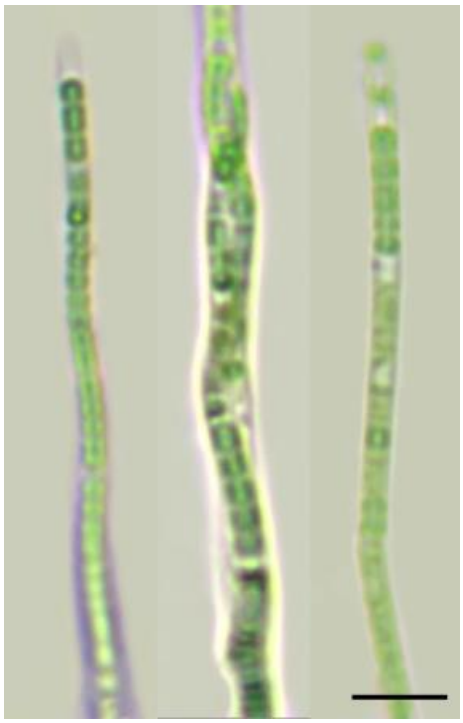


Fig. 34. Nodosilinea epilithica E19 (Vlachovice). Scale bar 5 μm.

***Nodosilinea epilithica* E20**

Morphology: Filaments of various lengths (≤ 875 μm), undulated, isopolar, rarely pseudobranched, forming nodules; sheath colorless, usually inconspicuous, sometimes prominent; trichomes blue green to pale green, distinctly constricted at the cross walls, 1.6−1.9 μm wide; cell isodiametric, often shorter than wide; apical cell rounded; hormogonia motile (trembling); necridic cells present (Fig. 35).

Habitat: Wet soil near a mineral stream.

Locality: Sivá Brada.

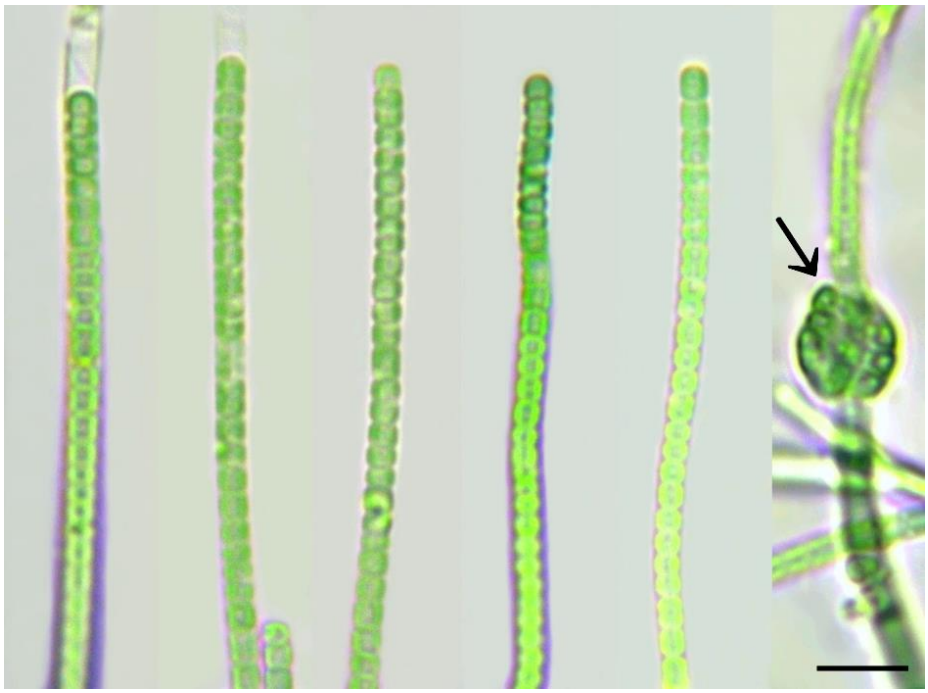


Fig. 35. *Nodosilinea epilithica* E20 (Sivá Brada). Arrow – nodule. Scale bar 5 μm.

***Nodosilinea epilithica* E22**

Morphology: Filaments of various lengths (100−625 μm), undulated, isopolar, occasionally pseudobranched; frequently forming nodules; sheath thin, colorless, inconspicuous or prominent; trichomes blue green to green, constricted at the cross walls, 1.6 μm wide; cells isodiametric; apical cell rounded; hormogonia immotile; necridic cells present (Fig. 36).

Habitat: Bare garden soil.

Locality: Vysoké Pole.

Fig. 36. *Nodosilinea epilithica* E22 (Vysoké Pole). Arrows – nodules. Scale bar 5 μm.

***Anagnostidinema carotinosum* E27**

Morphology: Trichomes of various lengths (≤ 875 μm), straight to undulated, green to blue green, not constricted at the cross walls, 1.4 μm wide, not or slightly attenuated towards the ends; cells longer than wide, characteristic reddish granules not observed; apical cell rounded to obtuse conical; hormogonia motile; necridic cells absent (Fig. 37).

Habitat: Wet soil.

Locality: Vysoké Pole.

Note: Determination at the species level is based on molecular data (16S rRNA gene).

Fig. 37. *Anagnostidinema carotinosum* E27 (Vysoké Pole). Arrows – carotenoid granules. Scale bar 5 μm.

5.2.5. Rock

Morphological features of strain E17 are summarized in Table 10.

***Leptolyngbya* s. l./*Schizothrix* sp. E17**

Morphology: Filaments usually 250−500 μm long, straight to undulated, frequently occurring in tight fascicles, sometimes pseudobranched; more filaments in one sheath not observed under laboratory conditions; trichomes green to blue green, constricted at the cross walls, 2.3−2.7 μm wide; cells isodiametric or shorter than wide, sometimes swollen, with dark granules; apical cell rounded; hormogonia motile (trembling); necridic cells present (Fig. 38).

Habitat: Rock.

Locality: Olomouc (Bezručovy sady).

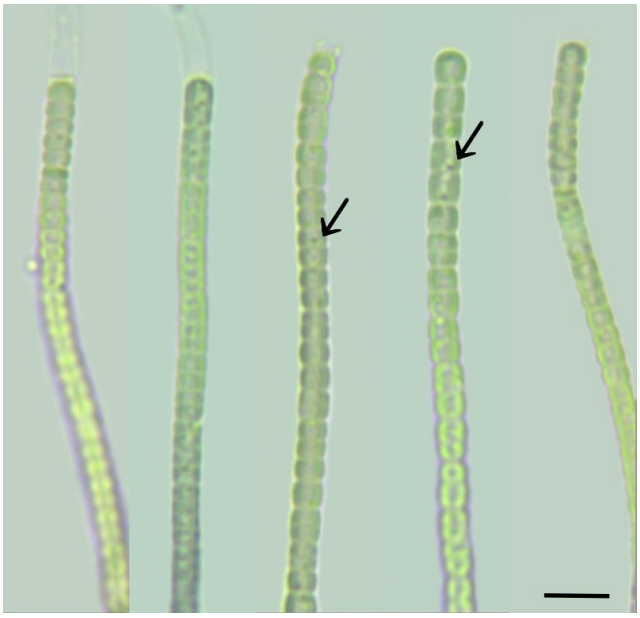


Fig. 38a. *Leptolyngbya* s. l./*Schizothrix* sp. E17 (Olomouc). Arrows – granules. Scale bar 5 μm.

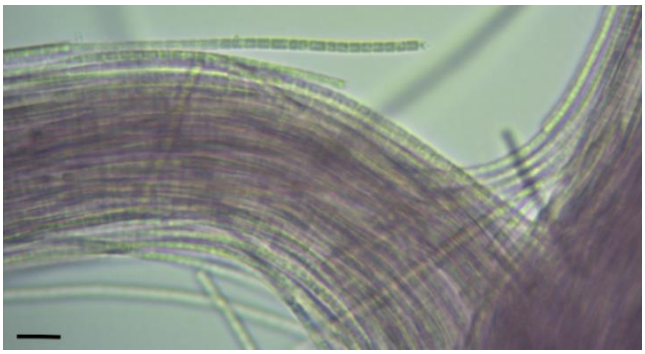


Fig. 38b. *Leptolyngbya* s. l./*Schizothrix* sp. E17 (Olomouc). Scale bar 10 μm.

Table 10. Table of strains isolated from soils and a rock. Sequenced strains are in **bold**.

Strain	Species	Width [μm]	Constrictions	Necridic cells	Hormogonia	Sheath	False branching	Cell dimensions	Apical cell	Habitat	Locality
E10	*Jaaginema* sp.	1.0–1.2	+	–	$+_i$	+	+	l/w	rounded	forest soil	Nezdín
E13	*Drouetiella lurida*	1.5	±	–	$+_i$	+	+	isodiametric or slightly l/w	rounded	meadow soil	Ploština
E15	*Nodosilinea epilithica*	1.9	+	+	$+_i$	+	+	isodiametric	rounded	soil	Olomouc
E19	*Nodosilinea epilithica*	1.7	+	+	$+_m$	+	+	isodiametric	rounded	meadow soil	Vlachovice
E20	*Nodosilinea epilithica*	1.6–1.9	+	+	$+_m$	+	+	isodiametric or s/w	rounded	soil near a min. stream	Sivá Brada
E22	*Nodosilinea epilithica*	1.6	+	+	$+_i$	+	+	isodiametric	rounded	garden soil	Vysoké Pole
E27	*A. carotinosum*	1.4	–	–	$+_m$	–	–	l/w	rounded/ conical	soil	Vysoké Pole
E17	*Leptolyngbya* s.l./ *Schizothrix* sp.	2.3–2.7	+	+	$+_m$	+	+	isodiametric or s/w	rounded	rock	Olomouc

+ present, – absent, $+_m$ present and motile, $+_i$ present and immotile, s/w – shorter than wide, l/w – longer than wide

5.3. DNA analysis

Phylogenic analysis based on partial 16S rRNA gene sequence revealed eight clades among the studied cyanobacterial strains in the resultant phylogenetic tree (Fig. 39). These clades mostly correspond to the genus level with one uncertain exception indicating the polyphyly of the genus *Leptolyngbya* s. s. The resulting tree contains leptolyngbyoid taxa from three families (Leptolyngbyaceae, Oculatellaceae, Prochlorotrichaceae) and nonleptolyngbyoid taxa (*Anagnostidinema* and *Pseudanabaena*) from two other families (Pseudanabaenaceae and Coleofasciculaceae).

Clade 1: *Nodosilinea* (Prochlorotrichaceae)

Six strains (E2, E5, E15, E19, E20, E22, Figs. 13, 16, 33, 34, 35, 36) were assigned to the genus *Nodosilinea* based on the 16S rRNA gene analysis. The similarity between these strains was above 97 % (Table 11), thus all six strains probably belong to one species – *Nodosilinea epilithica*. A variability in ITS secondary structures existed among these strains (Figs. 40, 41).

Clade 2: *Jaaginema* (cf. Oculatellacae)

One strain (E10, Fig. 31), originally collected from forest soil, was assigned to the currently unrevised genus *Jaaginema*. Species determination was not possible, as neither the morphology nor sequence fit to any previously described species of this genus. The clade contained also species *Tildeniella torsiva* which is morphologically similar, but several morphological features do not correspond with this genus.

Clade 3: *Drouetiella* (Oculatellaceae)

Two strains (E7, E13, Figs. 12, 32) were assigned to the genus *Drouetiella*. Both belong to *D. lurida* based on the 16S rRNA gene analysis (100% support). Predicted secondary structures and p-distance values confirm the strains belong to one species (Figs. 40, 41).

Clade 4: *Leptolyngbya* s.l./*Schizothrix* (?)

E17 (Fig. 20) is a strain lacking previous molecular characterization. The most similar BLAST hits were unspecified *Leptolyngbya* spp. Morphology partially resembles *Schizothrix*, as filaments tend to form tight fascicles. ITS secondary structures differ significantly from other *Leptolyngbya* s. s. spp. (see Figs. 42, 43).

Clade 5: *Leptolyngbya* (Leptolyngbyaceae)

Strains E1 (Fig. 19) and E23 (Fig. 27) belong to *Leptolyngbya* s. s., specifically to the type species *L. boryana*. ITS secondary structures show minor differences between these two strains (Figs. 42, 43). P-distance values indicate the same species (similarity 99.9 %, Table 11).

Clade 6: *Anagnostidinema* (?)

Clade 6 contains two strains (E24, E25 – Figs. 18, 24), determined as *Anagnostidinema amphibium* based on morphological and genomic data. This clade has a closer relationship to leptolyngbyoid taxa than to the rest of *Anagnostidinema* strains. ITS secondary structures and p-distance values indicate the strains belong to two separate species (Fig. 42, 43, Table 11).

Clade 7: *Anagnostidinema* (Coleofasciculaceae)

Three strains were assigned to *Anagnostidinema* s. s. ITS structures (Fig. 44, 45) confirm this classification. E26 (Fig. 29) belongs to *A. pseudacutissimum*, while E27 (Fig. 37) and E29 (Fig. 26) should belong to *A. carotinosum*. P-distance values (all > 99 %) and the similarity in ITS structures, however, indicate all strains belong to one species.

Clade 8: *Pseudanabaena* (Pseudanabaenaceae)

A separate clade was formed by *Pseudanabaena* spp. According to 16S rRNA sequence, strain E30 (Fig. 11) is *Pseudanabaena catenata*.

