Czech Society for Parasitology

Charles University, Faculty of Pharmacy

26TH HELMINTHOLOGICAL DAYS 2021

Programme & Abstracts

Editors: Pavlína Kellerová & Ivan Vokřál

Hradec Králové 2021

Czech Society for Parasitology

Charles University, Faculty of Pharmacy

26TH HELMINTHOLOGICAL DAYS 2021

Programme & Abstracts

Editors: Pavlína Kellerová & Ivan Vokřál

Hradec Králové 2021

26th Helminthological Days

Organised by:	Czech Society for Parasitology, Helminthological Section
Venue & date:	Horská chata Radost, Plasnice 124, Czechia, 20 – 24 September 2021
Organising committee:	Petra Matoušková (coordinator), Pavlína Kellerová, Ivan Vokřál, Lucie Raisová Stuchlíková, Martina Navrátilová, Linh Thuy Nguyen, Diana Dimunová, Markéta Zajíčková, Karolína Štěrbová, Martin Ambrož (Faculty of Pharmacy, Charles University, Prague, Czechia)
Conference website:	https://portal.faf.cuni.cz/HD2021
Conference email:	hd2021@faf.cuni.cz

ISBN 978-80-906644-9-4

PROGRAMME OF 26TH HELMINTHOLOGICAL DAYS

Invited talk: **25 min** + 5 min for discussion
Regular talk: **10 min** + 5 min for discussion
Poster talk: **2 min** and individual discussion at the poster

BSc/MSc or **PhD**

Oral and Poster Presentations included in a student competition will be evaluated by a conference committee. The winners will be announced and awarded during the closing ceremony.

MONDAY, SEPTEMBER 20

15:00 – 18:00	**Arrival and registration of the participants**
18.00 – 18:45	**Dinner**
19:00 – 21:00	Š. Mašová: An overview of known Antarctic parasitofauna in Prince Gustav Channel (James Ross Island, Weddell Sea) **(Workshop/Seminar)**
21:00 – 00:00	**Get-together-evening**

TUESDAY, SEPTEMBER 21

08:00 – 08:45	**Breakfast**
09:00 – 09:15	**Opening ceremony (*P. Matoušková, I. Vokřál*)**
Session I	**"Omics" Approaches in Helminthology (*Chairman: P. Matoušková*)**
09:15 – 09:45	R. Laing, S. Doyle, J. McIntyre, K. Maitland, I. Flis, A. Morrison, D. Bartley, U. Chaudhry, N. Sargison, R. Kaplan, M. Berriman, A. Tait, J. Cotton, C. Britton, E. Devaney: Genomic and transcriptomic analysis of ivermectin resistance in *Haemonchus contortus* **(invited talk)**
09:45 – 10:00	V. Vacek, T. Peterková, L. Panská, Š. Nedvědová, J. Dvořák: Transcriptomic and metabolomic analysis of M28B peptidases knock-out mutants of *Caenorhabditis elegans*
10:00 – 10:15	O. Vondráček, L. Mikeš, P. Talacko, R. Leontovyč, J. Bulantová, P. Horák: Laser-assisted microdissection and shotgun proteomic analysis of schistosome penetration glands

10:15 – 10:30	J. Ilgová, L. Škorpíková, J. Vorel, P. Roudnický, M. Horn, M. Kašný: Changes of peptidase profile during *Fasciola hepatica* embryonation
10:30 – 11:00	**Coffee break**
Session II	**Biochemistry of Anthelmintics and Anthelmintic Resistance *(Chairman: I. Vokřál)***
11:00 – 11:15	M. Navrátilová, D. Dimunová, I. Vokřál, P. Matoušková, L. Skálová: Environmental circulation of albendazole and its *in vivo* effect in sub-lethal doses on *Haemonchus contortus*
11:15 – 11:30	M. Zajíčková, L. Prchal, I. Vokřál, N. Vodvárková, M. Navrátilová, L. T. Nguyen, L. Skálová: Toxicity, efficacy, and biotransformation of sertraline as a new potential anthelmintic against *Haemonchus contortus* **(PhD)**
11:30 – 11:45	D. Dimunová, M. Navrátilová, P. Matoušková: UDP-glycosyltransferases in the metabolism of anthelmintics in *Haemonchus contortus* **(PhD)**
11:45 – 12:00	K. Štěrbová, P. Matoušková, L. Skálová: Constitutive expression of SDR genes throughout the life stages of *Haemonchus contortus* **(PhD)**
12:00 – 12:15	P. Kellerová, I. Vokřál, P. Matoušková: P-glycoprotein transporters in helminths zooming in on *Haemonchus contortus* **(PhD)**
12:30 – 13:15	**Lunch**
Session III	**Novel Methodology Approaches in Helminthology Research *(Chairman: P. Horák)***
13:30 – 13:45	L. T. Nguyen, M. Zajíčková, P. Matoušková, L. Skálová: Optimization of ATP assay for viability testing in the parasitic nematode *Haemonchus contortus* **(PhD)**
13:45 – 14:00	M. Žofka, L. T. Nguyen, L. Skálová: Automated motility assay in *Haemonchus contortus* using image recognition based on deep learning **(PhD)**
14:00 – 14:15	M. Majer, Z. Burdíková, B. Šmídová, J. Pačes, J. Novák, R. Leontovyč, T. Macháček, P. Horák: Seeing the whole: light sheet fluorescence microscopy elucidates the biology of schistosomes within the spinal cord **(PhD)**
14:15 – 14:30	J. Bulantová: Searching for ideal tool for monitoring of avian schistosomes: Research vs. Practice
14:30 – 15:00	**Coffee break**
Poster session	**Poster talks *(Chairman: L. T. Nguyen)*** **Individual discussion at the posters**
15:00 – 17:45	**P1** L. Anettová, D. Bílek, B. Fecková, R. Moyse, D. Modrý: Extended *Angiostrongylus cantonensis* L3 larvae survival in soil **(PhD)** **P2** D. Barčák, A. Alexovič Matiašová, L. Ihnacik, D. Uhrovič, M. Oros: Immunofluorescent detection of PCBs in fish parasites from heavily polluted locality in eastern Slovakia **P3** M. Benovics, C. Pantoja, Z. Žákovicová, P. Papežík, P. Mikulíček: Hidden in the plain sight: The unexpected genetic diversity of amphibian's paramphistomous trematodes

P4 M. Benovics, N. Reslová, L. Škorpíková, L. Seidlová, O. Mikulka: Morphological and molecular diversity of helminths of Eurasian beaver in the Czech Republic
P5 N. Bernardová, M. Chanová: Long-term infection of murine paratenic host by *Toxocara canis* larvae **(PhD)**
P6 N. Bušková, R. Kuchta, H. Mazanec: Extracellular vesicles during the ontogeny of the tapeworm *Schistocephalus solidus* **(BSc/MSc)**
P7 V. Funioková, P. Kellerová, P. Matoušková: Characterisation and localisation of P-glycoprotein-9.2 in *Haemonchus contortus* **(BSc/MSc)**
P8 L. Jedličková, M. Horn, K. Peterková, J. Dvořák: Regulation of host homeostatic mechanisms by proteolytic system of *Schistosoma mansoni*
P9 O. Košulič, Š. Mašová: First record of mermithid parasitism (Enoplea: Mermithidae) in a Southeast Asian spider (Araneae: Araneidae)
P10 L. Škorpíková, N. Reslová, J. Vadlejch, M. Kašný: Detection and semi-quantitative evaluation of gastrointestinal nematodes in faecal matrices using multiplex real-time PCR assays **(PhD)**
P11 N. Rychlá, L. Raisová Stuchlíková, P. Matoušková: Heterologous expression of SDR enzymes from *Haemonchus contortus* **(PhD)**

18:00 – 18:45 **Dinner**

19:00 – 00:00 **Campfire evening**

WEDNESDAY, SEPTEMBER 22

08:00 – 08:45 **Breakfast**

Session IV **Host-Helminth Interactions *(Chairman: L. Mikeš)***

09:00 – 09:15 V. Vajs, M. Schreiber, P. Horák: Larval cestode infection and tumour suppression **(BSc/MSc)**

09:15 – 09:30 M. Schreiber, O. Tolde, J. Brábek, D. Rösel, P. Horák: Effect of *Mesocestoides corti* and *Taenia crassiceps* larvae on melanoma tumors in mice **(PhD)**

09:30 – 09:45 T. Macháček, C. Schulz, M. Sombetzki: *Schistosoma mansoni* single-sex infection differentially affects the development of *Trichobilharzia regenti* in mice

09:45 – 10:00 A. Revalová, M. Majer, T. Macháček, P. Horák: Penetration of the mammalian skin by avian schistosomes: A comparative study of *Trichobilharzia regenti* and *T. szidati* related pathology and host reactions **(BSc/MSc)**

10:00 – 10:15 B. Šmídová, R. Leontovyč, M. Majer, O. Vondráček, T. Macháček, P. Horák: Eosinophils and M2-polarized macrophages/microglia dominate the immune response to *Trichobilharzia regenti* neuroinfection in mice **(PhD)**

10:15 – 10:45 **Coffee break**

Session V	**Molecular Biology and Biochemistry of Helminths *(Chairman: J. Dvořák)***
10:45 – 11:00	L. Panská, Š. Nedvědová, D. Křivská, L. Ulrychová, J. Dvořák: Glutamate carboxypeptidase-2 in *Caenorhabditis elegans*
11:00 – 11:15	Š. Nedvědová, J. Dvořák, K. Peterková: Structural and interaction study of *Schistosoma mansoni* MEG family proteins **(PhD)**
11:15 – 11:30	K. Peterková, J. Vorel, J. Illgová, L. Škorpíková, P. Ostašov, L. Konečný, M. Kašný, M. Horn, J. Dvořák: Proteases of *Schistosoma mansoni* and *Fasciola hepatica* eggs **(PhD)**
11:30 – 11:45	E. M. Boateng, K. Peterková, L. Ulrychová, M. Chanová, Z. Kutil, J. Kim, L. Jedličková, C. Bařinka, J. Dvořák: Trematode orthologs of human glutamate carboxypeptidase II share similar expression patterns
12:00 – 12:45	**Lunch**
13:15 – 17:15	**Half-day trip to the Family Brewery Rampušák in Dobruška**
17:30 – 18:15	**Dinner**
18:30 – 21:30	H. Ferklová: How to do a conference presentation **(Workshop in Czech)**

THURSDAY, SEPTEMBER 23

08:00 – 08:45	**Breakfast**
Session VI	**Biology and Diversity of Helminths *(Chairman: T. Scholz)***
09:00 – 09:30	J. Hernández-Orts: Helminths of marine mammals: Review of their diversity, zoonotic potential and future perspectives **(invited talk)**
09:30 – 09:45	T. A. Kuzmina, O. O. Salganskij, I. V. Dykyj, O. I. Lisitsyna, E. M. Korol, Y.I. Kuzmin: Helminths of the teleost fishes from the area of the Ukrainian Antarctic Station "Akademik Vernadsky", Argentine Islands, West Antarctica: Species diversity and parasite community structure
09:45 – 10:00	C. Kibet, R. Kuchta: Diversity of avian schistosomes in Europe **(PhD)**
10:00 - 10:30	**Coffee break**
Session VII	**Ecology and Evolution *(Chairman: R. Kuchta)***
10:30 – 10:45	T. Brázová, D. Miklisová, D. Barčák, D. Uhrovič, M. Orosová, M. Oros: Polychlorinated biphenyls in bream (*Abramis brama*) and its specific parasite (*Caryophyllaeus laticeps*) in a heavily contaminated environment
10:45 – 11:00	D. Barčák, M. Madžunkov, D. Uhrovič, M. Miko, T. Brázová, M. Oros: Asian tapeworm *Khawia japonensis* spreads in Central Europe, including feral population of common carp in polluted Bodrog River basin
11:00 – 11:15	N. Dedić, A. Vetešníková Šimková: Hybrid susceptibility to *Dactylogyrus* (Monogenea) infection: A measure of hybrid vigour **(PhD)**
11:15 – 11:30	E. Nosková, B. Pafčo, V. Baláž, D. Modrý: *Strongyloides* infections in mountain gorillas **(PhD)**

11:45 – 12:30	**Lunch**
Session VIII	**Monogenea: Diversity and Phylogeny *(Chairman: M. Gelnar)***
12:45 – 13:00	M. Benovics, L. Gettová, A. Šimková: *De novo* developed microsatellite markers for monogeneans and their application to study population genetics of generalist *Dactylogyrus* species
13:00 – 13:15	L. Seidlová, A. Šimková: Diversity and phylogeny of monogeneans parasitizing neotropical cichlids **(BSc/MSc)**
13:15 – 13:30	C. Rahmouni, M. P. M. Vanhove, S. Koblmüller, A. Šimková: Molecular phylogeny and speciation patterns in host specific monogeneans (C*ichlidogyrus*, Dactylogyridae) parasitizing cichlid fishes (Cichliformes, Cichlidae) in Lake Tanganyika
13:30 – 13:45	M. Seifertová, K. Francová, A. Šimková: Diversity and phylogeny of *Dactylogyrus* species (Monogenea: Dactylogyridae) parasitizing North American cypriniform fishes
13:45 – 14:00	F. Nejat, M. Benovics, A. Abodli, A. S. Tarkan, E. Ercan, A. Šimková: Species diversity and phylogeny genus *Dactylogyrus* (Monogenea) in the Middle East **(PhD)**
14:00 – 14:30	**Coffee break**
14:30 – 17:45	**Presentation of conference partners**
18:00 – 00:00	**Closing ceremony and social event**

FRIDAY, SEPTEMBER 24

08:00 – 08:45	**Breakfast**
09:00 – 10:00	**Departure of participants**

ABSTRACTS

The abstracts, ordered according to the conference programme, are published as received from the authors who are fully responsible for the content. No editing or corrections were made except for minor changes in formatting to keep the layout unified.

