Report on the Ph.D. thesis 'Towards a modern revision of the cyanobacteria, a critically important prokaryotic phylum' by Markéta Bohunická.

Submitted by: Dr Gabrielle Zammit on the 14 September 2015.

Overall, I thought this Ph.D. thesis included a considerable amount of research work that resulted in a remarkable number of six papers published in peer-reviewed scientific journals, with a seventh that has also been recently submitted. The papers deal with a revision of various cyanobacterial taxa, using the polyphasic approach with an emphasis on the application of molecular biology data. In the individual papers, each study was well thought out and very well written. The conclusions were substantiated and convincing.

However, I am uneasy with the title under which the collective work has been presented, since it is very vague and broad. The candidate should have specified at least which type of cyanobacteria are included in this Ph.D. research. I quote from pg. 8: 'The main group of interest were terrestrial soil and rock inhabiting taxa of cyanobacteria'. At the very least, then, this should have been reflected in the title like so: 'Towards a modern revision of *terrestrial* cyanobacteria, a critically important prokaryotic phylum '. Including in the title the actual number of taxa revised, would been a further improvement.

When one considers the title as presented, the Introduction chapter is extremely concise and does not reflect the vast topic. If the thesis deals with the revision of cyanobacterial taxa, then a number of recent studies, especially those dealing with modern phylogenies based on next generation sequencing data, should have been discussed in more detail. For instance, although the recent paper by Komarek, J., Kastovsky, J., Mares, J., & Johansen, J. R., entitled 'Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia, 86* (4), 295-335', is given a brief mention, its implications on the classification of the cyanobacterial taxa considered in this doctoral research is not discussed.

The thesis objectives, which are invariably the initial considerations that provide the incentive to initiate any research study, are also very short and not clearly presented in the Introduction chapter.

Another reservation includes the fact that the results obtained during this intensive research are not discussed collectively, vis-à-vis the implications to the classification of the phylum cyanobacteria as it currently stands. There is no general Discussion section or chapter that would have tackled questions, like for instance: Is this the ideal methodology for arriving at a suitable system for classifying cyanobacteria? Are there any limitations to our present knowledge? Can there be any improvements? In one of the published papers, for instance, the authors claim that the length and secondary folding structure of the internal transcribed spacer, the ITS, provides sufficient data for the description of new cryptic *Oculatella* species. It would have been interesting to read the candidate's opinion about whether this method is reliable enough to be recommended also in the description of new species from different orders of cyanobacteria.

Finally, it is customary for the publications resulting from Ph.D. research to include the name of the Ph.D. supervisor as the student's mentor and the primary investigator who is in responsible for overseeing the research. I was surprised that this was not the case for the work being reviewed. The scientific supervisor's name never figures in the authors' lists of any of the seven manuscripts presented in this thesis.

In conclusion, I do recommend this thesis for defense because it includes some important revisions for several cyanobacterial taxa. In addition to my considerations above, there are a couple of questions that I would like to personally ask the candidate during the *viva voce* interview.

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Review of the Ph.D. thesis of RNDr. Markéta Bohunická "Towards a modern revision of the cyanobacteria, a critically important prokaryotic phylum"

Overall assessment of the thesis

Cyanobacteria represent a unique, highly diverse and ubiquitous group of prokaryotic organisms. They exemplify one of the most ancient life forms known to inhabit Earth, with a fossil record of over 3.5 billion years. Evolutionarily, cyanobacteria played a crucial role in oxygenation of the early Earth atmosphere and by the mechanism of endosymbiosis, they gave rise to modern day plastids. Ecologically, Cyanobacteria are very important members of aquatic, terrestrial and marine ecosystems, performing oxygenic photosynthesis in a wide range of associations and symbiotic relationships. Despite their critical role in global ecosystem functioning (nitrogen cycles) and environmental change (toxic blooms), our knowledge of the Cyanobacterial diversity and evolution is still severely limited.

In the current age of NGS data expansion, we are faced with rapidly accumulating genetic data from meta-genomic approaches. However, this novel genetic information can only be understood from a functional and ecological point of view if it is linked to the phenotypic information about the organisms it comes from. As a consequence, taxonomic evaluation of the cyanobacterial genetic diversity is now extremely needed. I am therefore particularly happy that Markéta followed the famous school of the Czech Cyanobacterial taxonomy, and dedicated several years of her life to the valuable, though presently often marginalized, field of the Cyanobacterial research.

Although Markéta finally concluded that her work is just a drop in the ocean of yet unresolved part of the cyanobacterial taxonomy, a compilation of the seven papers presented in this thesis represents a significant, highly valuable taxonomic work. I enjoyed reading the papers, especially the *Oculatella* one where the authors insighted behind the taxonomy, discussing the species concept, speciation, and biogeography of Cyanobacteria. The Ph.D. thesis fulfils all the required formal and technical criteria, represents original work supported by publications in peer reviewed journals, and is therefore ready to be presented by the defendant in a public hearing.

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Questions to the defendant:

1. The first study listed in the thesis deals with the taxonomy of the genus *Scytonema*. After presenting the polyphyletic nature of the genus, one would expect that the authors will focus on delineation and formal description of particular generic-level lineages. Instead, two new *Scytonema* species are described despite the fact they clearly do not belong to the *Scytonema* s. str. lineage. In addition, no molecular data are presented for these two species. If you believed these two taxa do not belong to *Scytonema* s. str., why did you describe them as representatives of this genus? Why not to include them into the newly circumscribed genus *Myochrotes*?

2. In the second paper, the authors state that the particular species can be clearly delimited by the morphology, contradicting the notion that morphological diversity in Cyanobacteria is largely due to environmental stimuli. This is further emphasized by the statement that all "morphospecies are monophyletic in molecular phylogenies" (page 79). However, the vast majority of studied species were represented by a single investigated strain. Therefore, the statement concerning the species monophyly is simply wrong. What is the probability that further investigation of additional strains isolated from various habitats result in morphologically highly variable, cryptic species?

3. The secondary structures of the 16S-23S ITS rRNA molecules are frequently inferred in the papers. However, differences among these structures are compared only by their visual inspection, which is often very hard to follow by the reader. Is there any possibility how to statistically evaluate and clearly present the differences among the structures?

4. In the fifth paper, the newly described genus *Roholtiella* is mentioned to "form a highly supported compact clade within the Nostocaceae" (page 148). However, the monophyly of this genus has been highly supported by the Bayesian analysis only (PP 0.98). ML analysis inferred a very weak support for this clade (55), and MP analysis did not resolve it at all (page 163). Is the genus indeed molecularly supported? What is the reason to favour BI over ML and MP analyses? If favoured, is there a possibility to improve their bootstrap supports?

5. In the same paper, Markéta suggested to use the p-distance within the 16S-23S ITS region to recognize species boundaries in morphologically cryptic species (page 168). In my opinion, the p-distance value reflects only the evolutionary time elapsed since the speciation event. It has nothing to do with the speciation itself. In addition, the putative simple and general use of such "objective" criterion is very dangerous as it can be seemingly used across the lineages. Just imagine how the species boundaries recognized by any p-distance change in plants, where the speciation by polyploidy is considered to constitute about 15-31% of all speciation events. In my opinion, the application of any genetic distance to delimit species boundaries simply fails because of the incorrect assumption of the identical age of all species. So, why to use p-distances to delimit species as they are clearly confusing?