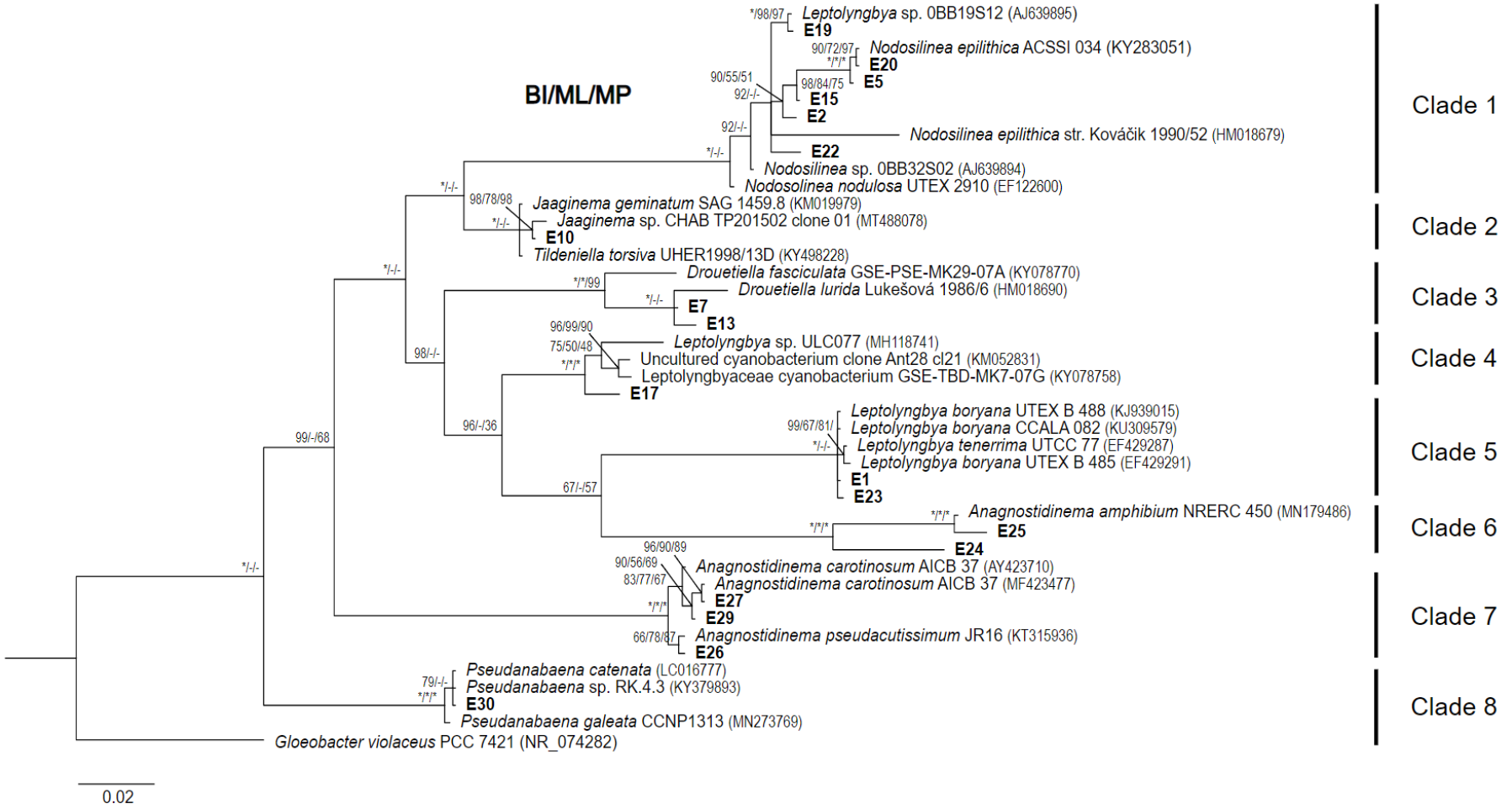


Fig. 39. Bayesian phylogenetic tree based on partial 16S rRNA gene sequence (942 bp). Isolated strains are E1−E30. Remaining sequences are the closest BLAST hits. The order of node supports is Bayesian Inference/Maximum Likelihood/Maximum Parsimony. Asterisk represents value 100. *Gloeobacter violaceus* was added as an outgroup.

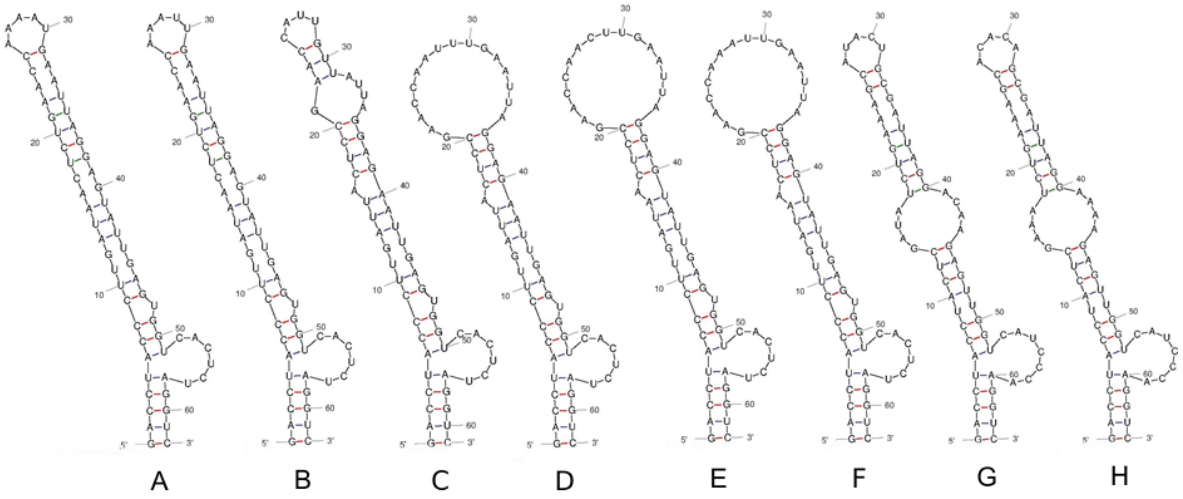


Fig. 40. 16S–23S ITS secondary structure (**D1-D1' helix**) of *Nodosilinea epilithica* (A, B, C, D, E, F – E5, E15, E22, E2, E19, E20) and *Drouetiella lurida* (G, H – E7, E13).

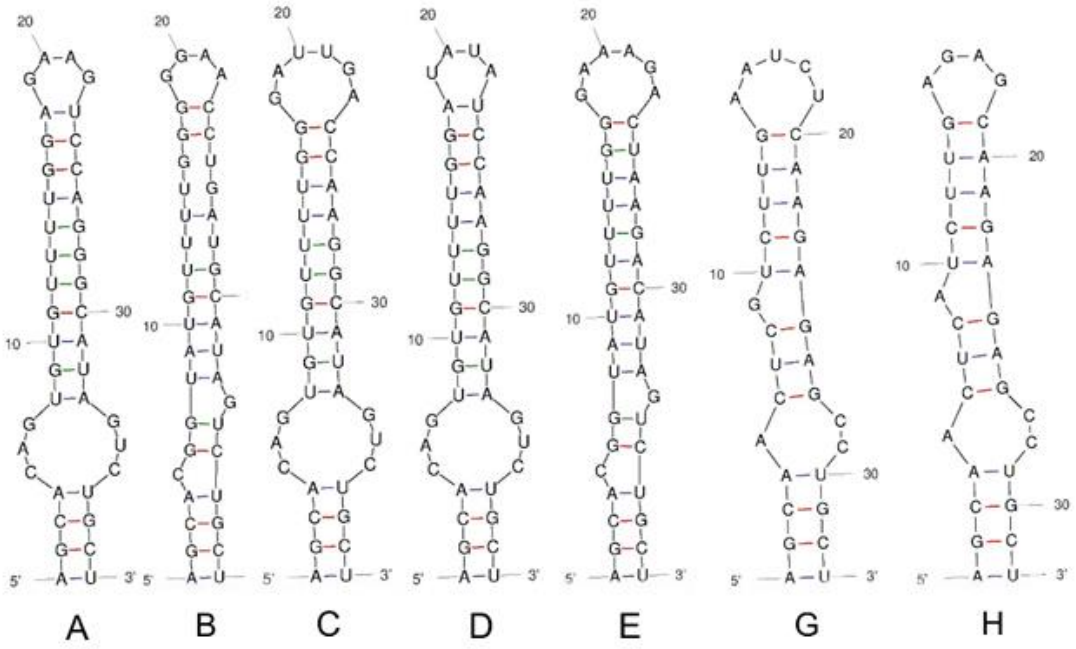


Fig. 41. 16S–23S ITS secondary structure (**Box B helix**) of *Nodosilinea epilithica* (A, B, C, D, E – E5, E15, E22, E2, E19) and *Drouetiella lurida* (G, H – E7, E13). Box B of strain E20 (F) is missing due to absence of sequence for this helix.

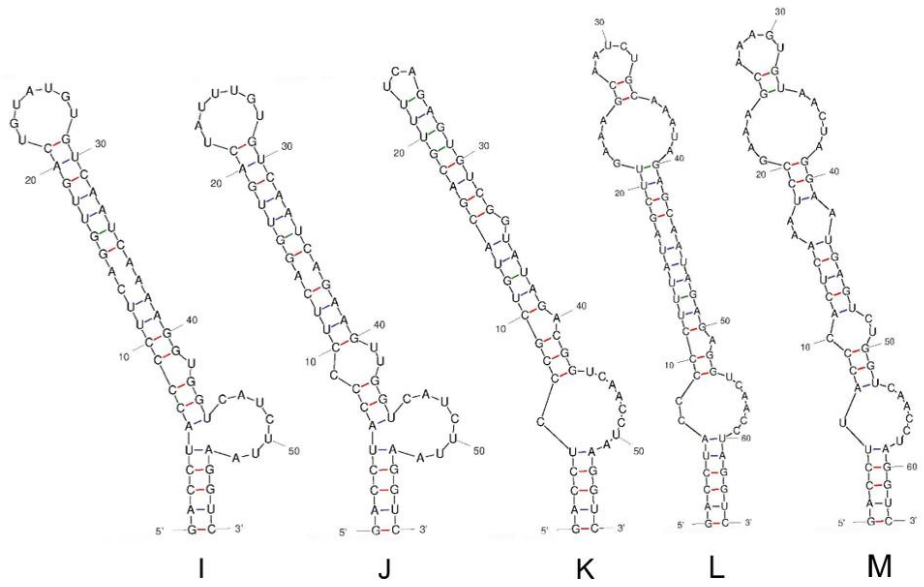


Fig. 42. 16S–23S ITS secondary structure (**D1-D1' helix**) of *Leptolyngbya boryana* (I, J – E1, E23), *Leptolyngbya* s. l./*Schizothrix* sp (K – E17), and *Anagnostidinema amphibium* (L, M – E24, E25).

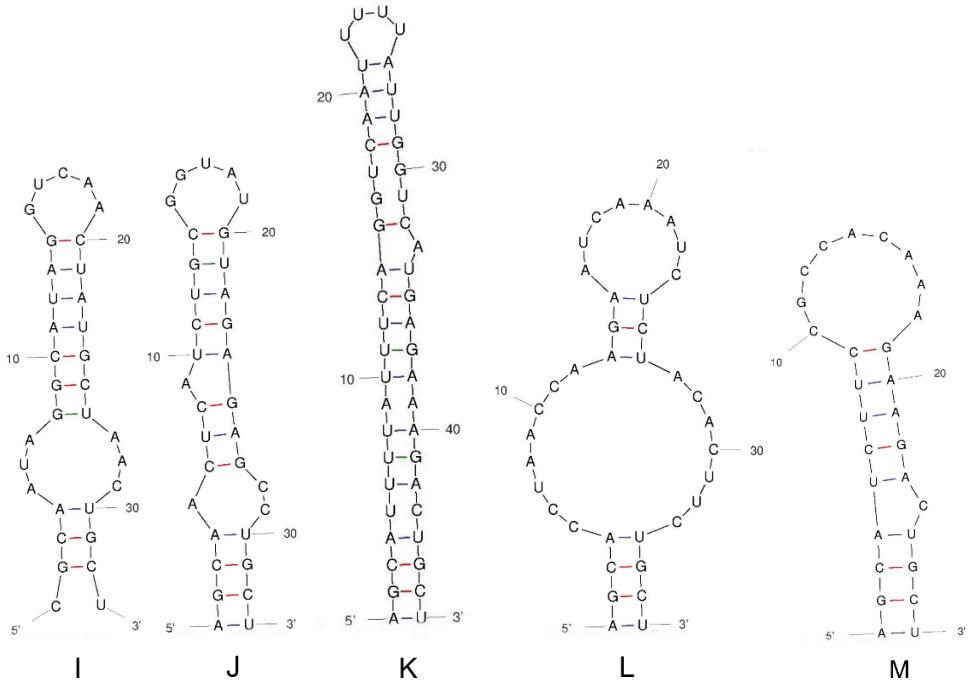


Fig. 43. 16S–23S ITS secondary structure (**Box B helix**) of *Leptolyngbya boryana* (I, J – E1, E23), *Leptolyngbya* s. l./*Schizothrix* sp. (K – E17), and *Anagnostidinema amphibium* (L, M – E24, E25).

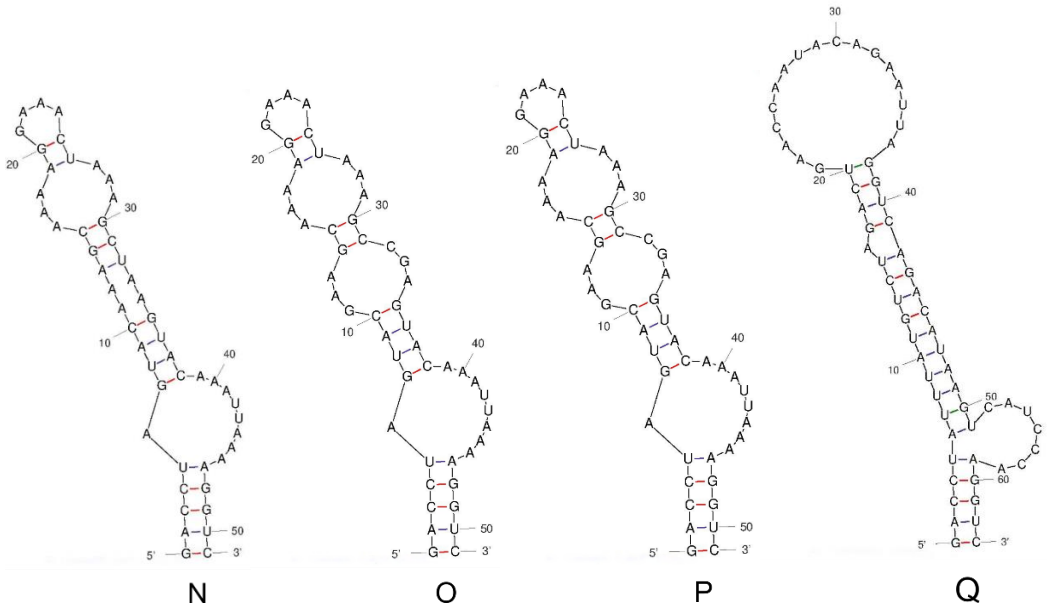


Fig. 44. 16S–23S ITS secondary structure (**D1-D1' helix**) of *Anagnostidinema pseudacutissimum* (N – E26), *A. carotinosum* (O, P – E27, E29), and *Pseudanabaena* sp. (Q – E30).

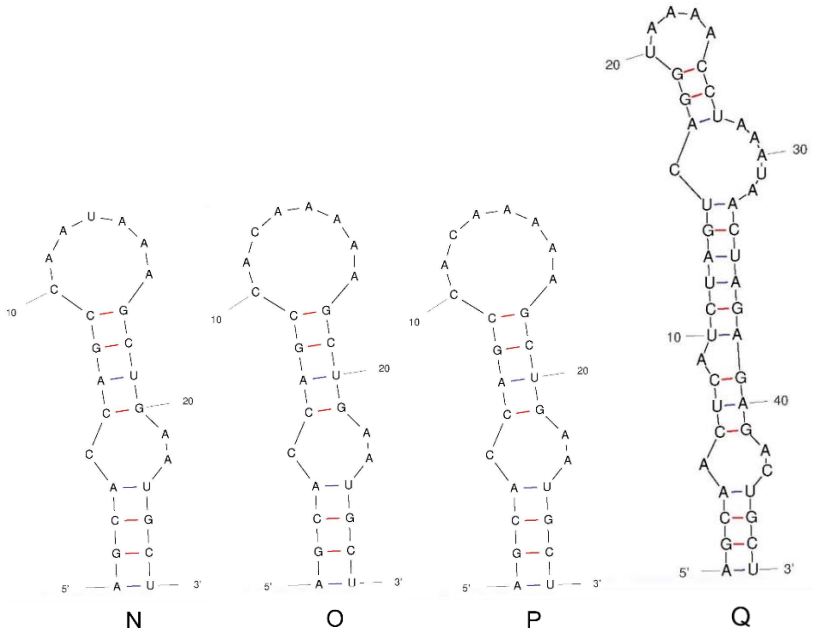


Fig. 45. 16S–23S ITS secondary structure (**Box B helix**) of *Anagnostidinema pseudacutissimum* (N – E26), *A. carotinosum* (O, P – E27, E29), and *Pseudanabaena* sp. (Q – E30).

