Institute of Parasitology

Biology Centre
of the Czech Academy of Sciences, v.v.i.
České Budějovice

Biennial Report

A Brief Survey of the Institute's Organisation and Activities

2012 – 2013

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Structure of the Institute

(*As of 1 July 2014*)

Director
(***Julius LUKEŠ***)

Deputy Director
(***Tomáš Scholz***)

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- **Laboratory of Molecular Biology of Protists** (*Julius Lukeš*)
- **Laboratory of Functional Biology of Protists** (*Alena Zíková*)
- **Laboratory of Molecular Genetics of Nematodes** (*M. Asahina-Jindrová*)
- **Laboratory of RNA Biology of Protists** (*Zdeněk Paris*)

Fish parasitology

- **Laboratory of Helminthology** (*Tomáš Scholz*)
- **Laboratory of Fish Protistology** (*Astrid Holzer*)

Folia Parasitologica
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Evolutionary Parasitology

- **Laboratory of Evolutionary Protistology*** (*Miroslav Oborník*)
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- **Laboratory of Molecular Phylogeny and Evolution of Parasites** (*Václav Hypša*)

Opportunistic diseases

- **Laboratory of Veterinary and Medical Protistology** (*Martin Kváč*)
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Supporting Facilities

- **Laboratory of Electron Microscopy** (*Jana Nebesářová*)
- **Animal Facility** (*Tomáš Douda*)
- **Administrative and technical services**

Scientific Council

Miroslav Oborník (Chairman)
Libor Grubhoffer
Astrid Holzer
Petr Kopáček
Tomáš Scholz
Alena Zíková
External members
Petr Horák
Petr Volf

Tick-Borne Diseases

- **Laboratory of Molecular Ecology of Vectors and Pathogens*** (*Libor Grubhoffer*)
- **Laboratory of Vector-Host Interactions** (*Jan Kopecký*)

Biology of Disease Vectors

- **Laboratory of Vector Immunology** (*Petr Kopáček*)
- **Laboratory of Genomics and Proteomics of Disease Vectors** (*Michalis Kotsyfakis*)
- **Laboratory of Tick Transmitted Diseases** (*Ondřej Hajdušek*)

Laboratory of Molecular Helminthology**
(*Jan Dvořák*)

* Joint research unit of Institute and Faculty of Science, University of South Bohemia; ** Laboratory closed in 2013

Editorial

It is my turn to write a brief entrée to the biennial brochure (2012–2013) of the Institute of Parasitology, Biology Centre, as I was appointed its director in July 2012. I took the helm from the previous director Tomáš Scholz, who kindly remains vice-director to ensure continuity, and am happy to say that the Institute is in a good shape. Within the last two years, we have opened four new laboratories, are in the process of adding new and modern lab space in one and half floor, which is being built on our animal house, and have more international community than ever before. The journal published by us – *Folia Parasitologica* – is getting a growing number of sound submissions. I believe that the combination of these factors gives the Institute a bright future.

In reaction on the trends of digitalisation, we decided to publish this biennial brochure only online, and plan to do so in the future. I hope that those interested will easily browse through it on the web, while we will save a few trees.

We are trying to be an open, vibrant and dynamic group of laboratories and teams, joint by the common interests in all aspects of parasitology. As we are looking forward to new collaborations, students and colleagues, I would like to encourage the reader to contact us in case of her/his interest.

Cheers,

Julius Lukeš

Director

Mission statement

The Institute of Parasitology is a research institution of the Czech Academy of Sciences, performing fundamental research on human and animal parasites at the organismal, cellular and molecular levels. The mission of the Institute is to acquire, advance and disseminate knowledge of the biology and host relationships of parasitic protists and related eukaryotic microorganisms, helminths and arthropods. The Institute pursues its mission through research, education and other activities at both the national and international levels. The results obtained have contributed to the prevention and control of human and animal parasitic diseases and had an impact on agriculture.

The Institute of Parasitology was established in Prague in 1962, but was relocated to České Budějovice in South Bohemia in 1985. The Institute represents a principal institution devoted exclusively to parasitological research in the Czech Republic. The main research areas encompass protistology, helminthology and medical entomology, including studies on the causative agents of the infections transmitted by arthropods. Investigations into fish parasites, life-cycles of helminths, parasitic arthropods as vectors of diseases, molecular biology of parasitic protists, phylogeny of parasites and their molecular ecology have remained long-term research priorities of the Institute.

Research areas

The mission of the Institute of Parasitology is primarily research in parasitology, with the aim to obtain and present new information about the biology of causative agents of parasitic diseases of man and animals. The main areas representing the priorities of the Institute's research are listed below:

1. **Molecular biology of parasitic protists and nematodes**
   - Functional genomics of the mitochondrion of the flagellate *Trypanosoma brucei*
   - Secondary endosymbiosis and evolution of complex plastids
   - Genetic analysis of the nuclear receptor function in the model nematode *Caenorhabditis elegans*

2. **Parasitic protists of man and animals with special reference to opportunistic parasites**
   - Human parasites with special attention to emerging opportunistic parasites
   - Immune response against microsporidia

3. **Parasites of fish**
   - Life-cycles, ultrastructure and phylogeny of myxosporeans
   - Diversity, systematics, ecology and phylogeny of helminth parasites related to aquatic environment, with special attention to helminths of fishes in temperate and tropical regions

4. **Biology of disease vectors: molecular interactions involved in pathogen transmission**
   - Immunology of host-vector interactions with respect to pathogen transmission
   - Molecular and cellular factors of pathogen transmission in ticks
   - Molecular ecology of Lyme borreliosis and tick-borne encephalitis with respect to the antigenic structures of the causative agents and protein-carbohydrate interactions

5. **Molecular taxonomy and phylogeny of parasites**
   - Phylogeny of parasitic protists, helminths and arthropods, and host-parasite co-evolution
   - Parasite distribution and host specificity as the result of coevolutionary and host-switching events
   - Molecular phylogeny of the bacteria associated with blood-feeding arthropods

Editorial activity

The Institute of Parasitology publishes the international journal *Folia Parasitologica* (www.paru.cas.cz/folia/). It is issued quarterly and publishes contributions from all branches of parasitology.

Facilities and capabilities

Laboratories of the Institute of Parasitology are well equipped with instruments to perform a vast array of methods such as scanning and transmission electron microscopy, biochemistry, molecular biology, genomics and tissue and cell cultures. Its facilities make it possible to study host-parasite interactions at the organismal, cellular and molecular levels. The Institute of Parasitology's research activities are augmented by equipment available in other research institutes within the Biology Centre of the Czech Academy of Sciences in České Budějovice. The Institute also possesses an Animal Facility accredited for experiments with laboratory animals as well as the most comprehensive parasitological library in the Czech Republic. It has been approved for work with genetically modified organisms.

The Institute of Parasitology maintains cultures of parasitic protists, cell lines, and laboratory colonies of ticks and mosquitoes. Extensive collections of parasites (about 3 000 species of protists, helminths and parasitic arthropods, including numerous types) are also deposited at the Institute of Parasitology.

Education

The Institute of Parasitology has a close relationship with the Faculty of Science of the University of South Bohemia in České Budějovice. The practical research experience and theses of undergraduate and graduate students of the Faculty of Science are facilitated by an established research program of the Institute. In order to facilitate scientific cooperation and participation of students in research performed at the Institute, two joint laboratories (see organisation chart on p. 4) have been established jointly with the University of South Bohemia.

