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To: Doc. RNDr. Jan Kaštovský, Supervisor

From: Dr. Dale Casamatta

Date: February 27, 2014

Re: Review of dissertation for RNDr. Jan Mareš

Dear Dissertation Committee,

The Kaštovský consortium is amongst the most productive, respected cyanobacterial research laboratories in the world, producing numerous manuscripts of excellent quality every year. Thus, I was already aware of, and had read, several of the papers from Dr. Jan Mareš dissertation work. Hence, it was with great pleasure that I agreed to review the totality of his Ph.D. dissertation.

Dr. Mareš' dissertation chiefly revolves around cyanobacterial taxonomy (mainly dealing with the rather enigmatic genus *Gloeobacter*) and systematics. He was the first author on five manuscripts and an important contributor on a sixth (Komárek *et al.* 2014). Thus, I will discuss Dr. Mareš' works individually and then as a whole body submitted for a dissertation.

Jan's dissertation begins with a thorough review of the state of cyanobacterial systematics. He begins with a discussion of the general ecological and environmental roles of cyanobacteria before segueing into a discussion of the trajectory and current history of cyanobacterial systematics. Jan includes all of the major, relevant milestones of cyanobacterial systematics in his review, thus illustrating a thorough knowledge of the field. His introduction sets the tone for his investigations into resolving some of the tangles of modern cyanobacterial systematics, while showing his grasp of the material.

The first chapter of Jan's dissertation concerns an investigation into the epilithic cyanobacterium *Gloeobacter*. This genus is rather interesting for two reasons. First, it lacks any type of thylakoid for photosynthesis and thus appears to be perhaps the most primitive cyanobacteria, making it an excellent candidate for rooting phylogenies. Second, while a common experimental taxon, very little is known concerning its ecology or life history. Further, there exists a good degree of confusion about the naming and taxonomy of *Gloeobacter*, although that will be addressed in subsequent papers by Jan.

In this manuscript, Jan provides detailed molecular (16S rDNA gene sequence data), morphological, and biochemical analyses of both *Gloeobacter* as well as a closely aligned, and often confused, taxon *Aphanothece caldariorum*. This work was published in *PloS ONE*, a well-regarded journal with a wide readership. The results of the paper are that *Gloeobacter* is probably a much more ubiquitous taxon (within permissible habitats) than previously anticipated, while additionally shedding light on the phenotypic plasticity, genetic identity and pigment composition of the genus.

The second chapter of Jan's dissertation concerns the validation of the name *Gloeobacter*. This is an important work since the original description was never valid under either of the competing codes employed for cyanobacteria (the ICN nor ICN/ICNP), which is rather surprising given the important taxonomic and laboratory status of this taxon. Jan *et al.* thus validated the name, established appropriate types, and clarified some of the convoluted history of this organism. Papers like this are impressive for two reasons. First, it is a rather labyrinthine task to validate an improperly erected taxon, especially one with such a long and convoluted history. Thus, it shows a great grasp of taxonomic knowledge and the application of the Codes of Nomenclature (both sets of codes). Second, it is important for the overall state of cyanobacterial taxonomic knowledge.

Jan's third and fourth chapters are very similar, and thus I will consider them together. These papers involved conserving two names: *Gloeobacter violaceus* and *Gloeothece*, spin-off projects from his validation manuscript. These two new papers, while not overly long, show that Jan has an excellent grasp of the rules for the Codes, and contributes to the body of cyanobacterial knowledge. In addition, it is always excellent to see important taxonomic conservation endeavors undertaken.

The fifth manuscript of Jan's dissertation concerned a phylogenetic assessment of a collection of culture resistant, massive sheathed cyanobacteria. This paper was very impressive for two reasons. First, it addressed a phylogenetically uncertain, poorly resolved collection of organisms, *Petalonema* and *Stigonema*, with variable placement depending on the phylogenetic analyses. To do so, Jan utilized 16S rDNA gene data and presented a morphological assessment, recovering a rather interesting phylogeny. While interesting in itself, the second major contribution of the paper was even more impressive: a clearly articulated method for the successful PCR amplification of single cells (or filaments) from strains with massive sheaths. These taxa have long been exceedingly difficult to cultivate, and are recalcitrant to PCR amplification due to their elaborate sheaths. Thus, the phylogenetic placement of these organisms is often subject to much debate. The method put forth in this paper will allow other researchers to undertake similar endeavors with other difficult to culture, mucilaginous taxa in the future (e.g., *Nostochopsis*). Hence, this paper will be of wide use to cyanobacterial researchers endeavoring to explore similarly difficult taxa in the future.