Table 11. Similarity matrix of isolated strains (based on p-distances of 16S rRNA gene sequences), *Anagnostidinema* spp. (yellow), *Leptolyngbya boryana* (blue), *Nodosilinea epilithica* (green) and *Drouetiella lurida* (violet). Values ≥ 97.5 (red) indicate the same species.

	E30	**E29**	**E27**	**E26**	**E25**	**E24**	**E23**	**E22**	**E20**	**E19**	**E17**	**E15**	**E13**	**E10**	**E7**	**E5**	**E2**	**E1**
E30	*																	
E29	87.93	*																
E27	87.74	**99.81**	*															
E26	88.12	**99.33**	**99.52**	*														
E25	88.40	88.02	87.83	87.93	*													
E24	88.21	88.97	89.16	89.45	94.11	*												
E23	88.02	87.36	87.17	87.17	88.97	90.02	*											
E22	89.26	87.93	88.12	88.31	89.54	89.35	88.50	*										
E20	88.97	87.64	87.83	88.02	89.54	89.45	88.40	**97.53**	*									
E19	89.35	87.93	88.12	88.31	89.16	89.26	88.21	**98.76**	**97.72**	*								
E17	90.02	88.59	88.40	88.50	89.92	90.49	91.83	90.30	89.54	90.21	*							
E15	89.83	88.21	88.40	88.59	89.07	88.97	88.21	**98.76**	**98.76**	**98.95**	90.59	*						
E13	89.54	88.12	88.31	88.40	87.83	89.73	90.11	90.21	89.45	90.02	91.92	90.30	*					
E10	91.44	89.45	89.26	89.26	90.21	90.02	89.92	92.68	91.92	92.68	93.73	92.97	93.54	*				
E7	89.35	88.02	88.21	88.31	87.45	89.54	90.40	89.83	89.26	89.64	91.54	90.11	**99.43**	93.35	*			
E5	88.88	87.74	87.93	88.12	89.64	89.54	88.40	**97.62**	**99.90**	**97.81**	89.64	**98.86**	89.35	92.02	89.16	*		
E2	89.92	87.93	88.12	88.31	89.35	89.07	88.40	**98.76**	**98.38**	**98.95**	90.49	**99.43**	90.40	92.87	90.02	**98.29**	*	
E1	88.12	87.36	87.17	87.17	89.07	89.92	**99.90**	88.50	88.40	88.21	91.83	88.21	90.11	89.92	90.40	88.40	88.40	*

5.4 Protein analysis

Protein analysis based on mass spectra of 16 strains revealed four main clades in the resultant dendrogram (Fig. 47) which slightly differs from the phylogenetic tree based on 16S rRNA gene (Fig. 46). The largest clade contains predominantly *Nodosilinea epilithica* strains. This clade is further divided into two subclades. The first one contains *N. epilithica* strains which share similar D1-D1' helices (Fig. 40). *Drouetiella lurida* E13 is also present in this clade. The second subclade consists of the rest of *N. epilithica* strains which also share similar D1-D1' helices (Fig. 40). *Anagnostidinema amphibium* E25 is also a part of this clade.

The second main clade is formed by *Leptolyngbya* s.l./*Schizothrix* sp. and *Drouetiella lurida* E7. The third clade contains single strain – *A. amphibium* E24.

The last clade contains the rest of *Anagnostidinema* strains (*A. carotinosum*, *A pseudacutissimum*), *Jaaginema* sp., and *Leptolyngbya boryana*. Strains of *L. boryana* are clustered together.

A split network of analyzed strains (Fig. 49) shows the arrangement of strains comparable to the results of the 16S rRNA gene analyses (Fig. 46, 48). Strains assigned to one species based on 16S rRNA gene analysis are also clustered together in this network. A split network analysis of peptide spectra divides *Nodosilinea epilithica* into two groups according to similarity in D1-D1´helices. It is the same result as in hierarchical clustering of MALDI-TOF MS spectra (Fig. 47). The phylogenetic tree based on the 16S rRNA gene does not show the same pattern (Fig. 46). Even if it is possible to recognize two branches, their D1-D1´ helices do not share the same structure. Both 16S rRNA and proteomic analyses support delimitation of *Leptolyngbya*/*Nodosilinea* genera. Similarly, the genus *Anagnostidinema* is located at one clade in both analyses except for strain E25 in hierarchical clustering (Fig. 47). Despite the same position in the phylogenetic tree (Fig. 46), the value of p-distances indicates a low similarity between E24 and E25 strains. Incongruity in these results requires detailed future studies. The position of *Leptolyngbya boryana* within the *Anagnostidinema* clade (hierarchical clustering, Fig. 47) is affected by a low number of analyzed strains and higher peptide similarity to *Anagnostidinema* than to *Nodosilinea*.

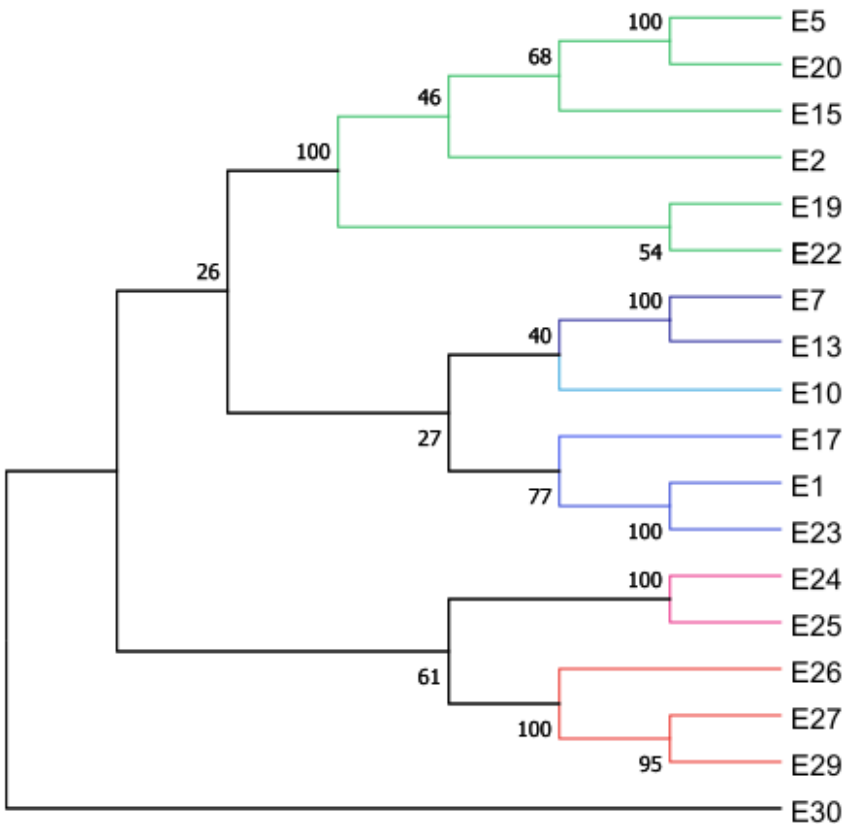


Fig. 46. Phylogenetic tree based on 16S rRNA gene sequences (1,066 bp). Node supports – Maximum likelihood.

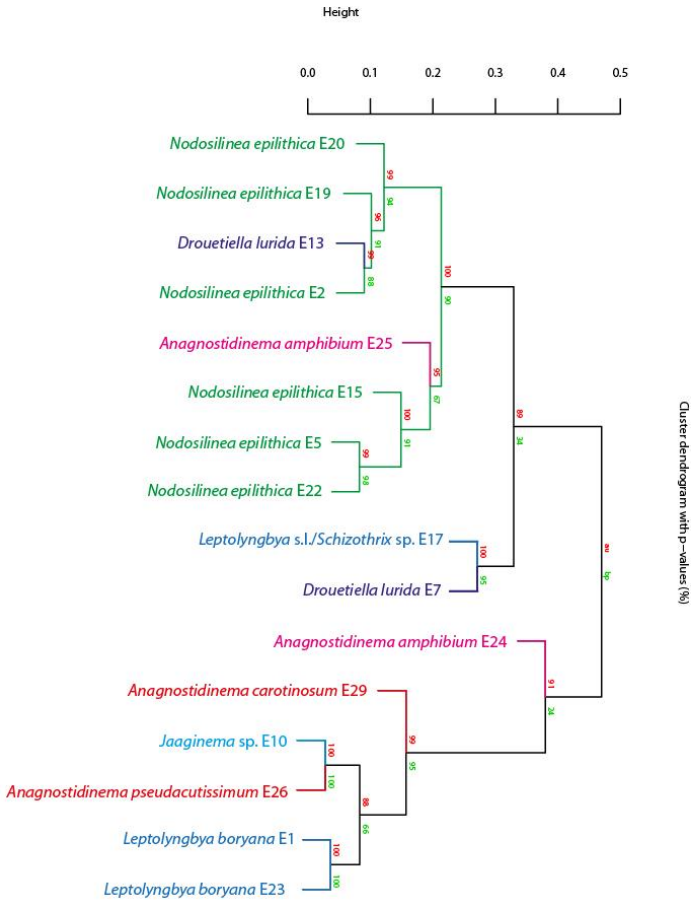


Fig. 47. Dendrogram based on MALDI-TOF mass spectra. Node supports: red – Approximately Unbiased (AU) p-values, green – Bootstrap Probability (BP) values.

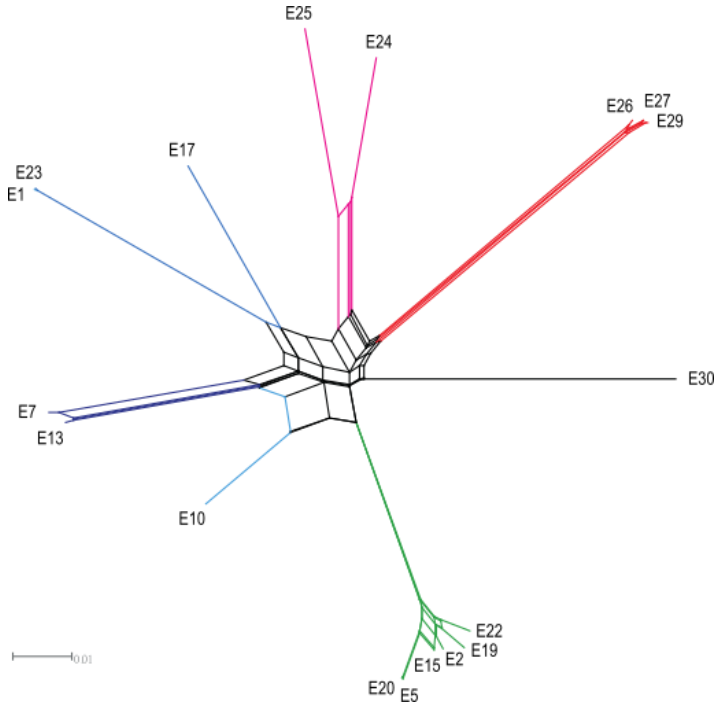


Fig. 48. A split network based on 16S rRNA gene (1,066 bp). Colored groups correspond to clades of the same color in the 16S rRNA phylogenetic tree (see Fig. 46).

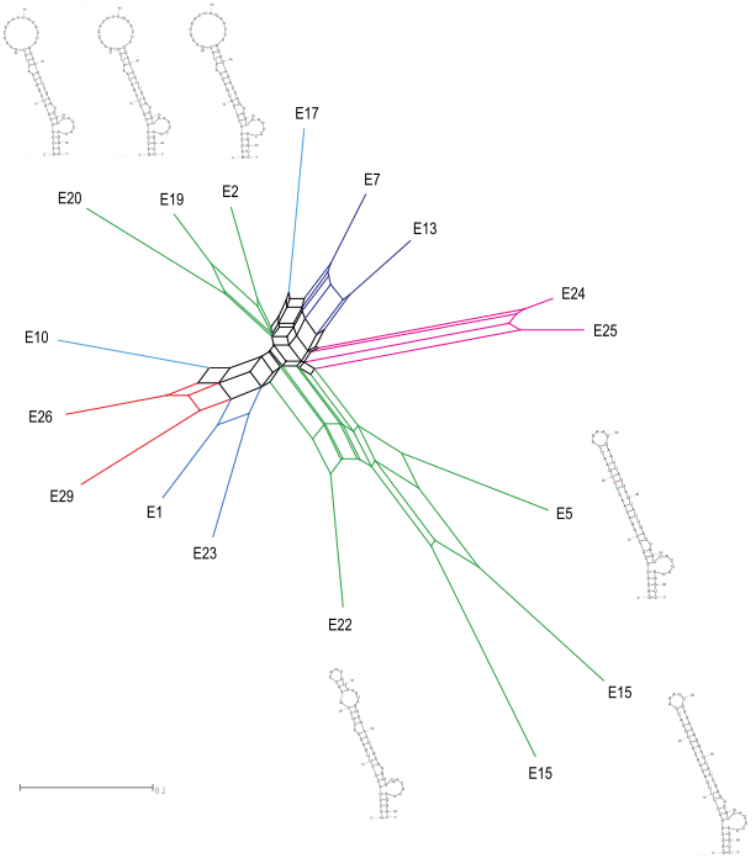


Fig. 49. A split network based on MALDI-TOF mass spectra. Colored groups correspond to clades of the same color in the MALDI-TOF MS dendrogram (see Fig. 47).

6. DISCUSSION

The River Continuum Concept validity

The validity of the RCC (Vannote et al. 1980) for the studied river is very limited since the river is strongly affected by anthropogenic activities (Procházková et al. 2020). The only unaltered reach of the river is the spring flowing through a forest. The absence of primary producers in the spring due to light unavailability is in concordance with the concept. However, the stream flows through a forest only for the first two kilometers and then continues through three stagnant water bodies to the town where there is ample availability of light (see Appendix 1). Further reaches flow alternately through municipalities and forests. The most shaded reaches are those between localities 7 and 9. Except for the impact on the light availability and primary production in the stream, the discontinuous presence of riparian vegetation may have additional consequences. It can be expected that the CPOM/FPOM ratio also does not change gradually, as well as the ratio between functional groups of macroinvertebrates which reflects the availability of carbon sources. Additional factors disrupting the river continuum are tributaries, stagnant water bodies, and point sources of pollution. For these reasons, the dynamics of the Svitava River appears to be patchy (Townsend 1989) rather than continuous.