The Institute of Parasitology is also involved in undergraduate and graduate teaching at Charles University in Prague, Masaryk University in Brno, and Veterinary and Pharmaceutical University in Brno; it has also been licensed for doctoral studies in parasitology for the Faculty of Science, Charles University in Prague. The staff of the Institute engage in teaching activities in parasitology, molecular and cellular biology, and zoology in both master and PhD programs at the University of South Bohemia.

The Institute of Parasitology offers opportunities for postdoctoral and residency training in parasitology. The staff of the Institute organises international training courses and its researchers participate as lecturers in parasitology courses abroad.

Other activities

An integral part of activities of the Institute of Parasitology is the organisation of scientific events such as international symposia and workshops. Scientists of the Institute also provide expert opinions to national and international agencies, professional societies and scientific granting agencies. Researchers of the Institute also serve as members of editorial and advisory boards of international journals in addition to being referees of submitted articles.

Organisation units and their research activities

1. Molecular Parasitology

1.1. Laboratory of Molecular Biology of Protists

Research scientists:	Prof. RNDr. **Julius LUKEŠ**, CSc. (head) RNDr. **Milan Jirků**; **Hassan Hashimi** MSc, PhD; RNDr. **Eva Horáková**, PhD; **Pavel Flegontov**, MSc, PhD, RNDr. **Drahomíra Faktorová**, PhD
Postdocs:	**Priscila Peña Diaz**, MSc, PhD (Venezuela); **Corinna Benz**, MSc, PhD (Germany)
PhD students:	**Somsuvro Basu**, MSc (India); **Piya Changmai**, MSc (Thailand); **Sameer Dixit**, MSc (India); **Zhenqiu Huang**, MSc (China); RNDr. **Lucie Novotná**; RNDr. **Pavel Poliak**; Mgr. **Tomáš Skalický**; Mgr. **Jiří Týč**; RNDr. **Zdeněk Verner**
Technicians:	Mgr. **Eva Kriegová**; **Gabriela Ridvanová**; RNDr. **Eva Stříbrná-Černotíková**
Undergraduate students:	Bc. **Lucie Kafková-Hanzálková**; Bc. **Julie Kovářová**; **Vojtěch David**; **Alexander Haindrich** (Austria); **Sabine Kaltenbrunner** (Austria)

Research priorities

Our primary interest is functional analysis of selected mitochondrial proteins of the kinetoplastid *Trypanosoma brucei*. Its mitochondrion is unique in many aspects and by knocking-down, tagging, overexpressing or knocking-in individual genes, we are trying to establish their function(s). We have focused primarily on: (i) proteins involved in RNA editing and regulation of stability of mitochondrial transcripts; (ii) subunits of respiratory complexes, (iii) iron/sulfur cluster assembly proteins; (iv) mitochondrial processing peptidases; and (v) proteins involved in heme metabolism. We are also interested in the evolution and biodiversity of free-living and parasitic kinetoplastid flagellates.

Selected publications

- Ammerman M.L., Downey K.M., **Hashimi H.**, Fisk J.C., Tomasello D.L., **Faktorová D.**, **Kafková L.**, King T., **Lukeš J.**, Read L.R. 2012: Architecture of the trypanosome RNA editing accessory complex, MRB1. *Nucleic Acids Research* 40: 5637–5650. [IF=8.278]
- **Flegontov P.**, Votýpka J., **Skalický T.**, Logacheva M.D., Penin A.A., Tanifuji G., Onodera N.T., Kondrashov A.S., Volf P., Archibald J.M., **Lukeš J.** 2013: *Paratrypanosoma* is a novel early-branching trypanosomatid. *Current Biology* 23: 1787–1793. [IF=9.916]
- Kořený L., Oborník M., **Lukeš J.** 2013: Make it, take it or leave it: heme metabolism of parasites. *PLoS Pathogens* 9: e1003088. [IF=8.057]
- Kořený L., Sobotka R., Kovářová J., Gnipová A., **Flegontov P.**, Horváth A., Oborník M., Ayala F.J., **Lukeš J.** 2012: Aerobic kinetoplastid flagellate *Phytomonas* does not require heme for viability. *Proceedings of the National Academy of Sciences of the United States of America* 109: 3808–3813. [IF=9.737]

- Pawlowski J., Audic S., Adl S., Bass D., Belbahri L, Berney C., Bowser S.S., Čepička I., Decelle J., Dunthorn M., Fiore-Donno A.-M., Gile H.G., Holzmann M., Jahn R., **Jirků M.**, Keeling P.J., Kostka M., Kudryavtsev A., Lara E., **Lukeš J.**, Mann G.D., Mitchell A.D.E., Nitsche F., Romeralo M., Saunders W.G., Simpson A.G.B., Smirnov V.A., Spouge J., Stern F.R., Stoeck T., Zimmermann J., Schindel D., de Vargas C. 2012: CBOL Protist Working Group: barcoding eukaryotic richness beyond the animal, plant and fungal kingdoms. *PLoS Biology* 10: e1001419. [IF=12.690]

Research projects

- **Genomic, transcriptomic and proteomic view of a photosynthetic algae (Chromerida) and evolutionary "missing link" to the human malaria parasites.** King Abdulah University of Science and Technology (FIC/2010/09, Saudi Arabia; P.I. Julius Lukeš; 2010–2013)
- **Characterization of iron-sulphur clusters components in *T. brucei*.** Grant Agency of the Czech Academy of Sciences (P305/11/2179; P.I. Julius Lukeš; 2011–2014)
- **Trypanosomiases in African great apes – quest for first data from the wild.** Grant Agency of the Czech Academy of Sciences (M200961204; P.I. Julius Lukeš; 2012–2015)
- **Posttranscriptional modification of tRNA in *Trypanosoma brucei*.** Ministry of Education, AMVIS (LH12104; P.I. Julius Lukeš; 2012–2015)
- **Characterization of the mitoproteome of the parasitic protist *Trypanosoma brucei* by means of recombinogenic engineering.** Grant Agency of the Czech Academy of Sciences (P305/12/2261; P.I. Julius Lukeš; 2012–2014)
- **RNPnet – RNP structure, function and mechanism of action.** EU – FP7, Marie Curie Actions (GA 289007, FP7-PEOPLE-2011-ITN; P.I. Julius Lukeš; 2011–2015)
- **Comparative imunogenetics of the Equidae family.** Grant Agency of the Czech Academy of Sciences (523/09/1972; P.I. Julius Lukeš; 2009–2012)
- **Characterization of a novel protein complex involved in RNA processing and editing in the mitochondrion of *Trypanosoma brucei*.** Grant Agency of the Czech Academy of Sciences (204/09/1667; P.I. Julius Lukeš; 2009–2012)

1.2. Laboratory of Functional Biology of Protists

Research scientist:	RNDr. **Alena ZÍKOVÁ**, PhD (head) **Brian Panicucci**, BSc; Mgr. **Eva Doleželová**, PhD; Mgr. **Ondřej Gahura** PhD; **David Wildridge**, MSc, PhD
PhD students:	Mgr. **Karolína Šubrtová**; Mgr. **Michaela Veselíková**
Undergraduate students:	**Martina Aistleitner** (Austria); **Matthias Guggenberger** (Austria); **Zuzana Kotrbová**; **Jan Martinek**; **Hana Váchová**

Research priorities

Our research projects contribute to the drug development against infectious diseases caused by unicellular parasites of the genera *Trypanosoma* and *Leishmania*. These medically and veterinary important pathogens cause significant disease burden in the third world with no potent cure or effective vaccines. Successful drug development is dependent on identification of good cellular drug targets. Unique and essential pathways in the parasitic cell represent potential and promising targets for chemotherapeutics.