MONDAY, SEPTEMBER 20

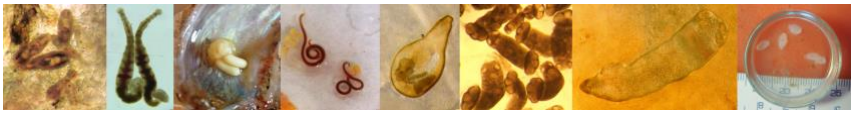
Invited Workshop/Seminar

AN OVERVIEW OF KNOWN ANTARCTIC PARASITOFAUNA IN PRINCE GUSTAV CHANNEL (JAMES ROSS ISLAND, WEDDELL SEA)

Š. Mašová

Department of Botany and Zoology, Faculty of Science, Masaryk University, Czech Republic

During the three Czech Antarctic expeditions in austral summer seasons between 2012 and 2014 in the coastal part of the James Ross Island and in the Prince Gustav Channel (Antarctica) were examined different host species for parasites by team from MUNI and Institute of Vertebrate Biology CAS. This contribution summarizes results and preliminary data of the research done by wide team of people who processed or participated in the processing of the imported parasitological material. The most of parasites were recorded from fishes of families Nototheniidae and Bathydraconidae (*Trematomus bernacchii*, *T. eulepidotus*, *T. hansoni*, *T. newnesi*, *T. pennellii*, *Gobionotothen gibberifrons*, *Parachaenichthys charcoti*, *Pagothenia borchgrevinki*, *Notothenia coriiceps*). Parasites from all groups (Nematoda, Trematoda, Cestoda, Acanthocephala, Monogenea, Hirudinea and Crustacea) were recovered from fins, gills, internal organs and tissues of Antarctic fishes. Fishes were collected up to 120m deep. A survey on anisakid nematode parasites collected from the skua's regurgitated pellet (South Polar Skua – *Catharacta maccormicki*, Stercorariidae) in February 2014 was conducted. One pellet was examined and among others parasites, 53 adults and larvae of genus *Contracaecum* were found. Also pinniped faeces collected in 2012 from wild living animals (Antarctic Fur Seal – *Arctocephalus gazella* and Weddell Seal – *Leptonychotes weddellii*) were examined. Samples were fixed immediately on the Station of Johann Gregor Mendel. Standard parasite concentration methods were used in Czechia. Sample of *L. weddellii* had diarrheal character. Infections with Nematoda, Trematoda, Acanthocephala, Amoebozoa and Ciliophora are reported in eggs/parasites/cysts per gram of faeces. Lot of Antarctic samples is not completely processed yet and still waiting for its parasitologist.



Acknowledgements to the Czech Antarctic Research Infrastructure and the crew of the Johann Gregor Mendel Station during the field expeditions for the help and companionship.

T

TUESDAY, SEPTEMBER 21

Session I

"Omics" Approaches in Helminthology

(*Chairman: P. Matoušková*)

GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF IVERMECTIN RESISTANCE IN *HAEMONCHUS CONTORTUS*

R. Laing[1], S. Doyle[2], J. McIntyre[1], K. Maitland[1], I. Flis[1], A. Morrison[3], D. Bartley[3], U. Chaudhry[4, 5], N. Sargison[4, 5], R. Kaplan[6], M. Berriman[2], A. Tait[1], J. Cotton[2], C. Britton[1], E. Devaney[1]

[1]University of Glasgow, Garscube Estate, Glasgow, UK
[2]Wellcome Sanger Institute, Hinxton, Cambridge, UK
[3]Moredun Research Institute, Penicuik, Edinburgh, UK
[4]University of Edinburgh, Penicuik, Edinburgh, UK
[5]Roslin Institute, Penicuik, Edinburgh, UK
[6]University of Georgia, Athens, Georgia, USA

Infections with parasitic nematodes are ubiquitous in grazing livestock throughout the world and are a major threat to global food security. Related nematodes cause debilitating diseases in billions of humans in some of the poorest countries in the world. Control of parasitic infections in animals and humans currently relies on mass drug administration of a limited number of anthelmintics. However, this is not sustainable due to the emergence and spread of anthelmintic resistance.

Haemonchus contortus is a highly pathogenic gastrointestinal nematode of small ruminants, which is becoming increasingly difficult to control due to multi-drug resistance. In the case of ivermectin, the mechanism(s) underlying resistance are poorly understood, with studies comparing resistant and sensitive parasites confounded by high levels of genetic diversity within and between and populations. To overcome this, we crossed a well-characterised multi-drug resistant isolate of *H. contortus* with a drug susceptible isolate to study ivermectin resistance while controlling for background variation. F2 adults were treated with ivermectin in vivo and pools of their L3 progeny pre and post treatment were sequenced. Bulk segregant analyses of these populations identified a major locus on chromosome V under ivermectin selection. This locus contains none of the previously studied 'candidate' resistance genes from the literature and no putative target genes, implicating a novel driver of resistance. Functional characterisation is ongoing in both *H. contortus* and transgenic *C. elegans* to identify which gene(s) in the locus confer ivermectin resistance. Transcriptomic analysis of the parental isolates and F2 adults with and without ivermectin treatment identified differential expression of a small number of genes associated with neuronal development or plasticity, including a single gene within the chromosome V locus that is highly upregulated in geographically separated resistant populations. This gene has been the focus of our investigations so far.

T

TRANSCRIPTOMIC AND METABOLOMIC ANALYSIS OF M28B PEPTIDASES KNOCK-OUT MUTANTS OF *CAENORHABDITIS ELEGANS*

V. Vacek[1], T. Peterková[1,2], L. Panská[1], Š. Nedvědová[1], J. Dvořák[1,3]

[1]Department of Zoology and Fisheries, Centre of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources Czech University of Life Sciences, Prague, Czech Republic
[2]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
[3]Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic

Glutamate carboxypeptidase 2 (GCP2) from the family M28B is long known as an important cancer marker and also as a therapeutical target for the treatment of several neurological disorders in humans. Despite these facts, the physiological roles of GCP2 in human cells are not fully understood. To better understand these roles we decided to study GCP2 orthologs in the less-complex model organism *Caenorhabditis elegans* as it offers a greater palette of available methods and possibilities for genetic manipulations. In the genome of *C. elegans* M28B peptidases, GCP2 orthologs, are encoded by three genes, here called *gcp-2.1* (R57.1), *gcp-2.2* (C35C5.2) and *gcp2.3* (C35C5.11). In *C. elegans*, each of the genes shows a distinctively different expression pattern. To elucidate the physiological roles of GCP2 orthologs in *C. elegans* we decided to sequence transcriptomes of three mutant strains – tm10889 (GCP-2.1), tm6541 (GCP-2.2) and tm13429 (GCP-2.3)- of *C. elegans* and compare them with the wildtype strain of *C. elegans.* For the transcriptomic analysis worms were synchronized and cultured to the L4 stage on NGM media plates. After reaching the L4 stage, worms were harvested and used for transcriptomic analysis. Transcriptomes for each mutant were sequenced by Illumina MiSeq. The resulting reads were aligned by STAR assembler to the genome of *C. elegans* and gene expression was analysed by featureCounts. Comparison of mutant strains to wildtype (WT) was done by DESeq2 package in R. Preliminary results show significant changes in gene expression between KO mutants and wild type. Additionally, metabolomic analyses of the L4 stages of *C. elegans* were also done. Preliminary results between tm10889 and WT show significant changes in at least 50 metabolites.

T

LASER-ASSISTED MICRODISSECTION AND SHOTGUN PROTEOMIC ANALYSIS OF SCHISTOSOME PENETRATION GLANDS

O. Vondráček[1], L. Mikeš[1], P. Talacko[2], R. Leontovyč[1], J. Bulantová[1], P. Horák[1].

[1] Department of Parasitology, Faculty of Science, Charles University, Czech Republic

[2] Proteomics Core Facility, BIOCEV, Faculty of Science, Charles University, Czech Republic

Schistosome invasive stages, cercariae, leave their intermediate snail hosts, penetrate the skin of vertebrate definitive hosts, and transform to schistosomula migrating to final localization. During the invasion process, cercariae employ histolytic and other bioactive products of specialized holocrine secretory cells – postacetabular (PAg) and circumacetabular (CAg) penetration glands. Although several studies attempted to characterize proteomes of the *in vitro* induced gland secretions in *Schistosoma mansoni* and *Schistosoma japonicum*, the results were partly inconsistent and largely dependent on the method of sample collection and processing. Products of both gland types that mixed during their secretion did not allow localization of the secreted proteins to a particular gland.

Here we compared proteomes of separately isolated cercarial gland cells of the bird schistosome *Trichobilharzia szidati* employing laser-assisted microdissection and shotgun LC-MS/MS, thus obtaining the largest dataset so far concerning the representation and localization of cercarial penetration gland proteins. We identified 3347 peptides assigned to 792 proteins, from which 461 occurred in at least 2 of 3 replicates in either gland type (PAg = 455, 40 exclusives; CAg = 421, 6 exclusives). Furthermore, 60 proteins differed significantly in their abundance between the glands. Other 355 proteins were detected in both types of the glands but the differences in their abundance between particular glands were below the level of significance. The highest numbers of proteins in both types of the glands were associated with the enzymes and exosome KEGG categories. Among 182 annotated enzymes, 29 % were exclusive or significantly more abundant in PAg while only 5 % were exclusive or more abundant in CAg. Peptidases of 5 catalytic types accounted for ca. 8 % and 6 % of reliably identified proteins in PAg and CAg, respectively. Invadolysin, nardilysin, cathepsins B2 and L3, and an elastase 2b ortholog were the major gland endopeptidases. Two cystatins and a serpin were the most abundant peptidase inhibitors in the glands. CAg were rich in cysteine-rich secretory proteins of the SCP/TAP family (venom allergen-like proteins).

The study was supported by the Czech Science Foundation (13-29577S and 18-11140S) and European Regional Development Fund and Ministry of Education Youth and Sports of the Czech Republic - "Centre for Research of Pathogenicity and Virulence of Parasites" (no. CZ.02.1.01/0.0/0.0/16_019/0000759).

CHANGES OF PEPTIDASE PROFILE DURING *FASCIOLA HEPATICA* EMBRYONATION

J. Ilgová[1], L. Škorpíková[1], J. Vorel[1], P. Roudnický[2], M. Horn[3], M. Kašný[1]

[1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2] Proteomics Core Facility – CEITEC, Masaryk University, Brno, Czech Republic
[3] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

Fasciola hepatica is a widespread foodborne trematode that parasitizes a broad range of mammals, including humans. Adults of this cosmopolitan species lay their propagation stages - unsegmented operculated eggs, into the bile duct of their mammalian host. Crucial aspects of *F. hepatica* life cycle, such as migration, reproduction and immune evasion, are directly dependent on the activity of proteolytic enzymes, also known as peptidases. Expression of these molecules have been intensely studied in metacercariae, newly excysted juveniles and adults, however embryonic stages of *F. hepatica*, have not yielded much scientific attention in this context.

We used combined transcriptomic/proteomic approach which led to the identification of expressed and translated peptidases in developing eggs of different age. Additionally, activity profiling with the substrate library and selective inhibitors identified presence of different peptidase classes in egg extracts.

Our results indicate that *F. hepatica* egg transcriptome remains mostly constant, while the proteome is being considerably adjusted throughout the embryonation. Threonine catalytic subunits of proteasome are constantly produced, while the cysteine peptidases prevailing in freshly laid eggs are supplemented by aspartic peptidases and metallopeptidases in the later stages of egg development.

The study was supported by the Czech Science Foundation GA19-17269S.

T

Session II

Biochemistry of Anthelmintics and Anthelmintic Resistance

(Chairman: I. Vokřál)

ENVIRONMENTAL CIRCULATION OF ALBENDAZOLE AND ITS *IN VIVO* EFFECT IN SUB-LETHAL DOSES ON *HAEMONCHUS CONTORTUS*

M. Navrátilová, D. Dimunová, I. Vokřál, P. Matoušková, L. Skálová

Department of biochemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

Anthelmintics administered to animals enter the environment as a parent compounds and their metabolites via excrements. In our previous project, we have traced the albendazole and its metabolites in the feces of sheep after the treatment in the surrounding soil and plants on the fertilized field by those excrements. We have fed another sheep with the plants and followed traces of the drug in the sheep plasma and feces again. The environmental circulation of albendazole was proven. Furthermore, the residuals of compounds may persist there and impact non-target organisms. Similarly, the exposure of lower development stages of parasitic helminths to non-lethal concentrations of anthelmintics in manure and soil may promote the development of drug-resistant strains. We suppose the continuous exposure of adult parasites living inside infected sheep to sub-lethal doses, even traces of the active compound, can slowly prepare them to survive the treatment. Our study was designed to test this hypothesis; we have fertilized the field with feces from ABZ treated sheep and fed new sheep infected with *H. contortus*. Eggs and adults were isolated for various experiments. No significant changes were observed in eggs sensitivity. However, significant differences were observed in the expression of several genes. Furthermore, the ability of isolated adults to metabolize albendazole was enhanced. Our results proved that not only improper and repeated treatment but also the environmental circulation of albendazole might lower the sensitivity of parasites and contribute to drug resistance development.

The study was supported by Charles University, project GAUK 1136120.

TOXICITY, EFFICACY, AND BIOTRANSFORMATION OF SERTRALINE AS A NEW POTENTIAL ANTHELMINTIC AGAINST *HAEMONCHUS CONTORTUS*

M. Zajíčková[1], L. Prchal[2], I. Vokřál[3], N. Vodvárková[1], M. Navrátilová[1], L. T. Nguyen[1], L. Skálová[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Biochemical Research Centre, University Hospital in Hradec Králové, Czech Republic
[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

The parasitic nematode of small ruminants *Haemonchus contortus* is well known around the world especially for its ability to develop resistance to all classes of available anthelmintics. For this reason, new effective drugs are highly needed. Based on previous studies, where the antipsychotic drug sertraline (SRT) had a negative impact on several nematodes such as *Caenorhabditis elegans*, *Trichuris muris* and *Ancylostoma caninum* we tested the efficacy of SRT against *H. contortus.* Moreover, we investigated the biotransformation of SRT in *H. contortus* and in ovine liver. Our findings showed that SRT decreased viability of *H. contortu*s adults, however no effect was observed on egg hatching. Identified metabolites of SRT in *H. contortus* were hydroxy- SRT, SRT-O-glucoside, dihydroxy-SRT, and SRT-ketone, but biotransformation was low and most of the parent drug stayed unmetabolized. Compared to *H. contortus*, ovine liver metabolized SRT more extensively via desmethylation and glucuronidation. Furthermore, the toxicity of SRT on ovine liver was also tested.

