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Evolution of cyanobacteria – difference (almost) without distinction

Habilitation thesis

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Abstract

During more than three billion years of evolution of cyanobacteria, they diversified into an amazing number of species and inhabited all environments with a sufficient light intensity to fuel their photosynthetic apparatus. In this thesis, I focus on the evolutionary patterns across all cyanobacterial groups using morphological and molecular approaches including whole-genome sequencing. I found that convergence of morphological features is much more frequent in the cyanobacterial evolution than previously expected. Actually, it seems to be rather typical for all cyanobacterial genera, and thus, they are polyphyletic. It led to a proposal of 10 genera and 4 new species. Using 16S rRNA phylogeny of all sequence of cyanobacteria in the GenBank, I also uncovered that only 13–21% of species are correctly identified there and 51% are uncultured. Further, cyanobacterial herbarium specimens were shown to be a fruitful source of possible data which can be used to shed a light on historical patterns of and evolution and diversity in bacteria.

Table of Contents

Introduction

Prokaryotes are the most abundant and the most diverse group of organisms inhabiting the Earth. The boldest estimates suggest tens of billions of species (Locey and Lennon 2016). One group of prokayotes stands out due to their importance as primary producers in many environments and as a group which gave a rise of all algae and plants as the predecessor of the chloroplast – cyanobacteria (also known as blue-green algae, Cyanophyceae, Cyanophytes, and Cyanoprokaryotes).

The evolution of cyanobacteria took over 2.7 billion years therefore they belong to the oldest groups of organisms inhabiting the biosphere. (Demoulin et al. 2019). During the time, cyanobacteria have diversified into a staggering morphological, ecological and genomic diversity (Whitton and Potts 2001, Dvořák et al. 2017a). We recognize up to 4769 species of cyanobacteria (https://www.algaebase.org/, accessed 16th June 2020). New species and genera of cyanobacteria are being described almost every day.

In this thesis, I will explore both recent and ancient events of diversification in cyanobacteria. I will focus on patterns of morphological and molecular evolution to shed some light on a question of what are the drivers of such amazing diversity. Moreover, my reconstructions of evolution showed that some proposed taxonomic treatments are outdated which led to taxonomic revisions and I also found several taxa new to the science.

Cyanobacteria

The phylum of bacteria with an ability of oxygenic photosynthesis is called cyanobacteria. Although bacteria are thought to have very simple morphology, cyanobacteria have evolved immense morphological variability including cell differentiation into three cell types: vegetative cell (photosynthesis and reproduction), heterocyst (anaerobic fixation of atmospheric nitrogen), and akinete (spore). Based on morphology, five basic groups were recognized – simple unicellular, complex unicellular, filamentous, filamentous with cell differentiation and filamentous with cell differentiation and true branching (Rippka et al. 1979, Castenholz et al. 2001). They are known simply by numbers – sections I-V or by names – Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales (see examples of each section in Figure 1). It should be noted here that none of the proposed orders is monophyletic and a more detailed discussion can be found below.

Cyanobacteria are distributed all around the globe and they thrive in all environments where they can utilize light including extremely cold (polar regions) and extremely hot habitats (e.g. hot springs and deserts) (e.g. Whitton and Potts 2001). Most of the species were identified in the temperate zone. The reason is nicely summarized by an old sarcasm saying that “the distribution of cyanobacterial diversity depends on the distribution of phycologists”. This trend has been rapidly changing during last two decades. Several phycological groups were established in tropical countries and they have made many large leaps forward in our knowledge of cyanobacterial diversity (Brasil, India, Costa Rica etc.). A short review on the diversity in tropics can be found in Dvořák et al. (2017a). The tropical regions seem to conceal an unprecedented amount of new species to science. This is apparent from the fact that most of the new species proposed are found in tropical countries (Dvořák et al. 2017a, Rigonato et al. 2017). Moreover, it can be expected that a large portion of cyanobacterial diversity remains to be discovered especially in tropical countries.