FoF1 ATPase in the *Trypanosoma* bloodstream forms

FoF1 ATP synthase/ATPase represents a splending molecular machine that is responsible for generation of ATP in the majority of all living cells. Importantly, its function, composition, structure and regulation differ between the parasite and the human host. The FoF1-ATPase in the infectious stage of the parasite works in a reverse mode, hydrolysing ATP to pump protons across the mitochondrial inner membrane to maintain the essential membrane potential. This essential ATPase activity can be specifically inhibited by a small inhibitory peptide TbIF1. We study the mechanism of its binding to and inhibition of F1 ATPase. Furthermore, we aim to decipher the crystal stucture of the inhibited TbIF1/F1-ATPase complex to identify the protein-protein interaction interface that can be used for structure-based drug design.

Gene expression regulation in *Leishmania*

The investigation of essential genes in many *Leishmania* species has proved challenging due to the lack of RNAi machinery and a regulatable gene expression system. It has been possible to control protein expression through the regulated degradation of a target protein fused to the FK506/rapamycin-binding protein destabilisation domain (DD). We are developing a novel method that utilises the bacteriophage T7 RNA polymerase fused to the DD to regulate expression of a gene of interest under the control of a T7 promoter at a transcriptionally silent locus in *Leishmania major*. The endogenous alleles of the gene of interest would then be targeted for deletion through homologous recombination. The major advantage of this approach is that stabilisation of the T7 RNA polymerase is independent of the protein of interest, thus providing a regulatable system for the investigation of essential genes, irrespective of their subcellular localisation.

Mode of action of selected trypanocidal drugs

In collaboration with several international and Czech laboratories we are investigating mode of action of some selected drugs that act on parasites in nanomolar amounts. Importantly, these compounds affect mitochondrial function and physiology. Studying the critical mitochondrial processes (membrane potential, ROS generation, oxygen consumption, activity of respiratory complexes, stability of mitochondrial DNA, protein import, redox metabolism, etc.), we are able to identify putative drug targets followed by their functional validation using RNA interference.

Purine salvage pathway in *T. brucei*

Purine nucleotides function in a variety of vital cellular processes. Unlike their mammalian hosts, *T. brucei* cannot synthesise the purine ring *de novo*, hence it absolutely relies on an external purine source. Consequently, a unique suite of salvage enzymes that enable purine acquisition has evolved in these medically important parasites. Since adenosine and hypoxanthine are the preferred purine source of the infectious bloodstream form of *T. brucei*, 6-oxo- and 6-aminopurine salvage enzymes offer an attractive paradigm for drug targeting. With a variety of molecular genetic and biochemical techniques, these enzymes are being studied to determine if they are essential and to examine their substrate specificity, activity and druggability.

Selected publication

- Gnipová A., **Panicucci B.**, Paris Z., Verner V., Horváth A., Lukeš J., **Zíková A.** 2012: Disparate phenotypic effects from the knockdown of various *Trypanosoma brucei* cytochrome c oxidase subunits. *Molecular and Biochemical Parasitology* 184: 90–98. [IF=2.734]

Research Projects:

- **Exploitation of the unique characteristics of the *Trypanosoma brucei* FoF1-ATP synthase complex for future drug development against African sleeping sickness.** Ministry of Education, Czech Republic (ERC CZ LL1205; P.I.: Alena Zíková; 2013–2017)
- **Comprehensive analysis of FoF1-ATP synthase in parasitic protozoa.** EMBO Installation grant (1965; P.I.: Alena Zíková; 2010–2014)
- **Inhibiting the essential FoF1-ATPase to eliminate the infectious stage of *Trypanosoma brucei*.** Grant Agency of the Czech Republic (P302/12/2513; P.I. Alena Zíková; 2012)

1.3. Laboratory of Molecular Genetics of Nematodes*

Research scientist: **Masako ASAHINA-JINDROVÁ**, PhD (head)
PhD student: **Nagagireesh Bojanala**, MSc

* Currently, the labarotary is temporarily closed because of long-term research stay of its head in San Francisco, USA.

Research priorities

Metazoan transcription factors (TFs) regulate distinct networks of genes in different tissues through combinatorial regulation. These transcription factors must interpret multiple inputs: (i) the physiological status of the tissue/organism; (ii) cues that indicate cell polarity/identity; and (iii) specific information dictated by the DNA sequence of the TF binding site. The nematode *Caenorhabditis elegans* is a genetically tractable model organism that has distinct tissue types and is amenable to visualising gene function at single-cell resolution. Our research here focuses to identify how a single TF, the nuclear receptor NHR-25, regulates target genes in tissue/cell-context dependent manner.

Post-translational modification SUMO modulates NHR-25/NR5A activity during *C. elegans* vulval development

We examined how a single regulatory input, sumoylation, differentially modulates the activity of a conserved *C. elegans* nuclear hormone receptor, NHR-25, in cell fate execution. Through a combination of yeast two-hybrid analysis and *in vitro* biochemistry, we identified the single *C. elegans* SUMO (SMO-1) as an NHR-25 interacting protein and showed that NHR-25 is sumoylated on at least four lysines. Some of the sumoylation acceptor sites are in common with those of the NHR-25 mammalian orthologs SF-1 and LRH-1, demonstrating that sumoylation has been strongly conserved within the NR5A family. NHR-25 binds canonical SF-1 binding sequences to regulate transcription and NHR-25 activity is enhanced *in vivo* upon loss of sumoylation. During vulval development, sumoylation of NHR-25 is critical for maintaining 3° cell fate. Moreover, SUMO also confer formation of a developmental time-dependent NHR-25 gradient across VPC daughters. We conclude that sumoylation operates at multiple levels to affect NHR-25 activity in a highly coordinated spatial and temporal manner.

NHR-25 is required for morphogenesis in *C. elegans*

Morphogenesis involves essential biological processes such as cell fate decision, determination of cell polarity and cell migration, cell fusion common to all eukaryotes. Vulva of *C. elegans* is a paradim of morphogenesis of animal development and various genetical regulatory pathways such as EGF, Ras, Notch, Runx, Wnt and Semaphorin have been intensively studied in this process. We show that the concerved nuclear receptor NHR-25 is required in vulval morphogenesis and cross talk with signaling pathways mentioned above is the key regulation for proper differentiation and formation of the tissue.

Selected publications

- Ward J.D., **Bojanala N.**, Bernal T., Ashrafi K., **Asahina M.**, Yamamoto K.R. 2013: Sumoylated NHR-25/NR5A regulates cell fate during *C. elegans* vulval development. *PLoS Genetics* 12: e1003992. [IF=8.167]

Research projects

- **Intercellular signalling in development and disease.** Grant Agency of the Czech Republic (204/09/H058; P.I.: V. Bryja; Co-I.: M. Asahina-Jindrová; 2009–2012)

1.4. Laboratory of RNA Biology of Protists

Research scientist: Mgr. **Zdeněk PARIS,** PhD (head)

Undergraduate students: **Helmut Stanzl** (Austria), **Rebecca Wolkerstorfer** (Austria)

Research priorities

Our group (established in February 2014, but included into the present biennial report) studies various aspects of RNA biology of the protistan parasite *Trypanosoma brucei* and related flagellates. In those early evolved unicellular organisms most genes are post-transcriptionally regulated. Consequently, posttranscriptional processing of RNA becomes of a great importance to regulate complex life cycles of these pathogens. We are mainly interested in processes such as tRNA modifications, nuclear tRNA export and role of the only intron containing tRNA in trypanosomes. Our long-term goal is an identification of unique mechanisms of RNA metabolism. We believe this will help us reveal new drug targets to combat diseases caused by trypanosomatid parasites.