The study was supported by Charles University, project GAUK 1568519, UNCE/18/SCI/012 and SVV 260 550 as well as by the project EFSA-CDN [CZ.02.1.01/0.0/0.0/16_019/0000841], co-funded by ERDF.

UDP-GLYCOSYLTRANSFERASES IN THE METABOLISM OF ANTHELMINTICS IN *HAEMONCHUS CONTORTUS*

D. Dimunová, M. Navrátilová, P. Matoušková

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

UDP-glycosyltransferases (UGTs) are important enzymes in the metabolism of xenobiotics and eobiotics. Glycosylation is often the most important conjugation reaction catalyzed by these enzymes in drug metabolism. This reaction results in enhanced elimination of the drug from the organism and protection from the toxic action. Our model organism is *Haemonchus contortus*, a gastrointestinal parasite of small ruminants that have a remarkable ability to develop resistance to anthelmintic drugs. The involvement of UGTs in resistance caused by biotransformation of anthelmintics was confirmed by observed differences in glycosides quantities between resistant and sensitive strains.

The activity of UGT enzymes was measured in subcellular fractions of *H. contortus* by the LC-MS. Anthelmintics – albendazole, ricobendazole, flubendazole and reduced flubendazole were used as substrate for UGTs. We have detected several glycosides. Albendazole and reduced flubendazole were metabolized to higher amounts of glycosides in comparison with ricobendazole and flubendazole. Furthermore, we have confirmed the involvement of UGTs by using several known UGT inhibitors (sulfinpyrazone, 4,6-dihydroxy-5-nitropyrimidine, 5´nitrouracil). The inhibitors in different concentrations reduced the enzyme activity and formation of glycosides. We believe UGTs can be further exploited as molecular targets for combination therapy.

T

CONSTITUTIVE EXPRESSION OF SDR GENES THROUGHOUT THE LIFE STAGES OF *HAEMONCHUS CONTORTUS*

K. Štěrbová, P. Matoušková, L. Skálová

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

Anthelmintic therapy in the agricultural industry is complicated by the rapid global development of drug resistance. Therefore, knowledge of the mechanisms of drug resistance and new drug development is at the forefront of scientific interest.

Haemonchus contortus, a gastrointestinal nematode of small ruminants, has developed resistance to all used anthelmintics. In this parasite, the resistance mechanism based on the increased expression and activity of drug-metabolizing enzymes (DMEs), e.g. cytochromes P450 (CYPs), UDP-glycosyltransferases (UGTs) and P-glycoprotein transporters (P-gps) has been described. Previous studies have shown significantly different levels of DMEs expression between drug-susceptible and drug-resistant strain of *Haemonchus contortus* and between various developmental stages.

DMEs from the short-chain dehydrogenases/reductases family (SDRs) catalyze the main metabolic transformation of many carbonyl-containing anthelmintics (e.g. flubendazole). The genome of *H. contortus* contains a relatively high number of genes from SDR family, but the information about constitutive expression, activity and their role in anthelmintic resistance is missing.

The expression of twenty SDRs showed significant differences between various developmental stages. In general, the most expressed were SDR1, SDR3, SDR5, SDR6, SDR14 and SDR18 genes. Furthermore, the changes in expression of the selected SDRs between drug-susceptible (ISE) and drug-resistant (IRE) strain of *H. contortus* were compared. SDR12 and SDR16 have shown increased expression in all the life stages of the resistant strain. Those candidates warrant further investigation since they might be responsible for the higher reduction of flubendazole contributing to the resistance in *H. contortus*.

The study was supported by Charles University, project GA UK No. 194421.

P-GLYCOPROTEIN TRANSPORTERS IN HELMINTHS ZOOMING IN ON *HAEMONCHUS CONTORTUS*

P. Kellerová[1], I. Vokřál[2], P. Matoušková[1]

[1]Department of Biochemical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

Parasitic worms place a tremendous burden on health and economics all over the world. The only effective treatment are anthelmintic drugs. There is only limited range of anthelmintics, which are extremely overused. It is not surprising that in recent times, many of the existing anthelmintics have begun to face problems of emerging resistance. Seeking new drugs and understanding expanding resistance is now urgent need. Therefore, this project focuses on studying one of the resistance mechanisms which is believed to be via efflux transporters. Efflux transporters like P-glycoprotein transporters (P-gps) are involved in the transport of structurally unrelated xenotoxins, and have been recognized as major players in resistance to drugs in mammals, bacteria and parasites. Although efflux transporters are little known in helminths, P-gps were linked with anthelmintic resistance several times. By exposing a sheep nematode *Haemonchus contortus* to various anthelmintics drugs and their concentration, we investigate the role of P-gps in anthelmintic resistance. We described P-gp homologs in *Haemonchus contortus* with theirs changes in the gene expression. Looking into expression of P-gps genes in all Haemonchus life stages and drug resistant and sensitive strains, and localizing their expression in the worm's body, we puzzle comprehensive information. It seems there are the same P-pg homologs across the nematodes which are associated with drug resistance. Finding its uniform mark could serve as molecular targets for pharmaceutical purposes and combination therapy.

This work was supported by Charles University Grant Agency, project No. 1171620.

T

Session III

Novel Methodology Approaches in Helminthology Research

(Chairman: P. Horák)

OPTIMIZATION OF ATP ASSAY FOR VIABILITY TESTING IN THE PARASITIC NEMATODE *HAEMONCHUS CONTORTUS*

L. T. Nguyen, M. Zajíčková, P. Matoušková, L. Skálová

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

Serious economic losses in small ruminant livestock industry are caused by the gastrointestinal parasite *Haemonchus contortus*. The treatment of haemonchosis rely mainly on the use of chemical anthelmintics. The drug screening for novel anthelmintics is ongoing also due to spreading drug resistance. Nowadays methods are mostly based on microscopic observations of larval motility or development. Moreover, none of the existing biochemical methods are performed in adults, the target stage of the anthelmintic treatment. In view of the need to establish alternatives for the viability testing of *H. contortus* which does not require microscopical counting, we provide bioluminescence assay of adenosine triphosphate (ATP) that can assess not only infectious third-stage larvae (L3) but also the adult stage. All the procedure steps were optimized to achieve maximal sensitivity and robustness. The optimized protocol for exsheathed L3 was applied to test the levamisole effect on larvae from the ISE strain (drug-susceptible) and the WR strain (drug-resistant). The optimized protocol for adults was used to test the effect of levamisole and monepantel on females and males. Based on the results, the novel method can be used as a complementary assay for the phenotypic screening, and additionally, it might be used for the detection of drug-resistant isolates.

The study was supported by GAUK 1568519, UNCE/18/SCI/012 and SVV 260 550 as well as by the project EFSA-CDN (CZ.02.1.01/0.0/0.0/16_019/0000841), co-funded by ERDF.

AUTOMATED MOTILITY ASSAY IN *HAEMONCHUS CONTORTUS* USING IMAGE RECOGNITION BASED ON DEEP LEARNING

M. Žofka, L. T. Nguyen, L. Skálová

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

The parasitic nematode *Haemonchus contortus*, often known as barber's pole worm, is a useful model organism for drug screening of potential novel anthelmintics and it is also used in drug resistance studies. The current gold standard for measuring drug effectiveness is the worm motility assays for some larval stages. Nowadays, the evaluation of motility assays is mainly manual, therefore time-consuming and hard to scale. However, recent advancements in computer vision, specifically Convolutional Neural Networks (CNN), have reached a level where individual instances in an image can be identified with a very high degree of precision, including complex shapes. These algorithms allow us to potentially automate the process and create high-throughput systems. Therefore, we applied a state-of-the-art method Mask-RCNN to analyze the motility videos and compared the performance to other automated approaches and to manual processing in order to evaluate the precision and potential of new CNN algorithms.

SEEING THE WHOLE: LIGHT SHEET FLUORESCENCE MICROSCOPY ELUCIDATES THE BIOLOGY OF SCHISTOSOMES WITHIN THE SPINAL CORD

M. Majer, Z. Burdíková, B. Šmídová, J. Pačes, J. Novák, R. Leontovyč, T. Macháček, P. Horák

Faculty of Science, Charles University, Czechia

The large body size of helminths and limited widefield microscopes' field of view usually hampers the study of host-helminth interactions *in toto*. Therefore, we adopted light sheet fluorescence microscopy (LSFM), which is based on optical sectioning and enables visualization of whole organs. We implemented LSFM to explore the migration of a neurotropic schistosome, *Trichobilharzia regenti*, within the spinal cord of mice 0, 7, and 14 days post-infection. Moreover, we localized and quantified the activated MHC-II+ immunocompetent cells using MHC-II-EGFP knock-in mice. The LSFM analysis of the cell volume revealed that the spinal cord invasion augmented MHC-II expression during the infection, as was indicated by the previous transcriptomic and flow cytometry analyses. The MHC-II+ cells around the parasite were partly represented by Iba1+ cells (i.e., microglia/macrophages), as shown by immunohistochemistry labeling. Moreover, the LSFM imaging revealed that MHC-II+ cells were localized not only in the close vicinity of the migrating parasite but also throughout the entire spinal cord, including perivascular areas. It suggests that the infection influenced the whole organ and not only the area damaged by parasite migration. In conclusion, the LSFM allows displaying the parasite surroundings and distant regions simultaneously. It leads to a better understanding of host-parasite interactions in a proper context and might contribute to thorough research of tissue helminthoses.

The study was supported by Czech Science Foundation (18-11140S), European Regional Development Fund, Ministry of Education, Youth and Sports and the state budget of Czechia (CZ.02.1.01/0.0/0.0/16_019/0000759, CZ.1.05/4.1.00/16.0347, CZ.2.16/3.1.00/21515), Charles University Grant Agency (1374119), Charles University institutional funding (PROGRES Q43, UNCE/SCI/012-204072/2018, SVV 260432/2018), Czech-BioImaging large RI project (LM2018129) and the project "e-Infrastruktura CZ" (e-INFRA LM2018140).

T

SEARCHING FOR IDEAL TOOL FOR MONITORING OF AVIAN SCHISTOSOMES: RESEARCH VS. PRACTICE

J. Bulantová

Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

In connection with the increasing occurrence of local epidemics of cercarial dermatitis in the world, Europe and also in the Czechia, there exists an effort to monitor this pathogenic skin allergic disease caused by larvae of avian schistosomes. Till now, monitoring of avian schistosomes was performed only on the basis of the collection and examination of intermediate snail hosts. Nevertheless, more complex monitoring methods are being developed using not only intermediate hosts, but also definitive hosts, or wild stages released into the aquatic environment. These highly sophisticated approaches are usually based on the principles of molecular biology. Properly optimized, they are capable to capture even very small amounts of DNA in the host or in the external environment. The optimization of these procedures is still at the beginning and therefore not commonly available. This fact, together with overall costs per analysis including high demands on expensive instrumentation, necessary reagents, and the evaluator's experience, mentioned methods still remains in the hands of research institutes. However, the causative agents of cercarial dermatitis are in Czech Republic newly included among the parameters monitored for the assessment of bathing water quality in Decree No. 258/2000 Coll. on the protection of public health. This fact means that methods mentioned above will be probably further developed to be applicable in practice. Till that time, it is possible to use a standardized methodology based on examination of intermediate snail host, which is described in ČSN 75 7737.

The study was supported by Czech Science Foundation (Grant No. 18-11140S), European Regional Development Fund/Ministry of Education, Youth and Sports of the Czech Republic ("Centre for Research of Pathogenicity and Virulence of Parasites" - Grant No. CZ.02.1.01/0.0/0.0/16_019/0000759; and Grant No. 8J21AT011), and Charles University institutional grants (PROGRES Q43, UNCE/SCI/012 - 204072/2018, and SVV 260432/2018).

T

Poster session

(Chairman: L. T. Nguyen)

P1: EXTENDED *ANGIOSTRONGYLUS CANTONENSIS* L3 LARVAE SURVIVAL IN SOIL

L. Anettová[1], D. Bílek[1], B. Fecková[2], R. Moyse[2], D. Modrý[1,2,3,4]

[1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Czech Republic
[2] Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Czech Republic
[3] Institute of Parasitology, Czech Academy of Sciences, Czech Republic
[4] Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources/CINeZ, Czech University of Life Sciences Prague, Czech Republic

The rat lungworm *Angiostrongylus cantonensis* is a metastrongylid zoonotic nematode causing neurological disorders in its accidental hosts, among others also humans. It is an invasive pathogen native to Southeast Asia, nowadays colonizing other parts of the world and forming new foci close to continental Europe. The parasite has a complex life cycle with range of gastropods serving as intermediate hosts. Broad spectrum of poikilotherm vertebrates and invertebrates can serve as paratenic hosts. Since it has already been proven that *A. cantonensis* third stage free larvae can survive for four weeks in water, our experiment was performed to evaluate how long can these larvae survive in both sterilized and natural soil, in 5 replicates for each type of soil and for each of three weeks. Larvae emerged from its natural source – dead snails *Subulina octona* into the soil in containers. Additionally, water solution of known larvae amount was applied onto the soil in other containers. Modified Baerman larvoscopy was performed each 7 days and larvae were quntified. In autoclaved soil, 1.6% (16/1000) live L3 larvae were obtained after the first week, none of larvae survived the second week. From the soil with natural inhabitants (e.g., ciliates and rhabditid nematodes), only 0.8% (13/1550) live larvae were obtained after the first week and none after the second week. Even though the survival of larvae in soil was shorter than in the water, our experiments proved that small percentage of *A. cantonensis* L3 larvae can survive in both types of soil at least one week after their emergence from the dead gastropod. This phenomenon may have important implications for the parasite's ecoepidemiology.

The study was supported by SEA-Europe Joint Funding Scheme for Research and Inovation.

