John Innes Centre

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To the Chairman of the Board of Examiners
Dr. Ivan Hrdý, Ph.D.
Department of Parasitology
Charles University
25250 Vestec

Re: Examiner's report for the PhD defence of Mr Vojtěch Vacek

Sir,

It was my pleasure to read the thesis on „Iron-sulfur cluster assembly in *Monocercomonoides exilis*" by PhD candidate Mr Vojtěch Vacek. It summarizes comprehensive bio-informatics analyses of *SUF* genes in this unusual organism and related species, together with experimental work to demonstrate the functionality of the SUF proteins. The work has been included in 4 papers that have been peer-reviewed and published in journals of medium – high impact. A 5th paper is still in preparation, for which a complete draft has been included.

The thesis is well written, with only minor mistakes in English. The relevant literature for this topic is covered in-depth and for the most part critically assessed. The methods are overall sufficiently described, and appropriate to address the scientific questions. The results are presented in well laid-out figures, although some of the labelling could have been better to facilitate a quicker grasp of the experimental set up.

Given the incorporation of the data in several published papers, the research presented in the thesis is an original contribution to science and merits the award of a PhD based on my previous experience as examiner.

After reading the thesis, in particularly Chapter 10 and the manuscript in preparation, I have a couple of questions for the candidate to discuss:
1. What could be the benefit of a fusion protein of SufDSU rather than separate proteins? And what the disadvantages? Could you speculate on the evolutionary drivers for having either fusion proteins or separate proteins for the many examples in biology?
2. The SufDSU protein seems to have a shorter SufD sequence. Please comment if this is real and not an incomplete sequence. If this is real, how is this likely to affect the assembly of the functional B_2CD complex? Could a B_2C_2 complex be functional in *M. exilis*?

3. For the results in Fig. 8, page 66 – why were different organisms used for the localization experiments? What causes the different localization pattern of SufB and SufC in (A) – shouldn't this be the same?
4. The thesis does not mention the paper by Ozer et al., 2015 (DOI: 10.1074/jbc.M115.682179) which addresses issues with previously published studies on Erv1. Ozer and colleagues show that a secondary mutation in a different gene in the original *erv1-1* strain caused a defect in Fe-S cluster assembly. Thus, the biological role of Erv1 is restricted to protein import into mitochondria. Please discuss whether this interpretation makes sense in the light of the phylogeny of Erv1 (presence or absence in different organisms with mitochondria, MROs or lacking MROs).
5. For the bacterial complementation (Fig 9, page 69 and Manuscript in preparation), IscR was chosen as an Fe-S reporter protein, linked to its activity as a transcription factor to drive lacZ expression. I hope you are aware of the complex regulation of IscR. Could this underly the issues with the large error bar in the empty vector control and the small difference between positive and negative control? What other Fe-S reporter proteins could be used? Were other proteins tried at all to set up a sensitive reporter system?

I wish the candidate good luck with the PhD thesis defence on 13 November 2020.

Yours sincerely,

Dr Janneke Balk