Queuosine biosynthesis in trypanosomes

Transfer RNAs are typical for the large number of post-transcriptional modifications. Most of the tRNA modifications are present in the anticodon loop, which have crucial role in proper translation of proteins. Queuosine is one of the most complex tRNA modifications. Despite its omnipresence among bacteria and eukaryotes, role of queuosine tRNA modification is not clear. The main aim of this project is to evaluate the function and subunit composition of the enzyme responsible for queuosine formation in *T. brucei*. Using the RNAi knock-down strategy, we want to address the principal question regarding the role of queuosine tRNA modification with respect to biology and physiology of this protistan parasite.

Role of the only tRNA intron in trypanosomatids

In yeast *Sacharomyces cerevisiae* and other model organisms, 20% of all tRNAs contain introns. Their removal is an essential step in the maturation of tRNA precursors. In *T. brucei*, there is only one intron containing tRNA: $tRNA^{Tyr}_{GUA}$. Since this tRNA is responsible for decoding all tyrosine codons, intron removal is essential for viability. Using molecular and biochemical approaches, several non-canonical editing events were identified within the intron-containing $tRNA^{Tyr}_{GUA}$. The RNA editing involves guanosine-to-adenosine transitions (G to A) and an adenosine-to-uridine transversion (A to U), which are both necessary for proper processing of the intron. We have been obtaining tRNA intron sequences from our collection of newly identified trypanosomatid species. We hope this will help us understand the process of RNA editing and ultimately identify biological function of the presence of the only intron containing tRNA in these organisms.

Nuclear export of tRNAs in trypanosomes

Regulation of tRNA export from the nucleus to the cytoplasm might be an additional post-transcriptional event involved in gene regulation. However, our knowledge of tRNA export in

trypanosomes is very limited. Although export factors of higher eukaryotes are reported to be conserved, only a few orthologs can be easily identified in the genome of *T. brucei*. Thus, we are going to employ methods of molecular biology and biochemistry to identify and characterize the tRNA export machinery in trypanosomes.

Selected publications (including those of Z. Paris before his lab was established)

- Gnipová A., Panicucci B., **Paris Z.**, Verner Z., Horváth A., Lukeš J., Zíková A. 2012: Disparate phenotypic effects from the knockdown of various *Trypanosoma brucei* cytochrome c oxidase subunits. *Molecular and Biochemical Parasitology* 184: 90–98. [IF=2.859]
- **Paris Z.**, Changmai P., Rubio M.A.T., Zíková A., Stuart K.D., Alfonzo J.D., Lukeš J. 2010: The Fe/S Cluster assembly protein Isd11 is essential for tRNA thiolation in *Trypanosoma brucei*. *Journal of Biological Chemistry* 285: 22394–22402. [IF=5.328]
- **Paris Z.**, Hashimi H., Lun S., Alfonzo J.D., Lukeš J. 2011: Futile import of tRNAs and proteins into the mitochondrion of *Trypanosoma brucei evansi*. *Molecular and Biochemical Parasitology* 176: 116–120. [IF=2.551]
- **Paris Z.**, Horáková E., Rubio M.A.T., Sample P., Fleming I.M.C., Armocida S., Lukeš J., Alfonzo J.D. 2013: The *T. brucei* TRM5 methyltransferase plays an essential role in mitochondrial protein synthesis and function. *RNA* 19: 649–658. [IF=4.622]
- Rubio M.A., **Paris Z.**, Gaston K.W., Fleming I.M., Sample P., Trotta C.R., Alfonzo J.D. 2013: Unusual noncanonical intron editing is important for tRNA splicing in *Trypanosoma brucei*. *Molecular Cell* 52: 184–192. [IF=14.464]

Research projects

- We acknowledge the use of research infrastructure that has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 316304.

2. Evolutionary Parasitology

2.1. Laboratory of Evolutionary Protistology

Research scientists:	Prof. Ing. **Miroslav OBORNÍK**, PhD (head) **Heather Esson**, MSc, PhD (Canada); Mgr. **Zoltán Füssy**, PhD; Mgr. **Aleš Horák**, PhD (till 2012); RNDr. **Eva Roubalová**, PhD; RNDr. **Aleš Tomčala**, PhD
PhD students:	Mgr. **Jaromír Cihlář**; Mgr. **Jitka Kručinská**; Mgr. **Jan Michálek**; Ing. **Ivana Schneedorferová**
Research assistant:	Mgr. **Kateřina Jiroutová**, PhD
Undergraduate students:	Bc. **Martina Jonáková**

Research priorities

Laboratory of Evolutionary Protistology (LEP) (formerly Laboratory of Molecular Taxonomy) was established in 2000 as a joint laboratory of the Institute of Parasitology and Faculty of Biological Sciences (now Faculty of Science), University of South Bohemia. At present the laboratory is designed to study evolution of protists and algae.

Genomics of chromerids

Chromerids are phototrophic algae isolated from Australian corals. Two species have been described so far, *Chromera velia* and *Vitrella brassicaformis*, which have been shown to represent the closest known phototrophic relatives to apicomplexan parasites. We have sequenced nuclear genomes of both chromerid species in cooperation with KAUST (Saudi Arabia) and worked on the annotation of those genomes, with the aim to understand the evolution of parasite from an algal ancestor.

Reduced respiratory chain in *C. velia* mitochondrion

Through investigation of genomic and transcriptomic sequences and those obtained from enriched mitochondrial fraction, we reconstructed respiratory chain of *C. velia*. This respiratory chain is non-canonically reduced, with such reduction not found in the mitochondrion of related *V. brassicaformis*. We propose that differences between respiratory chains of both chromerids likely evolved due to different ways how these algae store and utilise energy.

Localisations of enzymes involved in tetrapyrrole biosynthesis

Tetrapyrroles rank between most fundamental pathways in living organisms. We investigated origins of enzymes involved in the heme biosynthesis in chromerids and predicted their localisations in the cell. To confirm prediction results, we used xenotransfections of *C. velia* genes in the diatom *Phaeodactylum tricornutum* and apicomplexan *Toxoplasma gondii*. Since obtained results do not fully correspond to *in silico* predictions, we have generated antibodies against four selected enzymes of the pathway. It appears that the heme pathway is likely localised non-canonically in *C. velia* when compared to all other phototrophs.

Search for the essential function of non-photosynthetic plastid of *Euglena longa*

Euglena gracilis is an excavate alga, which can lose its secondary green plastid by the treatment with antibiotics, due to the presence of two pathways for heme biosynthesis. Close sibling of *E. gracilis*, non-photosynthetic *E. longa*, contains relic plastid essential for the algal cell. An investigation of the transcriptome suggests Calvin cycle as an essential metabolic route in non-photosynthetic plastid of *E. longa*.