Diversity of *Leptolyngbya* s. l. in the studied river

The identification key by Komárek & Anagnostidis (2005) comprises a high number of freshwater *Leptolyngbya* species. In contrast, the diversity and abundance of leptolyngbyoid taxa are low in the studied river. As described in the first chapters, factors diminishing biodiversity in running waters are e.g., the loss of heterogeneity, pollution, and connectivity disruption.

Heterogeneity appears to be essential, as various *Leptolyngbya* species possess various ecological demands. While some species require clear, nutrient-poor waters, others are capable of growth in waters of higher trophy or higher organic compounds content. Examples of the first group are *L. fontana* (Komárek & Anagnostidis 2005, Kaštovský et al. 2010) or *L. angustissima* (García & Aboal 2014). Species tolerant to impaired water quality are e.g., *L. boryana* (Loza et al. 2013) or *L. foveolarum* (Komárek & Anagnostidis 2005). As Svitava is heavily burdened by human activities (Procházková et al. 2020), it can host only taxa adapted to (especially nutrient) pollution, whereas demands on nutrient-poor water cannot be

met in any reach. The only exception is the unaltered initial reach. Here, however, no cyanobacteria were detected since the sunlight is reduced by the coniferous tree canopy.

The lack of heterogeneity can be observed in several other factors, for example altitude. While many Czech rivers (Morava, Labe, Vltava etc.) originate in mountains and continue to lowland regions, the difference in altitude between the spring and the confluence is only ± 280 m in the Svitava River (EDPP 2021). Variability was missing also in the pH of water (values 6.7−7.9).

Contrariwise, the greatest heterogeneity was observed in types of substrate (stones, mud, sand, leaves, branches). A certain amount of variability was also found in conductivity and temperature, but it should be noted that the temperature variability was partially caused by diurnal changes (some measurements were performed in the morning and some in the afternoon). Brown (1969), for example, states that diurnal temperature fluctuations in streams can be up to 9 °C. Moreover, it was observed that many periphytic cyanobacteria commonly grow in environments where the water temperature is up to 20 °C lower than in optimal conditions (Mosser & Brock 1979), thus temperature is not a reliable factor.

As regards pollution, the Svitava River was classified into the 4^{th} and 5^{th} class of overall water quality (1 – the best quality, 5 – the worst quality) based on the latest measurements (Procházková et al. 2020, summed in Appendix 5). The most problematic were high concentrations of phosphorus (classes 3 and 4). Similarly, concentrations of nitrogen were unsatisfactory in the majority of measured profiles. Nitrate concentrations were the worst in Brno (class 4), while concentrations of NH_3 were the highest in Moravská Chrastová and Letovice (class 5). At these two localities, also benzo(ghi)perylen was detected in harmful amounts. Such an unsatisfactory state of water excludes species which require clean water.

As in other Czech rivers, the connectivity between the river and the surrounding landscape is disrupted by various anthropogenic alterations. Moreover, the flow can become intermittent in periods of drought in initial reaches of the river (personal observations). *Leptolyngbya* is not a cyanobacterial genus capable of active movement, and thus relies on external ways of dispersal. The basic way how river species can spread to other reaches is via downstream water flow (Vis 2016). On the other hand, Ward et al. (1999) state that the biodiversity is highest at intermediate levels of connectivity, while high connectivity diminishes the species richness. In addition, dispersal of cyanobacteria is possible by wind,

animals and human as well (Kristiansen 1996, Vis 2016), so short-term flow cessation potentially should not reduce the cyanobacterial diversity in the studied river.

The above factors, however, explain only the lack of species diversity. Another question is why many localities did not contain leptolyngbyoid taxa at all. One explanation could be competition. There are a number of studies focusing on the competitive relationships between cyanobacteria and algae (van der Grinten et al. 2005) but it is not clear why some filamentous cyanobacteria dominate at a given locality, while others have only small populations or are not present at all even when conditions are favorable for their growth. The explanation could be the size and motility of the dominant taxa (e.g., *Phormidium*) which allow them to find optimal conditions for their growth (McCormick 1996). Effective growth of these taxa can then result in shading species of minor dimensions and with inability of motility, e.g., *Leptolyngbya*. An additional explanation could be the production of inhibitory compounds by the dominant cyanobacteria. The influence of competition is supported by the fact that in soil where there was lower species diversity, *Leptolyngbya* s. l. produced stronger populations than in aquatic species-rich communities.

Another question is how patchy the river really is. It is possible that conditions not far from the sampling site slightly differ, thus the species composition may be different too. Finally, there is the possibility that leptolyngbyoid species were present in collected samples but were overlooked due to their minor populations and inconspicuous dimensions.

Comparison of directly submerged environment, wet edges, and dry surrounding of the river

Leptolyngbyoid taxa were found in all three environments. Differences in the taxonomic composition existed among these environments.

Strains from directly submerged environment were assigned to *Leptolyngbya* cf. *margaritata* E33 and *Leptolyngbya* sp./*Nodosilinea bijugata* E4. *Leptolyngbya* cf. *margaritata* E33 was isolated from the mixture of mud and half-decayed leaves on the river bottom. According to the literature, this species occurs in metaphyton (Komárek & Anagnostidis 2005) or grows as an epiphyte on water macrophytes (Khanaev et al. 2020). The strain was isolated from the locality which was burdened with nutrient pollution, both N and P (Procházková et al. 2020). This is in concordance with findings of the species from the nutrient-rich littoral zone of Lake Baikal (Khanaev et al. 2020). *Leptolyngbya* sp. E4 was identified as *L. bijugata* according to the identification key (Komárek & Anagnostidis 2005). The species currently belongs to the genus *Nodosilinea* (Perkerson et al. 2011). This

determination is supported by the occasional presence of granules near the cell cross walls. However, the studied strain produced hormogonia, while Perkerson et al. (2011) state they are absent in this species. Nodules were also missing. In addition, the trichomes in strain E4 were narrower than in *N. bijugata*. Perkerson et al. (2011) suggested that the description of *L. bijugata* in the identification key by Komárek & Anagnostidis (2005) may be actually applicable for more species, thus the strain E4 does not have to be *N. bijugata*. To verify the species determination, gene sequences would be necessary. One additional sample from the aquatic habitat contained *Leptolyngbya*, but this species was lost during the purification process.

Strains from the boundary between river and surrounding soil differed from those obtained from directly submerged habitats. They included *Leptolyngbya foveolarum* E31, *Drouetiella lurida* E7 and *Anagnostidinema amphibium* E25. *L. foveolarum* is a species well known from moist soils (Komárek & Anagnostidis 2005). Like ecology, the morphology corresponds to the description in the identification key (Komárek & Anagnostidis 2005). The gene sequence for the confirmation of this identification has not been obtained yet. As regards *D. lurida* E7, the morphology of the strain agrees with the morphology described in Mai et al. (2018). The possible occurrence on moist soils was confirmed using the identification key (Komárek & Anagnostidis 2005, here under the name of *Leptolyngbya lurida*). ITS secondary structures (D1-D1' helix and Box B helix) were similar with slight differences (compare with Mai et al. 2018). *A. amphibium* is a species known from freshwater periphyton and wet soils (Komárek & Anagnostidis 2005, Johansen et al. 2017) which is in concordance with the ecology of the strain E25. ITS secondary structures did not agree with those shown in Johansen et al. (2017).

Soil samples contained species of *Leptolyngbya*, *Nodosilinea*, *Leibleinia*, and *Pseudanabaena*. Strain *Leptolyngbya* sp. E6 was not determined at the species level because molecular data are missing for this strain. As it was shown that strains possessing morphological features of *Leptolyngbya* s. s. may belong to *Nodosilinea* species, a proper determination is not possible based on morphology and ecology alone. The prominent feature of the strain *Leptolyngbya* sp. E3 was its conical, elongated apical cell. Filaments were mostly short. No suitable species possessing this morphology was found. Molecular data are not available, thus identification remains unresolved. Two strains (E5, E2) were assigned to *Nodosilinea epilithica* based on the 16S rRNA gene analysis. Although the majority of morphological features agreed with the morphology of *N. epilithica* (Perkerson et al. 2011),

characteristic nodules were not observed in these two strains. The rest of *Nodosilinea* strains possessed features agreeing with the morphology of this species (Perkerson et al. 2011). *Leibleinia* sp. E32 was not determined at the species level, as no *Leibleinia* species is known from soils. The most similar is *L. epiphytica*, which is a freshwater species (Komárek & Anagnostidis 2005, Kaštovský et al. 2010). Gene sequences have not been obtained so far. Similarly, *Pseudanabaena* strain E30 matched the description of *P. catenata* which is known only as an aquatic species (Komárek & Anagnostidis 2005). Here, however, molecular analyses confirmed the species identification.

The number of samples containing leptolyngbyoid taxa was not sufficient to properly evaluate their ecological valence. In addition, many strains have not been purified for molecular analyses, thus their correct identification has not been confirmed. On the other hand, several conclusions can be made based on findings within this thesis. It appears that some species have a broader ecological valence than previously thought. It is the case of *Pseudanabaena catenata* and perhaps also *Leibleinia epiphytica*. In contrast, *Nodosilinea epilithica* was found only in soils at a certain distance from the water which indicates it is strictly a terrestrial species.

Comparison of lotic, lentic, and terrestrial habitats

Several species were found in habitats with different ecological conditions. For example, *Leptolyngbya boryana* (strains E1 and E23) occurred both in ponds and puddles. These findings are consistent with previous reports (Anagnostidis & Komárek 2005, Kaštovský et al. 2010). Neither of the strains, however, grew in a metaphyton.

Similarly, strains of *Anagnostidinema pseudacutissimum* (E26, E38) were isolated from a pond and a puddle. These findings agree with the known habitat preferences (Johansen et al. 2017).

Strains identified as *Leptolyngbya foveolarum* (E11, E31, E35, E37) were found in the river, as well as in stagnant waters, including puddles. The identification key (Komárek & Anagnostidis 2005) suggests the species commonly grows as an epilithon in the submerged environment or it occupies soils in the terrestrial environment. While several strains (E11, E31, E37) met these requirements, one strain (E35) was isolated from a pond epipelon.

Anagnostidinema amphibium (E24, E25) was isolated from a river edge and from decaying organic matter in the pond. This species possesses a wide ecological valence (Komárek & Anagnostidis 2005, Kaštovský et al. 2010, Johansen et al. 2017).

Drouetiella lurida (E7, E13) was isolated from the boundary between soil and river and from a meadow soil. *D. lurida* is considered a freshwater species (Komárek & Anagnostidis 2005). Findings from a meadow soil indicate the species is more tolerant to various environmental conditions than is stated in the literature.

Strains of *Nodosilinea epilithica* were isolated from various soils. The species typically grows on stony substrates (Perkerson et al. 2011), but reports from soils also exist (Temraleeva 2018).

One nodule-forming strain of *Nodosilinea* was isolated from a pond. The strain could possibly be assigned to *N. bijugata* based on morphology and ecology (Komárek & Anagnostidis 2005, Perkerson et al. 2011). For this strain, however, molecular data for more proper determination are missing.

The findings described above indicate that many species can occur in various habitats with different environmental conditions.

Taxonomic issues

One of the most striking discrepancies was the presence of strains of *Anagnostidinema amphibium* (E24, E25) in the clade of *Leptolyngbya* s. l. in the phylogenetic tree. Morphological features of the studied strains indicate *A. amphibium* should not belong to *Anagnostidinema* s. s. The strains were capable of movement, as well as other *Anagnostidinema* species, but they resembled rather representatives of the subgenus *Protolyngbya* (longer cells, no necridic cells). ITS secondary structures supported the independence of this species. Nevertheless, the bootstrap support was not sufficient to prove the species should be transferred to the Leptolyngbyaceae family. It would also be necessary to add more strains to confirm the independence of this clade. Further revision is therefore desirable.

It is also notable that two analyzed strains assigned to *A. amphibium* possessed significant differences in ITS secondary structures (Fig. 41), their percentage of similarity was < 94 % (Table 11), and they were found in different habitats. One strain was sampled from the boundary between river and bank (wet soil), whereas the second one was isolated from a submerged stone in a pond. Hence, the species could possibly be split into two based on secondary structures, percentage of similarity, and ecology. Here, again, including more strains in molecular analyses would be necessary for such conclusions.

One remarkable observation is related to *Nodosilinea* species. From 18 strains isolated from various environments, six (i.e. 1/3) were assigned to the genus *Nodosilinea*. This is notewrothy considering the genus was established ten years ago (Perkerson et al. 2011). The high frequency of findings of *Nodosilinea* in soil samples indicates that the genus could fill the gap in knowledge about soil species of *Leptolyngbya* s. l. In the identification key by Komárek & Anagnostidis (2005), the only suggestion for soil species of the genus is *L. foveolarum* which is primarily an aquatic species. This conflicts with previous observations (Hajská 2019) that *Leptolyngbya* is very abundant in soils too. Analyses of morphology and DNA showed that *Nodosilinea* species do not always form distinctive nodules (Fig. 13, 16), thus the recognition of the genus is complicated. On the other hand, all sequenced strains possessing the morphology of *Leptolyngbya* s. s. which were obtained from soils were assigned to the genus. That could mean that *Nodosilinea* is a very common leptolyngbyoid genus in soils.

Identification of strains assigned to *Nodosilinea* at the species level turned out to be complicated. All six strains shared > 97.5 % similarity and were assigned to *N. epilithica* based on 16S rRNA analysis. However, there was a variability among ITS secondary structures, both D1–D1' helix and Box B helix, which did not correspond to helices of previously described *Nodosilinea* species (Perkersen et al. 2011, Heidari et al. 2018, Radzi et al. 2019, Vázquez-Martínez et al. 2018). Moreover, similar D1–D1' helices did not agree with similar Box B helices (Fig. 40, 41). A remarkable discovery was the congruence of proteomic data (Fig. 49) with D1–D1' helices. Both indicated there is a variability in *N. epilithica* at the infraspecific level and this variability cannot be detected based on 16S rRNA gene sequences only. As regards ecology, all strains were isolated from soil samples, which is not in conflict with the known distribution of *N. epilithica* (Temraleeva 2018). Also, no significant differences were found in morphology (the presence/absence of nodules did not correlate with the appearance of ITS secondary structures). These findings mean that there is a cryptic diversity in the genus *Nodosilinea*.

Leptolyngbya boryana and *L. foveolarum* represented another taxonomic problem. Both species frequently occur in freshwater ecosystems in central Europe (Komárek & Anagnostidis 2005, Kaštovský et al. 2010) but distinctive features are missing. *L. boryana* and *L. foveolarum* possess very similar morphology and their ecological demands are understudied. According to identification keys (e.g., Komárek & Anagnostidis 2005), *L. boryana* is a metaphytic species, while *L. foveolarum* mostly grows on submerged stones

or wet soil. This contrasts with observations within this thesis because *L. boryana* (confirmed by molecular analyses) was isolated from wet puddle edge and pond edge. Remaining strains with *L. boryana*/*L. foveolarum* have not been sequenced yet, thus this issue will require further studies.