P2: IMMUNOFLUORESCENT DETECTION OF PCBs IN FISH PARASITES FROM HEAVILY POLLUTED LOCALITY IN EASTERN SLOVAKIA

D. Barčák[1], A. Alexovič Matiašová[2], L. Ihnacik[1,2], D. Uhrovič[1], M. Oros[1]

[1] Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
[2] Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University in Košice, Slovak Republic

Anthropogenic pollution of an environment by persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) represents a serious threat for both human population and ecosystem. One of the most heavily PCB-contaminated area worldwide is located in eastern Slovakia, where these pollutants were produced and released to a nearby river system for decades. A detection of PCBs in water ecosystems is often performed by their indirect determination in tissues of fishes however our recent data suggest that some intestinal parasites are able to bioaccumulate higher concentrations of PCBs in their bodies than their fish hosts. The present study proposes an alternative method of PCBs detection based on immunofluorescence technique in the histological sections of tapeworm *Atractolytocestus huronensis* Anthony, 1958. They were isolated from the intestine of common carp (*Cyprinus carpio* L.), collected from a heavily polluted pond near a former PCB-producing factory. A fluorescence signal was recorded in the vitelline follicles and inner surface of middle part of the uterus. On the other hand, further structures such as testes, ovary and parenchyma surrounding the internal organs remained unreactive. Specific signal also lacked on sections used as negative controls, which were either incubated with primary anti-Vimentin antibody without an affinity to any tapeworm´s structures or primary antibodies were omitted during their incubation. These findings may contribute to understanding of a mechanism of PCB bioaccumulation in fish parasites and the immunofluorescence-based methodology might be used for rapid detection of these POPs in the environment.

The study was supported by the Slovak Research and Development Agency (project No. APVV-18–0467) and Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences (project Nos. VEGA 2/0126/20 and VEGA 1/0760/20).

P3: HIDDEN IN THE PLAIN SIGHT: THE UNEXPECTED GENETIC DIVERSITY OF AMPHIBIAN'S PARAMPHISTOMOUS TREMATODES

M. Benovics[1,2], C. Pantoja[3,4], Z. Žákovicová[1], P. Papežík[2], P. Mikulíček[2]

[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2]Department of Zoology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia
[3]Institut of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
[4]Institute of Ecology, Nature Research Centre, Vilnius, Lithuania

One of the common inhabitants of amphibians' digestive and excretory system are paramphistomous trematodes of the family Diplodiscidae. These robust parasitic worms are widely spread through the Palearctic region and their typical life-cycle includes a planorbid snail as the intermediate host and an amphibian as the final host. According to literature, a host range of these parasites is rather wide, however, in the western Palearctic, only two species are reported - *Diplodiscus subclavatus* (Pallas, 1760) and *Opisthodiscus diplodiscoides* Cohn, 1904. Even though there are some taxonomical controversies regarding the latter species, the whole distribution range of the diplodiscids is hardly known, even in Europe. Even less is known about their genetic diversity.

In the present study, we investigated one of the blank spots – the Balkan Peninsula, and compared diplodiscid diversity and distribution in the various sites in the region with their diversity and distribution in central Europe. The parasites were collected from four species of water frogs (*Pelophylax ridibundus, P. esculentus, P. epeiroticus, P. kurtmuelleri*) from nine sites in Slovakia, three sites in Greece, and one site in Albania over the years 2019 to 2021. Adult diplodiscid specimens extracted from hosts were morphologically examined, identified, and subsequently sequenced (COX I, 28S rDNA). In contrast to water frogs from Slovakia, which harboured both diplodiscid species; i.e., *D. subclavatus* and *O. diplodiscoides*, the water frogs in the Balkans were parasitized only by the former species. Moreover, the genetic diversity observed in the mitochondrial markers revealed a strong geographic genetic structure among investigated diplodiscid individuals. Even though the host specificity was not observed among diplodiscids collected from water frogs with the sympatric occurrence of one or more *Pelophylax* species, our observation revealed some degree of competition between *Diplodiscus* and *Opisthodiscus* individuals; i.e., no mixed infection was recorded.

The study was supported by the Slovak Research and Development Agency (APVV-19-0076) and the Scientific Grant Agency of the Slovak Republic (VEGA 1/0286/19).

P4: MORPHOLOGICAL AND MOLECULAR DIVERSITY OF HELMINTHS OF EURASIAN BEAVER IN THE CZECH REPUBLIC

M. Benovics[1,2], N. Reslová[1], L. Škorpíková[1], L. Seidlová[1], O. Mikulka[3]

[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2]Department of Zoology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
[3]Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University, Brno, Czech Republic

Eurasian beaver (*Castor fiber*) is well established faunal element in the Czech Republic, even though its populations were historically almost eradicated in this region due to damage control or harvesting of castoreum, meat, and fur. Nowadays, its distribution and population density are well monitored; nonetheless, the beaver's parasites as the potential threat to the environment, and especially to the sympatric fauna, are often neglected in wildlife management.

In the present study we investigated helminths of 15 beaver individuals that were found dead or were legally hunted in the Morava, Dyje, and Berounka river basins during years 2018 to 2020. A total of four helminth species were collected, and the highest prevalence, and also intensity of the infection observed for digenean *Stichorchis subtriquetrus*. The other three species were nematodes *Travassosius rufus*, *Trichostrongylus capricola*, and *Capillaria* cf. *hepatica* which are reported for the first time from the beavers in the Czech Republic. The investigation of the genetic diversity in the *S. subtriquetrus*, using in this species unprecedented mitogenomic markers, revealed a total of 9 unique *COI* haplotypes among 14 investigated individuals. The minor geographical structure was recognized, as similar haplotypes were observed in the individuals collected from the Berounka. Moreover, the identical haplotypes were observed in the individuals from Morava and Dyje, suggesting gene flow between populations in these two basins.

Results of our study suggest, that even when the common population-genetic markers (i.e., microsatellites) might not reveal any structure in the populations of the Eurasian beavers, genetic diversity of their specific parasites may shed more light on their population partition and historical migration routes.

This study was financially supported by the Specific University Research Fund of the FFWT Mendel University in Brno (LDF_VT_2018007).

P5: LONG-TERM INFECTION OF MURINE PARATENIC HOST BY *TOXOCARA CANIS* LARVAE

N. Bernardová, M. Chanová

Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University, Czech Republic

Infection of a paratenic host (e.g. rodents, also human) by ova or larvae of *Toxocara canis* can cause mild to severe illness, known as toxocariasis. The most severe variant is cerebral toxocariasis associated with invasion of parasite to the central nervous system. The recorded number of diagnosed cases is however low in comparison to high prevalence of specific antibodies against *T. canis* in population. Therefore, the presence of larvae in humans is considered to be asymptomatic. Larvae are known for their ability of long-term persistence in tissues of their host and thus some authors relate the long-term presence of larvae in central nervous system to development of chronic neurodegenerative illnesses. In addition, the infected mice within the experiments on *Toxocara canis* performed severe motor and neurological impairments (e.g. stereotypical motion) in the chronic phase of infection.

We focused on the chronic phase of infection before the development of severe motor impairment. Three groups of mice (in total n = 55 infected mice + controls) were infected by low, moderate and high dose (10, 100 and 1000 larvae / mouse) of infectious *Toxocara canis* larvae from different sources. Mice were regularly tested by motor tests until the late chronic phase of infection to reveal any motor changes that could not be detected by direct observation. Successful infection was checked by serological testing. As a result, the development of inconspicuous motor changes was observed in all groups of mice. Time of occurrence of these impairments correlated with infection dose – the higher dose, the earlier occurrence. Furthermore, mice infected by larvae from different sources showed variability in the time of onset of motor impairment.

Our work proved that the presence and act of *Toxocara canis* larvae in CNS is only seemingly asymptomatic before escalation into severe impairments. Therefore, more medical attention should be paid to this infection.

The study was supported by Charles University (SVV 260 520, Progress Q25).

P6: EXTRACELLULAR VESICLES DURING THE ONTOGENY OF THE TAPEWORM *SCHISTOCEPHALUS SOLIDUS*

N. Bušková[1,2], R. Kuchta[2], H. Mazanec[1,2]

[1]Department of Parasitology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
[2]Laboratory of Helminthology, Institute of Parasitology, Biology Center CAS, České Budějovice, Czech Republic

Extracellular vesicles (EVs) are small transport units derived from membranes and released by a variety of cells. Based on their biogenesis, two main classes were observed. First class, referred to as exosomes, are vesicles formed during an endosomal pathway and released by multivesicular bodies. The second class, microvesicles, are being formed by a direct budding of the plasma membrane. The main functions of extracellular vesicles are cell-cell communication and waste disposal. We focused on EVs of different life stages of the tapeworm *Schistocephalus solidus* (Cestoda: Diphyllobotridae), a parasite of fish-eating birds with a three-host life cycle. It is an important model organism in the studies of aquatic-life cycles of tapeworms and also for its manipulation with a fish-host organism.

For our experiments, a full life cycle of *S. solidus* was established under laboratory conditions. Coracidia from hatched eggs were used to infect copepods (*Macrocyclops albidus*). After 3 weeks, procercoids were fully developed in body cavity of copepods and subsequently used for infection of sticklebacks (*Gasterosteus aculeatus*). Three months post-infection, fish was dissected and recovered plerocercoids were kept in a cultivation medium in conditions simulating bird guts. Medium obtained this way was filtered for eggs and subsequent isolation of EVs.

Based on obtained material and data we have observed biogenesis of EVs in plerocercoids of *S. solidus* using transmission electron microscopy and successfully purified them from cultivation medium. Furthermore, we observe differences in secretory activity during the maturation of plerocercoids. Lastly, so far our results indicate no secretion of EVs by procercoids.

This study was supported by the Czech Grant Agency (GACR, Project No. 19-28399X).

P7: CHARACTERISATION AND LOCALISATION OF P-GLYCOPROTEIN-9.2 IN *HAEMONCHUS CONTORTUS*

V. Funioková, P. Kellerová, P. Matoušková

Department of biochemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

Resistance to anthelmintic drugs has become an alarming issue of the present. Important players of the anthelmintics resistance are ATP-binding-cassette transporters, most notably P-glycoproteins (P-gps). *Haemonchus contortus*, a pathogenic gastrointestinal nematode of small ruminants, has become resistant to all types of anthelmintic drugs. In this nematode, 10 homologs of P-gps have been identified and three of them localized (Hco-pgp-2, Hco-pgp-9.1, Hco-pgp-13). In our study, we focus on Hco-pgp-9.2, whose constitutive expression is significantly increased in resistant strain of *H. contortus*. The objective is to characterize and localize Hco-pgp-9.2. To define the transport activity of Hco-pgp-9.2 and its interaction with substrates and inhibitors, Rhodamine efflux assay and ATPase activity measurement will be applied.

Initially, RNA *in situ* hybridization will be carried out to localize the RNA expression of Hco-pgp-9.2. The place of target gene expression will be compared with the location of paralog Cel-pgp-9 in *C. elegans*, a model organism for studying drug resistance in parasitic nematodes. The findings could bring more clarity to the mechanisms of resistance in *H. contortus* and be useful in development of more efficient treatment in the future.

This work was supported by Charles University Grant Agency, project No. 1171620.

P8: REGULATION OF HOST HOMEOSTATIC MECHANISMS BY PROTEOLYTIC SYSTEM OF *SCHISTOSOMA MANSONI*

L. Jedličková[1], M. Horn[2], K. Peterková[1,3], J. Dvořák[1,2]

[1]Department of Zoology and Fisheries, Centre of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
[3]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

Proteolysis is an essential biochemical mechanism for numerous physiological processes such as homeostatic systems in vertebrates. Proteolytic enzymes (peptidases) involved in the hemostatic cascades belong to the group of serine peptidases and their activities must be strictly controlled by peptidase inhibitors. The blood-feeding parasites have to overcome the hemostasis of host blood. Therefore, they have molecular equipment containing an array of anti-hemostatic, anti-inflammatory, and immunomodulatory molecules that contribute to the suppression of the host protective mechanisms and enable parasite survival.

In this project, *Schistosoma mansoni* is as an experimental model organism. Adults *S. mansoni* are a highly adapted hematophagous parasite that reside in mesenteric veins for even decades and interfere with the host defense mechanisms. Therefore, our focus on proteolytic systems (peptidases and peptidase inhibitors) of this parasite is due to its involvement in the modulation of the host physiological mechanisms in the vessel. Based on the transcriptomic and proteomic analysis of *S. mansoni* adults and their eggs, we obtained several "hot" candidates belonging to the groups of cysteine peptidases, serine peptidases, and Kunitz-type inhibitors. Selected protein candidates are now produced in their recombinant forms, and will be biochemically, structurally, and functionally characterized and their interaction with the mammalian homeostatic system will be later analyzed.

The main aim of this project is to reveal the functions of these molecules and identify their role within the complex interaction mechanisms.

The study was supported by Czech Science Foundation grant no. 19-17269S and by the Charles University, project GA UK No. 6120.

P9: FIRST RECORD OF MERMITHID PARASITISM (ENOPLEA: MERMITHIDAE) IN A SOUTHEAST ASIAN SPIDER (ARANEAE: ARANEIDAE)

O. Košulič[1], Š. Mašová[2]

[1]Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic
[2]Faculty of Science, Department of Botany and Zoology, Masaryk University, Brno, Czech Republic

A new record of a parasitic nematode from the family Mermithidae (Nematoda) parasitising a female Bark Spider, *Caerostris sumatrana* Strand, 1915 (Araneae: Araneidae) from the Phethaburi province (Ban Lat district) in the south part of Central Thailand is described. The host spider belongs to the orb-web guild, occurring in shrubs and trees of lowland dipterocarp forests. The parasite was extremely long in respect to its spider host, length was about 28 cm, brownish in colour, slightly transparent at tapered rounded ends. Morphological features suggest this juvenile nematode belongs to the genus cf. *Aranimermis*. Due to the subadult stage of parasite, identification to species level was not possible. This finding is among the first record of a mermithid parasite from an orb web spider species living in higher vegetation. Moreover, our finding is among the first record of this host-parasite interaction from Southeast Asia.

This study was financially supported by the Specific University Research Fund of the FFWT Mendel University in Brno (Reg. Number: LDF_TP_2020006).