The sixth, and by far most elaborate, manuscript in Jan's dissertation was a project involving four researchers, led by J. Komárek. This paper is a very important one, surely to be highly cited by anyone interested in cyanobacterial taxonomy and systematics. The purpose of the paper was to articulate and present revisions to the totality of cyanobacteria using a polyphasic approach, namely 16S sequence data but also with great attention paid to the morphology and thylakoid arrangement (or lack thereof). The systematic assessments of cyanobacteria have undergone massive revisions in the past 10 years, and this paper is an excellent review and synthesis of those changes. Further, the clear articulation of the orders and families will greatly accelerate other cyanobacterial researchers.

Jan's contributions to cyanobacterial research are several fold. First, his conservation and validation of names solves and clarifies an urgently needed endeavor. Second, his work with the single cell/filament PCR amplification of reticent, culture resistant cyanobacteria will be of great use for others dealing with other similar, yet very interesting, taxa. Third, his contribution to the Komárek et al. 2014 paper will help the community as a whole move forward in a meaningful manner. Further, Jan clearly has the expertise to craft high quality, important manuscripts that add to the body of cyanobacterial research, and should be able to continue to explore questions pertaining to cyanobacterial systematics and biodiversity. The sum total of these endeavors is worthy of the awarding of a Ph.D.

It was a pleasure reviewing this dissertation, and if I may be of any further assistance in this manner please do not hesitate to contact me.

Respectfully submitted,

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After reviewing Jan's dissertation, it is clear that he has an excellent grasp of cyanobacterial systematics and taxonomy. The following questions are provided for addition inquiry:

Questions:

1. Cyanobacteria, as clonal organisms, are subject to different modes of speciation than eukaryotes. Recent papers have postulated on some of the potential mechanisms that cyanobacteria employ (or have thrust upon them) leading to speciation. Thus, what do you believe is the role of genomic rearrangements in

cyanobacterial evolution? Further, what do you hypothesize the role of viruses may be, and how prevalent might this process be in the evolution of this lineage?

2. While the 16S rDNA gene is clearly the "gold standard" for cyanobacterial phylogenies, there are some problems associated with its employment. What are three potential pitfalls of using just this one gene, and what are three potential remedies? How do you think that our picture of probable evolutionary relationships would be different if we employed other genes, and what might those genes be?

3. Cyanobacteria are present in nearly every ecosystem, and are typically very important primary producers. How does the ecology of cyanobacteria match the phylogenies recovered by molecular data sets? Why would (or would not) there be a dichotomy between the two? How would ecological specialization potentially drive cyanobacteria speciation?

4. Some recent papers have postulated about the total amount of cyanobacterial biodiversity, but the proposed number of taxa are widely divergent. What do you envision will be the actual biodiversity present, and why would (or would not) cyanobacteria have such robust diversity?

5. In your introduction, you mentioned a number of potential species concepts for cyanobacteria. What are some of the competing concepts, and what are the positives and negatives about such concepts? Why are species concepts so critical to untangling the biodiversity of cyanobacteria? Why is unraveling the systematic relationships of cyanobacteria so important from ecological, evolutionary, natural products, and human related perspectives?

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Oponentský posudok na dizertačnú prácu doktoranda RNDr. Jana Mareša „Polyphasic approach to the taxonomy of selected cyanobacteria".

Cyanobaktérie sú jedny z najúchvatnejších organizmov v našej biosfére. Ich zrod v rannom prekambriu bol jedným z najdôležitejších krokov v evolúcii. Z fylogenetického hľadiska je to výnimočná skupina baktérií s oxygennou fotosyntézou, ktoré celkom zásadným spôsobom ovplyvnili podmienky na Zemi. Práve im vďačíme za vznik kyslíkovej atmosféry v pôvodne anoxickom svete. Stali tiež hlavnými donormi chloroplastov pre heterotrofných eukaryotných hostiteľov, z ktorých sa rôznymi cestami vyvinuli všetky dnešné autotrofné eukaryotné organizmy s fotosyntézou rastlinného typu. Pozoruhodná je ich adaptabilita, ktorá im umožňuje kolonizovať rozmanité, často veľmi extrémne, biotopy v ekosystémoch Zeme. V zmysle „statickej evolúcie" a kombinácie s danou flexibilitou cyanobakteriálneho genómu aklimatizovať širokú škálu environmentálnych podmienok vyústilo do rozsiahlej a nezriedka aj veľmi komplikovanej diverzity cyanobaktérií s výskytom početných ekologicky špecializovaných genotypov a morfotypov. Súčasná fykológia sa tomu všetkému dnes snaží porozumieť použitím tradičných morfologických metód v kombinácii s molekulárnymi (fylogenetickými) a ekofyziologickými prístupmi v modernej taxonómi. Len to nám umožní pochopiť celú šírku biodiverzity cyanobaktérií na Zemi. A to je aj úsilie autora predloženej dizertačnej práce.