Species determination was also problematical in the case of *Leibleinia* sp. E32. The most similar species was *L. epiphytica* which is a common epiphytic *Leibleinia* species in central Europe. However, the strain *Leibleinia* sp. E32 was isolated from wet soil near a river. This means that either the strain belongs to a different species, or *L. epiphytica* possesses wider ecological valence than has been known.

The most similar BLAST hits for the strain E17 were unspecified Leptolyngbyaceae cyanobacteria, thus this strain was not assigned to a certain species. It is clear this strain does not belong to other *Leptolyngbya* s. s. species. As regards morphology, the strain resembles the genus *Schizothrix* since filaments tend to form tight fascicles, gradually narrowing towards the ends. However, more trichomes in one sheath were not observed. On the contrary, all trichomes seemed to possess their own sheath. That would mean the strain is not a *Schizothrix* species. Nevertheless, laboratory conditions could have caused changes in morphology and natural populations could have possessed this feature.

As with the strain E17, it was not possible to determine strain E10 even at the generic level. The closest BLAST hits were *Jaaginema* sp. and *Tildeniella torsiva*. The problem with *Jaaginema* is that this genus usually does not possess sheaths (Komárek & Anagnostidis 2005), while this strain was ensheathed. As regards *Tildeniella torsiva*, its morphology is very similar to the morphology of the studied strain, but its dimensions do not agree. In addition, hormogonia are not typical for *T. torsiva* (Mai et al. 2018), while the strain E10 produced them. The strain is currently labelled as *Jaaginema* sp., but the correct determination remains unresolved. In any case, a noteworthy observation was that *Jaaginema* species formed a cluster with the genus *Tildeniella* (Ocullatelaceae). This information is important because *Jaaginema* is currently an unrevised genus with an uncertain position in the cyanobacterial taxonomic system (Mai et al. 2018).

Comparison of morphological and molecular diversity

When comparing morphology with molecular data, it is apparent that the molecular diversity significantly overlaps the morphological one. The most striking difference between the variability of morphological and molecular data can be observed in *Nodosilinea*. It was

evidenced that the genus does not have to form its characteristic nodules. Consequently, it is not possible to determine soil species with the phenotype of *Leptolyngbya* s. s. even at the generic level (and not even at the level of family) without the use of molecular techniques. Moreover, ITS secondary structures and proteomic data revealed a hidden diversity in a single species (*N. epilithica*). A simililar phenomenon was observed in the strains of *Anagnostidinema*.

Many strains from the aquatic environment possessed the morphology of *Leptolyngbya* s. s. Although there were attempts to determine these strains at the species level in several cases, the precise determination was impossible without the availability of gene sequences and protein spectra. This was a problem especially when distinguishing between *L. boryana* and *L. foveolarum*, but also in strains of different morphology. It is probable that molecular analyses would reveal more significant differences among these strains.

The applicability of MALDI-TOF MS for taxonomy and species identification

Proteomic data were utilized to create a dendrogram and a split network which were both based on the similarities among protein spectra. Although the principle of clustering was similar, strains in the dendrogram were clustered differently in comparison with the split network (compare Figs. 47 and 49). Also, when the MALDI-TOF dendrogram was compared to the 16S rDNA phylogenetic tree, the differences were significant. On the contrary, when genomic and proteomic data were processed using the same software (Splitstree4), the results were comparable (Figs. 48 and 49). It shows the importance of using the same algorithm for comparing these two types of molecular data.

Although split networks based on genomic and proteomic data were in concordance, there was one prominent difference between them. Whereas *Nodosilinea* strains formed one cluster in the network based on the 16S rRNA gene, two separate clusters were formed in the network based on the protein spectra. These two clusters were in agreement with two types D1–D1´ helices of *Nodosilinea* strains. That indicates that 16S–23S ITS secondary structures and proteomic data are more sensitive to variability at the infraspecific level in comparison with the 16S rRNA gene.

The similarity between networks based on genomic and proteomic data and the sensitivity to intraspecific variability signal that proteomic data could be included in the taxonomic studies which use the polyphasic approach. These results follow up on previous

works which tested the potential of MALDI-TOF MS in other cyanobacteria (Sun et al. 2016, Imanishi et al. 2016, Šebela et al. 2018). These studies showed that MALDI-TOF MS is a simple, rapid, and sensitive method suitable for various purposes, e.g., distinguishing between toxic and non-toxic cyanobacterial strains (Sun et al. 2016), or for taxonomic studies as an alternative to the 16S rRNA gene analysis (Šebela et al. 2018).

7. CONCLUSION

Within this thesis, filamentous cyanobacteria of the genus *Leptolyngbya* s. l. were studied using the polyphasic approach. In addition to standard markers (i.e., morphology, ecology, 16S rRNA gene, and 16S–23S ITS secondary structures), proteomic data were included to verify their potential to be utilized in the taxonomic studies of cyanobacteria.

The diversity of *Leptolyngbya* s. l. in the studied river was rather low, since the river is heavily burdened by anthropogenic activities. However, strains of the studied taxonomic group were isolated from all types of environment (water, boundary between water and bank, and bank). Other strains were isolated from stagnant water bodies, soil, and a rock.

This thesis shows a considerable portion of the diversity in *Leptolyngbya* s. l. is cryptic, especially in the genus *Nodosilinea* and *Anagnostidinema*. For this reason, the identification at the species and often even generic level is impossible without the use of molecular analyses.

Several taxonomic issues were proposed and solutions for some of them were suggested. For instance, strains identified as *Anagnostidinema amphibium* E24 and E25 should probably be assigned to a novel genus. The issue of identifying soil *Leptolyngbya* species was partially clarified – genomic and proteomic data showed many soil species possessing leptolyngbyoid morphology belong to the genus *Nodosilinea*. The need for furter revisions of *Leptolyngbya* s. l. is proposed since distinguishing between taxa in this group is not clear. Revision is also necessary for the genus *Jaaginema*.

Proteomic data turned out to be sensitive even to intraspecific variability, and thus could be utilized as an additional marker in future taxonomic studies.

8. REFERENCES

Abed R. M. M., Garcia-Pichel F., Hernández-Mariné M. (2002): Polyphasic characterization of benthic, moderately halophilic, moderately thermophilic cyanobacteria with very thin trichomes and the proposal of *Halomicronema excentricum* gen. nov., sp. nov. Archives of Microbiology 177: 361−370.

Agentura ochrany přírody a krajiny České republiky (2020): O databázi [online]. [Cited October 29 2020] Retrieved from http://vodnitoky.ochranaprirody.cz/vodni-toky-o-databazi/.

Akagha S. C., Johansen J. R., Nwankwo D. I., Yin K. (2019): *Lagosinema tenuis* gen. et sp. nov. (Prochlorotrichaceae, Cyanobacteria): a new brakish water genus from Tropical Africa. Fottea 19(1): 1−12.

Albertano P., Grilli-Caiola M. (1988): Effects of different light conditions on *Lyngbya* sp. in culture. Algological Studies/Archiv für Hydrobiologie, Supplement Volumes No. 50−53: 47−54.

Anagnostidis K. (2001): Nomenclatural changes in cyanoprokaryotic order Oscillatoriales. Preslia 73: 359−375.

Anagnostidis K., Komárek J. (1988): Modern approach to the classification system of cyanophytes 3 – Oscillatoriales. Algological Studies 50−53: 327−472.

Becerra-Absalón I., Johansen J. R., Muñoz-Martín M. A., Montejano G. (2018): *Chroakolemma* gen. nov. (Leptolyngbyaceae, Cyanobacteria) from soil biocrusts in the semi-desert Central Region of Mexico. Phytotaxa 367(3): 201−218.

Becerra-Absalón I., Johansen J. R., Osorio-Santos K., Montejano G. (2020): Two new *Oculatella* (Oculatellaceae, Cyanobacteria) species in soil crusts from tropical semi-arid uplands of México. Fottea 20(2): 160−170.

Biggs B. J. F., Goring D. G., Nikora V. I. (1998): Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology 34: 598–607.

Boulton A. J. (2007): Hyporheic rehabilitation in rivers: Restoring vertical connectivity. Freshwater Biology 52: 632–650.

Boyer S. L., Flechtner V., Johansen J. R. (2001): Is the 16S−23S rRNA internal transcribed spacer (ITS) region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. Molecular Biology and Evolution 18: 1057−1069.

Boyer S. L., Johansen J. R., Howard G. L. (2002): Phylogeny and genetic variance in terrestrial *Microcoleus* (Cyanophyceae) species based on sequence analysis of the 16S rRNA gene and associated 16S−23S ITS region. Journal of Phycology 38: 1222−1225.

Brown B. L., Swan C. M. (2010): Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology 79: 571–580.

Brown G. W. (1969): Predicting temperatures in small streams. Water Resources Research 5(1): 68−75.

Burger-Wiesma T., Stal L. J., Mur L. R. (1989): *Prochlothrix hollandica* gen. nov., sp. nov., a filamentous oxygenic photoautotrophic prokaryote containing chlorophylls a and b: assignment to Prochlorotrichaceae fam. nov. and order Prochlorales Florenzano, Balloni, and Materassi 1986, with emendation of the ordinal description. International Journal of Systematic Bacteriology 39(3): 250−257.

Casamatta D. A, Hašler P. (2016): Blue-green algae (Cyanobacteria) in rivers. Pp. 5–34 in Necchi O. Jr. (ed.): River Algae. Springer.

Casamatta D. A., Johansen J. R., Vis M. L., Broadwater S. T. (2005): Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria). Journal of Phycology 41: 421−438.

Casamatta D. A., Vis M. L., Sheath R. G. (2003): Cryptic species in cyanobacterial systematics: a case study of *Phormidium retzii* (Oscillatoriales) using RAPD molecular markers and 16S rDNA sequence data. Aquatic Botany 77(4): 295−309.

Castresana J. (2000): Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540-552.

Chakraborty S., Maruthanayagam V., Achari A., Pramanik A., Jaisankar P., Mukherjee J. (2019): *Euryhalinema mangrovii* gen. nov., sp. nov. and *Leptoelongatus littoralis* gen. nov., sp. nov. (Leptolyngbyaceae) isolated from an Indian mangrove forest. Phytotaxa 422(1): 58−74.

Cole M., Lindeque P., Fileman E., Halsband C., Galloway T. S. (2015): The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environmental Science and Technology 49: 1130−1137.

Connell J. H. (1978): Diversity in tropical rain forests and coral reefs. Science 199: 1302−1310.

Cordeiro R., Luz R., Vasconcelos V., Gonçalces V., Fonseca A. (2020): Cyanobacteria phylogenetic studies reveal evidence for polyphyletic genera from thermal and freshwater habitats. Diversity 12(8): 298.

ČSÚ (2021): Počet obyvatel v obcích k 1. 1. 2021. [online]. [Cited May 1 2021] Retrieved from https://czso.cz/csu/czso/počet-obyvatel-v-obcich-k-112021?fbclid=lwAR1QylJHv_MEMh8YyMegwP_QaM8y6iqI7ZAX0Q16Xgc6rfZQgw94L3VA0bk.

Dadheech P. K., Mahmoud H., Kotut K., Krienitz L. (2012): *Haloleptolyngbya alcalis* gen. et sp. nov., a new filamentous cyanobacterium from the soda lake Nakuru, Kenya. Hydrobiologia 691: 269−283.

Doretto A., Piano E., Larson C. E. (2020a): The river continuum concept: lessons from the past and perspectives for the future. Canadian Journal of Fisheries and Aquatic Sciences 77(11): 1853−1864.

Doretto A., Bona F., Falasco E., Morandini D., Piano E., Fenoglio S. (2020b): Stay with the flow: How macroinvertebrate communities recover during the rewetting phase in Alpine streams affected by an exceptional drought. River Research and Applications 36: 91−101.

Doyle J. (1991): DNA protocols for plants. Pp. 283−293 in Hewitt G. M., Johnston A.W. B., Young J P. W. (eds.): Molecular techniques in taxonomy. NATO ASI Series (Series H: Cell Biology), vol. 57. Springer.

Dvořák P., Hašler P. (2007): Occurrence and morphological variability of *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya et Subba Raju (Cyanophyta, Nostocales) near Olomouc in 2006. Fottea 7(1): 39−42.

Dvořák P., Hašler P., Pitelková P., Tabáková P., Casamatta D. A., Poulíčková A. (2017): A new cyanobacterium from the Everglades, Florida – *Chamaethrix* gen. nov. Fottea 17(2): 269−276.

Dvořák P., Hindák F., Hašler P., Hindáková A., Poulíčková A. (2014): Morphological and molecular studies of *Neosynechococcus sphagnicola*, gen. et sp. nov. (Cyanobacteria, Synechococcales). Phytotaxa 170(1): 24−34.

Dvořák P., Jahodářová E., Hašler P., Gusev E., Poulíčková A. (2015): A new tropical cyanobacterium *Pinocchia polymorpha* gen. et sp. nov. derived from the genus *Pseudanabaena*. Fottea 15(1): 113−120.

Echenique-Subiabre I., Tenon M., Humbert J.-F., Quiblier C. (2018): Spatial and temporal variability in the development and potential toxicity of *Phormidium* biofilms in the Tarn River, France. Toxins 418. DOI: 10.3390/toxins10100418.

Edgar R. C. (2004): MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1732−1797.

EDPP (2021): Hydrologické údaje [online]. [Cited May 1 2021] Retrieved from https://www.edpp.cz/let_hydrologicke-udaje/.

ESRI (2016): Technical Support [online]. [Cited May 1 2021] Retrieved from https://support.esri.com/en/Products/Desktop/arcgis-desktop/arcmap/10-4-1.

Fox G. E., Wisotzkey J. D., Jurtshuk P. Jr. (1992): How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. International Journal of Systematic Bacteriology 42(1): 166−170.

García M. E., Aboal M. (2014): Environmetnal gradients and macroalgae in Mediterranean marshes: the case of Pego-Oliva marsh (East Iberian Peninsula). Science of the Total Environment 475: 216−224.

Garmo Ø. A., Kaste Ø., Arle J., Austnes K., de Wit H., Fölster J., Houle D., Hruška J., Indriksone I., Monteith D., Rogora M., Sample J. E., Steingruber S., Stoddard J. L., Talkop R., Trodd W., Ulańczyk R. P., Vuorenmaa J. (2020): Trends and patterns in surface water chemistry in Europe and North America between 1990 and 2016, with particular focus on changes in land use as a confounding factor for recovery. ICP Water report 142/2020.

Geitler L. (1932): Cyanophyceae. Pp. 1−1196 in Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz 14. Akademische Verlagsgesellschaft.

Gibb S., Strimmer K. (2012): MALDI-quant: a versatile R package for the analysis of mass spectrometry data. Bioinformatics 28(17): 2270–2271.

Ginebreda A., Muñoz I., López de Alda M., Brix R. (2010): Environmental risk assessment of pharmaceuticals in rivers: Relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environment International 36: 153–162.

Glibert P. M. (2017): Eutrophication, harmful algae and biodiversity – Challenging paradigms in a world of complex nutrient changes. Marine Pollution Bulletin 124(2): 591–606.