P10: DETECTION AND SEMI-QUANTITATIVE EVALUATION OF GASTROINTESTINAL NEMATODES IN FAECAL MATRICES USING MULTIPLEX REAL-TIME PCR ASSAYS

L. Škorpíková[1], N. Reslová[1], J. Vadlejch[2], M. Kašný[1]

[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2]Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

Diagnosis of strongyle nematodes is routinely based on traditional coprological methods involving morphological/morphometric analysis. However, such an approach is laborious, time-consuming, inaccurate, and requires a qualified specialist. Nowadays, molecular methods have become the basis for reliable diagnostics, allowing accurate and efficient identification of nematodes, which is necessary for the implementation of sustainable parasite control strategies.

Two multiplex real-time PCR assays for specific detection of six strongyle nematode species, including an internal amplification control to avoid false negative results, were designed. The assays were optimized and verified to detect *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Nematodirus battus*, *Chabertia ovina* and *Ashworthius sidemi* directly from sheep faeces. Semi-quantitative assessment of the infection intensity was enabled using a plasmid construct and a dilutions series of sheep faeces with known numbers of nematode eggs. The assays were performed on 44 individually collected faecal samples from three farms, and the results were compared with those from faecal egg counts using the Concentration McMaster technique and standard larval cultures.

Our assays showed great specificity to target species and proved higher sensitivity in strongylid-type egg detection over faecal egg counts techniques, while showing moderate agreement in evaluation of infection intensity. Further, the assays clarified species identification compared to larval cultures. We have proven that our assays are able to analyze faecal samples rapidly and accurately, allowing simultaneous and reliable identification and semi-quantitative estimation of egg numbers.

The study was supported by Ministry of Education, Youth and Sport of the Czech Republic (LTC19018) and the COST Action (CA16230).

P11: HETEROLOGOUS EXPRESSION OF SDR ENZYMES FROM *HAEMONCHUS CONTORTUS*

N. Rychlá, L. Raisová Stuchlíková, P. Matoušková

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

This project is based on heterologous expression of carbonyl-reducing enzymes, such as short-chain dehydrogenases/reductases (SDR) and aldo-keto reductases (AKR), from Barber´s pole worm, *Haemonchus contortus*, a gastrointestinal nematode of small ruminants. Carbonyl-reducing enzymes catalyze the first phase of xenobiotics biotransformation and thus participate in drug metabolism. Increased elimination leads to decreased toxicity and reduced efficacy of drugs in Barber´s pole worm. One of the proven mechanisms of deactivation of anthelmintics (e.g., flubendazole) is the reduction of the carbonyl group by these enzymes. Furthermore, the previous metabolism analysis demonstrated a higher ability of resistant strain of *H. contortus* to reduce flubendazole more effectively than sensitive strain. The genome of *H. contortus* contains approximately 70 SDR genes and 24 AKR genes; however, information about expression and function is still unknown. Selected enzymes chosen on previous quantitative analysis of gene expression in *H. contortus* will be cloned and expressed in two systems; in *E. coli* typical expression system for soluble enzymes and in eucaryotic cell lines an expression system enabling a higher level of posttranslational modifications. Expression of selected SDR or AKR, in a suitable system will allow us to characterize them, determine their enzyme activity, even test new potential inhibitors in the future.

This project was supported by Czech Science Foundation (Grant No. 20-14581Y).

WEDNESDAY, SEPTEMBER 22

Session IV

Host-Helminth Interactions

(Chairman: L. Mikeš)

LARVAL CESTODE INFECTION AND TUMOUR SUPPRESSION

V. Vajs, M. Schreiber, P. Horák

Department of Parasitology, Faculty of Science, Charles University, Prague, Czechia

In previous experiments, the *Taenia crassiceps* and *Mesocestoides corti* tapeworms have shown a potential to suppress the growth and metastasis of melanoma cells, reducing visibly the size and amount of tumours within the peritoneum of mice. It is yet to be determined how this ability comes into effect. Both of these tapeworm species possess the capacity to reproduce asexually within the host peritoneum, and can be cultivated in vitro, making for excellent laboratory subjects. This study aims to explore certain facets of this phenomenon. Previous studies have revealed structures on the *Mesocestoides* tapeworm, which are similar to abnormal glycosylations in certain cancer cells. Therefore, immunohistochemistry, ELISA and Western blot will be used to explore cross-reactivity and the immune response through the course of the infection of tumour-positive murine hosts. The developmental characteristics of the parasite and melanoma will also be described.

The study was supported by: European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/ 0000759), Charles University institutional funding (PROGRES Q43, UNCE/SCI/012-204072/2018, SVV 260432/2018.

EFFECT OF *MESOCESTOIDES CORTI* AND *TAENIA CRASSICEPS* LARVAE ON MELANOMA TUMORS IN MICE

M. Schreiber[1], O. Tolde[2], J. Brábek[2], D. Rösel[2], P. Horák[1]

[1]Department of Parasitology, Faculty of Science, Charles University, Prague, Czechia
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czechia

Several studies have shown that infection with helminths may affect the development of cancer. Some species like *Opisthorchis viverrini* or *Schistosoma haematobium* can promote the development or even be the causative agent of cancer. On the other hand, infections with other species, such as *Trichinella spiralis*, can reduce tumors and potentially have a protecting effect. Our work investigates the impact of infections by *Mesocestoides corti* and *Taenia crassiceps* in different strains of mice on the growth and metastasis of B16F10 melanoma tumors. *M. corti* and *T. crassiceps* larvae were used to infect BALB/c, C57BL/6J, or ICR mice which were then challenged with B16F10 melanoma cells administered intravenously, intraperitoneally, or subcutaneously. Although an increase in metastatic activities was observed after intravenous administration of melanoma cells to *M. corti*-infected mice, and no effect on subcutaneously localized tumors was noted, both tapeworms showed a strong suppressive effect on the size and number of tumors and metastases formed when the cells were administered intraperitoneally. In some cases, it led to the complete elimination of tumor cells. Although larval excretory-secretory products decreased the viability of melanoma cells *in vitro*, the observed effect in the murine peritoneum is most likely mediated by the immune response modulated via tapeworm larvae as indicated by an increase in peritoneal macrophages and granulocytes of *M. corti*-infected mice.

The study was supported by: European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/ 0000759), Charles University institutional funding (PROGRES Q43, UNCE/SCI/012-204072/2018, SVV 260432/2018).

SCHISTOSOMA MANSONI SINGLE-SEX INFECTION DIFFERENTIALLY AFFECTS THE DEVELOPMENT OF *TRICHOBILHARZIA REGENTI* IN MICE

T. Macháček[1,2], C. Schulz[1], M. Sombetzki[1]

[1]Department of Tropical Medicine and Infectious Diseases, Rostock University Medical Center, Germany
[2]Department of Parasitology, Faculty of Science,Charles University, Prague, Czech Republic

Immunomodulation by human schistosomes is a well-recognized phenomenon, which ensures their survival in mammals. Indeed, several molecules promoting Th2 or Treg immune responses, beneficial to the parasites, were identified in eggs or coupled adults. However, the sex-specific immunomodulatory effects have only recently been reported from mice infected with single-sex *Schistosoma mansoni* cercariae. Specifically, female schistosomes (contrary to males) suppressed the early innate immunity in the skin and mitigated hepatic fibrosis after secondary infection. Hence, we decided to test how these opposing effects of *S. mansoni* "pro-inflammatory" males and "anti-inflammatory/regulatory" females affect the course of other infections. Therefore, we co-infected mice with *S. mansoni* (males – Sm^{m}, females – Sm^{f}, or both – Sm^{mf}) and the neuropathogenic avian schistosome *Trichobilharzia regenti*, which is normally eliminated by mice. The anti-inflammatory/regulatory milieu, presumably induced by Sm^{f}, was expected to pave the way for *T. regenti* and enable its survival. Our preliminary data indicated that both Sm^{m} and Sm^{f} reduced *T. regenti* burden in the central nervous system, but Sm^{f} promoted the growth of surviving *T. regenti* schistosomula. Additionally, Sm^{m} inhibited the *T. regenti*-specific Th1 immune response, while Sm^{f} boosted mixed Th1/Th2 immune reactions. Collectively, these observations demonstrate the differential effects of male and female *S. mansoni* on *T. regenti* infection in mice. The hypothesis of "anti-inflammatory/regulatory females" was not confirmed in this setting. Specific immunological mechanisms leading to differential *T. regenti* outcomes should be further investigated.

The postdoctoral research fellowship of TM at Rostock University Medical Center, Germany was supported by the OP RDE project "International Mobility of Researchers at Charles University" (reg. n.: CZ.02.2.69/0.0/0.0/16_027/0008495).

PENETRATION OF THE MAMMALIAN SKIN BY AVIAN SCHISTOSOMES: A COMPARATIVE STUDY OF *TRICHOBILHARZIA REGENTI* AND *T. SZIDATI* RELATED PATHOLOGY AND HOST REACTIONS

A. Revalová, M. Majer, T. Macháček, P. Horák

Department of Parasitology, Faculty of Science in Prague, Charles University, Czechia

Penetration of the mammalian skin by avian schistosomes triggers cercarial dermatitis (CD), especially in the case of repeated exposures to the parasites. CD is regarded as a re-emerging allergic disease manifesting with maculo-papular eruptions, itching and edema. Avian schistosomes of the genus *Trichobilharzia* are the major causative agents of CD in Europe. While *T. regenti* is the most prevalent laboratory model, *T. szidati* seems to be a more frequent trigger of human CD as indicated by field observation. As the initial immune response largely orchestrates the skin pathology, we focused on detailed characterization and comparison of the host immune reaction against *T. regenti* and *T. szidati* in a mouse model. First, we estimated a cercarial penetration rate, which defines the amount of parasite antigens boosting immune reactions in the skin. Our experiment, using the infective dose of 100 cercariae, revealed that 45±14% (n = 8) and 38±6% (n = 8) cercariae of *T. regenti* and *T. szidati*, respectively, successfully penetrated the skin of mouse pinnae. Next, we examined the migration of schistosomula and histopathological changes in the infected pinnae at 5, 24, 48 hours post infection (pi) and 7 days pi. At 5 hours pi, schistosomula of both species reached beneath the *stratum corneum* but the onset of the inflammatory response, manifesting by thickening of skin and hyperkeratosis, was noticeable at later time points. Surprisingly, none of the schistosomula localized in the dermis were surrounded by granulomatous foci in the primo-infected pinnae. Our preliminary data hence suggest that *T. regenti* and *T. szidati* are comparable regarding the penetration rate and skin pathology in naïve mice. However, further comparative experiments are required to identify particular mechanisms responsible for trapping the schistosomula and to understand the immunopathological background of CD triggered by different *Trichobilharzia* species. The study will reveal variability inside the genus *Trichobilharzia* and test the eligibility of *T. regenti* as the model organism for CD.

The study was supported by European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/0000759) and institutional grants (Charles University PROGRES Q43, UNCE/SCI/012 - 204072/2018, SVV 260432/2018).

EOSINOPHILS AND M2-POLARIZED MACROPHAGES/MICROGLIA DOMINATE THE IMMUNE RESPONSE TO *TRICHOBILHARZIA REGENTI* NEUROINFECTION IN MICE

B. Šmídová, R. Leontovyč, M. Majer, O. Vondráček, T. Macháček, P. Horák

Faculty of Science, Charles University, Prague, Czechia

The avian neuropathogenic schistosome *Trichobilharzia regenti* is efficiently killed in the tissues of infected mice. After reaching the central nervous system (CNS), the migrating schistosomula are surrounded by the infiltrate of immune cells. The composition of the CNS infiltrating cells, presumably responsible for the parasite clearance, has only been characterized in the tissue surrounding the parasite. Hence, we focused on the dynamics of the myeloid cell populations infiltrating the whole CNS using flow cytometry to describe the cells responsible for the parasite clearance. The cellular immune response peaked at 14 days post-infection (dpi), with eosinophils predominating in all examined CNS segments (spinal cord, brain stem, cerebellum, and hemisphere). The rest of the infiltrating leukocytes consisted mostly of monocytes/macrophages and lymphoid cells. Transcriptomic analysis of the infected spinal cord revealed upregulation of genes associated with M2 polarization of macrophages/microglia (*Arg1*, *Chil3*, *Il10*, *Il13*, *Il4*, *Tgfb*) at 7 and/or 14 dpi. Immunohistochemical staining of selected M2 markers was then used to confirm the M2 polarization of macrophages/microglia and describe their distribution in the infected spinal cord. Indeed, we observed Arg-1, IL-4, and Iba-1 (marker of microglia/macrophages) signals around the schistosomula. Moreover, Arg-1 was the dominant protein in the microdissected tissue surrounding the parasite. These results showed that M2-polarized macrophages/microglia envelop the parasite in the spinal cord. Considering that most schistosomula are damaged at 14 dpi, which is also the peak of eosinophil infiltration, and M2-polarized cells rather participate in repairing the damaged tissue, it seems that not macrophages/microglia but eosinophils kill the parasite.

The study was supported by the Czech Science Foundation (18-11140S), European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/0000759), Charles University Grant Agency (1374119), and Charles University institutional funding (PROGRES Q43, UNCE/SCI/012-24072/2018, SVV 260432/2018).