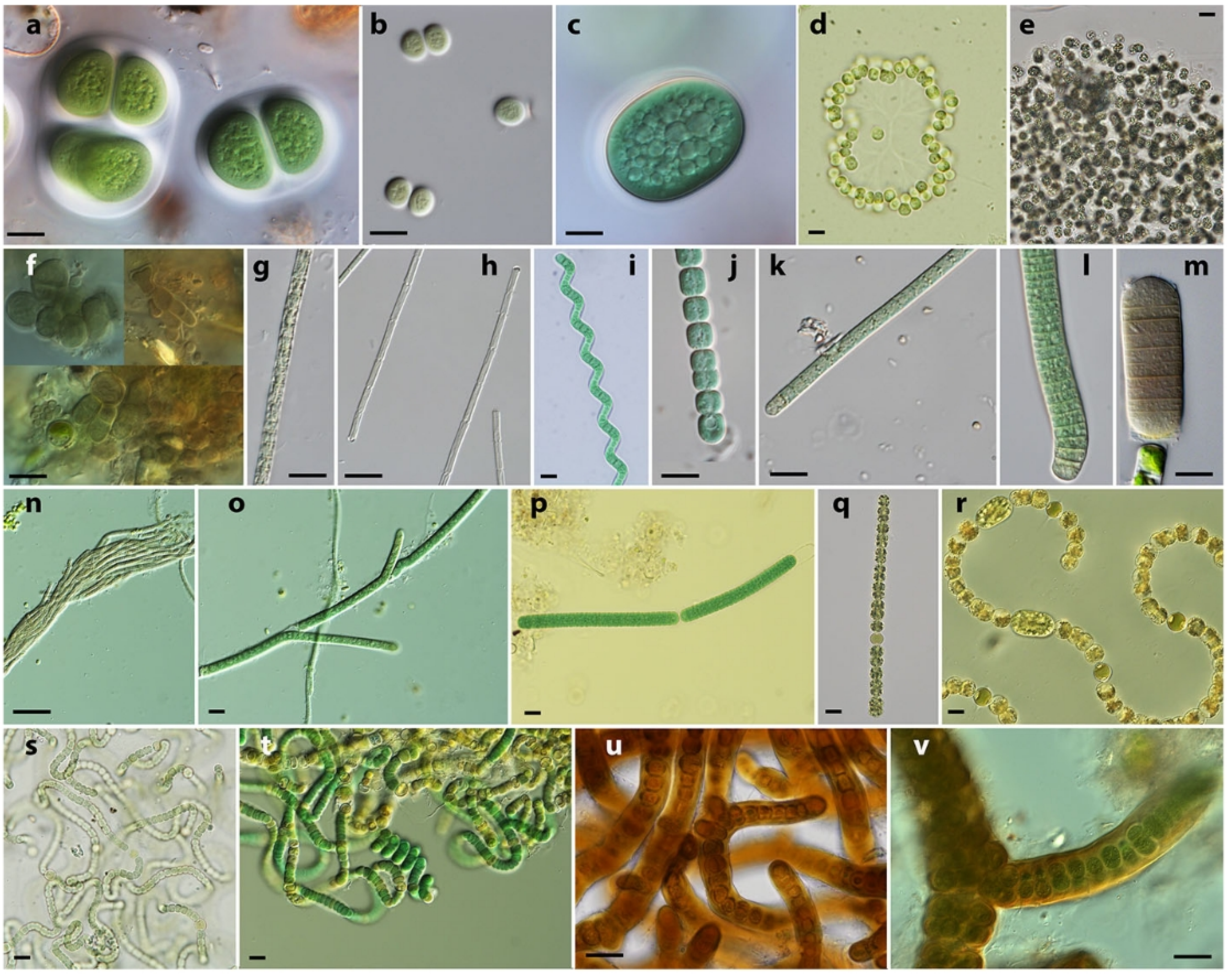

Figure 1. Examples of cyanobacteria from each order based on Rippka et al. (1979). The image was adopted from Dvořák et al. (2015). I. Chroococcales: a *Chroococcus subnudus*, b *Ch. limneticus*, c *Cyanothece aeruginosa*, d *Snowella litoralis*, e *Microcystis aeruginosa*. II. Pleurocapsales: f *Pleurocapsa minor*. III. Oscillatoriales: g *Planktothrix agardhii*, h *Limnothrix redekei*, i *Arthrospira jenneri*, j *Johanseninema constricum*, k *Phormidium* sp., l, m *Oscillatoria* sp., n *Schizothrix* sp., o *Tolypothrix* sp., p *Katagnymene accurata*., IV. Nostocales: q *Dolichospermum planctonicum*, r *Dolichospermum* sp., s *Nostoc* sp., t *Nodularia moravica*. V. Stigonematales: u, v *Stigonema* sp. Scale bar a–u = 10 µm, v = 20 µm.

With an ability of oxygenic photosynthesis, cyanobacteria play an important role in many environments as primary producers. The most conspicuous examples of all are tinny marine unicellular cyanobacteria *Prochlorococcus* and *Synechococcus* (it should be noted that taxonomic status has changed recently, however, I will keep these names because the revision in Coutinho et al. (2016) is not valid. *Prochlorococcus* and *Synechococcus* are the most abundant phototrophs with annual global mean abundances $2.9 \pm 0.1 \times 10^{27}$ and $7.0 \pm 0.3 \times 10^{26}$ cells, respectively (Flombaum et al. 2013). Cyanobacteria are also significant primary producers in drylands (Evans and Lange 2001). Here, the dominant genus is filamentous cyanobacterium *Microcoleus*. It is actually a complex of many species (Strunecký et al. 2013), although they are not often distinguished by ecologist interested in drylands. Finally, the third example, unicellular colonial *Microcystis*, invades fresh-water bodies all around the world and it seems to be spreading with a global change and eutrophication more and more localities in all latitudes. Although *Microcystis* is also an important primary producer, it grows very fast in unprecedented abundances, which causes world-wide problems with water bloom. *Microcystis* produces a variety of secondary metabolites, which are toxic and the best know is hepatotoxin microcystin. Water bloom causes decreased water quality, animal fatalities and change of species diversity (reviewed in Harke et al. 2016). Similar problems cause other cyanobacteria such as *Cylindrospermopsis* (recently moved to genus *Raphidiopsis*; (Aguilera et al. 2018)) or *Aphanizomenon* (Codd et al. 2005).

Cyanobacterial metabolism produces an immense variety of compounds with actual or possible importance for the human-kind. These bioactive compounds have antibacterial, antifungal, antiviral, and anticancer activity (Abed et al. 2009). Furthermore, cyanobacteria can produce hydrogen in large quantities as a potential source of alternative energy (Kopka et al. 2017). When genetically modified, *Synechococcus* strains can perform fermentation of sugars it has produced (Chow et al. 2015). Finally, experiments in mouse showed that a *Synechococcus* strain can grow in the heart and oxygenate the tissue in the case of ischemia (Cohen et al. 2017). The list of biotechnological applications of cyanobacteria is very long and it is beyond the scope of this thesis, however, the topic is reviewed extensively elsewhere (e.g. Abed et al. 2009).

Speciation and species concepts in cyanobacteria

Factors driving the diversification of lineages in prokaryotes are only poorly understood. Prokaryotes do not exhibit sexual reproduction and their genome properties significantly differ from eukaryotic genomes. Further, prokaryotes have small cells, but they have much larger population diversity and abundance than animals and plants, they can move very fast for long distances and their generation time is much faster (e.g. Cohan 2010, Shapiro et al. 2016, Shapiro and Polz 2015).

Prokaryotes possess small genomes (most often between 2 and 5 Mb; Koonin and Wolf 2008), and it is very dynamic. Although prokaryotes reproduce clonally, they are rather promiscuous with the DNA exchange. The most important is DNA exchange via homologous and non-homologous (horizontal gene transfer - HGT) recombination events.

The homologous recombination (HR) is horizontal genetic information transfer in which the DNA fragment transferred is almost identical to the recipient genome. HR can be found in almost all bacteria and its frequency varies among the clades (Bobay and Ochman 2017). It seems to be a significant factor driving speciation in bacteria and it can act as both a cohesive and disruptive force (Hanage et al. 2006, Fraser et al. 2009, Shapiro and Polz 2015, Shapiro et al. 2016). Moreover, it leaves signatures in the genome similar to HR during sexual reproduction in eukaryotes and it acts as a cohesive force in speciation. Thus, prokaryotes are often considered as quasi-sexual (Rosen et al. 2015).

Horizontal (lateral) gene transfer (HGT; also know as non-homologous recombination) is defined as a unidirectional foreign DNA integration into a recipient genome. The DNA fragment has a different nucleotide composition. HGT is likely to happen among genetically close lineages – its frequency negatively correlates with genome divergence in prokaryotes (Popa et al. 2011). However, the HGT can also happen among distantly related organisms including prokaryotes-eukaryotes transfers (Keeling and Palmer 2008). HGT is mostly concentrated in a small part of the genome called “hot-spots”, which comprises about one percent of the genome (Oliveira et al. 2017). The transferred fragments have often adaptive function and therefore they can drive divergence of new lineages (reviewed in Shapiro and Polz 2015). It has been suggested that HGT events affected at some point about 50% of gene families in cyanobacteria (Zhaxybayeva et al. 2006) and it has been observed in all cyanobacterial lineages (e.g. Dvořák et al. 2014, Dvořák et al. 2020; Zhaxybayeva et al. 2006).