Grabowska M., Mazur-Marzec H. (2011): The effect of cyanobacterial blooms in the Sieminówka Dam Reservoir on the phytoplankton structure in the Narew River. Oceanological and Hydrobiological Studies 40(1): 19–26.

Grime J. P. (1973): Competitive exclusion in herbaceous vegetation. Nature 242: 344–347.

Guiry M. D., Guiry G. M. (2021): AlgaeBase. World-wide electronic publication, National University of Ireland, Galway [online]. [Cited May 1 2021] Retrieved from https://www.algaebase.org.

Hajská E. (2019): *Leptolyngbya* sensu lato as a source of cyanobacterial diversity. Bachelor thesis (Bc). Palacký University in Olomouc, Faculty of Science.

Hašler P., Dvořák P., Johansen J. R., Kitner M., Ondřej V., Poulíčková A. (2012): Morphological and molecular study of epipelic filamentous genera *Phormidium*, *Microcoleus* and *Geitlerinema* (Oscillatoriales, Cyanophyta/Cyanobacteria). Fottea 12(2): 341–356.

Hašler P., Hindák F., Hindáková A. (2007): Phytoplankton of the Morava and Dyje Rivers in spring and summer 2006. Fottea 7(1): 49–67.

Heath M. W., Wood S. A., Ryan K. G. (2010): Polyphasic assessment of fresh-water benthic mat-forming cyanobacteria isolated from New Zealand. FEMS Microbial Ecology 73: 95–109.

Heidari F., Zima J. Jr., Riahi H., Hauer T. (2018): New simple trichal cyanobacterial taxa isolated from radioactive thermal springs. Fottea 18(2): 137−149.

Hein T., Schwarz U., Habersack H., Nichersu I., Preiner S., Willby N., Weigelhofer G. (2016): Current status and restoration options for floodplains along the Danube River. Science of the Total Environment 543: 778–790.

Hindák F., Hindáková A., Marvan P., Heteša J., Hašler P. (2006): Diversity, abundance and volume biomass of the phytoplankton of the Morava River (Czech Republic, Slovakia) and the Dyje River (Czech Republic) in November 2005. Fottea 6(1): 77–97.

Hoffman L. (1985): Quelques remarques sur la classification des Oscillatoriaceae. Cryptogamie, Algologie 6: 71–79.

Horsák M., Bojková J., Zahrádková S., Omesová M., Helešic J. (2009): Impact of reservoirs and channelization on lowland river macroinvertebrates: A case study from Central Europe. Limnologica 39(2): 140–151.

Humphries P., Keckeis H., Finlayson B. (2014): The river wave concept: Integrating river ecosystem models. Bioscience 64: 870–882.

Huston M. (1979): A general hypothesis of species diversity. The American Naturalist 113(1): 81–101.

Huson D. H., Bryant D. (2006): Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23(2): 254–267.

Imanishi S.Y., Nakayama T., Asukabe H., Harada K. (2016): Application of MALDI Biotyper to cyanobacterial profiling. Rapid Communications in Mass Spectrometry 31(4): 325−332.

Jahodářová E., Dvořák P., Hašler P., Poulíčková A. (2017a): Revealing hidden diversity among tropical cyanobacteria: the new genus *Onodrimia* (Synechococcales, Cyanobacteria) described using the polyphasic approach. Phytotaxa 326(1): 28−40.

Jahodářová E., Dvořák P., Hašler P., Holušová K., Poulíčková A. (2017b): *Elainella* gen. nov.: a new tropical cyanobacterium characterized using a complex genomic approach. European Journal of Phycology 53(1): 39−51.

Jarvie H. P., Neal C., Withers P. J. A. (2006): Sewage-effluent phosphorus: A greater risk to river eutrophication than agricultural phosphorus? Science of the Total Environment 360: 246–253.

Johansen J. R., Strunecký O., Bohunická M., Čapková K., Raabová L., Dvořák P., Komárek J. (2017): A revision of the genus *Geitlerinema* and a description of the genus *Anagnostidinema* gen. nov. (Oscillatoriophycidae, Cyanobacteria). Fottea 17(1): 114–126.

Jones K. C., de Voogt P. (1999): Persistent organic pollutants (POPs): state of science. Environmental Pollution 100: 209–221.

Jung P., Mikhailyuk T., Emrich D., Baumann K., Dultz S., Büdel B. (2020): Shifting boundaries: Ecological and geographical range extension based on three new species in the cyanobacterial genera *Cyanocohniella*, *Oculatella*, and *Aliterella*. Journal of Phycology 56(5): 1216−1231.

Junk W. J., Bayley P. B., Sparks R. E. (1989): The flood pulse concept in river-floodplain systems. Pp. 110–127 in Dodge D. P. (ed.): Proceedings of the International Large River Symposium. Canadian Special Publication of Fisheries and Aquatic Sciences.

Kanzari F., Syakti A. D., Asia L., Malleret L., Piram A., Mille G., Doumenq P. (2014): Distributions and sources of persistent organic pollutants (aliphatic hydrocarbons, PAHs, PCBs and pesticides) in surface sediments f an industrialized urban river (Huveaune), France. Science of the Total Environment 478: 141–151.

Kaštovský J., Hauer T., Komárek J., Skácelová O. (2010): The list of cyanobacterial species of the species of the Czech Republic to the end of 2009. Fottea 10(2): 245−249.

Kaštovský J., Hauer T., Geriš R., Chattová B., Juráň J., Lepšová-Skácelová O., Pitelková P., Puzstai M., Škaloud P., Šťastný J., Čapková K., Bohunická M., Mühlsteinová R. (2018): Atlas sinic a řas České republiky 1. Powerprint, Praha. Komárek J. (2007): Phenotype diversity of the cyanobacterial genus *Leptolyngbya* in the maritime Antarctic. Polish Polar Research 28(3): 211−231.

Khanaev I. V., Obolkina L. A., Belykh O. I., Nebsnyhk I. A., Sukhanova E. V., Fedotov A. P. (2020): Mass development of periphyton ciliates in the coastal zone of Southern Baikal in 2019-2020. Limnology and Freshwater Biology 3: 433−438.

Komárek J. (2016): A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. European Journal of Phycology 51(3): 346−353.

Komárek J., Anagnostidis K. (2005): Cyanoprokaryota 2 – Oscillatoriales. In Büdel B., Krienitz L., Gärtner G., Schagerl M. (eds.): Süsswasserflora von Mitteleuropa 19/2. Elsevier GmbH, Spektrum Akademischer Verlag, München.

Komárek J., Kaštovský J., Mareš J., Johansen J. R. (2014): Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86(4): 295−335.

Komárek J., Lukavský J. (1988): *Arthronema,* a new cyanophyte genus from Afro-Asian deserts. Archiv für Hydrobiologie (Algological Studies) 50−53: 249−267.

Konstantinou D., Voultsiadou E., Panteris E., Gkelis S. (2021): Revealing new sponge-associated cyanobacterial diversity: Novel genera and species. Molecular Phylogenetics and evolution 155: 106991.

Kristiansen J. (1996): Dispersal of freshwater algae – a review. Hydrobiologia 336: 151–*157.*

Kukučka P., Audy O., Kohoutek J., Holt E., Kalábová T., Holoubek I., Klánová J. (2015): Source identification, spatio-temporal distribution and ecological risk of persistent organic pollutants in sediments from the upper Danube catchment. Chemosphere 138: 777–783.

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547−1549.

Lahens L., Strady E., Kieu-Le T.-C., Dris R., Boukerma K., Rinnert E., Gasperi J., Tassin B. (2018): Macroplastic and microplastic assessment of a tropical river (Saigon River, Vietnam) transversed by a developing megacity. Environmental Pollution 236: 661–671.

Leibold M. A., Holyoak M., Mouquet N., Amarasekare P., Chase J. M., Hoopes M. F., Holt R. D., Shurin J. B., Law R., Tilman D., Loreau M., Gonzalez A. (2004): The metacommunity concept: A framework for multi-scale community ecology. Ecology Letters 7: 601–613.

Li X., Li R. (2016): *Limnolyngbya circumcreta* gen. & comb. nov. (Synechococcales, Cyanobacteria) with three geographical (provincial) genotypes in China. Phycologia 55(4): 478−491.

Loza V., Berrendero E., Perona E., Mateo P. (2013): Polyphasic characterization of benthic cyanobacterial diversity from biofilms of the Guadarrama River (Spain): Morphological, molecular, and ecological approaches. Journal of Phycology 49: 282–297.

Mai T., Johansen J. R., Pietrasiak N., Bohunická M., Martin M. P. (2018): Revision of the Synechococcales (Cyanobacteria) through recognition of four families including Oculatellaceae fam. nov. and Trichocoleaceae fam. nov. and six new genera containing 14 species. Phytotaxa 365(1): 1−59.

Malaj E., von der Ohe P. C., Grote M., Kühne R., Mondy C. P., Usseglio-Polatera P., Brack W., Schäfer R. B. (2014): Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proceedings of the National Academy of Sciences of the United States of America 111(26): 9549–9554.

Malone C. F. D. S., Genuário D. B., Vaz M. G. M. V., Fiore M. F., Sant'Anna C. L. (2020): *Monilinema* gen. nov., a homocytous genus (Cyanobacteria, Leptolyngbyaceae) from saline-alkaline lakes of Pantanal wetlands, Brazil. Journal of Phycology. DOI: 10.1111/jpy.13106.

Mani T., Hauk A., Walter U., Burkhardt-Holm P. (2015): Microplastics profile along the Rhine River. Scientific Reports 5: article no. 17988.

Mareš J. (2018): Multilocus and SSU rRNA gene phylogenetic analyses of available cyanobacterial genomes, and their relation to the current taxonomic system. Hydrobiologia 811: 19−34.

McAllister T. G., Wood S. A., Hawes I. (2016): The rise of toxic benthic *Phormidium* proliferations: A review of their taxonomy, distribution, toxin content and factors regulating prevalence and increased severity. Harmful Algae 55: 282−294.

McCormick P. V. (1996): Resource competition and species coexistence. Pp. 229−252 in Stevenson J. R., Bothwell M. L., Lowe R. L. (eds.): Algal Ecology: Freshwater Benthic Ecosystems (section two). Academic Press.

Minaudo C., Abonyi A., Leitão M., Lançon A. M., Floury M., Descy J.-P., Moatar F. (2021): Long-term impacts of nutrient control, climate change, and invasive clams on

phytoplankton and cyanobacteria biomass in a large temperate river. Science of the Total Environment 756: 144074.

Miscoe L. H., Johansen J. R., Vaccarino M. A., Pietrasiak N., Sherwood A. R. (2016): Novel cyanobacteria from caves on Kauai, Hawai. Pp. 75−152 in Miscoe L. H., Johansen J. R., Kociolek J. P., Lowe R. L., Vaccarino M. A., Pietrasiak N., Sherwood A. R. (eds.): The diatom flora and cyanobacteria from caves on Kauai, Hawaii. Gebr. Borntraeger Verlagsbuchhandlung, Stuttgart.

Mitrovic S M., Hardwick L., Dorani F. (2011): Use of flow management to mitigate cyanobacterial blooms in the Lower Darling River, Australia. Journal of Plankton Research 33(2): 229−241.

Mohamed Z. A., El-Sharouy H. M., Ali W. S. M. (2006): Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigational canals, Egypt. Toxicon 47: 584−590.

Mosser J. L., Brock T. D. (1979): Temperature optima for algae inhabiting cold mountain sreams. Arctic and Alpine Research 8(1): 111−114.

Mühlsteinová R., Johansen J. R., Pietrasiak N., Martin M. P., Osorio-Santos K., Warren S. D. (2014): Polyphasic characterization of *Trichocoleus desertorum* sp. nov. (Pseudanabaenales, Cyanobacteria) from desert soils and phylogenetic placement of the genus *Trichocoleus*. Phytotaxa 163(5): 241−261.

Nei M., Kumar S. (2000): Molecular evolution and phylogenetics. Oxford University Press.

Newbold J. D., Elwood J. W., O'Neill R. V., Winkle W. V. (1981): Measuring nutrient spiraling in streams. Canadian Journal of Fisheries and Aquatic Sciences 38(7): 860−863.

O'Neil J. M., Davis T. W., Burford M. A., Gobler C. J. (2012): The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14: 313−334.

Osorio-Santos K., Pietrasiak N., Bohunická M., Miscoe L. H., Kováčik L., Martin M. P., Johansen J. R. (2014): Seven new species of *Oculatella* (Pseudananbaenales, Cyanobacteria): Taxonomically recognizing cryptic diversification. European Journal of Phycology 49(4): 450−470.

Palarea-Albaladejo J., McLean K., Wright F., Smith D. (2018): MALDIrppa: quality control and robust analysis for mass spectrometry data. Bioinformatics 34(3): 522−523.

Palmer M. A., Menninger H. L., Bernhardt E. (2010): River restoration, habitat heterogeneity and biodiversity: a failure of theory to practice? Freshwater Biology 55: 205−222.

Park H.-K., Lee H.-J., Heo J., Yun J.-H., Kim Y.-J., Kim H.-M., Hong D.-G., Lee I.-J. (2021): Deciphering the key factors determining spatio-temporal heterogeneity of cyanobacterial bloom dynamics in the Nakdong River with consecutive large weirs. Science of the Total Environment 7555: 143079.

Patel J. B. (2001): 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Molecular Diagnosis 6(4): 313−321.

Pearl H. W., Otten T. G. (2013): Harmful cyanobacterial blooms: Causes, consequences and controls. Microbial Ecology 65: 995−1010.

Perkerson III. R. B., Johansen J. R., Kováčik L., Brand J., Kaštovský J., Casamatta D. A. (2011): A unique pseudanabaenalean (cyanobacteria) genus *Nodosilinea* gen. nov. based on morphological and molecular data. Journal of Phycology 47(6): 1397−1412.

Pietrasiak N., Osorio-Santos K., Shalygin S., Martin M. P., Johansen J. R. (2019): When is a lineage a species? A case study in *Myxacorys* gen. nov. (Synechococcales: cyanobacteria) with the description of two new species from the Americas. Journal of Phycology 55: 976−996.

Pietrasiak N., Reeve S., Osorio-Santos K., Lipson D. A., Johansen J. R. (2021): *Trichotorquatus* gen. nov. – a new genus of soil cyanobacteria discovered from American drylands. Journal of Phycology. DOI: 10.1111/jpy.13147.

Pinevich A., Velichko N., Ivanikova N. (2012): Cyanobacteria of the genus *Prochlorothrix*. Frontiers in Microbiology 3: article 173.

Posada D. (2008): jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25(7): 1253−1256.

Procházková L., Lošťáková Z., Kosour D., Geriš R., Jahodová D., Husák V. (2020): Souhrnná zpráva o vývoji jakosti povrchových vod v povodí Moravy ve dvouletí 2018−2019 Povodí Moravy.

Raabová L., Kováčik L., Elster J., Strunecký O. (2019): Review of the genus *Phormidesmis* (Cyanobacteria) based on environmental, morphological, and molecular data with description of a new genus Leptodesmis. Phytotaxa 395(1): 1−16.