Session V

Molecular Biology and Biochemistry of Helminths

(Chairman: J. Dvořák)

GLUTAMATE CARBOXYPEPTIDASE-2 IN *CAENORHABDITIS ELEGANS*

L. Panská[1], Š. Nedvědová[1], D. Křivská[2], L. Ulrychová[3], J. Dvořák[1]

[1]Department of Zoology and Fisheries, Centre of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic
[2]Department of Tropical Medicine and Infectious Diseases, Rostock University Medical Center, Rostock, Germany
[3]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

Glutamate carboxypeptidase 2 (GCP-2) is a membrane-bound zink-dependent metallopeptidase with high pharmacological potential. The pharmaceutical interventions in humans correspond to areas of the highest expression levels which are found in the nervous and prostatic tissue. GCP-2 serves as a therapeutic target associated with various neurological disorders or is used as a marker for imaging/therapy of prostate cancer. Despite its therapeutical use, its physiological role in humans has not been completely discovered. GCP-2 is expressed across all phyla, in plants, invertebrates, vertebrates. *Caenorhabditis* elegans, as a less complex and well-described organism, represent a suitable model to study GCP-2 orthologs in nematodes. GCP-2 is coded by three genes, *gcp-2.1*, *gcp-2.2*, *gcp-2.3*, in *C. elegans*. Knock-out (KO) of these genes led to changes in fertility, pharyngeal pumping and *gcp-2.2* KO worms possessed a fragile phenotype. In this case, transmission electron microscopy revealed structural changes in the cuticle of these worms. Also, the RNAi in wild-type, as well as mutant strains, gave several phenotypes in the range from mild to strong manifestation. Localization of GCP-2.1, GCP-2.2, and GCP-2.3 differs in various tissues of *C. elegans*. Examination of wild-type strain worms carrying GFP reporter constructs showed GCP-2.1 expression in the somatic muscles and some neurons and their processes. GFP signal corresponding to GCP-2.2 was exclusively observed in phasmids neurons and their processes in the tail and GCP-2.3 was localized in the large H-shape excretory cell and its bilateral canals. This is the first study describing so clear phenotypes of GCP-2 in the animals. Nevertheless, the exact role of GCP-2 in the physiology of Nematodes is waiting to be fully discovered.

The study was supported by The Czech Science Foundation (18-14167S) and The Ministry of Education, Youth and Sports (LTAUSA19023).

STRUCTURAL AND INTERACTION STUDY OF *SCHISTOSOMA MANSONI* MEG FAMILY PROTEINS

Š. Nedvědová[1, 2, 3], J. Dvořák[1, 4], K. Peterková[1, 5]

[1]Department of Zoology and Fisheries, Centre of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic
[2]Department of Chemistry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic
[3]BIOSYS, Institut des Sciences Analytiques, UMR CNRS 5280 - Université Lyon1, France
[4]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
[5]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

There are about 188 proteins that have been identified in the *Schistosoma mansoni* egg secretome – some of them are already well-characterized (IPSE/alpha-1, Omega-1, Sm-p40, however, the functions of many other highly expressed molecules are yet not known. One category of these is represented by an enigmatic group of genes with around 80 % of the protein-coding region comprising short symmetric exons from 6 - 36 bp termed MEGs (Micro-Exon Genes) that are indeed coded by several genes with micro-exon rich sequence structure. They represent a novel system for generating protein variation by alternative splicing; this phenomenon is accompanied by the formation of a variety of isoforms at the transcription level. So far, more than 30 MEG families have been described; MEGs in schistosomes are generally divided into 18 families (by protein sequence homology), but only three of MEG families proteins were identified in egg transcriptome and they had highly upregulated expression in mature eggs. For the schistosome egg, secretion of most abundant MEG proteins may be associated with escaping the portal vein into the tissues. Based on transcriptomic analysis of the egg transcriptome, candidate molecules were selected to be subjected to biochemical, biophysical and structural analyzes. The aim is to perform a comprehensive range of interaction studies to determine the host-parasite relationship.

The study was supported by: Barrande Fellowship Program (Structural and interaction study of MEG family proteins and their role in liver fibrosis onset.) and University grant competition of CZU n. 35/2021.

PROTEASES OF *SCHISTOSOMA MANSONI* AND *FASCIOLA HEPATICA* EGGS

K. Peterková [1,2], J. Vorel [3], J. Illgová [3], L. Škorpíková [3], P. Ostašov [4], L. Konečný [1,2], M. Kašný [3], M. Horn [5], J. Dvořák [2,5]

[1] Department of Parasitology, Faculty of Science, Charles University, Prague, Czechia
[2] Department of Zoology and Fisheries, Centre of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czechia
[3] Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czechia
[4] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czechia
[5] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czechia

Eggs of blood fluke *Schistosoma mansoni* are the main causative agent of clinical manifestations of a disease called schistosomiasis. In the definitive host, eggs are laid in the mesenteric veins and then migrate through the intestinal wall to the gut and outer environment. A large proportion of eggs, however, become entrapped in host tissues, typically the liver, causing inflammation and tissue damage. During this process, eggs secrete molecules to modulate host immune response. We hypothesize that proteases and protease inhibitors play an important role during the infection. Our focus in this project is to identify them and measure their transcriptional levels in undeveloped and developed eggs. Additionally, we included a comparison with eggs of another trematode *Fasciola hepatica*. These eggs pass from host bile ducts to the gut lumen and outer environment without any known pathological consequences for the host and without penetrating the tissues. Therefore, we employ this organism as a useful comparative subject to decipher which proteases and inhibitors are common and which are unique for each of them. We isolated RNA from several developmental stages of eggs of both organisms, prepared cDNA libraries, performed Illumina sequencing and analyzed transcripts using a pipeline of bioinformatics tools. By synthesis of database identifications and manual curation, we obtained a dataset of expressed proteases and protease inhibitors. Next, we identified orthologous proteins for both species and compared expression levels between them.

The study was supported by Charles University, project GA UK No. 6120 and Czech Science Foundation grant No. 19-17269S.

TREMATODE ORTHOLOGS OF HUMAN GLUTAMATE CARBOXYPEPTIDASE II SHARE SIMILAR EXPRESSION PATTERNS

E. M. Boateng[1#], K. Peterková[1,2#], L. Ulrychová[2,3], M. Chanová[4], Z. Kutil[5], J. Kim[1], L. Jedličková[1], C. Bařinka[5], J. Dvořák[1]

[1] Department of Zoology and Fisheries, Center of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Czech Republic.
[2] Department of Parasitology, Faculty of Science, Charles University, Czech Republic.
[3] Institute of Organic Chemistry and Biochemistry, The Czech Academy of Sciences, Czech Republic
[4] Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University
[5] Laboratory of Structural Biology, Institute of Biotechnology CAS, Czech Republic
[#] Equal contribution.

Metallopeptidases from the clan MH, subfamily M28B are membrane-bound di-zinc proteolytic enzymes with amino- or carboxypeptidase activities. In mammals, several M28B orthologs including the most studied glutamate carboxypeptidase II are involved in many not fully elucidated physiological processes including neuro-signaling, metabolism and cancer. We investigated orthologs found in *Schistosoma mansoni* and *Fasciola hepatica* not only as instrumental molecules for investigating their basal function but also as putative drug targets against these parasites. Gene coding M28B peptidases were identified in *S. mansoni* and *F. hepatica* genomes and sequences were fully annotated. Gene expression profile was evaluated by RT-qPCR for life stages of *S. mansoni*, and by RNAi we exploited the possible impact of knockdown on the viability of schistosomula and adults. Both *S. mansoni* and *F. hepatica* peptidases were cloned for recombinant expression. Polyclonal antibodies and RNA *in situ* hybridization (ISH) were used for localization studies in both parasite species. Substrate activities were tested using aminopeptidase and carboxypeptidase library screening. Gene knockdown did not lead to any phenotypic manifestations *in vitro* and *in vivo*. No relevant substrate activity was detected however, important residues essential for coordinating Zn^{2+} ions catalytic activity in human GCPII are conserved in *S. mansoni* and *F. hepatica* orthologs. The localization pattern of trematode M28B peptidases is similar to the expression pattern in functionally similar organ structures in the mammals but specific physiological roles of M28B peptidases in our model organisms could not be determined.

The study was supported by Czech Science Foundation (GACR).

THURSDAY, SEPTEMBER 23

Session VI

Biology and Diversity of Helminths

(Chairman: T. Scholz)

HELMINTHS OF MARINE MAMMALS: REVIEW OF THEIR DIVERSITY, ZOONOTIC POTENTIAL AND FUTURE PERSPECTIVES

Jesús S. Hernández-Orts

Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

Marine mammals comprise a diverse group of species, including cetaceans, pinnipeds, sea otters, sirenians and polar bears. Marine animals play crucial roles in aquatic environments, but anthropogenic activities drastically reduced their populations, with numerous species becoming vulnerable or critically endangered. These iconic animals are hosts for diverse groups of helminths (trematodes, cestodes, nematodes and acanthocephalans) which usually exhibit a wide geographical distribution. Some of these parasites are important pathogens and can regulate the populations of marine mammals, representing a potential threat to endangered species. Moreover, they may be implicated in mass stranding events of some whales and dolphins. Complex life cycles are common in these helminths and in almost all species, different life stages occur in invertebrates (first intermediate hosts), fish or cephalopods (second intermediate or paratenic hosts), and marine mammals (definitive hosts). Some larval stages of these parasites are recognised as causative agents of fish-borne zoonoses in humans and also represent a costly cosmetic problem for fish processors. At present, studies on helminths of marine mammals have serious obstacles due to the non-accessibility of hosts to be sampled. Collection of parasitological material is challenging, particularly because marine mammals are protected, inhabit remote locations or are very labour-intensive to sample. Moreover, species identifications of their helminths have been problematic due to their general morphological uniformity or high degree of intraspecific variability. Future research combining non-invasive sampling methods, integrative taxonomy and metagenomic approaches will be a potential tool to fill gap in our knowledge on the diversity, biology and ecology of helminths of these aquatic animals.

HELMINTHS OF THE TELEOST FISHES FROM THE AREA OF THE UKRAINIAN ANTARCTIC STATION "AKADEMIK VERNADSKY", ARGENTINE ISLANDS, WEST ANTARCTICA: SPECIES DIVERSITY AND PARASITE COMMUNITY STRUCTURE

T. A. Kuzmina[1], O. O. Salganskij[2], I. V. Dykyj[2,3], O. I. Lisitsyna[1], E. M. Korol[4], Y.I. Kuzmin[1]

[1]I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine
[2]State Institution National Antarctic Scientific Center, Kyiv, Ukraine
[3]Ivan Franko Lviv National University, Lviv, Ukraine
[4]National Museum of Natural History NAS of Ukraine, Kyiv, Ukraine

Monitoring studies of the species diversity in marine ecosystems provide important data on the current state of these ecosystems and reveal the ecological changes caused by global warming and anthropogenic influence. This work was aimed to analyze the modern state the species diversity of helminth fauna of main teleost fish species inhabiting the area near the Ukrainian Antarctic Station "Akademik Vernadsky", Argentine Islands, West Antarctica. During April – January of 2014–2015 and 2019–2021, 238 specimens of six teleost fish species (*Notothenia coriiceps, N. rossii, Chaenocephalus aceratus, Parachaenichthys charcoti, Trematomus bernacchii, Harpagifer antarcticus*) were examined. In total, 28,904 specimens of 33 helminth species belonging to five taxonomic groups: Monogenea (1 species), Digenea (10), Nematoda (5), Cestoda (4), and Acanthocephala (12) were collected. In *N. coriiceps*, 28 helminth species were recorded; 11 species (aniskid nematodes, cestodes and acantocephalans of the genus *Corynosoma*) were on larval stages. In *N. rossii*, 14 helminth species were found; in *P. charcoti*, 27 helminth species were found; in *Ch. aceratus*, 23 helminth species were found; in *T. bernacchii*, 16 helminth species were found; in *H. antarcticus*, only 6 species were found. Five groups of helminths: dominant (prevalence 80.1–100 %), subdominant (50.1–80 %), background (20.1–50 %), rare (1–20 %), and occasional (< 1 %) species were separated in the fish helminth community. These data on the species diversity of helminth communities can be used as a baseline for further long-term monitoring studies of fish parasites in the region of Argentine Islands, West Antarctica.

This study was partially supported by the National Research Foundation of Ukraine (project number 2020.02/0074) and by the National Antarctic Scientific Center, Ministry of Education and Science of Ukraine (project number H/03-2021).

DIVERSITY OF AVIAN SCHISTOSOMES IN EUROPE

C. Kibet[1,2], R. Kuchta[1]

[1] Laboratory of Helminthology, Institute of Parasitology, Biology Center CAS, České Budějovice, Czech Republic
[2] Department of Parasitology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

In recent years, avian schistosomes have been of concern due to increased cases of human cercarial dermatitis (HCD), worldwide. Avian schistosomes have a complex life cycle involves pulmonated snails as intermediate host and aquatic birds as definitive hosts. The free-swimming cercaria liberated from infected snails may infect also mammals, including humans, and caused HCD. In Europe 17 species of the 7 genera (*Anserobilharzia*, *Allobilharzia*, *Bilharziella*, *Dendritobilharzia*, *Gigantobilharzia*, *Ornithobilharzia*, and *Trichobilharzia*) have been reported. However, only 10 species and additional 12 unidentified genotypes have been recognised in Europe using molecular data. So far, snails of 19 species in 12 genera (Lymnaeidae, Phyidae, Planorbidae, and Valvatidae) have been recognised as their intermediate host of 30 bird schistosome species and aquatic birds of 22 species in 16 genera (Anatidae, Ardeidae, Gruidae, Laridae, Podicipedidae and Rallidae) as the definitive host. Adult trematodes are localised in visceral or nasal cavities of the definitive host. Despite extensive research on avian schistosomes in Europe, recent studies based on molecular techniques have shown that the actual diversity of bird schistosomes is much higher including several undescribed species.

This study was supported by the Czech Grant Agency (GACR, Project No. 19-28399X).

Th

Session VII

Ecology and Evolution

(Chairman: R. Kuchta)

POLYCHLORINATED BIPHENYLS IN BREAM (*ABRAMIS BRAMA*) AND ITS SPECIFIC PARASITE (*CARYOPHYLLAEUS LATICEPS*) IN A HEAVILY CONTAMINATED ENVIRONMENT

T. Brázová, D. Miklisová, D. Barčák, D. Uhrovič, M. Orosová, M. Oros

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic

The interrelationships between fish and their parasites were studied in the Zemplínska Šírava water reservoir in eastern Slovakia, heavily polluted with polychlorinated biphenyls (PCBs). The concentrations of these contaminants were measured in various fish matrices (dorsal and abdominal muscle tissues, hepatopancreas and intestine wall) of the freshwater bream, *Abramis brama* (Cyprinidae), and in its intestinal parasite *Caryophyllaeus laticeps* (Cestoda). Tissue analyses showed that the intestinal wall had the highest capacity to biomagnify the mixture of PCB congeners, followed by the hepatopancreas and muscle tissues. The amounts of PCBs were higher in abdominal muscles than in their dorsal parts. Concentrations of ΣPCBs above the limits set by European regulations were detected in both muscle parts in the fish, showing that the consequences of the old environmental heritage persist and still pose significant risks to the environment, and the consumption of fish from this site is risky to human health. PCBs reached much higher levels in intestinal parasites compared to bream matrices. Some significant differences in PCB amounts between infected and uninfected bream were determined. Fulton's condition factor (CF) significantly differed in infected and non-infected fish, with values of CF surprisingly lower in fish free of parasites compared to parasitized fish, which suggests a "synergistic" relationship between the parasite and its host.