The genome of bacteria is a dynamic system, which can be rapidly changed by gaining and loosing pieces of DNA. A complete set of DNA of a species is called a pan-genome (see e.g. Sherman and Salzberg 2020). Genes, which are common to all individual genomes, are called core genes and the rest of the genes is called accessory genes. The size of the pan-genome can be exemplified by the following estimates. Marine cyanobacterium *Prochlorococcus* was estimated to have a pan-genome size of 5407 genes in total based on 40 genomes (Ding et al. 2017). There were only 1047 core genes. It should be noted that *Prochlorococcus* has the second smallest genome among cyanobacteria (~1.6–2.7 Mbp and ~2000–3000 genes; Berube et al. 2018). Such incredible variation provide a wide field for innovation and adaptation. The pan-genome studies are still in its infancy especially in free-living prokaryotes, but it is already apparent that its dynamics is a very important driver of evolution (e.g. Sherman and Salzberg 2020).

One crucial question remains. Are bacterial species coherent? The dynamic nature of the bacterial genome suggested that the barrier between the cyanobacterial species can be “fuzzy” some authors advocated the idea that there are no species in prokaryotes (Hanage et al. 2005). However, homologous recombination and horizontal gene transfer seems are likely to be happen among closely related lineages (genetic distance negatively correlates with the number of events). Moreover, the core genes which encodes basic metabolic pathways seem to be stable during the speciation. Thus, most of the bacterial species seem to be coherent (Shapiro et al. 2012, 2016, Shapiro and Polz 2015).

Allopatric speciation (speciation with geographical isolation) was considered to be a major mode of diversification of new lineages in plant and animals. Speciation in sympatry (no geographical isolation between populations) was, on the other hand, thought to be rare. However, more and more pieces of evidence were gathered that sympatry can be common (reviewed in Bolnick and Fitzpatrick 2007). The most colorful example of all are cichlid fish, which have been diverging in African lakes such as Malawi (e.g. Malinsky et al. 2018). Thousands of beautiful fish species evolved here without any geographical barrier.

Contrary to the animals and plants, microorganisms (including prokaryotes) were supposed to have a prevailing mode of speciation in sympatry, because they have much greater population sizes and capabilities of dispersal. These patterns were observed based on morphological variability, which suggested that all species are everywhere (or their spores) and only the local environment selects local species diversity (summarized by Finlay 2002). In cyanobacteria, we can find evidence for both modes of speciation. Signatures of geographical differentiation were identified in filamentous heterocystous toxic invasive cyanobacterium *Raphidiopsis* (Ribeiro et al. 2020), and *Microcoleus* (Dvořák et al. 2012). No geographical differentiation was exhibited by cyanobacterium *Microcystis* (Ribeiro et al. 2020). However, these results were inferred based on a single or two DNA sequence markers, which have only limited resolution. The genome diversity within the cyanobacterial lineages seems to be high. Single-cell sequencing of hundreds of *Prochlorococcus* cells revealed hundreds of coexisting sub-populations (Kashtan et al. 2014). This suggests that to grasp the global biogeographical patterns in cyanobacteria, both local and global genome diversity of the analyzed lineages must be described. Otherwise, it will remain unclear what causes the observed geographical pattern. Such a study is not yet available in cyanobacteria.

The patterns of speciation are also forming how we conceptualize a unit “species”. At least 27 different species concepts were recognized (Wilkins 2009). This exemplifies that defining species can be a subjective matter and a search for a universal species concept has been unsuccessful. Of course, the biological species concept proposed by Mayr (Mayr 1942) is the most favorite one among biologists (e.g. Wilkin 2009). This concept requires organisms to have sexual reproduction and cyanobacteria are clonal. However, the gene flow among cyanobacterial lineages seems to be frequent enough that they can be considered as quasi-sexual. Thus, the biological species concept can be theoretically used. In practice, it is complicated to measure the gene flow among cyanobacteria cost-effectively therefore other concepts must be applied. Phenetic species concept was the most wide spread before the advent of the molecular methods especially DNA sequencing. Species were recognized based on their morphology (recently reviewed by Dvořák et al. 2015). However, as I will show below, the morphological variability does not correspond with genetic variability, which is much higher. Other species concept must be applied to describe the exiting diversity more realistically.

The most popular species concept among cyanobacteriologists is the monophyletic species concept sensu Johansen and Casamatta (2005). Species are monophyletic groups of taxa (showed usually by a phylogeentic reconstruction) and it is supported by one or more apomorphies. This concept can be found in most of the recent taxonomic papers, although it should be noted that it is not usually explicitly stated. Its popularity stems from a wide use of species definition so-called “polyphasic approach”, which combines morphological, molecular and ecological features for a species definition.

Phylogenetic reconstruction of cyanobacterial diversification

Evolutionary relationships among cyanobacteria can be visualized using phylogenetic reconstruction as in other organisms. Firstly, the relationships were inferred based on restriction enzyme-based markers such as RFLP. However soon, attention was moved to sequencing – most frequently 16S rRNA coding a small subunit of the ribosome. 16S rRNA is ubiquitous among organisms and thus provide an excellent tool for phylogeny. One of the first attempts of phylogenetic inference of cyanobacterial evolution revealed that cyanobacterial taxa can be defined by the 16S rRNA sequence, but at the same time the phylogeny did not concur with the morphology. All five sections in cyanobacteria defined above were poly and paraphyletic (Giovannoni et al. 1988). This evidence was the first signature that the evolution of morphology in cyanobacteria has complicated patterns. Later, it has been shown that most of the major morphological features are polyphyletic in cyanobacteria (reviewed in Dvořák et al. 2015).

The most extreme evidence of the morphological polyphyly is the genus *Synechococcus.* It is a simple unicellular cyanobacterium and the second most abundant cyanobacterium after *Prochlorococcus*. It is usually composed of one or two rod-like cells. Simple morphology covers an immense genome variability which has been evolving perhaps over three billion years (Dvořák et al. 2014a, Dvořák 2017). At least 12 polyphyletic lineages have diverged in *Synechococcus* (Figure 2; Dvořák et al. 2014a). Some of those lineages were formally recognized. For example, the genus *Neosynechococcus* was found to be a new lineage among cyanobacteria. It is morphologically almost identical to *Synechococcus elongatus* (the type species of *Synechococcus*), but it diverged out of the type species clade (Dvořák et al. 2014b). Nevertheless, most of the other lineages were proposed invalidly (see example here: Coutinho et al. 2016). Similar patterns can be observed on a smaller scale in most of the large cyanobacterial genera – *Phormidium* (Hašler et al. 2012, Strunecký et al. 2013, Hašler et al. 2014), *Leptolyngbya* (Dvořák et al. 2017, Jahodářová et al. 2017, Jahodářová et al. 2018), and *Nostoc* (Hrouzek et al. 2013).

