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Dear Prof. Ivan Hrdy (Chair of the committee),

It was my pleasure to be the examiner for **Mgr. Vojtech Vacek's** PhD thesis entitled "Iron-sulfur cluster assembly in *Monocercomonoides exilis*". The candidate has presented an almost holistic view of the current literature on the Fe/S cluster assembly machineries and proteins/enzymes that are carrying these factors as well. I have been very impress with the publication record of the candidate, since I have previously read all the publications assigned with this thesis and I would really like to see the manuscript on the functional characterisation of the SUF machinery published as well. As seen from the candidate's publication record and his involvement in the manuscripts, during his project he has developed multiphasic expertise, from sophisticate bioinformatic analyses to cell biological, complementation studies and biochemistry in very challenging systems as well. The aims provided throughout the thesis have been answered through the various publications provided.

I have the following questions/suggestions and I would greatly appreciate if the candidate would be able to respond during his defence:

1. What would you considered to be your biggest contribution to the scientific community throughout your PhD?

2. What is the idea that binds your thesis together?

3. If you had the chance to start your PhD from the beginning, what would you have done differently and why?

4. What is the relevance of the section 2 (Fe-S clusters and origin of life) to the whole thesis? This is not something that the candidate has tackled throughout any of the publications.

5. What were the most challenging parts/experiments that you have included or not included in your thesis?
    a. Are there any experiments left behind?

6. Do you think there might be another species of Eukaryotes that lacks mitochondrion? If yes, from which phylogenetic group?

7. How is your thesis relevant to the Archezoa hypothesis?

8. From the list of proteins that require Fe-S clusters in order to function, which ones would you considered the most important and why?

9. Which form of iron is toxic to the cell and why?

10. Why a section on the plastid SUF machinery was not included under section 7, which is probably more relevant to this thesis, than any of the other machineries?

11. Tsaousis 2019 introduced three theories regarding the acquisition of SufCB gene by the anaerobic protists (*Blastocystis, Proteromonas, Pygsuia, Stygiella*). Which one do you support and why?

12. While the summary section provided an overview of all the results that came out from the publications of the candidate, I would have liked to see a better connection between them and present the whole story of the thesis.

13. What do you think are the next steps of your PhD work? What is the future for *M. exilis?*
    a. If you had more time, what else would you wanted to include on your thesis/

Minor suggestions:

- Abstract needs to be rewritten, since it lacks structure and does not provide a flow of the story.
- Non-published sections require proof-reading since there a few issues with grammar and lack of punctuation.
- Avoid the use of the phrase “Thanks to” across the thesis. This should be replaced with “Due to”.
- Some additional figures on the Fe/S cluster machineries could be included in dedicated sections, along with the operons when describing the bacterial homologous machineries.
- Why Fig. 4 is under section 6.4?

I am looking forward to the candidate’s defence.

Kind regards,

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