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To
RNDr. Ivan Hrdy, Ph.D.
Chair of the Committee

Evaluation of the of the dissertation of
Mgr. Roman LEONTOVYC

Dear Ladies and Gentlemen!

It is a pleasure for me to provide an evaluation of the thesis of Roman Leontovyc with the following title: *Molecular adaptations of neurotropic and visceral bird schistosomes during the infection of the avian definitive host.*

The work of Roman Leontovyc focused on an important question of bird schistosome biology, infection and migration behaviour in the final host. As model parasites, *Trichobilharzia regenti* and *Trichobilharzia szidati* were chosen due to their different life styles in the final host. Similar to human schistosome species, *T. szidati* has adapted the visceral way of migration via bloodstream and lungs. In contrast, *T. regenti* has specialized on another route, migrating through the peripheral nerves and the spinal cord. This way of neurotropic migration is a unique feature of this bird schistosome species, and therefore of general scientific interest. Against this background, the aim of the work of Roman Leontovyc was to unravel the molecular mechanisms linked with visceral versus neurotropic life styles of these schistosome species. To this end, transcriptomics approaches were performed using the relevant life-cycle stages as targets, cercaria, as the free-living and infective stage, and schistosomulum, the first stage within an infected final host staring the migration process. Roman Leontovyc followed three main aims, (i) to sequence, reconstruct and annotate the first transcriptomes of visceral and neurotropic avian schistosomes *T. szidati* and *T. regent*, to (ii) identify key biological and metabolic pathways specifically occurring in the cercaria and schistosomulum stages, and to (iii) identify molecular mechanisms

possibly linked to visceral and neurotropic schistosomiasis with special focus on nutrition preferences of *T. szidati* and *T. regenti* schistosomula.

As planned, transcriptome data of *T. szidati* and *T. regenti* were generated and published in different articles. In one of these, comparative bioinformatics analyses showed common molecular mechanisms between cercariae of *T. regenti* and human schistosomes such as carbohydrate and energy meta-bolism. This was expected as it reflects the energy need of cercariae to fulfil their role as free-living infectious stage. Although a predicted cercarial elastase was not found, evidence for the potential involvement of carbohydrate meta-bolism, translation, amino acid metabolism, and calcium metabolism were obtained for *T. regenti* cercariae. For schistosomula, indications for cell adhe-sion molecules, microaerobic metabolism, peptidases, and further enzymes were found, which could be involved in migration processes through neural tissues in the avian host.

In the second paper, molecular aspects of the nutritional requirements of *T. szidati* versus *T. regenti* were discussed. Again, the cercaria and schistosomulum stages were in focus. In cercariae of both species, pathways associated with carbohydrate metabolism, oxidative phosphorylation and ribosome biogenesis, exosome production, and lipid biogenesis were highlighted based on bioinformatics. Schistosomules of both species showed pathways associated with signal transduction, cell turnover and motility, DNA replication and repair, molecular transport and/or catabolism. By a comparative approach, peptidases and secretory proteins were detected to be expressed in schistosomules, and they may be involved in degrading macromolecules in both *T. szidati* and *T. regenti*. However, also species-specifically or predominantly occurring molecules were found, which could be involved in selective nutrient uptake, which may contribute to migration differences. Among these are micro exon genes (MEGs), which were found to be differently transcribed between *T. regenti* and *T. szidati.*

The third paper reported on the characterization of one specific factor, the enzyme cathepsin B1 of *T. regenti* (TrCB1), and it putative role in in migration and the nutrient digestion of schistosomula. Interestingly, multiple isoforms of this enzyme were found, TrCB1.1-TrCB1.6, which showed varying substrate specificities. Gene expression analyses showed most abundant transcript levels for the potentially inactive paralogs TrCB1.5 and TrCB1.6. The catalytically active enzymes were able to complex with TrCB1.6 suggesting that inactive paralogs may be able to regulate the activity of the active forms. Furthermore, inactive paralogs can bind cystatins, which may be accom-plished by specific mutations. This suggests roles of these inactive paralogs in sequestration of inhibitors and/or protection of the parasite against active cysteine peptidases of the host.

During his PhD studies, Roman Leontovyc was able to successfully perform a number of different, in part difficult and complex experiments. He used a variety of molecular, biochemical, and bioinformatics techniques and obtained novel and interesting results. The quality of his work is high-ranking, which is supported and documented by the fact that three papers originating from his work were published in internationally recognized, peer-reviewed journals. The thesis is well written, although not free of some mistakes in grammar and orthography. The results were presented in a condensed way. Here I missed some information of all important data, which have been found and published. I found this information in the abstracts and the result sections of the listed papers but not in the thesis summary.

Besides the remarkable results obtained in his work, Roman Leontovyc also deserves credit for the fact that he worked with a complex parasite, which is difficult to handle in the laboratory. Compared to model organisms such as *C. elegans* or *Drosophila*, working with schistosomes is highly demanding and requires specific skills and patience.

Among the questions for future research aspects in this research field is, how Roman Leontovyc could imagine to continue in characterizing the poten-tial roles of cathepsins and their isoforms as well as other genes/proteins found to be differentially expressed between *T. szidati* and *T. regent*. With respect to the fact that transcription is not always a hint for immediate trans-lation, another question would be how (and with what methods) the transcript-tome data could be substantiated by proteome data to get an idea of stored transcripts versus immediately translated/expressed ones. A subsequent question is whether there are any possibilities (or whether he could conceive such possibilities) to functionally characterize genes of interest of the schisto-some species in an animal model? Finally, is there any practical or theoretical possibility to establish *in vitro* models to follow all these interesting research activities under controlled condition, avoiding animal experiments, in the future.

With respect to the facts that (i) Roman Leontovyc has performed an original and elegant thesis, and (ii) against the background that three papers were published with new results from his work demonstrated his capacity as a researcher, and that he significantly contributed to an increase of knowledge about bird schistosomes. Therefore, I highly recommend him for awarding the PhD title.

With best regards,