Selected publications

- Curtis B.A., Tanifuji G., Burki F., Gruber A., Irimia M., Maruyama S., Arias M.C., Ball S.G., Gile G.H.,Hirakawa Y.,Hopkins J.F.,Kuo A.,Rensing S.A., Schmutz J.,Symeonidi A., Elias M., Eveleigh R.J.M., Herman E.K., Klute M.J., Nakayama T., **Oborník M.**, Reyes-Prieto A., Armbrust E.V., Aves S.J., Beiko R.G., Coutinho P., Dacks J.B., Durnford D.G., Fast N.M., Green B.R., Grisdale C.J., Hempel F., Henrissat B., Hoppner M.P., Ishida K.I., Kim E., Kořený L., Kroth P.G., Liu Y., Malik S.B., Maier U.G., McRose D., Mock T., Neilson J.A.D., Onodera N.T., Poole A.M., Pritham E.J., Richards T.A., Rocap G., Roy S.W., Sarai C., Schaack S., Shirato S., Slamovits C.H., Spencer D.F., Suzuki S., Worden A.Z., Zauner S., Barry K., Bell C., Bharti A.K., Crow J.A., Grimwood J., Kramer R., Lindquist E., Lucas S., Salamov A., McFadden G.I., Lane C.E., Keeling P.J., Gray M.W., Grigoriev I.V., Archibald J.M. 2012: Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. *Nature* 492: 59–65. [IF=38.597]
- Janouškovec J., Sobotka R., Lai D.-H., Flegontov P., Koník P., Komenda J., Ali S., Prášil O., Pain A., **Oborník M.**, Lukeš J., Keeling P.J. 2013: Split photosystem protein, linear-mapping topology and growth of structural complexity in the plastid genome of *Chromera velia*. *Molecular Biology and Evolution* 30: 2447–2462. [IF=14.308]
- Kořený L., Sobotka R., Kovářová J., Gnipová A., Flegontov P., Horváth A., **Oborník M.**, Ayala F.J., Lukeš J. 2012: Aerobic kinetoplastid flagellate *Phytomonas* does not require heme for viability. *Proceedings of the National Academy of Sciences of the United States of America* 109: 3808–3813. [IF=9.737]
- **Oborník M.**, Lukeš J. 2013: Cell biology of chromerids, the autotrophic relatives to apicomplexan parasites. *International Review of Cell and Molecular Biology* 306: 333–369. [IF=4.522]
- **Oborník M.**, Modrý D., Lukeš M., Černotíková-Stříbrná E., Cihlář J., Tesařová M., Kotabová E., Vancová M., Prášil O., Lukeš J. 2012: Morphology, ultrastructure and life cycle of *Vitrella brassicaformis* n. sp., n. gen., a novel chromerid from the Great Barrier Reef. *Protist* 163: 306–323. [IF=4.140]

Research Projects

- **Genomic, transcriptomic and proteomic view of a photosynthetic algae, Chromerida, and evolutionary "missing link" to the human malaria parasites.** King Abdulah University of Science and Technology (project FIC/2010/09, Saudi Arabia, 2010–2013)
- ***Chromera velia* – newly discovered live photosynthetic ancestor of parasites of the group Apicomplexa.** Grant Agency of the Academy of Sciences of the Czech Republic (IAA601410907, Co-P.I.: M. Oborník, 2009–2012)
- **Photosynthesis Research Centre.** Grant Agency of the Czech Republic (P501/12/G055, Co-P.I. M. Oborník, 2012–2018)
- **Evolution of tetrapyrrole synthesis in phototrophic eukaryotes.** Grant Agency of the Czech Republic (P506/12/1522, P.I.: M. Oborník, 2012–2015)
- **A genomic approach to unravelling the biology and evolution of eustigmatophyte algae.** Grant Agency of the Czech Republic (13-33039S, P.I.: M. Oborník, 2013–2015)

2.2. Laboratory of Environmental Genomics

Research scientists: Mgr. **Aleš HORÁK**, PhD (head)

PhD students: **Olga Flegontova**, MSc (Russia)

Research priorities

Study on biodiversity and biology of uncultivable unicellular eukaryotes using power of next-generation sequencing.

Early stages of evolution of parasitism in Apicomplexa

Apicomplexans are probably the most diverse and successful group of parasitic protozoans, with millions of dollars spent on the research of the key players (*Plasmodium*, *Toxoplasma*, coccidia, etc.). Yet, we know very little about the early phases of their evolution. Therefore, we are characterizing the diversity and the genomes of representatives of several enigmatic apicomplexan clades (archigregarines, blastogregarines and agammococcidians) in order to reveal the evolution of non-photosynthetic plastid (apicoplast) and composition and evolution of the surface proteins associated with the infection of host. Collaboration: Sonja Rueckert, Edinburgh Napier University (UK).

Diversity and ecology of marine diplonemids

Tara Oceans is an international project of unprecedented scale, which aimed to investigate prokaryotic and eukaryotic planktonic diversity of the world oceans. During 2009–2012, almost 28 thousand samples were obtained from 154 locations of the World Ocean. Preliminary analysis of V9 region of the 18S rRNA gene has revealed that some stations are dominated by diplonemid-like kinetoplastid excavates. We aim to elucidate the role of these mysterious organisms in the global ocean ecosystem. We also aim to analyze the metabarcode V9 data to assess diversity and distribution-pattern of marine excavates. Collaboration: Tara Consortium, namely Colomban de Vargas Station Biologique de Roscoff (France).

Selected publications

- Janouškovec J., **Horák A.**, Barott K.L., Rohwer F.L., Keeling P.J. 2012: Global analysis of plastid diversity reveals apicomplexan-related lineages in coral reefs. *Current Biology* 22: R518–R519. [IF=9.494]
- Janouškovec J., **Horák A.**, Barott K.L., Rohwer F.L., Keeling P.J. 2013: Environmental distribution of coral-associated relatives of apicomplexan parasites. *ISME Journal* 7: 444–447. [IF=9.267]
- Okamoto N., **Horák A.**, Keeling P.J. 2012: Description of two species of early branching dinoflagellates, *Psammosa pacifica* n. g., n. sp. and *P. atlantica* n. sp. *PLoS ONE* 7: e34900. [IF=3.730]
- Pomajbíková K., Oborník M., **Horák A**., Petrželková K.J., Grim J.N., Levecke B., Todd A., Mulama M., Kiyang J., Modrý D. 2013: Novel insights into the genetic diversity of *Balantidium* and *Balantidium*-like cyst-forming ciliates. *PLOS Neglected Tropical Diseases* 7: e2140. [IF=4.489]
- Votýpka J., Klepetková H., Yurchenko V.Y., **Horák A.**, Lukeš J., Maslov D.A. 2012: Cosmopolitan distribution of a trypanosomatid *Leptomonas pyrrhocoris*. *Protist* 163: 616–631. [IF=4.140]

Research Projects

- **Evolution of surface proteins in transition to intracellular parasitism: case of Apicomplexa.** Grant Agency of Czech Republic (P506/12/P931; P.I.: Aleš Horák, 2012–2014)

2.3. Laboratory of Molecular Phylogeny and Evolution of Parasites

(at present, head J. Štefka)

Research scientists:	Prof. RNDr. **Václav HYPŠA**, CSc. (head) MVDr. **Jana Kvičerová**, PhD; RNDr. **Eva Nováková**, PhD; RNDr. **Jan Štefka**, PhD
PhD students:	RNDr. **Tomáš Chrudimský**; RNDr. **Filip Husník**; Mgr. **Marie Krausová**; Mgr. **Anna Mácová**; RNDr. **Jana Martinů**
Technician:	**Lenka Štifterová**
Undergraduate students:	Bc. **Michaela Matějková**; Bc. **Jakub Vlček**; **Aneta Hoblíková**; **Pavlína Kočová**; **Aneta Maršíková**; **Eva Šochová**; **Lukáš Vejsada**

Research priorities

Our research is mainly focused on molecular phylogenetic analysis of the origin, evolution and relationships of parasitic and symbiotic organisms. It further involves investigation into their co-evolution, biogeography and other bionomical features, including intraspecific variability and genealogy. The research is carried out on several models of parasitic and symbiotic associations.