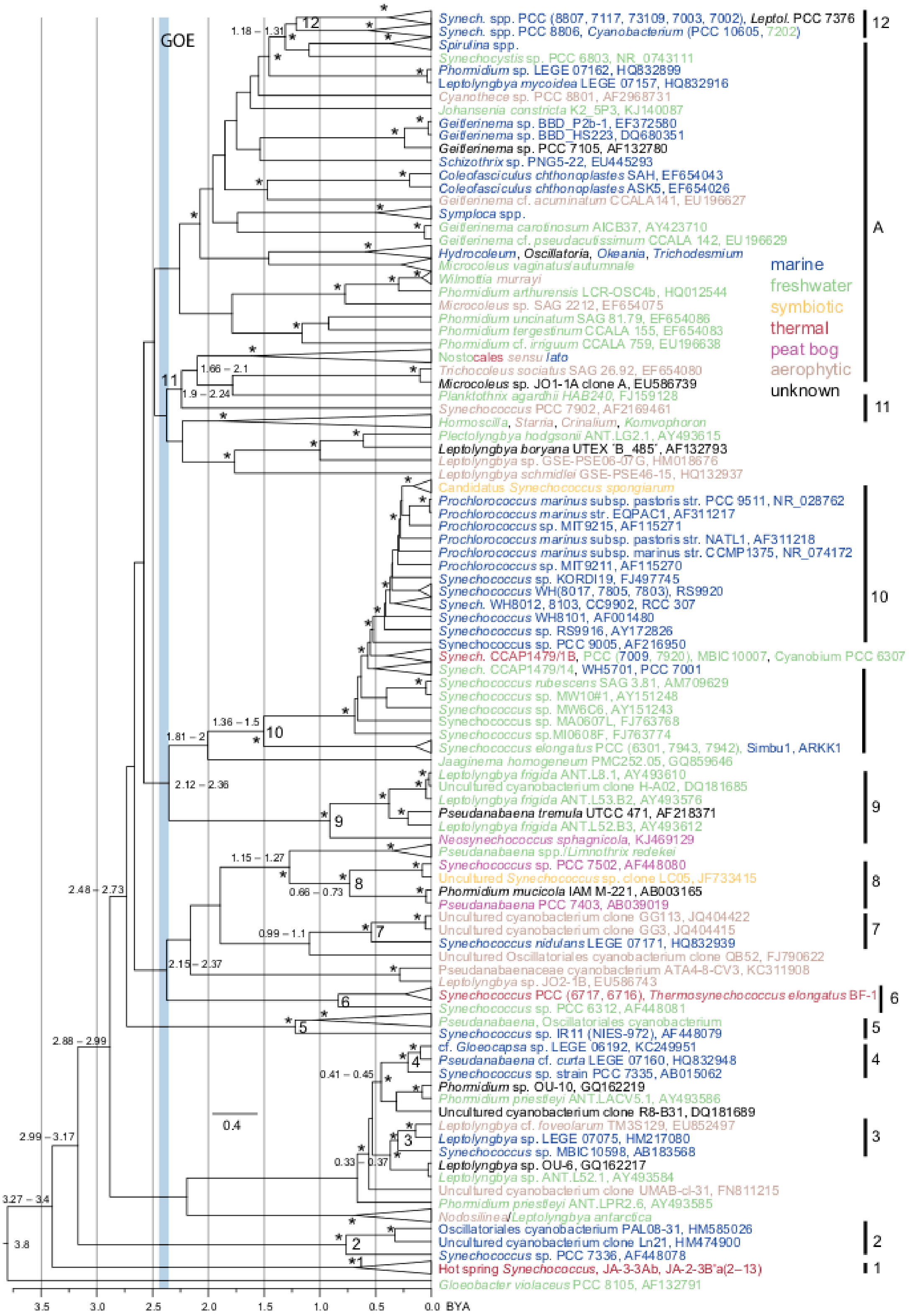

Figure 2. A dating of *Synechococcus* evolution based on 16S rRNA gene. The tree was adopted from Dvořák et al. (2014a). Asterisk represents posterior probabilities ≥0.9. Twelve lineages are numbered at the nodes and habitats of strains is colored and explained in the legend.

On the other hand, a very important innovation in the cyanobacterial evolution has remained monophyletic with ever-growing number of sequenced species – cell differentiation. Akinetes heterocytes have likely appeared only once before 2.1 – 2.45 billion years (Tomitani et al. 2006).

The diversity below the genus level is complicated as well, because the molecular diversity is larger than morphological diversity. Similar to algae, there seem to be many cryptic species in cyanobacteria. Cryptic species cannot be recognized without the molecular data (Boyer et al. 2001, Dvořák et al. 2015, Osorio-Santos et al. 2014 and many others). For example, 7 cryptic species (Osorio-Santos et al. 2014) were recognized in the genus *Oculatella*, which was described by Zammit et al. (2012).

Herbarium sequencing

Herbarium collections have been recently recognized as an important source of ancient DNA data, which can be used to study evolution and taxonomy. The herbarium specimens have low DNA yield and it is shredded into small pieces, therefore long-read sequencing is impossible. However, the DNA has a quality for short-read sequencing of second generations. One whole issue of The Philosophical Transactions of the Royal Society, B (2018) was dedicated to studies of herbarium specimens, but prokaryotes were not included (Meineke et al. 2018).

Cyanobacteria have been traditionally included into botanical code (see details below) therefore many type species were stored in the herbarium collections as dried samples. Only one paper was focused on 16S rRNA sequencing of several herbarium specimens. Authors were able to obtain sequences and reconstruct phylogeny (Palinska et al. 2006). They found that the herbarium specimens have almost identical sequence to the recent one. Until recently, no whole-genome sequence was obtained from the cyanobacterial herbarium.

In 2015, I aimed to challenge this and I visited Botanical Museum in Berlin, which has extensive herbarium collection, which is unfortunately uncatalogued. The museum was bombed during the second world war and everything was torn to the ground except for the algal herbarium collection located in the basement and few specimens which were on loan. All the documentation was lost. I went through the whole collection and found that there are specimens with biomass suitable for DNA extraction. I extracted DNA and two those specimens which had successful 16S rRNA amplification were chosen for the DNA sequencing using Illumina. They were identified as *Nostoc* sp. After the data filtering, contamination removal, assembly and annotation, I was able to do phylogenomic reconstruction (Dvořák et al. 2020). This a first evidence that herbarium specimens can be used to reconstruct historical evolutionary events in prokaryotes using herbarium specimens.

Taxonomy of cyanobacteria

Although cyanobacterial cells have microscopic dimensions, cyanobacteria have caught the attention of botanists for at least two centuries. Cyanobacteria were included under the International Code of Nomenclature for algae, fungi, and plants (ICN) due to their green pigments resembling plants. The botanist pioneers cyanobacterial diversity during the 19th century in Europe especially in Germany, France and Switzerland. The work of Bornet, Flahault, Kützing, Nägeli and several others built a strong foundation for our current understanding of the cyanobacterial diversity (Kutzing 1849, Nägeli 1849, Bornet and Flahaut 1888). These pioneers were armored only with their enthusiasm and simple microscopes, and thus, they were focused only on morphological characters. Nevertheless, their finding have still a large impact on our understanding of the cyanobacterial taxonomy.