The study was supported by the Slovak Research and Development Agency, project No APVV-18-0467 and Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences (VEGA), project No 2/0126/20.

ASIAN TAPEWORM *KHAWIA JAPONENSIS* SPREADS IN CENTRAL EUROPE, INCLUDING FERAL POPULATION OF COMMON CARP IN POLLUTED BODROG RIVER BASIN

D. Barčák[1], M. Madžunkov[2], D. Uhrovič[1], M. Miko[3], T. Brázová[1], M. Oros[1]

[1] *Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic*
[2] *University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic*
[3]*Institute of Histology and Embryology, Comenius University, Bratislava, Slovak Republic*

The Asian tapeworm *Khawia japonensis* (Yamaguti, 1934), a specific parasite of common carp (*Cyprinus carpio* L.), was originally described in Japan. In Europe, it was recorded for the first time in the Po River basin (Italy) in 2010. Few years later, this allochthonous tapeworm was also confirmed in breeding fishponds in Slovakia (2014) and the Czech Republic (2016). The present study reports new data on its distribution in Central Europe, including its first record in Hungary (Danube River basin) and Poland (Vistula River basin). In addition, *K. japonensis* was confirmed for the first time in the feral population of common carp in eastern Slovakia, which were caught in heavily polluted Zemplínska šírava water reservoir and in the Laborec River (Bodrog River basin) during an ecotoxicological survey for polychlorinated biphenyls in fish and their parasites. The highest prevalence (47%) of *K. japonensis* was found in a carp breeding facility in eastern Slovakia and the highest intensity of infection (8 parasites per fish) was recorded in a common carp from Laborec River. The tapeworms isolated from both cultured and feral carps were identified based on their morphological similarity with specimens of *K. japonensis* from Italy, Asia and North America. Histopathological examination of infected carp showed only mild signs of chronic inflammation of intestinal mucosa. These data show that although *K. japonensis* currently does not represent a dangerous pathogen, its ability to colonize new regions is remarkable. Its relatively quick spreading in Europe also reflects insufficient veterinary controls during fish transports, which increases a risk of transmission of other fish pathogens.

The study was supported by the Slovak Research and Development Agency (project No. APVV-18–0467) and Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences (project No. VEGA 2/0126/20).

HYBRID SUSCEPTIBILITY TO *DACTYLOGYRUS* (MONOGENEA) INFECTION: A MEASURE OF HYBRID VIGOUR

N. Dedić, A. Vetešníková Šimková

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

Hybridization is a common phenomenon reported in fish species. The present study was focused on the analyses of monogenean parasite infection (i.e., the intensity of infection of the dominant parasite group – *Dactylogyrus* species) in two species with higher evolutionary divergence, common bream (*Abramis brama*) and roach (*Rutilus rutilus*) and their hybrids representing F1 generations and backcross generations. We hypothesized low parasite intensity of infection in F1 generation in line with hybrid heterosis and high parasite intensity of infection in backcross generation in line with hybrid breakdown. We found that hybrids (F1 and backcross) harbored more species of *Dactylogyrus* when compared to each of the parental species. In contrast, abundance of *Dactylogyrus* species was higher in parental species and backcrosses when compared with F1 hybrids. In addition, backcross generations harboured a majority of the specific parasites of both parental species; however, only one roach-specific *Dactylogyrus* parasite, *D. fallax*, was completely absent in backcross generations. Our results may indicate gene disruption in backcross generations due to mitochondrial-nuclear genome incompatibilities and highlight that hybrid genomes limit their susceptibility to these parasites that primarily coevolve with their hosts.

The study was supported by Czech Science Foundation, project n. 19-10088S.

STRONGYLOIDES INFECTIONS IN MOUNTAIN GORILLAS

E. Nosková[1], B. Pafčo[2], V. Baláž[3], D. Modrý[1,4,5,6]

[1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Czech Republic
[2] Institute of Vertebrate Biology, The Czech Academy of Sciences, Czech Republic
[3] Department of Ecology and Diseases of Zoo Animals, Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences, Czech Republic
[4] Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary Sciences, Czech Republic
[5]Institute of Parasitology, The Czech Academy of Sciences, Czech Republic
[6]Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources/CINeZ, Czech University of Life Sciences Prague, Czech Republic

The increasing anthropogenic pressure leads to spillover of parasites from humans and domestic/feral animals into populations of free-ranging mammals, especially in close-contact situations. Diseases caused by parasites can represent a threat to endangered species such as mountain gorillas (*Gorilla b. beringei*). The genus *Strongyloides* includes at least fifty species of parasitic rhabditid nematodes. Species as *S. stercoralis*, *S. fuelleborni*, *S. cebus* and several unidentified *Strongyloides* sp. are known to infect primates, however, these species greatly differ in their host spectrum. *Strongyloides stercoralis* is considered a generalist species that affects a range of hosts including humans, carnivores, and mainly captive non-human primates (NHPs), while the host spectrum of *S. fuelleborni* is restricted to NHPs with occasional spillover to humans. Until now, *S. cebus* has only been detected in primates in South Americas NHPs. Other closely unidentified *Strongyloides* species have been listed in both Old and New world NHPs. Using molecular tools (PCR, qPCR) we detected *S. stercoralis* in the feces of wild mountain gorillas and feral dogs in the Virunga Mountains in Rwanda. Based on phylogenetic analysis, we identified two clades of *S. stercoralis* in a bayesian inference/maximum likelihood cox1 tree. Sequences from mountain gorilla's samples cluster within the first clade infecting humans and dogs. Feral dogs could be a source of parasitosis potentially altering health of mountain gorillas.

The study was supported by Czech Science Foundation project 18-24345S.

Session VIII

Monogenea: Diversity and Phylogeny

(Chairman: M. Gelnar)

DE NOVO DEVELOPED MICROSATELLITE MARKERS FOR MONOGENEANS AND THEIR APPLICATION TO STUDY POPULATION GENETICS OF GENERALIST *DACTYLOGYRUS* SPECIES

M. Benovics[1], L. Gettová[1], A. Šimková[1]

[1]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

Over the last few decades, genetic markers have been intensively developed to study the population genetics in a wide range of organisms; however, they are weakly applied in parasites' population studies. Microsatellite markers (together with mitochondrial DNA) are considered as a golden standard for population genetic studies. These highly polymorphic short tandem repeats are due to their unique characteristics (e.g., high allelic variance, codominance, and Mendelian inheritance) usually applied to infer gene flow rate, hybridization, or mating patterns on the intra- and interpopulation levels. Even though one set of such microsatellite markers was already developed for the monogeneans (more specifically for the *Gyrodactylus*), they were not previously applied to assess the population structure and diversity of these ectoparasitic helminths. In the present study, we *de novo* developed set of 24 microsatellite markers and used them to investigate the genetic diversity of the generalist monogenean species *Dactylogyrus vistulae* parasitizing cyprinoid fish. The analyzed parasite specimens were collected from 13 cyprinoid species from 11 sites in the Apennine and Balkan peninsulas. A total of 159 specimens were genotyped at each of the loci and the number of alleles per locus ranged from 2 to 16, with a mean number of 6.958 alleles per locus. Exceptionally high genetic diversity was observed among *D. vistulae* individuals in the southern Balkans, suggesting that this region might represent the centre of diversification of *Dactylogyrus* species in Europe, from where *Dactylogyrus* parasites expanded to the north of Europe. The initial clustering analysis divided all investigated specimens into three major clusters; however, the results of the subsequent analyses revealed the existence of various subpopulations, suggesting that the population structure of *D. vistulae* is associated with the diversification of their cyprinoid hosts. In addition, partition of the parasite population was observed in regions of the sympatric occurrence of two host species, indicating that these hosts may represent a barrier to gene flow, even for generalist parasite species.

The study was supported by Czech Science Foundation, project n. GA20-13539S.

DIVERSITY AND PHYLOGENY OF MONOGENEANS PARASITIZING NEOTROPICAL CICHLIDS

L. Seidlová, A. Šimková

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

Secondary freshwater fish of the Cichlidae exhibit disjunctive distribution covering tropical and subtropical areas including Africa, South and Central America, Madagascar, and the parts of southern India and the Middle East. In Neotropical America cichlids represent one of the most numerous fish group harbouring the second-highest diversity of monogeneans following Africa. Cichlids in this geographical area are parasitized by 5 genera of gill monogeneans: *Sciadicleithrum*, *Gussevia*, *Tucunarella*, *Trinidactylus,* and *Parasciadicleitrum*; all representatives of the Dactylogyridae. The knowledge of the diversity and phylogenetic relationships among parasites in this area is limited. We investigated monogenean fauna in selected cichlid species representing divergent phylogenetic lineages, focusing on the species richness and phylogeny of parasites, and coevolution in this host-parasitic system. For the presence of monogeneans, we examined 31 species, of which 20 were parasitized by monogeneans. The collected monogeneans represent 30 species of genera *Gussevia*, *Sciadicleithrum* and *Trinidactylus*; of which 17 species have not been described yet. Reconstruction of phylogenetic relationships performed using partial 28S rDNA revealed three well-supported monophyletic groups of Neotropical monogeneans parasitizing cichlids, however, the genus *Sciadicleithrum* was polyphyletic. Cophylogenetic analyses performed using concatenated data of partial 28S rDNA, partial 18S rDNA and complete ITS1 revealed a statistically significant cophylogenetic signal; 6 host-parasite associations contributed to the cophylogenetic structure. Simulations of host-parasite coevolution revealed host switch and duplication as the main speciation and diversification processes.

MOLECULAR PHYLOGENY AND SPECIATION PATTERNS IN HOST SPECIFIC MONOGENEANS (*CICHLIDOGYRUS*, DACTYLOGYRIDAE) PARASITIZING CICHLID FISHES (CICHLIFORMES, CICHLIDAE) IN LAKE TANGANYIKA

C. Rahmouni[1], M. P. M. Vanhove[1,2], S. Koblmüller[3], A. Šimková[1]

[1]Department of Botany and Zoology, Masaryk University, Brno, Czech Republic
[2]Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity & Toxicology, Diepenbeek, Belgium
[3]Institute of Biology, University of Graz, Graz, Austria

Cichlidogyrus (Dactylogyridae) represents the monogenean genus with the highest species diversity in African and Levantine cichlid fishes (Cichlidae). In the present study, the phylogeny of *Cichlidogyrus* parasites was inferred based on nuclear 28S, 18S, and ITS1 rDNA genes. Results revealed that *Cichlidogyrus* parasitizing mainly West African cichlid tribes is paraphyletic with respect to species parasitizing hosts belonging to the East African cichlid radiation, which constitute a well-supported monophylum. Members of *Cichlidogyrus* from tylochromine and oreochromine hosts that colonised Lake Tanganyika (LT) only recently, cluster with their non-LT relatives, indicating that they colonized the lake with their current host species, thus these fish were not host-switched by *Cichlidogyrus* from any of the numerous cichlid species already present in the lake. The diversification of *Cichlidogyrus* in LT seems to be driven by failure to diverge in old lineages of cichlids, cospeciation in more recently evolved ones, and host-switching followed by parasite duplication at the level of the various host tribes. Evaluation of host specificity and structural evolution of haptoral and reproductive organs in LT *Cichlidogyrus revealed that* strict specialist species with larval hook size and lacking a sclerotized vagina represent the ancestral states of haptor configuration and vaginal sclerotization, respectively, suggesting that members of *Cichlidogyrus* in this system evolved from a very simple form to a more complex one like in their West African congeners. Generalist species among *Cichlidogyrus* with a sclerotized vagina parasitizing ancient LT lineages seem to have developed a different hook configuration, most probably to ensure successful colonization of new, phylogenetically unrelated hosts.

This research was funded by the Czech Science Foundation project no. P505/12/G112 - European Centre of Ichtyoparasitology (ECIP) and the Special Research Fund of Hasselt University (BOF20TT06, personal costs to M.P.M.V.).

DIVERSITY AND PHYLOGENY OF *DACTYLOGYRUS* SPECIES (MONOGENEA: DACTYLOGYRIDAE) PARASITIZING NORTH AMERICAN CYPRINIFORM FISHES

M. Seifertová, K. Francová, A. Šimková

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

The genus *Dactylogyrus* Diesing, 1850 comprises gill ectoparasite species of cyprinid fishes exhibiting a high degree of host specificity. Although 163 species of *Dactylogyrus* are described from the North American cypriniform fishes, however, no molecular data useful for phylogenetic reconstruction of parasite history are currently available. Here we present the first molecular phylogeny of *Dactylogyrus* parasites using samples collected from cypriniform host fishes (Catastomoidei and Cyprinoidei) in North America. Our aims were to characterize the morphological and molecular diversity of North American *Dactylogyrus* and to reveal their evolutionary relationship with congeners sampled from cyprinoids in Europe, Asia, and Africa. *Dactylogyrus* parasites were collected in 2018 and 2019 from the gills of 17 host species in four areas in North America including Arkansas, Mississippi, New York, and Wisconsin. Using combined morphological and molecular approaches in species delimitation, a total of 33 *Dactylogyrus* species were recognized, 16 of them were identified as new for science. Phylogenetic analyses based on partial sequences of 28S rDNA, ITS1 and 18S rDNA revealed two well-supported clades of North American *Dactylogyrus* species and their close relationship with European *Dactylogyrus* species parasitizing Leuciscidae. Our results expand the knowledge of the monogenean parasitic fauna of North American freshwater fish and represent the first step in addressing the host-parasite coevolution and revealing the biogeography of fish using host-specific parasites.