Evolution of symbiotic bacteria associated with arthropods

The main goal of our research is complex characterisation of symbiotic systems in several model insect groups using microscopy, genomic, transcriptomic and phylogenomic methods. Our main questions involve genome evolution of both the host and its symbionts, their phylogeny and populational structure, and host-symbiont-pathogen interactions. For example, the smallest reported bacterial genome belongs to *Tremblaya princeps*, a symbiont of *Planococcus citri* mealybugs (PCIT). *Tremblaya* PCIT not only has about 120 genes, but possesses its own bacterial endosymbiont, *Moranella endobia*. Genome and transcriptome sequencing, including genome sequencing from a *Tremblaya* lineage lacking intracellular bacteria, reveals that the extreme genomic degeneracy of *Tremblaya* PCIT likely resulted from acquiring *Moranella* as an endosymbiont. In addition, at least 22 expressed horizontally transferred genes from multiple diverse bacteria to the mealybug genome likely complement missing symbiont genes. However, none of these horizontally transferred genes are from *Tremblaya*, showing that genome reduction in this symbiont has not been enabled by gene transfer to the host nucleus. Our results thus indicate that the functioning of this three-way symbiosis is dependent on genes from at least six lineages of organisms and reveal a path to intimate endosymbiosis distinct from that followed by organelles.

Coevolution between Galápagos mockingbirds and their ectoparasites

We are studying the character of coevolution between Galápagos mockingbirds and their parasites. The research focuses on determining the factors responsible for formation of

population structure, reconciling the mutual evolutionary history and identifying genes under selection in the hosts. With the use of parallel amplicon sequencing of the MHC class IIB locus, we found evidence for lowered genetic diversity in threatened populations of mockingbirds. Microsatellite study of one of the louse parasites showed decreased heterozygosity and potential inbreeding in louse populations on individual hosts. Such genetic pattern indicates that host individuals of a single species play role in creating population structure of their parasites.

Phylogeny and host specificity of coccidia

Using a broad geographic sample of *Apodemus*-associated *Eimeria*, we revealed a complex phylogenetic arrangement of the lineages showing that eimerian fauna associated with a host group, in some cases even a single host species, may be composed of several distant genetic lineages. Comparing the phylogenetic patterns with morphological and biological features, we also documented a rapid diversification of these traits in the early stages of the speciation. For comparative reasons, we performed a similar study on a little known genus *Hemolivia*. We collected molecular data for *Hemolivia* from a broad geographic area and host spectrum and provided detailed morphological description of the included samples. We demonstrated monophyletic origin of the genus from a broad geographic span and various groups of hosts, and confirmed its close relationship to the genus *Hepatozoon*. Our results provide for the first time a reliable background for an unequivocal placement of new samples into the *Hemolivia*/*Hepatozoon* complex.

Selected publications

- Bouzid W., **Štefka J.**, Bahri-Sfar L., Beerli P., Loot G., Lek S., Haddaoui N., **Hypša V.**, Scholz T., Dkhil-Abbes T., Meddour R., Ben Hassine O.K. 2013: Pathways of cryptic invasion in a fish parasite traced using coalescent analysis and epidemiological survey. *Biological Invasions* 15: 1907–1923. [IF=2.716]
- **Chrudimský T.**, **Husník F.**, **Nováková E.**, **Hypša V.** 2012: Candidatus *Sodalis melophagi* sp. nov.: phylogenetically independent comparative model to the tsetse fly symbiont *Sodalis glossinidius*. *PLoS ONE* 7: e40354. [IF=3.730]
- **Husník F.**, Nikoh N., Koga R., Ross L., Duncan R.P., Fujie M., Tanaka M., Satoh N., Bachtrog D., Wilson A.C.C., von Dohlen C.D., Fukatsu T., McCutcheon J.P. 2013: Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. *Cell* 153: 1567–1578. [IF=33.116]
- **Kvičerová J.**, **Hypša V.** 2013: Host-parasite incongruences in rodent *Eimeria* suggest significant role of adaptation rather than cophylogeny in maintenance of host specificity. *PLoS ONE* 8: e63601. [IF=3.546]
- **Nováková E.**, **Hypša V.**, Klein J., Foottit R.G., von Dohlen C.D., Moran N.A. 2013: Reconstructing the phylogeny of aphids (Hemiptera: Aphididae) using DNA of the obligate symbiont *Buchnera aphidicola*. *Molecular Phylogenetics and Evolution* 68: 42–54. [IF=4.018]

Research project

- **Evolutionary hitchhiking: co-evolution of Galápagos mockingbirds and their ectoparasite populations.** Grant Agency of the Czech Republic (P506/12/P529; P.I.: J. Štefka; 2012–2014)
- **Population structure and evolutionary relationships of the intracellular parasite *Hemolivia mauritanica* (Sergent and Sergent, 1904).** Grant Agency of the Czech Republic (P506/11/1738; P.I.: V. Hypša; 2011–2014)
- **Molecular evolution of *Arsenophonus*, an emerging group of symbiotic bacteria with a broad host distribution.** Grant Agency of the Czech Republic (P505/10/1401; P.I.: V. Hypša; 2011–2013)
- **Population genetics, demography and molecular evolution in interspecific associations: comparative study of two complex parasitic/symbiotic systems.** Grant Agency of the Czech Republic (P505/12/1620; P.I.: V. Hypša; 2012–2015)
- **Evolutionary and ecological factors in genome evolution of bacterial symbionts in insects.** Grant Agency of the Czech Republic (13-01878S; P.I.: V. Hypša; 2013–2016).

3. Ticks and Tick-Borne Diseases

3.1. Laboratory of Molecular Ecology of Vectors and Pathogens

Research scientists:	Prof. RNDr. **Libor GRUBHOFFER**, CSc. (head) **Nataliia Rudenko**, MSc, PhD (Ukraine) (deputy head); **Maryna Golovchenko**, MSc (Ukraine); **Ryan O.M. Rego**, MSc, PhD (India); Mgr. **Ján Štěrba**, PhD (Slovakia)
PhD students:	**Annika Brinkmann**, MSc (Germany); Mgr. **Jiří Černý**; Mgr. **Tereza Chrudimská**; Mgr. **Václav Hönig**; Mgr. **Martin Selinger**; RNDr. **Jarmila Štěrbová-Dupejová**; **Miray Tonk**, DVM (Turkey); Mgr. **Hana Tykalová-Šťastná**; RNDr. **Pavlína Věchtová**
Research assistants:	Mgr. **Zuzana Vavrušková**
Undergraduate students:	**Alejandro Cabezas Cruz**, DVM (Cuba); Bc. **Radka Hobizalová**; Bc. **Helena Mondeková**; Bc. **Lucia Ouředníková**; Bc. **Martin Strnad**; **Eva Dršková**; **Hana Hájková**; **Paula Helebrandová**; **Nelly Keplová**; **Hana Slabá**; **Lucie Tichá**
Laboratory worker:	**Zuzana Němcová**

Research priorities

Our group studies molecular and cellular factors involved in the mechanism of pathogen transmission by ticks. Lyme borreliosis spirochetes as well as tick-borne encephalitis virus are the main focus of research, with respect to protein-carbohydrate interactions and their interaction with the inner environment of the tick *Ixodes ricinus*.

Molecular epidemiology of Lyme disease spirochetes

We confirmed that the genetic diversity of spirochetes from the *Borrelia burgdorferi* s.l. complex in Europe and in the USA is higher than previously reported. We showed a close relationship of European and North American spirochete populations, confirming the genetic endemism, recent transoceanic migration and existence of recombinant genomic types.

Borrelia factors of sense and survival in vertebrate host and tick vector

Diverse interaction that occurs between spirochete, the tick vector and the host makes *Borrelia* an elusive pathogen and is essential to understand the molecular mechanisms that affect transmission of pathogen by vector, development of *Borrelia* infection, or triggers the host reaction to tick bite and infection. We search why the Lyme borreliosis system so successful.