Radzi R., Muangmai N., Broady P., Omar W. M. W., Lavoue S., Convey P., Merican F. (2019): *Nodosilinea signiensis* sp. nov. (Leptolyngbyaceae, Synechococcales), a new terrestrial cyanobacterium isolated from mats collected on Signy Island, South Orkney Islands, Antarctica. PLOS ONE 14(11): e0224395.

Read D. S., Bowes M. J., Newbald L. K., Whiteley A. S. (2014): Weekly flow cytometric analysis of riverine phytoplankton to determine seasonal bloom dynamics. Environmnetonal Science Processes and Impacts 16: 594–603.

Rippka R, Deruelles J., Waterbury J. B., Herdman M., Stanier R. Y. (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111(1): 1−61.

Ronquist F. & Huelsenbeck J. P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572−1574.

Rulík M., Helešic J., Maršálek B. (2014): Znečišťování kontinentálních vod. Pp. 47–121 in Adámek Z., Helešic J., Maršálek B., Rulík M. (eds.): Aplikovaná hydrobiologie.

Rulík M., Opatřilová L., Jurajda P., Špaček J., Grulich V. (2020): Rivers in the Czech Republic. Pp. 39–69 in Zelenakova M., Fialová J., Negm A. M. (eds.): Assessment and Protection of Water Resources in the Czech Republic.

Quiblier C., Wood S. A., Echenique-Subiabre I., Heath M., Villeneuve A., Humbert J.-F. (2013): A review of current knowledge on toxic benthic freshwater cyanobacteria – Ecology, toxin production and risk management. Water Research 47(15): 5464–5479.

Sabater S., Vilalta E., Gaudes A., Gausch, H., Muñoz I., Romaní A. (2003): Ecological implications of mass growth of benthic cyanobacteria in rivers. Aquatic Microbial Ecology 32: 175–184.

Sanchez W., Bender C., Porcher J.-M. (2014): Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: Preliminary study and first evidence. Environmental Research 128: 98–100.

Sauvageau C. (1892). Sur les algues d'eau douce recoltées en Algérie pendant le session de la Societé Botanique en 1892. Bulletin de la Société Botanique de France 39: 104-128, pl. 6.

Schmera D., Árva D., Boda P., Bódis E., Bolgovics Á., Borics G., Csercsa A., Deák C., Krasznai E. Á., Lukács B. A., Mauchart P., Móra A., Sály P., Specziár A., Süveges K., Szivák I., Takács P., Tóth M., Várbíró G., Vojtkó A. E., Erös T. (2018): Does isolation influence the relative role of environmental and dispersal-related processes in stream networks? An empirical test of the network position hypothesis using multiple taxa. Freshwater Biology 63: 74–85.

Sciuto K., Moro I. (2016): Detection of the new cosmopolitan genus *Thermoleptolyngbya* (Cyanobacteria, Leptolyngbyaceae) using the 16S rRNA gene and 16S−23 ITS region. Molecular Phylogenetics and Evolution 105: 15−35.

Sciuto K., Moschin E., Moro I. (2017): Cryptic cyanobacterial diversity in the giant cave (Trieste, Italy): the new genus *Timaviella* (Leptolyngbyaceae). Cryptogamie, Algologie 38(4): 285−323.

Scott J. T., Marcarelli A. M. (2012): Cyanobacteria in freshwater benthic environments. Pp. 271−289 in Whitton B. (ed.): Ecology of cyanobacteria II. Springer.

Šebela M., Jahodářová E., Raus M., Lenobel R., Hašler P. (2018): Intact cell MALDI-TOF mass spectrometric analysis of *Chroococcidiopsis* cyanobacteria for classification purposes and identification of possible marker proteins. PloS ONE 13(11): e0208275.

Singhal N., Kumar M., Kanaujia P. K., Virdi J. S. (2015): MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Frontiers in Microbiology 6: 791.

Song G.-F., Jiang Y.-G., Li R.-H. (2015): *Scytolyngbya timoleontis*, gen. et sp. nov. (Leptolyngbyaceae, Cyanobacteria): a new false branching cyanobacteria from China. Phytotaxa 224(1): 72−84.

Staub R. (1961): Ernährungphysiologish-autökologische Untersuchung an den planktonischen Blaualge *Oscillatoria rubescens* DC. Schweizerische Zeitschrift für Hydrologie 23: 82−198.

Strunecký O., Raabová L., Bernardova A., Ivanova A. P., Semanova A., Crossley J., Kaftan D. (2020): Diversity of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella* and *Shackletoniella*. FEMS Microbiology Ecology 96(3): fiz189.

Sun L.-W., Jiang W.-J., Sato H., Kawachi M., Lu X.-W. (2016): Rapid classification and identification of *Microcystis aeruginosa* strains using MALDI-TOF MS and polygenetic analysis. PLOS ONE 11(5): e0156275.

Sussarellu R., Suquet M., Thomas Y., Lambert C. Fabioux C., Pernet M. E. J., Le Goïc N., Quillien V., Mingant C., Epelboin Y., Corporeau C., Guyomarch J., Robbens J., Paul-Pont I., Soudant P., Huvet A. (2016): Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences of the United States of America 113(9): 2430–2435.

Suzuki R., Shimodaira H. (2006): Pvclust: an R package for assessing the uncertainty in hierarchical clustering. Bioinformatics 22(12): 1540–1542.

Svobodová J., Matěna J., Kopáček J., Poláková S., Vrba J. (2012): Spatial and temporal changes of benthic macroinvertebrate assemblages in acidified streams in the Bohemian Forest (Czech Republic.). Aquatic Insects 34: 157–172.

Sweeney B. W., Bott T. L., Jackson J. K., Kaplan L. A., Newbold J. D., Standley L. J., Hession W. C., Horwitz R. J. (2004): Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proceedings of the National Academy of Sciences of the United States of America 101(39): 14132–14137.

Taton A., Wilmotte A., Šmarda J., Elster J., Komárek J. (2011): *Plectolyngbya hodgsonii*: a novel filamentous cyanobacterium from Antarctic lakes. Polar Biology 34(2): 181–191.

Temraleeva A. D. (2018): Cyanobacterial diversity in the soils of Russian steppes and semidesserts. Microbiology 87: 249–260.

Thorp J. H., Delong M. D. (1994): The riverine productivity model: a heuristic view of carbon sources and organic processing in large river ecosystems. Oikos 70: 305–*308.*

Tockner K., Pusch M., Borchardt D., Lorang M. S. (2010): Multiple stressors in coupled river-floodplain ecosystems. Freshwater Biology 55: 135–151.

Townsend C. R. (1989): The patch dynamics concept of stream community ecology. Journal of the North American Benthological Society 8(1): 36–50.

Turicchia S., Ventura S., Komárková J., Komárek J. (2009): Taxonomic evaluation of cyanobacterial microflora from alkaline marshes of northern Belize. 2. Diversity of oscillatorialean genera. Nova Hedwigia 89(1−2): 165−200.

van der Grinten E., Janssen A. P. H. M., de Mutsert K., Barranguet C., Admiraal W. (2005): Temperature- and light-dependent performance of the cyanobacterium *Leptolyngbya foveolarum* and the diatom *Nitzschia perminuta* in mixed biofilms. Hydrobiologia 548: 267−278.

Vannote R. L., Minshall G. W., Cummins K. W., Sedell J. R., Cushing C. (1980): The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130–137.

Vaz M. G. M. V., Genuário D. B., Andreote A. P. D., Malone C. F. S., Sant'Anna C. L., Barbiero L., Fiore M. F. (2015): *Pantanalinema* gen. nov. and *Alkalinema* gen. nov.: novel pseudanabaenacean genera (Cyanobacteria) isolated from saline-alkaline lakes. International Journal of Systematic and Evolutionary Microbiology 65(1): 298−308.

Vázquez-Martínez J., Gutierrez-Villagomez J. M., Fonseca-García C., Ramírez-Chávez E., Mondragón-Sánchez M. L., Partida-Martínez L., Johansen J. R., Molina-Torres J. (2018): *Nodosilinea chupicuarensis* sp. nov. (Leptolyngbyaceae, Synechococcales) a subaerial cyanobacterial isolated from a stone monument in central Mexico. Phytotaxa 334(2): 167−182.

Vilela C. L. S., Bassin J. P., Peixoto R. S. (2018): Water contamination by endocrine disruptors: Impacts, microbiological aspects and trends for environmental protection. Environmental Pollution 235: 546–559.

Vis M. (2016): Biogeography of river algae. Pp. 219–243 in Necchi O. Jr. (ed.): River Algae. Springer.

Wang J., Li Y., Lu L., Zheng M., Zhang X., Tian H., Wang W., Ru S. (2019): Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (*Oryzias melastigma*). Environmental Pollution 254: 113024.

Ward J. W. (1989): The four-dimensional nature of lotic ecosystems. Journal of the North American Benthological Society 8(1): 2–8.

Ward J. W., Stanford J. A. (1983): The serial discontinuity concept of lotic ecosystems. Pp. 29–42 in Fontaine T. D., Bartell S. M. (eds.): Dynamics of lotic ecosystems. Ann Arbor Science.

Ward J. W., Stenford J. A. (1988): Thy hyporheic habitat of river ecosystems. Nature 335: 64–66.

Ward J. W., Stanford J. A. (1993): An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. Journal of the North American Benthological Society 12(1): 48–60.

Ward J. W., Stanford J. A. (1995): The serial discontinuity concept: Extending the model to floodplain rivers. Regulated rivers: Research and Management 10: 159-168.

Ward J. W., Tockner K., Schiemer F. (1999): Biodiversity of floodplain river ecosystems: ecotones and connectivity. Regulated Rivers: Research and Management 15: 125–139.

Webster J. R., Waide J. B., Patten B. C. (1975): Nutrient recycling and the stability of ecosystems. Pp. 1–27 in Howel F. G., Gentry J. B., Smith M. H. (eds.): Mineral cycling in southeastern ecoystems. ERDA Symposium Series CONF-740513.

Wen Y., Schoups G., van de Giesen N. (2017): Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. Scientific Reports, 43289.

Windsor F. M., Tilley R. M., Tyler C. R., Ormerod S. J. (2019): Microplastic ingestion by riverine macroinvertebrates. Science of the Total Environment 646: 68–74.

Wohl E. (2017): Connectivity in rivers. Progress in Physical Geography 41(3): 345–362.

Wood S. A., Depree C., Brown L., McAllister R., Hawes I. (2015b): Entrapped sediments as a source of phosphorus in epilithic cyanobacterial proliferations in low nutrient rivers. PLOS ONE 10(10): e0141063.

Wood S. A., Hawes I., McBride G. Truman P., Dietrich D. (2015a): Advice to inform the development of a benthic cyanobacteria attribute. Prepared for Ministry for the Environment. Cawthron Report No. 2752.

Wood S. A., Kelly L. T., Bouma-Gregson K., Humbert J.-F., Laughinghouse H. D. IV., Lazorchak J., McAllister T. G., McQueen A., Pokrzywinski K., Puddick J., Quiblier C., Reitz L. A., Ryan K. G., Vadeboncoeur Y., Zastepa A., Davis T. W. (2020): Toxic benthic freshwater cyanobacterial proliferations: Challenges and solutions for enhancing knowledge and improving monitoring and mitigation. Freshwater Biology 65: 1824–1842.

Xu J. (2004): A study of anthropogenic seasonal rivers in China. Catena 55: 17–32.

Xu J., Xu Y., Wang H., Guo C., Qiu H., He Y., Zhang Y., Li X., Meng W. (2015): Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river.

Yonkos L. T., Friedel E. A., Perez-Reyes A. C., Ghosal S., Arthur C. D. (2014): Microplastics in four estuarine rivers in the Chesapeake Bay, USA. Environmental Science and Technology 48(24): 14195–14202.

Zammit G., Billi D., Albertano P. (2012): The subaerophytic cyanobacterium *Oculatella subterranea* (Oscillatoriales, Cyanophyceae) gen. et sp. nov.: a cytomorphological and molecular description. European Journal of Phycology 47(4): 341−354.

Zhu F., Yang M., Luo Z., Yu R., Hu G., Yan Y. (2020): Bioaccumulation and biotransformation of arsenic in *Leptolyngbya boryna*. Environmental Science and Pollution Research 27: 29993−30000.

Zuker M. (2003): Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Research 31: 3406−3415.

9. APPENDICES

Appendix 1. Map of sampling sites in the town of Svitavy. Created in the ArcGIS 10.4 (ESRI 2016).

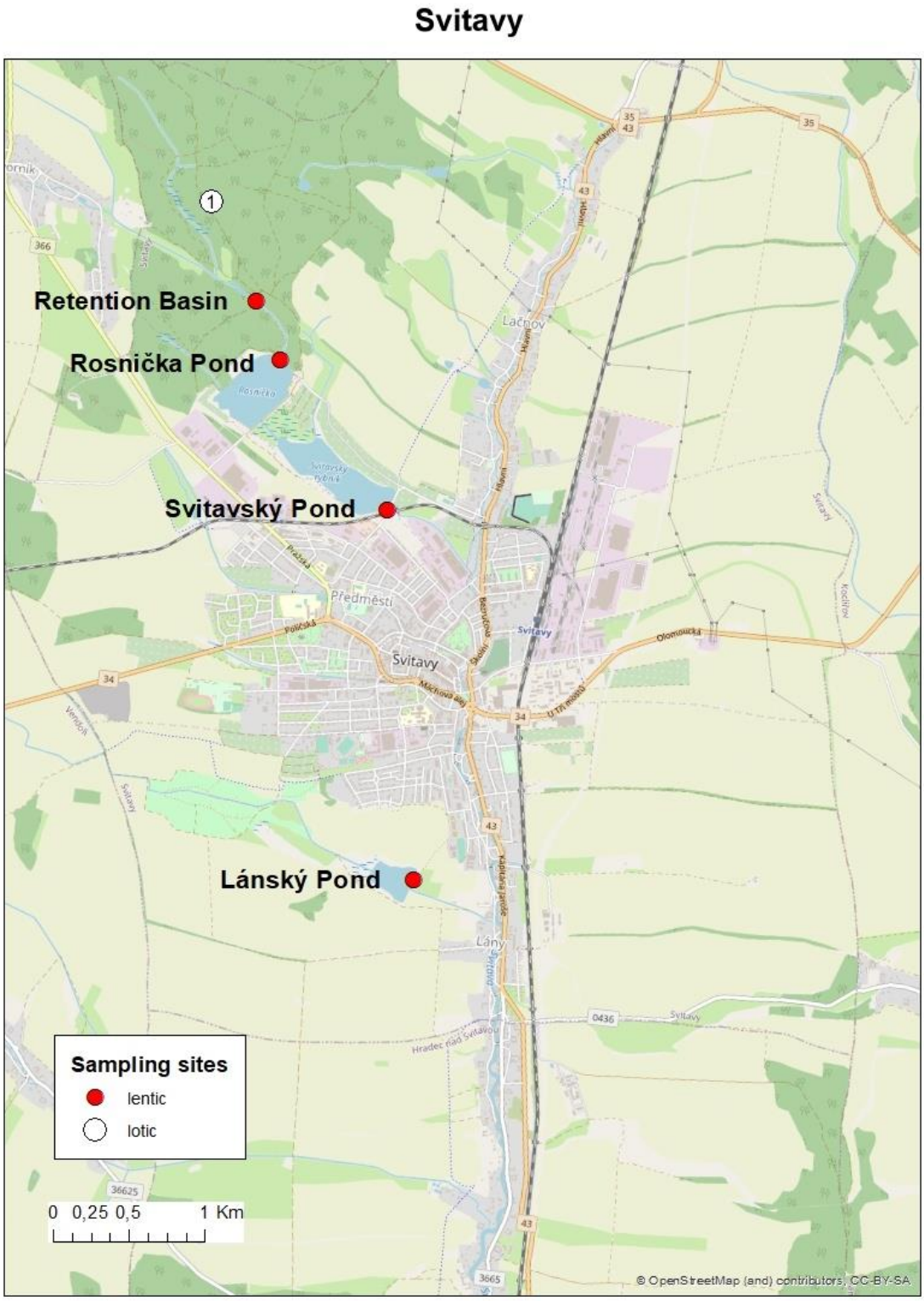
Appendix 2. Map of sampling sites in the town of Letovice. Created in the ArcGIS 10.4 (ESRI 2016).

