Review report on Ph. D. thesis of Mgr. Eva Pyrihova

I was very excited to receive and read the PhD thesis of Mgr. Eva Pyrihova. The research presented in this thesis extends our current understanding the diversity and composition of the mitochondrial protein import. The candidate utilized *Giardia intestinalis*, a highly diverged anaerobic protist, as a model organism to explore some of the minimalistic composition and function of the mitochondrial protein import. *Giardia's* remnant mitochondrion, so called mitosome, has many unique characteristics and is one of the most difficult organelle to work with. The candidate has clearly acquired unique expertise with highly advanced technology in the field of proteomics in order to tackle aspects of her thesis. As seen from the presented publications, these have provided an important contribution in the fields of cell biology and biochemistry of mitochondria, under the umbrella of anaerobic parasites/protists.

The candidate includes 4 publications in support of the degree, although there is no statement on the contribution of the candidate in writing these, with the exception of the last manuscript:

Eukaryotic Cell, 2014: 3rd author out of 7.
This manuscript provides valuable information regarding the eukaryotic distribution of cyt*b5* proteins and the function of *Giardia*'s homologue. This work demonstrates that *Giardia's* homologue binds to heme, which is a unique attribute for a anaerobic eukaryote, spearheading further research in this field.

Molecular and Cellular Biology, 2015: 1st author out of 8.
This work demonstrates a novel method for *in vivo* enzymatic tagging of mitosomes in *Giardia*, which subsequently enable the identification of novel or highly diverged proteins; in this case Tim44.

Mol Microbiol, 2016: 2nd author out of 11.
This work demonstrated dual localisation, both in the cytosol and mitosome, of two proteins involved in the cytosolic iron-sulfur cluster assembly. In model organisms (e.g. yeast, humans), these proteins are found only in the cytosol, where in *Giardia* they seem to be present in the intermembrane space of the mitosome, suggesting an intermediate role of these proteins between the mitochondrial and cytosolic Fe-S cluster assembly pathways.

Single Tim translocase in the mitosomes of Giardia intestinalis illustrates the convergence of the protein import machines in anaerobic eukaryotes. *Manuscript in preparation*: 1st author out of 11.
This work uses multidisciplinary approaches to characterise the mitochondrial protein import in anaerobic protists, where it's been demonstrated, using *Giardia* as a model, that unlike the other eukaryotes, there is a "single Tim17 family protein translocase" functional system, using Tim17 instead of Tim23 as a protein backbone.

Critical comments:

My main criticism is in regards to the structure of the thesis. The thesis contains an Abstract, an Introduction, a Discussion, and the experimental part of the thesis consisting of four publications, one of which is unpublished. The short abstract is uninformative, and does not provide any information neither on the results of the thesis, nor conclusions or impact of the whole work. The introduction lucks of structure as well, and is focusing only in general aspects of the mitochondrial protein import, while introducing some pathways or functions, that are not very well explained. While two of the experimental (results) chapters refer to Fe-S proteins and function, it would have been ideal that these pathways would have also been introduced and explained in the introduction section as well. I would have liked to see some additional information in regards to the cell biology of *Giardia* and the current *status quo* on proteomic analyses of its mitosomes. In addition, I have some concerns in regards to the literature review and particular referencing. There are several missed citations and various statements that have been wrongly cited. For example, in page 12, the references in regards to the discoveries of the mitosomes in microsporidia and in *Cryptosporidium* are incorrect. There are also some factual inaccuracies: the candidate refers to *Brevimastigomonas motovehiculus* as a recent discovery that could be the transition organelle between hydrogenosomes and mitochondria, while many other organelles, including the MROs of *Nyctotherus* and *Blastocystis* were also considered intermediates stages and have been discovered over a decade ago. In another example, while the thesis introduced the remnant mitochondria of anaerobic protists including *Entamoeba*, microsporidian, *Cryptosporidium* and *Giardia* and their unique mitochondrial protein import, they have then talked about Trypanosomes' unique mitochondrial protein import acquisition, which seemed a bit out of place. Lastly, I would have liked to see a conclusions' section that could provide a concise overview and assessment on the results achieved during the candidate's PhD. Also, a figure/cartoon demonstrating the mitochondrial protein import in yeast vs *Giardia*, would have been really useful either in the introduction or the discussion section.

Despite some criticism expressed above, overall, I would recommend the candidate for the award of a Ph.D degree.

Canterbury, on the 7th of February, 2018

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Questions to be addressed during the PhD thesis defense:

1. What is/are the most important function(s) of *Giardia*'s mitosomes and which do you think is the reason for their existence?

2. Are there any differences between the central and peripheral mitosomes in *Giardia* and what do you think could be their functions?

3. How does the mitosomal protein work from Rout et al 2016 (PLoS Pathogens) fits with your current data on the protein import machinery in *Giardia* mitosomes?

4. Since there is neither an ATP/ADP carrier nor an ISC export machinery found in *Giardia* mitosome, could you discuss the possibility of the protein import been involved in either of these functions?

5. You have mentioned that *Giardia* serves as a great model to study mitochondrial evolution, since its mitosomes are one of the simplest MROs. Could you elaborate on this statement?