When the prokaryotic nature of cyanobacterial cell was recognized, cyanobacteria were included in the International Code of Nomenclature of Prokaryotes (ICNP) along with the rest of prokaryotes. Thus, cyanobacteria are still covered under both botanical and bacteriological code. This great cyanobacterial schism brought a lot of confusion into the taxonomy, because both codes have quite a distinctive view on the rules of species description. ICNP requires two axenic type cultures stored in two culture collections. The task to obtain an axenic culture represents long-lasting and tedious endeavor with uncertain results. Thus, it has been criticized for lengthiness before a species description. New bacterial species are found in metagenomic data practically every day and it led to the proposal of the genome sequence as a type material (Whitman et al. 2019). This will speed up new species descriptions in prokaryotes. On the other hand, ICN does not accept culture as the type material. A herbarium specimen or fixed sample (e.g. using formaldehyde) is required. Thus, species can be described in non-axenic cultures or even only based on DNA sequences (any sequence including 16S rRNA) and herbarium specimens from the type locality as was used, for example, for the proposal of the genus *Johanseninema* (Hašler et al. 2014a, 2014b).

Thus, describing a species or other taxon is rather painful under the provisions of ICNP and many cyanobacteriologists are keen on the ICN. On the other hand, there are hundreds of species proposed under ICN with no type material and sequence data. It is almost impossible to connect a species description with any material. While some authors see epitypification as a solution, other prefer careful searching for the type material or at least type locality. A rather lengthy discussion was held on the last IAC meeting in Brisbane Australia in 2019 (the meeting of International Association for Cyanophyte/Cyanobacterial research) with no specific conclusion.

Two decades of the great taxonomic boom of cyanobacteria

As the result of a decreased price of sequencing and enhanced sampling effort hundreds new taxa in cyanobacteria were proposed in the last two decades. Only in the last five years, over a hundred of genera were proposed mostly under the provision of the ICN. Several taxa are proposed every week. Most of them were found in tropical areas. This can be caused either by the assumption that tropical diversity is much higher than in the temperate zone or that more cyanobacteriologist sampled in the tropical zone.

Only in the last five year, 110 new genera were proposed among cyanobacteria. Most of them were separated from existing morphologically defined genera and providing evidence that they are polyphyletic. On the other hand, many of them also failed to fulfill some requirements of the ICN (talk and subsequent discussion by prof. Jeffrey J. Johansen at IAC meeting in Brisbane Australia in 2019). If this trend continues, the system of cyanobacteria will be atomized into many more species and genera than previously expected. The main reason behind this trend lies that it is very often impossible to connect type material of the particular genus. Type materials are stored usually as herbarium specimen, which are, however, quite small and therefore they are not suitable for destructive sampling. Moreover, it is often impossible to evaluate morphological characters using light microscopy.

Another taxonomic system was described in (Parks et al. 2018, 2020) and it is stored at https://gtdb.ecogenomic.org/about. The system uses whole-genome phylogenetic approach to classify whole-genome sequences including incomplete assemblies of metagenomes. Thus, it relies on the genome sequence as type material. No physical specimen is needed. The database is well-curated, but since only whole genomes are accepted, its use is still limited, because only a small part of cyanobacterial species have their genome sequenced. However, I suspect that with the cheap sequencing, which allows to sequence all strains stored in a culture collection, this approach will grow on popularity. Eventually, it may bring the desired stability in the cyanobacterial and bacterial taxonomy.

GenBank taxonomy – a tricky business

DNA sequence databases contain millions of sequences of microbes. The largest one, NCBI GenBank (https://www.ncbi.nlm.nih.gov/), has its own taxonomic scheme which mostly follows the most recent taxonomic revisions. It was suggested on many occasions that most of the sequences are incorrectly identified.

To test this question quantitatively, I took the following approach. I downloaded all sequences of 16S rRNA of cyanobacteria from the GenBank. I reconstructed the phylogenetic relationships. Further, I used Poisson Tree Process (PTP) to define species among all the sequences. It was impossible to quantify the correctness of identification based only on sequences, because some lineages are over-sampled and some lineages have only one sequence available. I will leave technical details aside. I found that GenBank contains 2741 PTP-defined species of which only 571 were connected to the existing name. Thus, only 13-21% (depending on the taxonomic scheme) of sequences in GenBank are correctly identified (Dvořák et al. 2018).

Another interesting issue which rose from my analysis was that 49% of PTP-defined species were from the cultured strains (Dvořák et al. 2018). This strongly contrasts with the widely spread tenet that only ~1% of bacteria can be cultured (Amann et al. 1995). The reasons may lie, for example, in the fact that cyanobacteria were studied more intensively than other bacteria groups and for a long time. Moreover, it may be easier to culture cyanobacteria using standard media.

Conclusions

The diversity of cyanobacteria is immense and it will remain far from being fully understood for a long time. I used tools of molecular and evolutionary biology to shed some new light in the field. I showed that cyanobacterial evolution is entangled by many events of convergent evolution. It was exemplified on genera *Synechococcus*, *Leptolyngbya*, *Pseudanabaena*, *Geitlerinema* and *Phromidium*. An extensive sampling effort, molecular and morphological analyses resulted a description of new genera: *Neosynechococcus*, *Anagnostidinema*, *Ammassolinea*, *Elainella*, *Onodrimia*, *Chamaethrix*, *Lightfootiella*, *Reptodigitus*, *Johanseninema*, and *Jacksonvillea*, and species: *Chroococcidiopsis linchenoides*, *Brasilonema lichenoides*, *Brasilonema geniculatum*, and *Calothrix dumus*.

I also obtained first full metagenome sequence of the cyanobacterial herbarium specimen, which stresses an importance of the herbarium collections for study of evolution and taxonomy.

Moreover, I obtained genomic data from several lineages. I reconstructed phylogenetic relationships among cyanobacteria using the whole genome dataset. It showed that that they are similar to those reconstructed by 16S rRNA. Moreover, the dataset also allowed to estimate amount of horizontal gene transfer among all cyanobacteria and within the genus *Nostoc*. My analyses showed that HGT is very frequent among cyanobacteria and it differ among lineages. Frequency of HGT among *Nostocs* is low.

Finally, 16S rRNA analysis of all cyanobacterial sequences in the GenBank revealed that about half of cyanobacterial species are culturable and that about only 20% of species in cyanobacteria are correctly identified.

References

Abed, R.M.M., Dobretsov, S. & Sudesh, K. 2009. Applications of cyanobacteria in biotechnology. *J. Appl. Microbiol.* 106:1–12.

