CENTER FOR PATHOPHYSIOLOGY,
INFECTIOLOGY AND IMMUNOLOGY
MEDICAL UNIVERSITY OF VIENNA
Institute of Specific Prophylaxis
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Dear Prof. RNDr. Petr Horak, Ph.D., FRSC,

Enclosed please find my evaluation of the dissertation of Mgr. Lenka Ulrychova entitled: "Characterization of serine proteases of *Schistosoma mansoni* involved in interaction with host", written under the supervision of Mgr. Martin Horn, CSc. RNDr. Jan Dvořák, Ph.D. had the role of an advisor. Mgr. Ulrychova began her doctoral studies at Charles University and in parallel at the Czech University of Life Sciences in 2011.

Mgr. Ulrychova's doctoral thesis examines the proteolytic enzymes (proteases) of the blood fluke *Schistosoma mansoni*, which is endemic in over 50 countries and is responsible for schistosomiasis, a neglected tropical disease. Around 130,000 people die every year from an infection with *S. mansoni*. Therefore, this parasite poses a major public health challenge that requires intensive research to better understand its biology and to develop more effective treatments and preventive measures against schistosomiasis. The dissertation provides a comprehensive insight into the serine proteases of *S. mansoni*. The main focus is on their characterisation and their importance for the biology of the parasite and the interaction between parasite and host. The results could potentially pave the way for new vaccines against schistosomiasis.

The cumulative dissertation comprises seven (!) publications. Mgr. Ulrychova is first author of a scientific paper published in Parasites & Vectors (2021: IF 3.2 according to Web of Science 2024; rank 9/37 in the category Parasitology) and a book chapter in Methods in Molecular Biology (2020). In addition, she is co-author of three articles published in the journal PLOS Neglected tropical diseases, one article published in Biochimie and one book chapter in

Methods in Molecular Biology. All publications were peer-reviewed. The work is of great interest given the high number of citations. Four of these articles were cited more than 20 times.

The literature review is extensive, demonstrating a broad and consistent analysis of the current state of research in the field. A total of 238 articles were cited in the thesis.

The research is well designed and the technical quality of this work is impressive. The candidate has developed and applied a wide range of techniques (e.g. cultivation and isolation of developmental stages of *S. mansoni* (adults, eggs and schisosomula), isolation of secretory/excretory products of developmental stages of *S. mansoni*, fluorescence in situ hybridisation, RNA probe design, recombinant protein expression, protein biochemistry, RNA and protein isolation, basic and advanced histology, immunohistochemistry, and various microscopy techniques) to achieve the objectives of the thesis. The corresponding methods were performed at a high level of quality and development and are detailed in detail in the publications.

Results are presented in the form of published articles. Each published paper is briefly introduced, followed by a short explanation of the candidate´s contribution. The results are significant and original. The data in the published articles are presented clearly and concisely. In my review, I will focus primarily on the two first author publications and also on the non-peer-reviewed text of the dissertation.

The first paper describes the spatial expression pattern of five serine proteases in adult male and female *S. mansoni*. Highly sensitive fluorescent RNA in situ hybridisation enabled the visualisation of mRNA with low expression potential. The authors suggest that this method could also be applicable to other trematodes. The study identified previously unknown sites of *S. mansion* serine protease (SmSPs) expression, such as the syncytial part of the tegument or tegumental tubercles. The authors hypothesise that these proteases play a physiological role of these proteases in host-parasite communication, such as "regulation of the host vascular system, repair of stress-induced damage, and/or adaptation to changed conditions in the external environment". Can you elaborate on how the proteases might work? What would be the proposed mechanism? Please give us an example for each of the proposed functions.

The second publication, for which Mgr. Ulrychova is first author, describes an advanced RNA in situ hybridisation protocol for detailed spatial analysis of gene expression in *S. mansoni* tissue. It uses digoxigenin-labelled RNA probes and a fluorescence-based detection system enhanced by tyramide signal amplification for precise visualisation of mRNA transcripts, including those with low abundance. The method includes improved tissue preservation, sectioning, signal amplification and pre-hybridisation treatment. This optimised protocol represents a significant advance in molecular parasitology and enables the visualisation of gene expression in fragile structures. Indeed, this method has been tested to identify genes encoding proteases in *Fasciola hepatica* or *Eudiplozzon nipponicum*; Mgr. Ulrychova were involved in these studies. What were the main limitations of this technique in these organisms? Did some species-specific adaptations have to be made? What advantages does this method have over other methods, such as standard in situ hybridisation or RNA sequencing?

The Discussion chapter summarises the main findings of all thesis papers, which are further discussed in the light of the relevant literature. The Conclusion chapter summarises the fulfilled objectives of the thesis.

The thesis follows the expected formal structure of a dissertation and is provided with an extensive bibliography. Whilst I have no reservations about the published work, there are some issues in the sections that have not been peer-reviewed, particularly in relation to inconsistent terminology, formatting and areas that could benefit from further proofreading to improve readability.

General remarks:

Formatting: To improve the readability of the work, it is important that style and formatting are consistent across all chapters and sub-chapters. Not only each chapter, but also each subchapter should have a clear and consistent numbering system that reflects its position within the chapter. The font and style for chapters and subchapters should be differentiated to visually distinguish the hierarchical levels of the text.

Abbreviations: There are many abbreviations missing from the list, such as ISH, WISH, MEROPS, or POP. Also, I do not think RNA needs to be explained, and if it does, then the same applies to DNA. When an abbreviation appears in the text for the first time, it should be preceded by

the full term with the abbreviation in brackets. After that, only the abbreviation is used. This rule has not been applied consistently.

To improve the readability and scientific rigour of the text, some sentences should be reworded:

- Abstract: "all stages secreted significant serine protease activities"?
- Page 9: "A vaccine utilizing protein or mRNA mechanisms"
- Page 3: "Compared to the large number of trematodes, about a hundred of digenetic flukes are known..." This implies that flukes are not trematodes.

Specific remarks:

- Page 1: "Praziquantel and oxamniquine - are recommended for the treatment and prophylaxis" What is the mechanism of action of praziquantel? How might it work in prophylaxis?
- Page 4: Was the S. japonicum protease inadvertently mentioned in the chapter "The life cycle of S. mansoni"
- Page 6: Replace "persons" with humans; correct "Schistosomiasis mansoni"
- Page 7: Can proteolytic enzymes be considered metabolites?
- Page 13: "S. mansoni, S. japonicum and S. haemotobium are known to produce such RNAs [130-132]" The RNA content within extracellular vesicles from S. haematobium has not been described in the paper 132.

The candidate has presented her work at several national and international conferences (oral and poster presentations) and completed five research stays at three host institutions in Germany, Switzerland and the USA, thus demonstrating her mobility. She has also participated in five national and international scientific workshops, focussing on microscopy, bioinformatics, mass spectrometry and RNA. As the main applicant, she also received funding to carry out experiments and for scientific travel. She is also co-author of five original papers that were published in peer-reviewed journals such as the International Journal of Parasitology but were not included in the dissertation.

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Overall, the submitted work demonstrates that Mgr. Lenka Ulrychova has fulfilled all the criteria for the award of the doctoral degree.

I confirm that I have no conflict of interest with regard to the assessment of this dissertation.

To conclude my review, I would like to congratulate Mgr. Lenka Ulrychova on her excellent work! It is really impressive how she have deepened our understanding of the role of proteases in parasitism. I wish her all the best for the future.

Yours sincerely

Irma Schabussova

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