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Ph.D. study programme: Parasitology
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Mgr. Roman Leontovč

**Molecular adaptations of neurotropic and visceral bird schistosomes
during the infection of the avian definitive host.**

**Molekulární adaptace neurotropních a viscerálních schistosom během
infekce ptačího hostitele.**

Ph.D. thesis/Dizertační práce

Supervizor/Školitel: RNDr. Martin Kašný, Ph.D.

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Curriculum vitae

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Education:

- 2011 – present Ph.D. study in Parasitology
Department of Parasitology, Faculty of Science, Charles University
Title of the Ph.D. thesis: “Molecular adaptations of neurotropic and visceral bird schistosomes during the infection of the avian definitive host “
Supervisor: RNDr. Martin Kašný, Ph.D
- 2009 - 2011 Master’s degree study
Department of Parasitology, Faculty of Science, Charles University
Study program: Biology, field of study: Parasitology
Title of the master thesis: “Species of the genus *Radix* in transmission of trematodes in the Czech Republic”
Supervisor: RNDr. Petr Horák, Ph.D
- 2005 – 2009 Bachelor’s degree study
Faculty of Agrobiolology, Food and Natural Resources, Czech University of Life Sciences
Title of the bachelor thesis: “Occurrence of *Echinococcus multilocularis* in red fox in the Czech Republic”
Supervisor: Ing. Ivana Jankovská, Ph.D

Work experience

- 2010 – 2017 Research scientist
Department of Parasitology, Charles University, Czech Republic

2017 – present Assistant professor
Department of Parasitology, Charles University, Czech Republic

2016 – present Bioinformatician
Distributed Computing Department, CESNET, association of legal entities

Professional experience

2017 Faculty of Veterinary Science, The University of Melbourne, Parasite Genetics and Genomics, Dr. Neil D. Young , Prof. Robin B. Gasser (1,5 months)

2014 Faculty of Veterinary Science, The University of Melbourne, Parasite Genetics and Genomics, , Dr. Neil D. Young, Prof. Robin B. Gasser , (2,5 months)

Grant Projects:

Principal investigator - The Charles University Grant Agency (*Grant No. 243-253411*):
Digestive enzymes of blood-feeding trematodes and monogeneans (2012 – 2015)

Publications (overview):

Total number of publications: 9 research articles

H index: 4, Sum of the Times Cited without self-citations: 51 (according to Web of Science).

- Dvořáková H., **Leontovyč R.**, Macháček T., O'Donoghue A. J., Šedo O., Zdráhal., Craik C. S., Caffrey C. R., Horák P., Mikeš L. (2020) 'Isoforms of Cathepsin B1 in Neurotropic Schistosomula of *Trichobilharzia regenti* Differ in Substrate Preferences and a Highly Expressed Catalytically Inactive Paralog Binds Cystatin', *Frontiers in Cellular and Infection Microbiology*, 10, p. 66. doi: 10.3389/fcimb.2020.00066.
- Nezhybová V., Blažek R., Kašný M., Slamková D., **Leontovyč R.**, Ondračková M. Morphological and molecular characterization of *Apatemon* sp. infecting killifish in Mozambique. *Parasitol Int.* Netherlands; 2019;73: 101967. doi:10.1016/j.parint.2019.101967
- **Leontovyč R.**, Young N. D., Korhonen P. K., Hall R. S., Bulantová J., Jeřábková V., Kasny M., Gasser R. B., Horák P. (2019) 'Molecular evidence for distinct modes of nutrient acquisition between visceral and neurotropic schistosomes of birds', *Scientific Reports*, 9(1), p. 1347. doi: 10.1038/s41598-018-37669-2
- **Leontovyč R.**, Young N. D., Korhonen P. K., Hall R. S., Tan P., Mikeš L., Kašný M., Horák P., Gasser R. B. Comparative Transcriptomic Exploration Reveals Unique Molecular Adaptations of Neuropathogenic *Trichobilharzia* to Invade and Parasitize Its Avian Definitive Host. *PLoS Negl Trop Dis.* United States; 2016;10: e0004406. doi:10.1371/journal.pntd.0004406

- Ma J., He J. J., Liu G. H., **Leontovyč R.**, Kašný M., Zhu X.Q. (2016). Complete mitochondrial genome of the giant liver fluke *Fascioloides magna* (Digenea: Fasciolidae) and its comparison with selected trematodes. *Parasites and Vectors* 9, 1-7

- Pankrác J., Novobilský A., Rondelaud D., **Leontovyč R.**, Syrovátka V., Rajský D., et al. Effect of *Fascioloides magna* (Digenea) on fecundity, shell height, and survival rate of *Pseudosuccinea columella* (Lymnaeidae). *Parasitol Res.* 2016; doi:10.1007/s00436-016-5068-4

- **Leontovyč R.**, Košťáková M., Siegelová V., Melounová K., Pankrác J., Vrbová K., Horák P., Kašný M. (2014). Highland cattle and *Radix labiata*, the hosts of *Fascioloides magna*
 • *BMC Vet Res.*; 10: 41., doi: 10.1186/1746-6148-10-41

- Huňová K., Kašný M., Hampl V., **Leontovyč R.**, Kuběna A., Mikeš L, Horák P. (2012): *Radix* spp.: Identification of trematode intermediate hosts in the Czech Republic. *Acta Parasitologica* 57, 273-284 .