Aguilera, A., Gómez, E.B., Kaštovský, J., Echenique, R.O. & Salerno, G.L. 2018. The polyphasic analysis of two native *Raphidiopsis* isolates supports the unification of the genera *Raphidiopsis* and Cylindrospermopsis (Nostocales, Cyanobacteria). *Phycologia*. 57:130–46.

Amann, R.I., Ludwig, W. & Schleifer, K.H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59:143–69.

Berube, P.M., Biller, S.J., Hackl, T., Hogle, S.L., Satinsky, B.M., Becker, J.W., Braakman, R. et al. 2018. Data descriptor: single cell genomes of *Prochlorococcus*, *Synechococcus*, and sympatric microbes from diverse marine environments. *Sci. Data*. 5:1–11.

Bobay, L.-M. & Ochman, H. 2017. Biological Species Are Universal across Life's Domains. *Genome Biol. Evol.* 9:491–501.

Bolnick, D.I. & Fitzpatrick, B.M. 2007. Sympatric Speciation: Models and Empirical Evidence. *Annu. Rev. Ecol. Evol. Syst.* 38:459–87.

Bornet, E. & Flahaut, C. 1888. Note sur deux nouveaux genres d'algues perforantes. 2:161–5.

Boyer, S.L., Flechtner, V.R. & Johansen, J.R. 2001. Is the 16S-23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. *Mol. Biol. Evol.* 18:1057–69.

Castenholz, R.W., Wilmotte, A., Herdman, M., Rippka, R., Waterbury, J.B., Iteman, I. & Hoffmann, L. 2001. Phylum BX. Cyanobacteria. *In Bergey's Manual of Systematic Bacteriology*. Springer New York, pp. 473–599.

Chow, T.J., Su, H.Y., Tsai, T.Y., Chou, H.H., Lee, T.M. & Chang, J.S. 2015. Using recombinant cyanobacterium (*Synechococcus elongatus*) with increased carbohydrate productivity as feedstock for bioethanol production via separate hydrolysis and fermentation process. *Bioresour. Technol.* 184:33–41.

Codd, G.A., Lindsay, J., Young, F.M., Morrison, L.F. & Metcalf, J.S. 2005. Harmful Cyanobacteria. *In Harmful Cyanobacteria*. Springer-Verlag, pp. 1–23.

Cohan, F.M. 2010. Bacterial Species and Speciation. *Syst. Biol.* 50:513–24.

Cohen, J.E., Goldstone, A.B., Paulsen, M.J., Shudo, Y., Steele, A.N., Edwards, B.B., Patel, J.B. et al. 2017. An innovative biologic system for photon-powered myocardium in the ischemic heart. *Sci. Adv.* 3:e1603078.

Coutinho, F., Tschoeke, D.A., Thompson, F. & Thompson, C. 2016. Comparative genomics of *Synechococcus* and proposal of the new genus *Parasynechococcus*. *PeerJ* 4:e1522.

Demoulin, C.F., Lara, Y.J., Cornet, L., François, C., Baurain, D., Wilmotte, A. & Javaux, E.J. 2019. Cyanobacteria evolution: Insight from the fossil record. Free Radic. Biol. Med. 140:206–23.

Ding, W., Baumdicker, F. & Neher, R.A. 2017. panX: pan-genome analysis and exploration. *Nucleic Acids Res.* 46:e5.

Dvořák, P. 2017. Genome-wide Analysis of Cyanobacterial Evolution: The Example of *Synechococcus*. *In Cyanobacteria: Omics and Manipulation.* Caister Academic Press, United Kingdom, pp. 35-54.

Dvořák, P., Casamatta, D.A., Hašler, P., Jahodářová, E., Norwich, A.R. & Poulíčková, A. 2017a. Diversity of the Cyanobacteria. *In Modern Topics in the Phototrophic Prokaryotes.* Springer, Cham., pp. 3-46.

Dvořák, P., Casamatta, D.A., Poulíčková, A., Hašler, P., Ondřej, V. & Sanges, R. 2014a. *Synechococcus*: 3 billion years of global dominance. *Mol. Ecol.* 23:5538–51.

Dvořák, P., Hašler, P., Pitelková, P., Tabáková, P., Casamatta, D.A. & Poulíčková, A. 2017b. A new cyanobacterium from the everglades, Florida - *Chamaethrix* gen. nov. *Fottea*. 17:269–76.

Dvořák, P., Hašler, P. & Poulíčková, A. 2012. Phylogeography of the *Microcoleus vaginatus* (Cyanobacteria) from Three Continents – A Spatial and Temporal Characterization. *PLoS One*. 7:e40153.

Dvořák, P., Hašler, P. & Poulíčková, A. 2020. New insights into the genomic evolution of cyanobacteria using herbarium exsiccatae. *Eur. J. Phycol.* 55:30–8.

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

Dvořák, P., Jahodářová, E., Casamatta, D.A., Hašler, P. & Poulíčková, A. 2018. Difference without distinction? Gaps in cyanobacterial systematics; when more is just too much. *Fottea*. 18:130–6.

Dvořák, P., Poulíčková, A., Hašler, P., Belli, M., Casamatta, D.A. & Papini, A. 2015. Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. *Biodivers. Conserv.* 24:739–57.

Evans, R.D. & Lange, O.L. 2001. Biological Soil Crusts and Ecosystem Nitrogen and Carbon Dynamics. *In Biological Soil Crusts: Structure, Function, and Management*. Springer, Berlin, Heidelberg, pp. 263–79.

Finlay, B.J. 2002. Global dispersal of free-living microbial eukaryote species. *Science* 296:1061–3.

Flombaum, P., Gallegos, J.L., Gordillo, R.A., Rincón, J., Zabala, L.L., Jiao, N., Karl, D.M. et al. 2013. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proc. Natl. Acad. Sci. U. S. A.* 110:9824–9.

Fraser, C., Alm, E.J., Polz, M.F., Spratt, B.G., Hanage, W.P. & Bacteria, T. 2009. The Bacterial Species Challenge: Making Sense of Genetic and Ecological Diversity. *Science* 323:741–6.

Giovannoni, S.J., Turner, S., Olsen, G.J., Barns, S., Lane, D.J. & Pace, N.R. 1988. Evolutionary relationships among cyanobacteria and green chloroplasts. *J. Bacteriol.* 170:3584–92.

Hanage, W.P., Fraser, C. & Spratt, B.G. 2005. Fuzzy species among recombinogenic bacteria. *BMC Biol.* 3:6.

Hanage, W.P., Fraser, C. & Spratt, B.G. 2006. The impact of homologous recombination on the generation of diversity in bacteria. *J. Theor. Biol.* 239:210–9.

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A. & Paerl, H.W. 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae* 54:4–20.

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*. 12:341–56.

Hašler, P., Dvorák, P. & Poulícková, A. 2014a. A new genus of filamentous epipelic cyanobacteria, *Johansenia*. *Preslia*. 86:81–94.