Identification of *Borrelia* virulence factors during its infectious cycle

We are adapting and developing genetic tools to understand the varied and segmented genome of various European strains, including *B. afzelii*. By using the tick-mouse infectious model, we are aiming to delineate the requirements of particular plasmids/genes and to investigate

interaction of infective strains with specific factors of the innate immune system of ticks.

Molecular virology of tick-borne encephalitis virus (TBEV)

We have shown that expression of two host antiviral proteins, OASL and viperin, is induced in human neural cell lines infected with TBEV. *In vitro* overexpression of wild-type viperin resulted in temporary reduction in level of selected viral proteins, but not in progeny production.

Defence peptides from tick vectors as potential antibiotics of new generation

One of the facets of the tick defence is a rapid and transient synthesis of a set of potent antibacterial peptides (AMPs) following infection or trauma. Recently, we isolated the genes encoding tick immune proteins involved in digestion, transmission of *B. burgdorferi*, antioxidant defense (ROS), pathogen recognition and defence.

Selected publications

- Antunes S., Galindo R.C., Almazán C., **Rudenko N.**, **Golovchenko M.**, **Grubhoffer L.**, Shkap V., do Rosário A., de la Fuente J., Domingos A. 2012: Functional genomics studies of *Rhipicephalus* (*Boophilus*) *annulatus* ticks in response to infection with the cattle protozoan parasite, *Babesia bigemina*. *International Journal for Parasitology* 42: 187–195. [IF=3.404]
- Menten-Dedoyart C., Faccinetto C., **Golovchenko M.**, Dupiereux I., Van Lerberghe P.B., Dubois S., Desmet C., Elmoualij B., Baron F., **Rudenko N.**, Oury C., Heinen E., Couvreur B. 2012: Neutrophil extracellular traps entrap and kill *Borrelia burgdorferi* sensu stricto spirochetes and are not affected by *Ixodes ricinus* tick saliva. *Journal of Immunology* 189: 5393–5401. [IF = 5.520]
- **Rudenko N.**, **Golovchenko M.**, **Hönig V.**, Mallátová N., Krbková L., Mikulášek P., Fedorova N., Belfiore N., **Grubhoffer L.**, Lane R, Oliver Jr. J.H. 2013: Detection of *Borrelia burgdorferi* sensu stricto ospC alleles associated with human Lyme borreliosis worldwide in non-human-biting tick *Ixodes affinis* and rodent hosts in Southeastern United States. *Applied and Enviromental Microbiology* 79: 1444–1453. [IF = 3.952]
- Schwarz A., **Hönig V.**, **Vavrušková Z.**, **Grubhoffer L.**, Balczun C., Albring A., Schaub G.A. 2012: Abundance of *Ixodes ricinus* and prevalence of *Borrelia burgdorferi* s.l. in the nature reserve Siebengebirge, Germany, in comparison to three former studies from 1978 onwards. *Parasites & Vectors* 5: 268.[IF=3.246]
- Vancová M., **Štěrba J.**, **Dupejová J.**, Simonová Z., Nebesářová J., Novotný M.V., **Grubhoffer L.** 2012: Uptake and incorporation of sialic acid by the tick *Ixodes ricinus*. *Journal of Insect Physiology* 58: 1277–1287. [IF=2.379]

Research projects

- **POSTICK – Training Network on Ticks and Tick-Borne Diseases.** FP7-PEOPLE-ITN Marie-Curie project (P.I.: L. Grubhoffer; 2010–2013)
- **Population structure of the Lyme borreliosis spirochete *Borrelia burgdorferi* sensu stricto in the southeastern United States.** National Institute of Allergy and Infectious Diseases, USA (CZB1-2963-CB-09; P.I.: M.Golovchenko; 2010–2012)
- **Distribution and diversity of *Borrelia bissettii* in the southeastern United States.** National Institute of Allergy and Infectious Diseases, USA (CZB1-2966-CB-09; P.I.: N. Rudenko; 2010–2012)
- **Antimicrobial peptides in the immune system of competent and noncompetent vectors of the *Borrelia burgdorferi* spirochetes.** Grant Agency of the Czech Republic (302/11/1901; P.I.: L. Grubhoffer; 2011–2013)
- **Tick-borne encephalitis virus-host interaction on the molecular, cellular and organismal level.** Grant Agency of the Czech Republic (302/12/2490; P.I.: L. Grubhoffer; 2012–2014)
- **ANTIGONE – ANTIcipating the GlobalOnset of Novel Epidemics.** FP7 HEALTH project (278976; P.I.: L. Grubhoffer; 2011–2016)
- **ANTIDotE (Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe).** FP7 EU-HEALTH 2013.2.3.4-1 (P.I.: L. Grubhoffer; 2013–2018)

3.2. Laboratory of Vector-Host Interactions

(at present, Laboratory of Arbovirology; head D. Růžek)

Research scientists:	Prof. RNDr. **Jan KOPECKÝ**, CSc. (*head*) Mgr. **Jaroslava Lieskovská**, PhD; doc. RNDr. **Daniel Růžek**, PhD; RNDr. **Jiří Salát**, PhD; RNDr. **Jindřich Chmelař**, PhD; RNDr. **Helena Langhansová-Horká**, PhD
PhD students:	Mgr. **Jana Elsterová**; Mgr. **Jana Páleníková**; Mgr. **Martin Palus**
Research assistant:	Bc. **Veronika Slavíková**
Technicians:	**Jan Erhart**, **Eva Výletová**
Laboratory worker:	**Lenka Marešová**
Undergraduate students:	Bc. **Hana Chytráčková**; Bc. **Adéla Harcubová**; Bc. **Helena Huspeková**; Bc. **Šárka Koudelková**; Bc. **Šárka Pospíšilová**; Bc. **Jana Širmarová**; **Adéla Chlastáková**; **Eliška Maršálková**; **Ivana Pokorná**

Research priorities

Immunomodulatory effects of *Ixodes ricinus* tick saliva on the host and their role in tick-borne pathogen transmission. Investigation of the pathogenesis and molecular epidemiology of tick-borne encephalitis.

Effect of tick cystatins on experimental asthma

Ticks developed a multitude of different immune evasion strategies to obtain a blood meal. Sialostatin L is an immunosuppressive cysteine protease inhibitor present in the saliva of the hard tick *Ixodes scapularis*. We demonstrated that sialostatin L strongly inhibits the production of IL-9 by Th9 cells. Since we showed recently that Th9-derived IL-9 is essentially involved in the induction of asthma symptoms, sialostatin L was used for the treatment of experimental asthma. Application of sialostatin L almost completely abrogated airway hyperresponsiveness and eosinophilia. Our data suggest that sialostatin L can prevent experimental asthma, most likely by inhibiting the IL-9 production of Th9 cells. Thus, sialostatin L provides the basis for the development of innovative therapeutic strategies to treat asthma.

Effect of tick saliva on cell signalling pathways

Type I interferons (IFN-α and IFN-β) are crucial determinants of the host immune response and tick saliva modulates this response, thus facilitating the transmission of tickborne pathogens. Our study examines the effect of saliva *Ixodes ricinus* saliva on IFN-β signalling in murine dendritic cells using lipopolysaccharide (LPS) and *Borrelia afzelii* spirochaetes as inducers. Activated dendritic cells secret IFN that activates Signal Transducer and Activator of Transcription 1 (STAT-1). Our results show that *Borrelia*-induced activation of STAT-1 was suppressed by tick saliva. As the amount of secreted IFN-β was not influenced by tick saliva, the results indicated that saliva affected the interferon pathway at the IFN receptor or downstream of it. By using recombinant IFN-β, we show that tick saliva attenuates IFN-triggered STAT-1 activation.

