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Review Report on Ph.D. thesis of Jaromír Cihlár

entitled

Evolution of the Heme Biosynthetic Pathway in Eukaryotic Phototrophs

The PhD thesis submitted by Jaromír Cihlár focuses on the heme biosynthetic pathway and its evolution in chloroplast-bearing eukaryotes. The central part of the thesis is a published paper 'Evolution of the Tetrapyrrole Biosynthetic Pathway in Secondary Algae: Conservation, Redundancy and Replacement' on which Jaromír is a shared leading author. Other parts consist of two other papers, which only marginally relate to the main topic of the thesis and the candidate's contribution to these publications is relatively minor. The number of papers the candidate co-authored justifies the format of the thesis, which is basically a compilation of the three papers supplemented with an introduction and a conclusion, and which is the customary thesis format at the Department of Molecular Biology and Genetics at the Faculty of Science of the University of South Bohemia. The introduction sufficiently covers the topic of heme metabolism and the current state of knowledge of the evolution of heme biosynthesis in photosynthetic eukaryotes as contributed to by the candidate's own research as well as by that of other scientists in the field. The introduction reads well and is very clear and the final conclusion recapitulates well the major findings.

The grammar of the text is very good overall and largely devoid of typos and while I noticed several errors these are rather rare and I do not intent to dwell on them here.

All results that the candidate includes in the thesis have been published in respected journals and went through a peer-review process and I also have not found anything that would challenge the validity of the data and conclusions presented in the publications. I appreciate that the candidate's share in the performance of experiments and analyses is clearly delineated for each of the research paper presented, as the publications are a result of a collective effort. The major contribution of the candidate is the generation of various bioinformatics data, namely the performance of the phylogenetic analyses of the genes for heme biosynthesis and the *in silico* predictions for protein targeting for two of the papers and identification of contaminated sequences and phylogenetic analyses for the third paper. The wet lab experiments that are listed in the candidate's contributions consist of DNA and RNA extractions, library preparation and sequencing for the genome project paper.

Although the findings presented in the thesis significantly enrich our knowledge in the field of evolutionary protistology, I do not find the amount of candidate's contributions very impressive and I would expect more data to be generated in more than six years of PhD studies. I would especially value if the candidate could support some of the hypotheses derived from the *in silico* analyses by cell biology or biochemical data. For example, in the study 'Evolution of the Tetrapyrrole Biosynthetic Pathway in Secondary Algae: Conservation, Redundancy and Replacement' the major outcomes are hypotheses on the presence of multiple enzymes for the same reaction in different cell compartments and in some cases the authors suggest dual targeting or relocation of some of the enzymes during evolution, yet no experimental evidence to back up these claims is provided. So my first question to the candidate is whether it was never his intention to try and obtain the wet lab data to supplement the bioinformatics analyses or he did not pursue such experiments due to difficulties of a technical nature.

Also, for some of the statements in the introduction, the candidate refers to Cihlár et al. unpublished (page 10 and 19) and in the conclusion he states that some of the work that he has done will be published as separate papers. Can the candidate explain why none of these experiments were included in the thesis?

Despite the criticism above, I believe that the presented amount of data is sufficient and that the PhD thesis meets all the criteria required by the Department of Molecular Biology and Genetics at the Faculty of Science of the University of South Bohemia. I have therefore no reservations in recommending the thesis for a passing grade and as a basis for awarding Mgr. Jaromír Cihlár the degree of Doctor of Philosophy.

Sincerely,

Luděk Kořený

Specific questions and comments:

Top of page 5: The statement "The consecutive steps of heme biosynthetic pathway are common to all organisms that are able to synthesize heme" is not true as an alternative pathway has been described in Archaea and it was recently revealed that many bacterial groups use yet another deviation from the 'classical' heme pathway. Can the candidate make up for this omission and describe these alternative pathways?

On page 14 the candidate states that "*Lepidodinium chlorophorum* is the only example of dinoflagellate where the original red-derived plastid was replaced by a secondary green plastid". I wonder whether there is enough evidence to suggest this was indeed a plastid replacement rather than the two plastids still co-existing. Can the candidate comment on this?



February 5, 2018

**Re: Ph.D. thesis "Evolution of the heme biosynthesis pathway in eukaryotic phototrophs" by
Mgr. Jaromír Cihlář**

The work under review ("Evolution of the heme biosynthesis pathway in eukaryotic phototrophs" by Mgr. Jaromír Cihlář) includes a broad introduction to the topic, followed by conclusions, a collection of 3 published (1 first-authored, PLOS One) papers, and final conclusions. The overall structure of the thesis is well organized and the work is presented in a concise and comprehensive manner. I also liked the language of the thesis (f.e. "imaginary shopping bag of enzymes", page 25). The published papers represent a significant step in our exploration of evolution of the heme biosynthesis pathway in eukaryotic phototrophs. All data presented there are solid and very much bullet-proof.

My concerns are mainly of the "decorative" nature.

- 1) It puzzles me why there are two conclusion sections?
- 2) List of references is formatted rather sloppy.
 - 2a) Why some titles are selectively capitalized (f.e. Delwiche, 1999)?
 - 2b) Italic font is not used for species/generic names in numerous instances (f.r. van Doorten et al, 2006 and some others).
 - 2c) One of the references on page 33 (Plant Physiol, 2007) states just the journal info, but no author(s) or title is provided.
- 3) I was not able to find several supplemental figures or tables (f.e. Fig. S2 from the POS One manuscript – by the way, I am also curious why this is the only instance where ML tree is presented (versus Bayesian trees elsewhere)?; all supplementary Tables from the same manuscript; all supplementary materials from the GBE manuscript). I think all these materials are crucial and must be presented in the thesis.

The following 2 questions are rather general and intend to provoke some discussion.

- 1) Can you elaborate on the experimental approaches currently used to study heme biosynthesis pathway in the wet lab? I understand the axenic cultivation of some organisms, studied by the author, is still an issue.
- 2) Author has referred to (Cihlár et al., unpublished) in several places. Do you plan to publish these observations?

All the above mentioned remarks are minor, and I wholeheartedly recommend Mgr. Jaromír Cihlár to be awarded a Ph.D. title.

Sincerely,

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