- Kašný M., Beran L., Siegelová V., Siegel T., **Leontovyč R.**, Beránková K., Pankrác J., Košťáková M., Horák P. (2012). Geographical distribution of the giant liver fluke (*Fascioloides magna*) in the Czech Republic and potential risk of its further spread. *Veterinary Medicine* 57: 101-109.

Abstract

Genus *Schistosoma* is one the most studied group of helminths due to the importance of several representatives in terms of veterinary and human health. The advent of the modern sequencing technologies, as well as the increasing computational capacities, enabled large-scale screening of nucleic acids and thus deep exploration of complex transcriptome and genome information.

To date the main attention of leading molecular parasitological “Schistosoma” research teams was focused on serious human pathogens *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium*. In the term of molecular/biochemical research, the other schistosomatids were mostly neglected and general knowledge was limited to characterization of particular genes/proteins without further link to biological functional complex.

Presented thesis summarises the first large-scale insights into the molecular basis of biological principles of two bird schistosomes *Trichobilharzia regenti* and *Trichobilharzia szidati* during their invasion of the definitive avian host. While *T. szidati* uses the “classical” visceral way of migration - bloodstream and lungs (same as human schistosomes), *T. regenti*, migrates trough the peripheral nerves and spinal cord. Neurotropic migration is unique among schistosomes and it is also extremely rare within helminths. We aimed to determine the molecular mechanisms linked with visceral and neurotropic life strategy of both *Trichobilharzia* species using transcriptomic profiling of two consecutive developmental stages – cercariae (free living stage) and schistosomula (tissues migrating stage).

Our work started with the transcriptomic analysis of cercariae and schistosomula of neurotropic *T. regenti* leading to the identification of protein classes and biological pathways related to important physiological processes (publication No 1). In order to more accurately identify the molecular mechanisms linked to neurotropism of *T. regenti* schistosomula, we sequenced and reconstructed transcriptome of visceral *T. szidati* and performed comparative analysis. The numerous links to particular visceral or neurotropic strategies of these two schistosomes were identified (publication No 2). Our further research was related to functional characterisation of the important proteolytic enzyme cathepsin B - peptidase of *T. regenti*, including also a detailed analysis of expression of different isoforms (publication No 3).

Aims of the thesis

Five years of the research aimed to provide the first complex overview of the molecular biology of the two avian schistosomes differing in the life strategy during the infective phase of the definitive host. Specific focus was on the molecular mechanisms linked to visceral and neurotropic schistosomiasis using the transcriptomic-bioinformatic approach.

Specific aims

1. Sequence, reconstruct and annotate the first transcriptomes of visceral and neurotropic avian schistosomes *T. szidati* and *T. regenti*.
2. Identify the key biological and metabolic pathways as well as the protein classes specific to cercarial and schistosomulum stages.
3. Identify the molecular mechanisms possibly linked to visceral and neurotropic schistosomiasis with special focus on nutrition preferences of *T. szidati* and *T. regenti* schistosomula.

Concluding remarks

Schistosomes have a complex life cycle requiring dramatic changes of particular developmental stages. One of the critical steps is the infection of the definitive host, where the cercariae have to swiftly react on transition between the aquatic environment and the environment inside the definitive host. It has to deal with the different osmotic pressure, different aerobic conditions, nutrition uptake, host immunity etc. Schistosomula migrate through the various tissues, actively feed, grow and develop and finally navigate through the tissues to reach the final localisation. During this process schistosomula are under constant pressure of the host immune system. The adaptations enabling successful infection of the definitive host can be observed on the molecular level using transcriptomic profiling of the different developmental stages and differential gene expression analysis. To date the large-scale transcriptomic studies were focus on few representatives of the genus *Schistosoma*. Presented thesis broadened the knowledge of the molecular biology of the schistosomatids with avian species. We explored the transcriptomic landscape of cercarial and schistosomulum stages of two schistosomes – visceral *T. szidati* and neurotropic *T. regenti* and compared the data with already published findings on human schistosomes. We found that the molecular mechanisms of cercarial stage possess common molecular mechanisms across different species such as carbohydrate and energy metabolism which is linked with the same aim of cercariae (regardless on species) – to cover the energy demands during the active seeking of the host and utilising same source of energy – glycogen stores. The differences between the cercariae of the human and avian schistosomes may be observed in

mechanisms linked to different definitive hosts they penetrate to. For example different proteolytic enzymes used for penetration into mammal (*S. mansoni*) or bird (*T. regenti*). Regarding to schistosomula we confirmed the different modes of nutrient acquisition using of light and electron microscopy – nervous tissue and blood for *T. regenti* and *T. szidati* respectively. We linked the microscopic observations with the transcriptional data and we observed different expression profiles of peptidases as well as the products of Micro Exon Genes (MEGs) and therefore we predicted the different role of these enzymes in the digestion of the blood and the nervous tissue.

