

Master Thesis Review

Title: New insights in the morphology and phylogeny of heterocytous cyanobacteria from Peru, including the description of new taxa

Author: Leonardo Humberto Mendoza Carbajal

Reviewer: RNDr. Jan Mareš, Ph.D.

The thesis includes combined molecular and morphological taxonomic analysis of 36 new heterocytous cyanobacterial strains, mostly isolated from Peru. The polyphasic evaluation applied in the study meets international standards for this kind of work. In particular, I appreciate careful and comprehensive molecular and phylogenetic analyses and their well-arranged graphical presentation. This part of the thesis is better than many papers published in impact-factor journals. The morphological analysis and photographic documentation are also good. The thesis, although perhaps a bit too long, is well written and the text is logically organized. I was especially satisfied with the discussion part, which elucidated almost all unclear points and questions raised by myself after reading the results. The presented data have potential for future description of several new genera and species, as expected for a high-quality survey of an underexplored tropical country. There are numerous small errors in English grammar and style as well as some typos throughout the text, which however do not affect the scientific clarity.

This said, the thesis unfortunately also has a couple of weak points. First of all, the Introduction part is only two and half pages long, which seems less than I would expect in this kind of work. The introduction is only very general, and more detailed literature is missing, for example dealing with taxonomic concepts in cyanobacteria, individual taxa under study and their recent taxonomic treatment, or the diversity of cyanobacteria in the tropics. The second flaw is the description of the new genus „*Skacelovkia*“. The name is created incorrectly, the suffix should not be „kia“ but „ia“ or „ea“, in this case probably „*Skacelovaea*“, although the honoured Olga is no longer Skácelová, but Lepšová. Furthermore the name would have been published invalidly due to the author's failure to deposit preserved material as required by Art. 8 of the International Code of Nomenclature for algae, fungi, and plants (ICN). Luckily a thesis without ISSN/ISBN is not considered an effective publication under the ICN, so these problems can be solved when later published in a scientific journal.

In summary, the thesis fulfils all requirements placed on this type of work, and I recommend it to be defended. I am inclined to recommend classification „**EXCELLENT**“ in case of successful defense presentation.

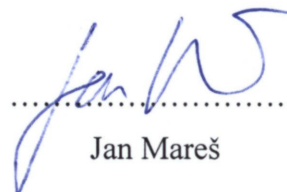
I have following additional questions and comments:

- 1) p. 7 – Observations of cultures for morphology analysis were made every three to six months. This seems very sparse, and I recommend additional and more frequent analysis to cover the whole morphological variability. This could e.g. help to find the missing akinetes in strain T044, which is in my opinion closer to *Rivularia* than *Calothrix*.
- 2) p. 8 – The biomass for DNA extraction was dried and then pulverized using beads and lysis buffer. This does not make sense, because you cannot pulverize something liquid. Please explain. Why was the biomass dried and not frozen if it was then homogenized in a lysis buffer?
- 3) p. 11 and elsewhere – Why did you include a single strain from each Czech Republic, USA, and Kenya? It looks quite weird in the context of the thesis that otherwise addresses the unexplored cyanobacterial diversity of Peru.
- 4) The author seems to have sequenced the complete ITS regions of all strains. The necessity of ITS analysis for species delimitation is mentioned on numerous occasions in Discussion. Why was it not

applied at least in some cases, where ITS data of close relatives are available from NCBI for comparison? Can the author provide some examples how exactly such analysis could look in the case of his taxa and what it could tell?

5) I would hesitate to call strains T008, T017, and T048 *Dichothrix*, even in quotation marks. Based on presented data such as the presence of geminate heterocytes, prostrate filaments, and sequence similarity, I would rather call it „*Scytonematopsis*“. There are also more *Scytonematopsis*-like strains in this clade, partly published (Johansen et al. 2017, cited in the thesis), partly unpublished (M. Bohunická). I also doubt, based on the photographs, that the radial-like colonies are formed by filaments that grow from clustered basal heterocytes. Have you tried to inspect the colonies in greater detail? It rather looks that densely entangled creeping parts of filaments form the centre of the colony, and erect terminal branches extend radially from this cluster.

May 12, 2018 in České Budějovice



Jan Mareš



A review of a diploma thesis by Leonardo Humberto Mendoza Carbajal (University of South Bohemia, supervisor: Esther Berrendero Gómez, Ph.D.):

New insights in the morphology and phylogeny of heterocytous cyanobacteria from Peru, including the description of new taxa

Tropical regions have been omitted by cyanobacteriologist for a long time, because they were concentrated on cyanobacteria near their home (predominantly in Europe and North America). However, some pioneer studies of e.g. Gardner and Desikachary showed that tropical species diversity is immense especially in heterocystous cyanobacteria. This thesis brings fresh new data about cyanobacteria from Peru – another omitted region with respect to the diversity of cyanobacteria.

The diploma thesis is focused on morphological analysis of 36 cyanobacterial strains isolated from Peru and a reconstruction of phylogeny of their 16 rRNA gene. The phylogeny also contains strains of other heterocystous cyanobacteria and the closest relatives to investigated strains.

A number of analyzed strains and careful morphological characterization must have been extremely time consuming and it makes this thesis valuable for a scientific community although most of the strains were only identified to existing taxa. The strains belong to a variety of clades inside heterocystous cyanobacteria therefore author had to reconstruct a phylogeny of the whole group. Author also added data involved in ecology of strains, but he does not discuss any general patterns in the thesis (the information is only used for taxa identification), which is a pity, because it may provide interesting evidence in macroevolution in cyanobacteria.

The thesis starts with an introduction which is rather short but it contains most of the important information except the part involved in cyanobacteria of Peru. It should have been elaborated in more detail, although I understand that there are not many source of information, but there might be data about cyanobacterial diversity in surrounding countries. Dispersal abilities of cyanobacteria are high therefore county borders are not relevant in most cases. Material and methods are comprehensively written and there is not much to add.

In results, I really appreciate how carefully author described morphology of the strains and also the microphotographs. The description of strains is in all cases concise and complete.

Morphological data are synthesized with phylogenetic and taxonomic identification for each strain is provided. Moreover, author found one possibly new genus, which he provisionally called *Skacelovkia*. I think that proposal of this genus will be successful in future and can be published.

Discussion repeats results too much and this makes this part really long and hard to read. However, it is also comprehensive and an amount of literature, which was used here, is sufficient.

Stylistics and language of the thesis is very good for a non-native speaker, but typos are quite common and easily noticeable.

Altogether the thesis an example of hard work both in lab and by a computer when analyzing data and writing the thesis. Some parts of thesis are worth to publish especially new genus *Skacelovkia*. I recommend this thesis for the defense with a mark A.

In Olomouc, 14th May 2018



Petr Dvořák, Ph.D.
Palacký University Olomouc

Questions:

Author mentions many times that ITS shed more light into taxonomic position of many taxa, for example in case of *Brasilonema* sp. T076 and *B. angustatum*. Why do you think that it helps here? These taxa are quite distant in 16S rRNA phylogeny therefore ITS structure might differ just by chance not by evolution.

Is genetic similarity a good tool for taxonomy?

The phylogeny contains information about ecology of strains. Can you infer any evolutionary pattern? This could have been discussed in the thesis.

Horizontal gene transfer is common in cyanobacteria. How much or if at all does it influence one gene phylogenetic reconstructions (16S rRNA)?