Review report on Ph. D. thesis from Mgr. Neritza Campo Beltrán

The PhD thesis of Mgr. Beltrán addresses a significant biological topic, what are the functions of mitochondrion-derived organelles, via analyses of proteomes of the hydrogenosomes of *Trichomonas vaginalis*. The main aim of this thesis was to analyze changes in the hydrogenosomal proteome caused by fluctuation of iron availability and during development of resistance to metronidazole *in vitro*. In this context, to develop a method for isolation of the highly purified hydrogenosomes and mitosomes as another aim of the thesis is a meaningful contribution to these studies. In my opinion, the aims of the thesis as listed on p. 27 are fulfilled and the results of this thesis certainly contribute to our understanding how the pathogenic trichomonads cope with changes in their natural habitat, how they cause chronic infection and eventually, how they resist to the treatment.

Mrs. Beltrán´s thesis consists of four papers and shortly-described unpublished results that cover several aspects of the hydrogenosomal proteome, and of one paper on the reduced proteome of *Giardia* mitosomes. The experimental design of all studies is of a high quality, innovative approaches combine mass spectrometry, bioinformatics, reverse genetics, etc. The first two papers, “Iron-induced changes in the proteome of *Trichomonas vaginalis* hydrogenosomes” published in PLOS ONE (Neritza C. Beltrán as the first author) and “Transcriptomic identification of iron-regulated and iron-independent gene copies within the heavily duplicated *Trichomonas vaginalis* genome” published in Genome Biology and Evolution (Neritza B. Campo as the fifth author) reveal that in cells of *T. vaginalis*, availability of external iron (i) modifies expression of iron-containing hydrogenosomal proteins and of proteins involved in assembly of Fe-S clusters, and (ii) affects, in a specific way, a regulation of expression of multi-copy genes. These are very interesting findings necessary for further studies e.g., on adaptability of the pathogens to host environments. The unpublished results of the thesis describe changes in the hydrogenosomal proteome of a highly metronidazole resistant cell line of *T. vaginalis* similar to those found under iron restricted conditions. The paper analyzing the hydrogenosomal membrane proteins required for protein import and exchange of metabolites (Neritza Beltrán as the ninth author) nicely supports relationship between the mitochondrion and the hydrogenosome but also reveals specificities in the hydrogenosomal membrane transport. In my opinion, the findings on the hydrogenosomes of *T. vaginalis* and their proteomes presented in the thesis will substantially contribute to our knowledge on biology and evolution of the mitochondrion-derived organelles and will stimulate further research. Integration of the paper on the mitosomal proteome of *G. intestinalis* to the thesis of Mrs. Beltrán (the eleventh author)

likely corresponds with the aim of a development of a method of the isolation of highly purified mitosomes, because the *Giardia* mitosomes are not covered in a literature review of the thesis (as mentioned below).

My criticism concerns a structure of the thesis. The thesis contains Abstract, Introduction, a review of the literature, an experimental part of the thesis consisting of five publications and unpublished results, and Conclusions. A short abstract is not informative describing mainly the experimental approach. The Introduction more likely represents the abstract of that part of the thesis which addresses proteomic analysis of hydrogenosomes (but not findings on proteome of *Giardia* mitosomes). The literature review, although a common title of this section is absent, covers in a simple, but more or less comprehensive way, what is currently known about hydrogenosomes, hydrogenosomal metabolism, and significance of the organelles for *T. vaginalis* as a human pathogen. The references cited show a good knowledge of Neritza Beltrán in the field. To my surprise, there is a very few information about *Giardia* mitosomes, although proteomic analysis of this organelle is included to the results of the thesis. My criticism regarding this part of the thesis concerns several formal aspects, including subdivision of the text into paragraphs that sometimes breaks coherency and clarity of the information, and the absence of sorting style when groups of references are cited (neither alphabetically, nor chronologically). There are also some factual inaccuracies, e.g., phagocytosis of host proteins (page 4), nitrite formulated as NO_2 (page 7). The literature review is followed by a list of aims of the PhD thesis, the publications and the well written Conclusions that provide a concise overview and assessment on the results achieved during PhD studies of Mrs. Beltrán.

My overall view is that Mgr. Beltrán presents formally acceptable PhD thesis which fulfils criteria required for granting the PhD degree at Charles University. Her PhD thesis is built on five articles of a high quality published in well-reputed journals, with her significant scientific contribution declared by her supervisor but has some deficiencies in structure and organization. Despite some criticism expressed in my review report, after successful defense including adequate responses to questions I recommend Mrs. Beltrán thesis for the award of a Ph.D. degree.

Prague, September 7, 2016 Eva Nohýnková, Ph.D.

Questions to be addressed during the Ph. D. thesis defense:

In a study on hydrogenosomal membrane proteins, functional components necessary for biogenesis of this organelle have been identified (among others). Could you summarize what is currently known about biogenesis of hydrogenosomes and what can be a particular function of the identified membrane proteins in the process?

In a highly purified hydrogenosomal fraction prepared from a metronidazole-sensitive parent *T. vaginalis* isolate and from cell lines with different levels of metronidazole resistance derived from this isolate, mass spectrometry (MS) analysis identified about 700 proteins, of which 140 proteins were of a hydrogenosomal origin. Could you explain a nature of residual 560 proteins identified by MS in the hydrogenosomal fraction?

Are there any biological consequences (other than resistance to metronidazole) of downregulation of hydrogenosomal enzymes in the highly metronidazole resistant MR100 *T. vaginalis* cell line?

Is it known from other species that transcription of individual copies of a gene which is present in several copies in a genome is differently affected e.g., by metal ions (iron, zinc, cooper)?

Genome of *T. vaginalis* contains many genes of bacterial origin suspected to be received by a lateral gene transfer. In vagina of fertile females, representing a natural habitat of *T. vaginalis*, lactate producing lactobacilli are far the most and usually the only bacterial species. Is there any evidence for genes coming from this particular genus in a genome of *T. vaginalis*?