We used transcriptomic-bioinformatics approach for our investigations, which is powerful tool for global overview, however subtle details may stay hidden. Also the predictions and hypothesis should be put into the context with parasite biology and already published wet-lab experiments. Nevertheless we strongly believe that our data provides new and significant insights into molecular biology of avian schistosomes, elucidate some aspects of the visceral and neurotropic schistosomiasis and can serve as a valuable resource for comparative studies of schistosomatids and other trematodes.

Publication No.1

Leontovyč R., Young N. D., Korhonen P. K., Hall R. S., Tan P., Mikeš L., Kašný M., Horák P., Gasser R. B. (2016): **Comparative transcriptomic exploration reveals unique molecular adaptations of neuropathogenic *Trichobilharzia* to invade and parasitize its avian definitive host.** PLoS Neglected Tropical Diseases 10: e0004406. DOI: 10.1371/journal.pntd.0004406 [IF2016=3.834]

Trichobilharzia regenti is unique among schistosomes due to its neurotropic migration in the definitive host. Infection may cause serious neuromotor disorders of the permissive avian and accidental mammalian hosts. This publication is the first large-scale analysis utilizing transcriptomic-bioinformatic approach to identify key biological pathways and protein classes specific to two consecutive stages - free-living cercariae and parasitic schistosomula of both species.

Based on gene expression analysis cercariae of the *T. regenti* didn't much differ from well-characterized human schistosomes. They use the aerobic metabolism where limited stores of glycogen are utilised by glycolysis where the Krebs cycle is the main terminal of the carbohydrate breakdown followed by oxidative phosphorylation to cover high-energy demands of cercariae. Screening of cercarial peptidases didn't confirm the transcription of cercarial elastase - the main penetration enzyme of *S. mansoni*. The transcriptome of schistosomula revealed the microaerobic metabolism and an overview of the schistosomula peptidases provided valuable information about enzymes involved in digestion and migration of the worm.

My contribution:

- Conceived and designed the experiments
- Performed the experiments
- Analyzed the data
- Wrote the manuscript

Publication No.2

Leontovyč R., Young N., Korhonen P., Hall R., Bulantová J., Jeřábková V., Kašný M., Gasser R., Horák P. (2019): **Molecular evidence for distinct modes of nutrient acquisition between visceral and neurotropic schistosomes of birds.** Scientific Reports 9: 1347. DOI: 10.1038/s41598-018-37669-2 [IF2018=4.011]

The results of the larval transcriptome of *T. regenti* indicated mechanisms/molecules possibly involved in neurotropic life strategy of the *T. regenti* schistosomula. We constructed the larval transcriptome of related visceral schistosome *T. szidati* to identify the biological processes linked to visceral and neurotropic schistosomiasis. In this study, we particularly focused on distinct modes of nutrient acquisition. We linked the light microscopy and transmission electron microscopy (TEM) with *in silico* analysis and predicted the molecular mechanisms of nutrients uptake by blood-feeding and neurotropic schistosomes.

Using comparative transcriptomics we obtained data of different expression of micro exon genes (MEGs) between the *T. regenti* and the *T. szidati* to date only known from human schistosomes. Our data support the hypothesis of the involvement of the MEGs in blood digestion of visceral schistosomes. Detailed analysis of expression of peptidases in both species revealed the expression profiles of peptidases linked to the different life strategies of *T. regenti* and *T. szidati*.

My contribution:

- Conceived and designed the experiments
- Performed the experiments
- Analyzed the data
- Wrote the manuscript

Publication No.3

Dvořáková H., Leontovyč, R. Macháček T., O'Donoghue A., Šedo O., Zdráhal Z., Craik Ch., Caffrey C., Horák P., Mikeš L. (2020) **Isoforms of Cathepsin B1 in Neurotropic Schistosomula of *Trichobilharzia regenti* Differ in Substrate Preferences and a Highly Expressed Catalytically Inactive Paralog Binds Cystatin.** *Frontiers in Cellular and Infection Microbiology* 10: 66. doi:10.3389/fcimb.2020.00066 [IF2020=3.518]

Long-term research of *T. regenti* pointed out the prominent role of the cathepsin B1 (TrCB1) by schistosomula. It is possibly involved in migration and the nutriment digestion by the parasitic developmental stages. Previously several isoforms of TrCB1 have been identified including two TrCB1.5, TrCB1.6 with amino acid substitution of the catalytic cysteine in the active site. Those two isoforms appeared to be inactive.

This part of our research is focused on the in-depth characterization of the active and inactive recombinant forms of isoforms of the TrCB1 including substrate specificity, macromolecular substrate digestion and inhibition tests by host inhibitors. Transcriptomic data obtained from previous part of the research were used for detailed analysis of gene expression of particular isoforms, which revealed the surprising result of high expression of the inactive isoform.

My contribution:

- Bioinformatic analysis – gene expression of cathepsin B isoforms