Appendix 3. Map of sampling sites in the town of Rájec-Jestřebí. Created in the ArcGIS 10.4 (ESRI 2016).

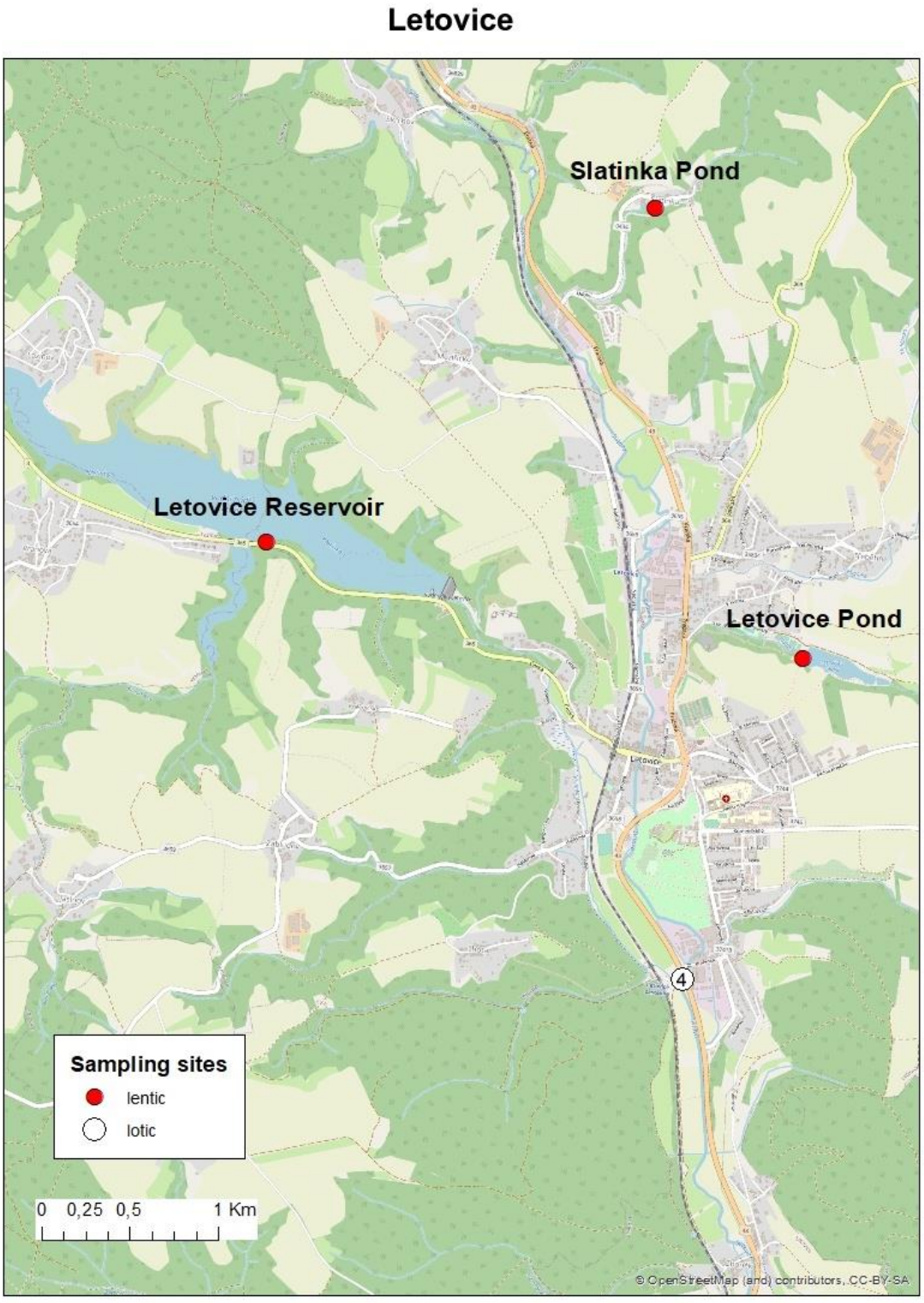
Appendix 4. Map of sampling sites in the town of Blansko. Created in the ArcGIS 10.4 (ESRI 2016).

Appendix 5. Values of water quality parameters at sampling sites according to Procházková et al. 2020.

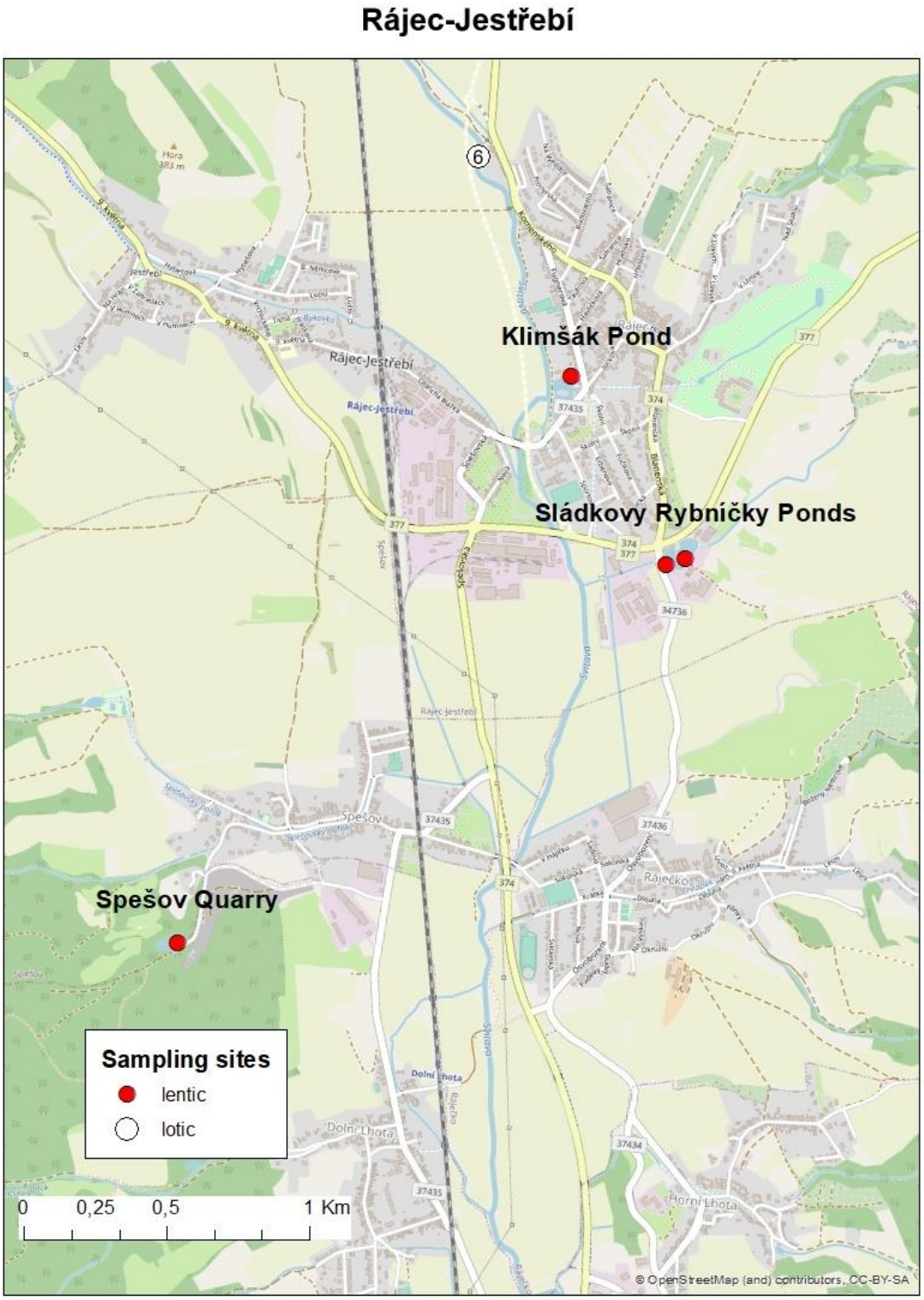
Appendix 1. Map of sampling sites in the town of Svitavy. Created in the ArcGIS 10.4 (ESRI 2016).



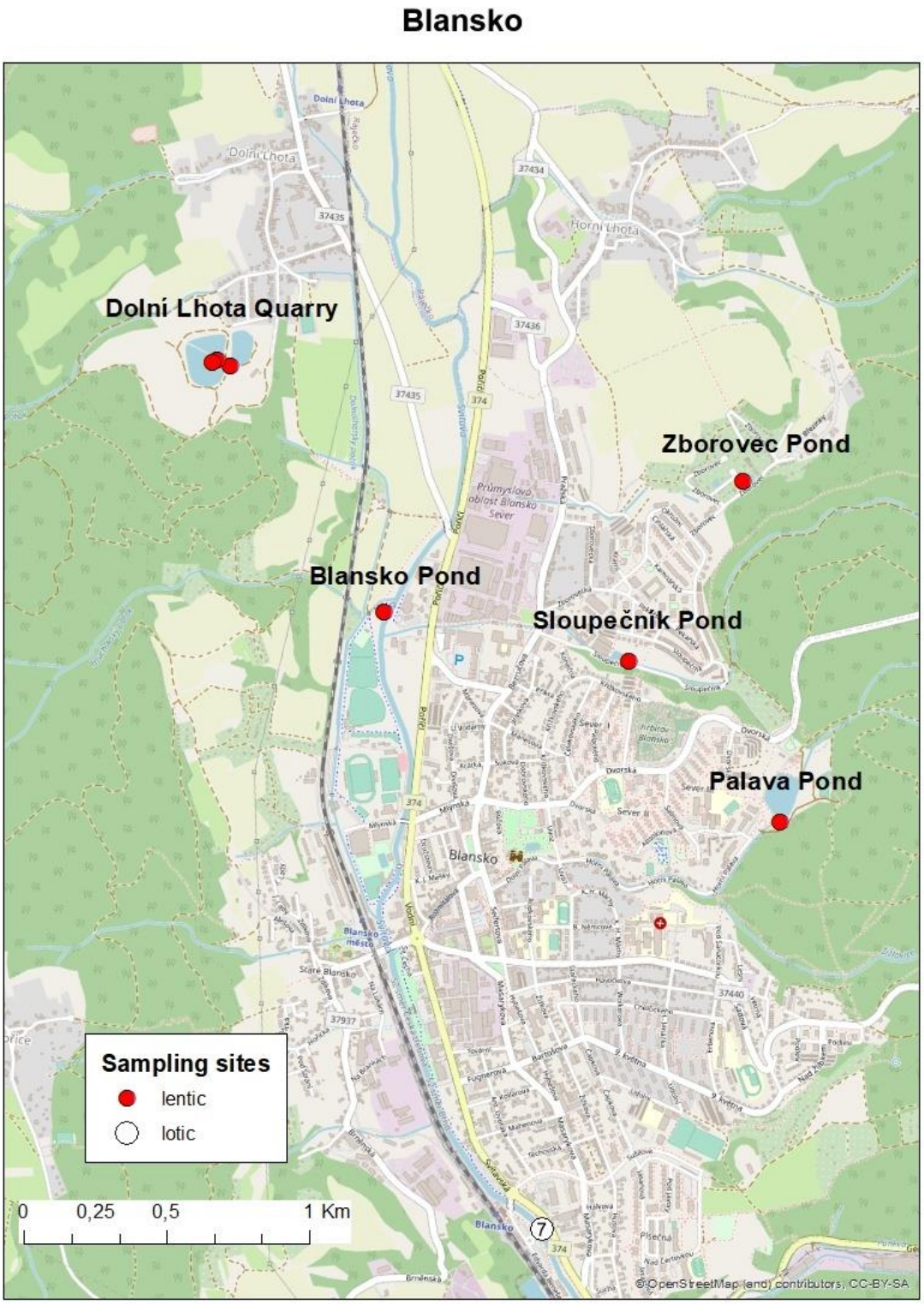
Appendix 2 Map of sampling sites in the town of Letovice. Created in the ArcGIS 10.4 (ESRI 2016).



Appendix 3. Map of sampling sites in the town of Rájec-Jestřebí. Created in the ArcGIS 10.4 (ESRI 2016).



Appendix 4. Map of sampling sites in the town of Blansko. Created in the ArcGIS 10.4 (ESRI 2016).



Appendix 5. Values of water quality parameters at sampling sites according to Procházková et al. 2020. The best water quality – 1, the worst quality – 5. Check marks – satisfactory values, cross marks – unsatisfactory values, dashes – missing data.

	Sampling site	Basic parameters						Heavy metals					AOX	Benzo(ghi)perylen
		BOD_5	COD_{Cr}	$N\text{-}NO_3$	$N\text{-}NH_4$	$P_{tot.}$	Total score	As	Cd	Cr	Hg	Pb		
1	Svitavy – spring	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Hradec nad Svitavou	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Moravská Chrastová	2	1	3	5	3	5	1	1	1	1	1	-	-
		✓	✓	×	×	×	-	✓	✓	✓	✓	✓	-	×
4	Letovice	2	2	3	5	4	5	2	1	1	-	1	-	-
		✓	✓	✓	×	×	-	✓	-	✓	-	-	-	×
5	Mladkov	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Rájec-Jestřebí	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Blansko	2	2	3	3	4	4	2	1	1	-	1	-	-
		✓	✓	✓	×	×	-	✓	-	✓	-	-	-	✓
8	Adamov	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Bílovice nad Svitavou	2	2	3	2	4	4	2	1	1	-	1	1	-
		✓	✓	✓	✓	×	-	✓	✓	✓	✓	✓	-	-
10	Brno	2	2	4	2	4	4	2	1	1	1	1	1	-
		✓	✓	✓	✓	×	-	✓	-	✓	-	-	-	✓

BOD_5 – Biochemical oxygen demand, COD_{Cr} – Chemical oxygen demand, AOX – Halogenated organic compounds