The study was supported by MŠMT Inter-Excellence Project No. LTAUSA18010.

SPECIES DIVERSITY AND PHYLOGENY GENUS *DACTYLOGYRUS* (MONOGENEA) IN THE MIDDLE EAST

F. Nejat[1], M. Benovics[1], A. Abodli[2], A. S. Tarkan[3], E. Ercan[3], A. Šimková[1]

[1] Department of Botany and Zoology, Faculty of Science in Brno, Masaryk University, Czech Republic
[2] Environmetal Science Research Institute in Tehran, Shahid Beheshti University, Iran
[3] Faculty of Fisheries in Muğla, Muğla Sıtkı Koçman University, Turkey

The Middle East is one of the most underexplored regions regarding the diversity of *Dactylogyrus* monogeneans parasitizing cyprinoid fish. Although there were different monogenean species described from the cyprinoids of this region, molecular data are not available for the fish parasites. The Middle East is an important crossroad region for Asian, European and North African fauna. We aimed to study the diversity and phylogenetic relationships of host-specific *Dactylogyrus* hypothesizing that host-specific monogeneans contribute to the knowledge of their hosts' historical dispersion.

Field collection was conducted from 2018 to 2020 in Iran, Iraq, and Turkey. During field collection 224 cyprinoid specimens were processed, and all *Dactylogyrus* were collected for further morphological and molecular analyses. Twenty-two *Dactylogyrus* species from 26 host species were identified. For assessing phylogenetic relationships of *Dactylogyrus* species, partial regions of 18S rDNA, 28S rDNA and compete ITS1 region were amplified. The phylogenetic reconstruction revealed 5 different lineages of the *Dactylogyrus* species in the Middle East. Both morphological and molecular phylogeny revealed 3 potentially new species for science. Furthermore, the unexpected intraspecific genetic variability was found within *D. linstowi* and *D. dyki*. Surprisingly, *D. anchoratus* was observed on *Capoeta aydinensis,* an endemic freshwater fish in Turkey, suggesting possible host switching from the introduced *Carassius gibelio* (the main host of *D. anchoratus*). The position of endemic Middle East *Dactylogyrus* species within the phylogeny of *Dactylogyrus* including also European and North African species shed new light on the historical dispersion of these host-specific parasites in cyprinoids from the Middle East.

The study was supported by Czech Science Foundation, project n. GA20-13539S.

Conference partners

PREPARATIONS OF NGS OF PARAZITIC SAMPLES

Mgr. Petr Táborský

BioTech a.s.

Progress in NGS methods in the last decade strongly reduce cost and decries time needed for preparation of NGS libraries leads to emergence huge data collections. Collecting data process begins by isolation quality RNA/DNA from investigated organisms. Isolation of RNA/DNA can be done using older way phenol-chloroform extraction or by using manufactured kits containing lyse step, bind to membrane of columns step, washing step and finally elution step. RNA/DNA obtained by this way has to undergo quality check using nanodrop or bianalyzer. After quality check step you can continue to library preparation and sing some sequencing machine. The last step is Bioinformatic analysis. Definitely in the NGS preparation it is possible to enrich your sample using enrichment kits for example for prokaryotes sequences or you can get rid of non-eukaryotes sequences.

PARTICIPANTS CONTACT INFORMATION

Ambrož Martin, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, ambrozm@faf.cuni.cz

Anetta Krištof, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, kristof.anetta@gmail.com

Anettová Lucia, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, lucia.anettova@gmail.com

Barčák Daniel, Laboratory of Ichthyoparasitology, Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovakia, barcak@saske.sk

Benovics Michal, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, michal.benovics@gmail.com

Bernardová Nicol, Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Studničkova 7, 128 00 Praha 2, Czechia, nicol.bernardova@lf1.cuni.cz

Bobek Lumír, Animalab s.r.o., Lysolajské údolí 53, 16500 Praha 6, Czechia, lb@animalab.cz

Brázová Tímea, Laboratory of Ichthyoparasitology, Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovakia, brazova@saske.sk

Bulantová Jana, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, bulantov@natur.cuni.cz

Bušková Nikol, Institute of Parasitology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice, Czechia, buskova.nikol@email.cz

Dedić Neira, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, neiradedic@mail.muni.cz

Dimunová Diana, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, dimunovd@faf.cuni.cz

Dvořák Jan, Department of Zoology and Fisheries, Centre for Infectious Animal Diseases, Czech University of Life sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czechia, hdvorak76@outlook.com

Ferklová Helena, Faculty of Science, Charles University, Albertov 6, 128 43 Praha, Czechia, helena.ferklova@natur.cuni.cz

Funioková Valentýna, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, funiokov@faf.cuni.cz

Gelnar Milan, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, gelnar@sci.muni.cz

Hernandez-Orts Jesus, Institute of Parasitology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice, Czechia, jesus.hernandez.orts@gmail.com

Horák Petr, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, petr.horak@natur.cuni.cz

Ilgová Jana, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, ilgova@sci.muni.cz

Jedličková Lucie, Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czechia, jedlickoval@af.czu.cz

Kašný Martin, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, martin.kasny01@gmail.com

Kellerová Pavlína, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, voborilp@faf.cuni.cz

Kibet Caroline, Institute of Parasitology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice, Czechia, caroline.kibet@paru.cas.cz

Kim Juan, Second faculty of Medicine, Charles University, V Úvalu 84, 150 06 Prague 5, Czechia, kjuan1215@gmail.com

Konečný Lukáš, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, lukkas.konecny@gmail.com

Kuchta Roman, Institute of Parasitology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice, Czechia, krtek@paru.cas.cz

Kuzmina Tetiana I. I., Department of Parasitology 15, Schmalhausen Institute of Zoology NAS of Ukraine, Bogdan Khmelnytsky street, 01030 Kyiv, Ukraine, taniak@izan.kiev.ua

Laing Roz, IBAHCM Garscube Campus, University of Glasgow, G61 1QH Glasgow, UK, Rosalind.Laing@glasgow.ac.uk

Leontovyč Roman, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, leontovyc.roman@seznam.cz

Macháček Tomáš, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, tomas.machacek@natur.cuni.cz

Majer Martin, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, majermar@natur.cuni.cz

Mašová Šárka, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czechia, Masova@sci.muni.cz

Matoušková Petra, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, matousp7@faf.cuni.cz

Mensah Boateng Enoch, Department of Zoology and Fisheries, Centre for Infectious Animal Diseases, Czech University of Life sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czechia, boateng@af.czu.cz

Mikeš Libor, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, mikes@natur.cuni.cz

Navrátilová Martina, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, navratimart@faf.cuni.cz

Nedvědová Štěpánka, Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czechia, nedvedovas@af.czu.cz

Nejat Pashaki Farshad, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czechia, farshad.nejat@gmail.com

Nguyen Linh Thuy, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, nguyenli@faf.cuni.cz

Nosková Eva, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, ev.nosek@gmail.com

Panská Lucie, Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czechia, panska@af.czu.cz

Rahmouni Chahrazed, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, rahmouni.chahrazed@gmail.com

Revalová Alena, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, alena.revalova@natur.cuni.cz

Rychlá Nikola, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, Rychlan@faf.cuni.cz

Seidlová Lucie, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, lucie.seidlova135@gmail.com

Seifertova Maria, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, seifertovamaria@gmail.com

Scholz Tomáš, Institute of Parasitology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice, Czechia, tscholz@paru.cas.cz

Schreiber Manfred, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, schreibm@natur.cuni.cz

Skálová Lenka, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, lenka.skalova@faf.cuni.cz

Slánková Natálie, Biotech a.s., Služeb 3056/4, 10800 Praha, Czechia, slankova@ibiotech.cz

Soldánová Miroslava, Institute of Parasitology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice, Czechia, soldanova@paru.cas.cz

Szotáková Barbora, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, szotakova@faf.cuni.cz

Škorpíková Lucie, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia, skorpikovaLucie@seznam.cz

Šmídová Barbora, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czechia, barbora.smid@natur.cuni.cz

Štěrbová Karolína, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, sterbovak@faf.cuni.cz

Táborský Petr, BioTech a.s., Služeb 4, 108 00 Praha 10, Czechia, taborsky@ibiotech.cz

Vacek Vojtěch, Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czechia, vacekvojtech@af.czu.cz

Vajs Vojtěch, Faculty of Science, Charles University, Viničná 7, 128 44 Praha, Czechia, vojte.vajs@gmail.com

Vokřál Ivan, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, vokral@faf.cuni.cz

Zajíčková Markéta Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, zajickm@faf.cuni.cz

Žofka Martin, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czechia, zofkm7as@faf.cuni.cz

Total number of registered participants: **61**

CONFERENCE PARTNERS

Eppendorf Czech & Slovakia s.r.o.
Voděradská 2552/16, 215 01 Říčany u Prahy
www.eppendorf.com/CZ-cs

Generi Biotech s.r.o.
Machkova 587/42, 500 11 Hradec Králové 11
www.generi-biotech.com/cs/

KRD - Obchodní společnost s.r.o.
Pekařská 12, 155 00 Praha 5
www.krd.cz

BioTech a.s.
Služeb 4, Praha 10, 108 00
www.ibiotech.cz

Trigon Plus s.r.o.
Západní 93, 251 01 Čestlice
www.trigonplus.cz

Animalab s.r.o.
Lysolajské údolí 53, 165 00 Praha 6
www.animalab.cz

Schoeller Instruments, s.r.o.
Vídeňská 1398/124, 148 00 Praha 4
www.instruments.cz

Laboratorní přístroje a vybavení
příprava, ochrana, kultivace, analýza a skladování vzorků
centrifugace, laminární a ochranné boxy, mrazicí a chladicí zařízení, readery a bioimaging, ohřev a kultivace, anaerobní a hypoxická kultivace, koncentrátory a lyofilázátory, myčky, sterilizace, dekontaminace, plničky, autoklávy, spotřební materiál

BAKER

ThermoFisher SCIENTIFIC

Přístroje a vybavení pro laboratorní chovy
bariérové technologie, IVC a konvenční chovy, mytí a dekontaminace, logistika konvenční nádoby, IVC technologie, izolátory, technologie pro chov ryb a obojživelníků, přestýlací a laminární boxy, prokládací boxy, technologie pro welfare, analýza a digitální technologie chovů, malé, komorové a tunelové myčky, dekontaminační komory, vzduchové sprchy, skladovací technologie

TECNIPLAST

IVT

www.trigonplus.cz

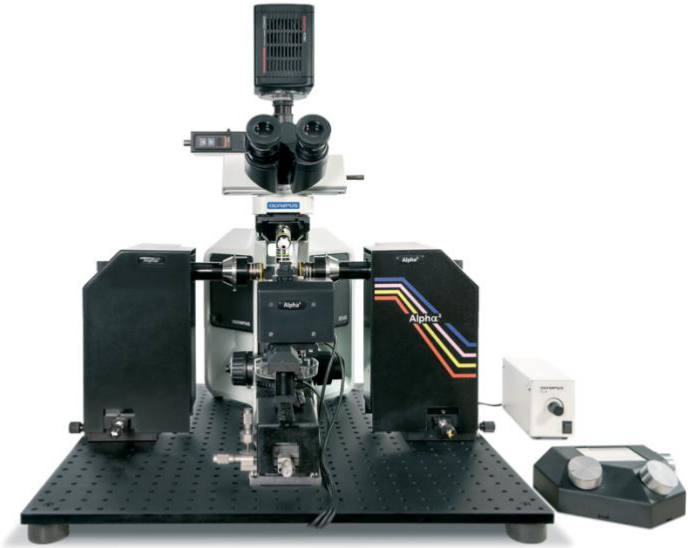
prodej aplikační podpora	autorizovaný servis	validace prevence	akreditovaná kalibrační a zkušební laboratoř

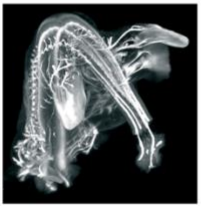
OLYMPUS

Flexibility Meets Quality Performance

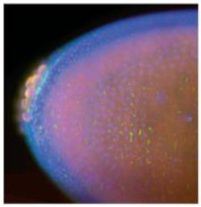
Light Sheet Microscope Alpha^3 - High-Resolution 3D Images with Low Phototoxicity

The Alpha^3 light sheet microscope system from PhaseView® combines speed and high-quality Olympus optics for high-resolution 3D imaging of both live and fixed biological samples. The Alpha^3 enables volume acquisitions at up to 75 images/second with the Smart 3D Scanning module, delivering high spatial and temporal resolution while minimizing phototoxicity. The system's dual illumination configuration combined with real-time laser focus sweeping technology permits very thin optical sectioning with illumination homogeneity, increased axial resolution, and enhanced signal-to-noise response compared to traditional confocal techniques. The Alpha^3 system can thus provide enhanced subcellular resolution without compromising speed or excitation efficiency.

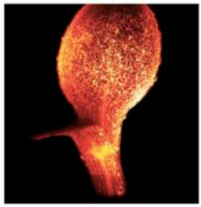




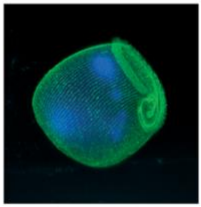
Maximum intensity projection of mouse embryo



Fixed drosophila egg, 3 channels at 20x



Membrane stained Arabidopsis leaf at 10x



Fixed Stentor, GFP cilia and DAPI polynuclei at 10x

Olympus Czech Group, s.r.o., člen koncernu
Evropská 176/16, Praha 6, 160 41
Tel.: +420 221 985 495 • e-mail: info@olympus.cz • www.olympus-lifescience.com

26th Helminthological Days 2021, Programme & Abstracts

Edited by: Pavlína Kellerová, Ivan Vokřál
Published by: Charles University, Faculty of Pharmacy
Printed by: TAH reklamní agentura, s.r.o., S. K. Neumanna 726, 500 02 Hradec Králové

1st edition, 2021
Number of copies: 80

ISBN 978-80-906644-9-4

ISBN 978-80-906644-9-4