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Evaluation of the graduate thesis of PhD candidate Mgr. Luboš Voleman, Charles University of Prague

Dear Prof. Dr. Hrdý,

With this letter, I wish to submit my review of the graduate thesis written by PhD candidate Mgr. Luboš Voleman, Charles University in Prague, submitted to the Faculty of Science.

a) Assessment of the thesis

The PhD candidate's thesis is centered around mitochondria and mitochondria-like organelle (MLO) dynamics, with a clear focus on parasitic protists as models for mitochondrial evolution. This topic carries high scientific merit from several perspectives which include eukaryotic cell biology, organelle evolution and subcellular diversification. Research on the cell biology, evolutionary history and biochemical diversification of reduced and non-model MLOs has gathered significant momentum in the past years, and the work presented in this thesis is a substantial contribution to the field. The proposed experimental design and choice of methods to investigate mitosomes in *Giardia lamblia* and hydrogenosomes in *Trichomonas vaginalis* are, to a large extent, suitable. As befits the subject matter, the interpretation of the data is appropriately conservative and is coupled to a good quality of the documentation. Altogether, I found this thesis well-written, except for some grammatical and spelling errors which can be easily corrected. Overall, I would judge this thesis suitable for presentation in a PhD defense examination.

b) Specific critical comments

The candidate's introduction to the topic of mitochondrial dynamics was well-organized and included a very informative section on current knowledge in protist parasites. However, this thesis lacks a clear statement of the hypothesis or hypotheses that inform the aims of this thesis. A simple text search yields no mention of the word "hypothesis", except in one of the published papers. A research thesis usually contains a central hypothesis that informs the aims, the methods and the interpretation of any acquired data. Furthermore, there is no specific section on "Conclusions" which are drawn only in the summary.

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c) Questions to the PhD candidate

- **Question 1:** Could the PhD candidate state and elaborate on the central hypothesis informing this thesis ?

- **Question 2:**
  From the thesis, page 19:
  « …the metazoan homologue of Gem1, Miro1, was also localized to several foci per mitochondria and consistently coincided with the ER tubules, a pattern reminiscent of the ERMES components [125]. Interestingly, Miro1 homologues are absent in organisms lacking mitochondrial DNA [144] which corresponds to the phenotype of yeast cells lacking gem1, which rapidly lose mtDNA [126]. »

  Given that the PhD candidate suggests this may be a mechanism to obtain genome-free mitochondria (such as mitosomes), what other factors would then ensure maintenance of genome-free organelles? What kind of selective pressure would there be?

- **Question 3:** Amongst the parasites the PhD candidate lists in his introduction, he omitted *Entamoeba histolytica*, despite the fact we know this organism harbours mitosomes. Could he elaborate on *Entamoeba* mitosomes in terms of fusion and fission during this parasite's life cycle, similarly to what was done for other parasites in the introduction?

- **Question 4:**
  From the thesis, page 29 :
  « According to our data, disruption of GlDRP GTPase fuction has no effect on mitosomal division. Encysting cells expressing non-functional version of GlDRP, K43E GlDRP [199], harbor the same number of mitosomes as the wild type encysting cells [210]. »

  What are the candidate's suggestions on how to explain this discrepancy, given previous findings in Rout et al., 2016?

  Question 5: In figure 1 of the publication Martincová et al., 2015, biotinylation and enrichment of outer mitosomal membrane proteins such as Tom40 and MOMP35 is shown. How does the PhD candidate explain this result, given that the mtBirA construct is enriched in the mitosomal matrix and is therefore physically separated from outer membrane components ?

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Unfortunately, I will not be able to physically participate in the defense due to a non-postponable family commitment. For this reason, I look forward to reviewing the PhD candidate's answers to my questions, by email. As *per* instructions, I will then review the candidate's answers and send you my comments.

My review will be mailed out this week as a signed hard copy, in line with the instructions sent to me by Dr Helena Kulíková.

Yours sincerely,
Carmen Faso, PhD

Carmen Faso