Ako už zo samotného názvu vyplýva, celá doktorská dizertačná práca je napísaná v anglickom jazyku a obsahuje celkom 109 strán formátu A4 zviazaných v pevnej väzbe. Nejde o klasickú formu rukopisu, ale ťažiskom práce je šesť pôvodných vedeckých prác „in extenso". Všetky tieto práce majú kolektív autorov, pričom sám autor je uvedený ako prvý pri piatich z nich a už boli riadne publikované v rozpätí rokov 2013 - 2015 v prestížnych svetových botanických, či fykologických časopisoch a s najvyšším IF v danom odbore.

Z hľadiska obsahu je práca rozdelená do šiestich kapitol. V prvej časti autor sa venuje úvodu do predmetnej témy, kde rozoberá jednotlivé aspekty doterajších vedomostí o cyanobaktériách z pohľadu, ktoré sú známe súčasnej vede a obzvlášť si všíma implementáciu molekulárnych dát do ich systematiky a taxonomického konceptu. Autor sa tu odvoláva celkove na 128 už publikovaných cudzích prác k tejto téme, ale z dikcie jednotlivých formulácií viet a obsahových celkov je očividné, že je v problematike zorientovaný, disponuje rozsiahlymi vedomosťami a citom pre zmysluplnú vedeckú prácu.

V nasledujúcich kapitolách je vložených už spomenutých šesť publikácií v plnom znení. Obsahujú polyfázickou metódou rozanalyzované niektoré vybrané rody cyanobaktérií s primitívnou morfológiou a dlhodobo sporným taxonomickým postavením, ale aj testovanie molekulárnych metód len ojedinele využívaných doposiaľ v cyanobakteriálnej taxonómii, ako je sekvenovanie jednotlivých buniek/vlákien z prírodných zberov takých druhov, ktoré sa nám nedarí laboratórne kultivovať na klonálnej báze kvôli veľkej slizovej pošve. Predstavený protokol môže už v blízkej budúcnosti vniesť výrazne zmeny v molekulárnej taxonómii cyanobaktérii pri celom rade doteraz problematických rodov. Posledná publikácia z roku 2014 sumarizuje doterajšie vedomosti z polyfázických štúdií v celosvetovo a predstavuje najaktuálnejší stav klasifikácie cyanobaktérií, ktorý je založený na tomto v súčasnosti preferovanom prístupe k ich štúdiu. Posledná kapitola je stručný sumár celej dizertačnej práce.

Keďže všetky práce už boli publikované v prestížnych vedeckých časopisoch a teda prešli dôkladným recenzným konaním v príslušných redakciách, preto mi neprináleží akokoľvek spochybniť ich vedeckú hodnotu. Značnú prácu v tomto smere bezpochyby už odviedli oponenti spomenutých článkov a takto ich ja beriem. Všetky práce ale aj samotná dizertačná práca odzrkadľujú autora ako cieľavedomého bádateľa zanieteného pre vednú problematiku, medzinárodne komunikatívneho, schopného zaujať a nadchnúť kolegov. Autor sa úspešne prejavil aj pri schopnosti nielen si vedecké ciele stanoviť, zvládnuť zložité metodické postupy, získané výsledky analyzovať a účelne pretaviť do záverečnej syntézy. Je bezpochyby nádejným prínosom pre českú fykológiu.

Záverom rád konštatujem, že autor splnil požiadavky kladené na dizertačnú prácu, preukázal schopnosť samostatnej vedeckej práce, syntézy výsledkov a pod. Prácu odporúčam k obhajobe v odbore Botanika a po jej úspešnom zvládnutí navrhujem udeliť RNDr. **Janu Marešovi** vedecko-akademickú hodnosť **PhD** (philosophiae doctor) v zmysle zákona a platných predpisov.

V Bratislave 16. 3. 2015

Ľ. Kováčik

RNDr. Ľubomír Kováčik, PhD.