Hašler, P., Dvořák, P. & Poulíčková, A. 2014b. *Johanseninema*, a corrected name for a recently described genus of filamentous epipelic cyanobacteria. *Preslia*. 86:293–294.

Hašler, Petr, Dvořák, P., Poulíčková, A. & Casamatta, D.A. 2014. A novel genus *Ammassolinea* gen. nov. (Cyanobacteria) isolated from sub–tropical epipelic habitats. *Fottea*. 14:241–8.

Hrouzek, P., Lukešová, A., Mareš, J. & Ventura, S. 2013. Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*. *Fottea*. 13:201–13.

Jahodářová, E., Dvořák, P., Hašler, P., Holušová, K. & Poulíčková, A. 2018. *Elainella* gen. nov.: a new tropical cyanobacterium characterized using a complex genomic approach. *Eur. J. Phycol.* 53:39–51.

Jahodářová, E., Dvořák, P., Hašler, P. & Poulíčková, A. 2017. Revealing hidden diversity among tropical cyanobacteria: The new genus *Onodrimia* (synechococcales, cyanobacteria) described using the polyphasic approach. *Phytotaxa*. 326:28–40.

Johansen, J.R. & Casamatta, D.A. 2005. Recognizing cyanobacterial diversity through adoption of a new species paradigm. *Algol. Stud. für Hydrobiol. Suppl. Vol.* 117:71–93.

Kashtan, N., Roggensack, S.E., Rodrigue, S., Thompson, J.W., Biller, S.J., Coe, A., Ding, H. et al. 2014. Single-cell genomics reveals hundreds of coexisting subpopulations in wild *Prochlorococcus*. *Science* 344:416–20.

Keeling, P.J. & Palmer, J.D. 2008. Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet*. 9:605–18.

Koonin, E. V. & Wolf, Y.I. 2008. Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. *Nucleic Acids Res.* 36:6688–719.

Kopka, J., Schmidt, S., Dethloff, F., Pade, N., Berendt, S., Schottkowski, M., Martin, N. et al. 2017. Systems analysis of ethanol production in the genetically engineered cyanobacterium *Synechococcus* sp. PCC 7002. *Biotechnol. Biofuels* 10:1–21.

Kutzing, F.T. 1849. Species Algarum. Leipzig, Germany. 922 pp.

Locey, K.J. & Lennon, J.T. 2016. Scaling laws predict global microbial diversity. *Proc. Natl. Acad. Sci. U. S. A.* 113:5970–5.

Malinsky, M., Svardal, H., Tyers, A.M., Miska, E.A., Genner, M.J., Turner, G.F. & Durbin, R. 2018. Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. *Nat. Ecol. Evol.* 2:1940–55.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia Univ. Press, New York.

Meineke, E.K., Davies, T.J., Daru, B.H. & Davis, C.C. 2018. Biological collections for understanding biodiversity in the Anthropocene. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 374:20170386.

Nägeli, C. 1849. Gattungen einzellinger Algen. *Neue Denkschriften der Allg. Schweizerischen Gesellschaft für die Gesammten Naturwissenschaften* 10:1–139.

Oliveira, P.H., Touchon, M., Cury, J. & Rocha, E.P.C. 2017. The chromosomal organization of horizontal gene transfer in bacteria. *Nat. Commun.* 8:1–11.

Osorio-Santos, K., Pietrasiak, N., Bohunická, M., Miscoe, L.H., Kováčik, L., Martin, M.P. & Johansen, J.R. 2014. Seven new species of *Oculatella* (Pseudanabaenales, Cyanobacteria): taxonomically recognizing cryptic diversification. *Eur. J. Phycol.* 49:450–70.

Palinska, K. a, Thomasius, C.F., Marquardt, J. & Golubic, S. 2006. Phylogenetic evaluation of cyanobacteria preserved as historic herbarium exsiccata. *Int. J. Syst. Evol. Microbiol.* 56:2253–63.

Parks, D.H., Chuvochina, M., Chaumeil, P.A., Rinke, C., Mussig, A.J. & Hugenholtz, P. 2020. A complete domain-to-species taxonomy for Bacteria and Archaea. *Nat. Biotechnol.* 38:1079–86.

Parks, D.H., Chuvochina, M., Waite, D.W., Rinke, C., Skarshewski, A., Chaumeil, P.A. & Hugenholtz, P. 2018. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat. Biotechnol.* 36:996.

Popa, O., Hazkani-Covo, E., Landan, G., Martin, W. & Dagan, T. 2011. Directed networks reveal genomic barriers and DNA repair bypasses to lateral gene transfer among prokaryotes. *Genome Res.* 21:599–609.

Ribeiro, K.F., Ferrero, A.P., Duarte, L., Turchetto-Zolet, A.C. & Crossetti, L.O. 2020. Comparative phylogeography of two free-living cosmopolitan cyanobacteria: Insights on biogeographic and latitudinal distribution. *J. Biogeogr.* 47:1106–18.

Rigonato, J., Alvarenga, D.O. & Fiore, M.F. 2017. Tropical cyanobacteria and their biotechnological applications. *In Diversity and Benefits of Microorganisms from the Tropics*. Springer International Publishing, pp. 139–67.

Rippka, R., Deruelles, J. & Waterbury, J.B. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111:1–61.

Rosen, M.J., Davison, M., Bhaya, D. & Fisher, D.S. 2015. Fine-scale diversity and extensive recombination in a quasisexual bacterial population occupying a broad niche. *Science* 348:1019–23.

Shapiro, B.J., Friedman, J., Cordero, O.X., Preheim, S.P., Timberlake, S.C., Szabó, G., Polz, M.F. et al. 2012. Population genomics of early events in the ecological differentiation of bacteria. *Science* 335:48–51.

Shapiro, B.J., Leducq, J.-B. & Mallet, J. 2016. What is speciation? *PLOS Genet.* 12:e1005860.

Shapiro, B.J. & Polz, M.F. 2015. Microbial Speciation. *Cold Spring Harb. Perspect. Biol.* 7:a018143.

Sherman, R.M. & Salzberg, S.L. 2020. Pan-genomics in the human genome era. *Nat. Rev. Genet.* 21:243–54.

Strunecký, O., Komárek, J., Johansen, J., Lukešová, A. & Elster, J. 2013. Molecular and morphological criteria for revision of the genus *Microcoleus* (Oscillatoriales, Cyanobacteria). *J. Phycol.* 49:1167–80.

Tomitani, A., Knoll, A.H., Cavanaugh, C.M. & Ohno, T. 2006. The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. *Proc. Natl. Acad. Sci. U. S. A.* 103:5442–7.

Whitman, W.B., Sutcliffe, I.C. & Rossello-Mora, R. 2019. Proposal for changes in the international code of nomenclature of prokaryotes: Granting priority to *Candidatus* names. *Int. J. Syst. Evol. Microbiol.* 69:2174–5.

Whitton, B.A. & Potts, M. 2001. Introduction to the Cyanobacteria. *In The Ecology of Cyanobacteria*. Kluwer Academic Publishers, Dordrecht, pp. 1–11.

Wilkin, J.S. 2009. Species: A history of the idea. University of California Press, Berkely, USA.

Zammit, G., Billi, D. & Albertano, P. 2012. The subaerophytic cyanobacterium *Oculatella subterranea* (Oscillatoriales, Cyanophyceae) gen. et sp. nov.: a cytomorphological and molecular description. *Eur. J. Phycol.* 47:341–54.

Zhaxybayeva, O., Gogarten, J.P., Charlebois, R.L., Doolittle, W.F. & Papke, R.T. 2006. Phylogenetic analyses of cyanobacterial genomes: Quantification of horizontal gene transfer events. *Genome Res.* 16:1099–108.

Appendixes

1. **Dvořák, P.** 2017. Genome-wide Analysis of Cyanobacterial Evolution: The Example of *Synechococcus*. In Cyanobacteria: Omics and Manipulation. Caister Academic Press, United Kingdom, pp. 35-54.
2. **Dvořák, P.,** Casamatta, D.A., Hašler, P., Jahodářová, E., Norwich, A.R. & Poulíčková, A. 2017a. Diversity of the Cyanobacteria. *In Modern Topics in the Phototrophic Prokaryotes.* Springer, Cham., pp. 3-46.
3. **Dvořák, P.,** Poulíčková, A., Hašler, P., Belli, M., Casamatta, D.A., Papini, A. 2015. Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. *Biodivers. Conserv.* 24: 739–57.
4. **Dvořák, P.,** Jahodářová, E., Casamatta, D.A., Hašler, P., Poulíčková, A. 2018. Difference without distinction? Gaps in cyanobacterial systematics; when more is just to much. *Fottea* 18: 130–6.
5. **Dvořák, P.,** Casamatta, D.A., Poulíčková, A., Hašler, P., Ondřej, V., Sanges, R. 2014. *Synechococcus*: 3 billion years of global dominance. *Mol. Ecol.* 23: 5538–51.
6. **Dvořák, P.,** Hašler, P., Poulíčková, A. 2012. Phylogeography of the *Microcoleus vaginatus* (Cyanobacteria) from Three Continents – A Spatial and Temporal Characterization. *PLoS ONE* 7:e40153.
7. 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* 12:341–56.
8. Strunecký, O., Bohunická, M., Johansen, J.R., 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* 17:114–26.
9. **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* 170:24–34..
10. **Dvořák, P.,** Jahodářova, E., Hašler, P., Gusev, E., Poulíčková, A. 2015. A new tropical cyanobacterium *Pinocchia polymorpha* gen. et sp. nov. derived from the genus Pseudanabaena. *Fottea* 15:113–20.
11. Poulíčková, A., **Dvořák, P.,** Mazalová, P., Hašler, P. 2014. Epipelic microphototrophs: an overlooked assemblage in lake ecosystems. *Freshw. Sci.* 33:513–23.
12. Hašler, P., **Dvořák, P.,** Poulíčková, A., Casamatta, D.A. 2014. A novel genus *Ammassolinea* gen. nov. (Cyanobacteria) isolated from sub–tropical epipelic habitats. *Fottea* 14:241–248.
13. Jahodářová, E., **Dvořák, P.,** Hašler, P., Holušová, K., Poulíčková, A. 2018 *Elainella* gen. nov.: a new tropical cyanobacterium characterized using a complex genomic approach. *Eur. J. Phycol.* 53: 39–51.

14. Jahodářová, E., **Dvořák, P.,** Hašler, P., Poulíčková, A. 2017. Revealing the hidden tropical diversity among cyanobacteria using polyphasic approach, new genus *Onodrimia* (Synechococcales, Cyanobacteria). *Phytotaxa* 326:25–40.

15. **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* 17:269–76.

16. **Dvořák, P.,** Hašler, P. 2007. Occurrence and morphological variability of *Cylindrospermopsis raciborskii* (WOLOSZ.) SEENAYYA et SUBBA RAJU (Cyanophyta, Nostocales) near Olomouc in 2006. *Fottea* 7:39–42.

17. Hašler, P., Pentecost, A., Jahodářová, E., **Dvořák, P.,** Poulíčková, A. 2018. Taxonomic revision of *Ulva montana* (Lightfoot 1777) and description of a new genus of *Lightfootiella* (Cyanophyceae, Chroococcaceae). *Phytotaxa* 362:173–186.

18. Villanueva, C.D., Garvey, A.D., Hašler, P., **Dvořák, P.,** Poulíčková, A., Norwich, A.R., Casamatta, D.A. 2019. Descriptions of *Brasilonema geniculatum* and *Calothrix dumus* (Nostocales, Cyanobacteria): two new taxa isolated from cemetery tombstones. *Phytotaxa* 387:1–20.

19. Casamatta, D.A., Villanueva, C.D., Garvey, A.D., Stocks, H.S., Vaccarino, M., **Dvořák, P.,** Hašler, P., Johansen, J.R. 2020. *Reptodigitus Chapmanii* (Nostocales, Hapalosiphonaceae) gen. nov.: a unique nostocalean (Cyanobacteria) genus based on a polyphasic approach. *J. Phycol.* 56:425–36.

20. Hašler, P., **Dvořák, P.,** Poulíčková, A. 2014. A new genus of filamentous epipelic cyanobacteria, *Johansenia*. *Preslia* 86: 81–94.

21. Hašler, P., **Dvořák, P.,** Poulíčková, A. 2014. *Johanseninema*, a corrected name for a recently described genus of filamentous epipelic cyanobacteria. *Preslia* 86:293–294.

22. Hašler, P., Casamatta, D.A., **Dvořák, P.,** Poulíčková, A. 2017. *Jacksonvillea apiculata* (Oscillatoriales, Cyanobacteria) gen. & sp. nov.: a new genus of filamentous, epipsamic cyanobacteria from North Florida. *Phycologia* 56:284–95.

23. Hašler, P., **Dvořák, P.,** Ondřej, V., Kitner, M., Hloušková, P., Poulíčková A. (2011): The importance of the polyphasic approach in a comparative study of *Nodularia* (Nostocales, Cyanobacteria). *Preslia*, 83:167–182.

24. **Dvořák, P.,** Hašler, P., Poulíčková, A. (2020): New insights into the genomic evolution of cyanobacteria using herbarium exsiccatae. *Eur. J. Phycol.* 55:30–8.

25. Villanueva, C.D., Hašler, P., **Dvořák, P.,** Poulíčková, A., Casamatta, D.A. 2018. *Brasilonema lichenoides* sp. nov. and *Chroococcidiopsis lichenoides* sp. nov. (cyanobacteria): two novel cyanobacterial constituents isolated from a tripartite lichen of headstones. *J. Phycol.* 54:224–233.