Pathogenesis of tick-borne encephalitis

Tick-borne encephalitis (TBE) is the most important viral neuroinfection in Europe and north-eastern Asia. In our laboratory, we investigate the interaction between TBE virus with human neural cells (neurons, astrocytes and pericytes), role of the blood-brain barier in the development of TBE and the effect of host genotype on the severity of TBE. Moreover, we also participated in ecological and phylogeographical studies focused on TBE in the Czech Republic, Germany, Switzerland and Russia, and in the description of the first case of TBE in Australia.

Selected publications

- **Horká H.**, Staudt V., Klein M., Taube C., Reuter S., Dehzad N., Andersen J.F., **Kopecký J.**, Schild H., Kotsyfakis M., Hoffmann M., Gerlitzki B., Stassen M., Bopp T., Schmitt E. 2012: The tick salivary protein sialostatin L inhibits the Th9-derived production of the asthma-promoting cytokine IL-9 and is effective in the prevention of experimental asthma. *Journal of Immunology* 188: 2669–2676. [IF=5.520]
- **Lieskovská J.**, **Kopecký J.** 2012: Tick saliva suppresses IFN signaling in dendritic cells upon *Borrelia afzelii* infection. *Parasite Immunology* 34: 32–39. [IF=2.208]
- **Palus M.**, Vojtíšková J., **Salát J.**, **Kopecký J.**, Grubhoffer L., Lipoldová M., Demant P., **Růžek D.** 2013: Mice with different susceptibility to tick-borne encephalitis virus infection show selective neutralizing antibody response and inflammatory reaction in the central nervous system. *Journal of Neuroinflammation* 10: 77. [IF=4.902]
- **Růžek D.**, Dobler G., Niller H.H. 2013: May early intervention with high dose intravenous immunoglobulin pose a potentially successful treatment for severe cases of tick-borne encephalitis? *BMC Infectious Diseases* 13: 306. [IF=2.561]
- Weidmann M., Frey S., Freire C.C., Essbauer S., **Růžek D.**, Klempa B., Zubrikova D., Vögerl M., Pfeffer M., Hufert F.T., Zanotto P.M., Dobler G. 2013: Molecular phylogeography of tick-borne encephalitis virus in central Europe. *Journal of General Virology* 94: 2129–2139. [IF=3.529]

Research projects

- **Effect of tick saliva cystatins on Th9 cells and the development of experimental asthma.** Grant Agency of the Czech Republic (P302/11/J029; P.I.: Jan Kopecký; 2011–2013)
- **Effect of tick saliva on signalling pathways activated by *Borrelia* pathogen in dendritic cells.** Grant Agency of the Czech Republic (P302/12/2208; P.I.: Jan Kopecký; 2012–2015)
- **Role of the blood-brain barrier in the neuropathogenesis of tick-borne encephalitis.** Grant Agency of the Czech Republic (P302/10/P438; P.I.: D. Růžek; 2010–2012)
- **Differences in the clinical course of tick-borne encephalitis and their genetic determination.** Grant Agency of the Czech Republic (P502/11/2116; P.I.: D. Růžek; 2011–2015)

4. Biology of Disease Vectors

4.1. Laboratory of Vector Immunology

Research scientists:	RNDr. **Petr KOPÁČEK**, CSc. (head) RNDr. **Lenka Grunclová**, PhD; RNDr. **Daniel Sojka**, PhD; RNDr. **Radek Šíma**, PhD*; RNDr. **Veronika Urbanová-Burešová**, PhD*
PhD students:	RNDr. **Marie Jalovecká***; RNDr. **Zdeněk Franta**; RNDr. **Helena Frantová-Pěničková** (maternity leave since 2012), Mgr. **Jan Perner**
Undergraduate students:	Bc. **Petr Franta**; Bc. **David Hartmann**; Bc. **Jitka Konvičková**; Bc. **Matěj Kučera**; Bc. **Jana Schrenková**

* Also members of the research team of Ondřej Hajdušek.

Research priorities

Molecules involved in the tick innate immunity playing a role at the tick-pathogen interface. Molecular physiology of blood digestion in ticks as a potential target for efficient anti-tick intervention.

The role of primordial complement system in the tick immunity

The hard tick *Ixodes ricinus* possesses components of priomordial complement system such as thioester-containing proteins, fibrinogen-related lectins (ixoderins) and putative convertases. In the years 2012–2013, we focused mainly on the characterisation and function of two putative C3-complement convertases from *I. ricinus* referred to as Factor C (IrFC) and Factor B/C2 (IrFB/C2). Both molecules are multi-domain serine proteases containing several sushi (CCP) domains. IrFC is related to *Limulus* factor C (IrFC) and is responsive to the injury suggesting that it plays a role in hemolymph clotting or wound healing. Further continued the study of all nine tick TEPs in the phagocytosis of microbes including the yeast *Candida albicans* and the Lyme diseases spirochete *Borrelia afzelii*.

Blood digestion in ticks – what is haemoglobin good for in the tick diet?

We have completed the characterisation of the multi-enzyme network involved in the digestion hemoglobin during the feeding phase of *I. ricinus* on the host (review paper in *Trends in Parasitology*). Implementation of the *in vitro* feeding of adult *I. ricinus* on artificial membrane made it possible to feed ticks on full or hemoglobin-depleted blood (serum). Surprisingly, the ticks fed on serum were capable to fully engorge and layed equal amount of eggs as ticks fed on the full blood. However, no larvae hatched from the eggs from the serum-fed ticks. Further experiments revealed that hemoglobin is vitally important for tick development as a source of heme but not amino-acids or iron.

‘Anti-tick’ vaccine development

An attempt continued to develop a vaccine based on recombinant tick ferritin-2 from *I. ricinus* and *Rhipicephalus* (*Boophilus*) *microplus*. An important achievement in this applied research activity was the implementation of the complete laboratory transmission model for *Borellia afzelii* and several strains of *Borelia burgdorferi* sensu stricto. This transmission models will allow us or potential partners from the R&D of veterinary pharma companies to investigate the potential of other vaccine candidates based either on concealed (from the midgut) or exposed (from the saliva) antigens against transmission of Lyme disease.

Selected publications

- Hajdušek O., **Šíma R.**, Ayllon N., **Jalovecká M.**, **Perner J.**, de la Fuente J., **Kopáček P.** 2013: Interaction of the tick immune system with transmitted pathogens. *Frontiers in Cellular and Infection Microbiology* 3: 26. [IF=2.620]
- **Kopáček P.**, Hajdušek O., **Burešová V.** 2012: Chapter 9. Tick as a model for the study of a primitive complement system. In: E. Mylonakis, F.M. Ausubel, M. Gilmore and A. Casadevall (Eds.), *Advances in Experimental and Medical Biology* 710. Springer-Verlag, Berlin, pp. 83–93. [IF=1.825]
- **Sojka D.**, **Franta Z.**, **Frantová H.**, Bartošová P., Horn M., Váchová J., O’Donoghue A.J., Eroy-Reveles A.A., Craik C.S., Knudsen G.M., Caffrey C.R., McKerrow J.H., Mareš M., **Kopáček P.** 2012: Characterization of gut-associated cathepsin D hemoglobinase from tick *Ixodes ricinus* (IrCD1). *Journal of Biological Chemistry* 287: 21152–21163. [IF=4.651]
- **Sojka D.**, **Franta Z.**, Horn M., Caffrey C.R., Mareš M., **Kopáček P.** 2013: New insights into the machinery of blood digestion by ticks. *Trends in Parasitology* 29: 276–285. [IF=6.217]

Research projects

- **The digestive system of ticks – the target for rational development of vaccine against ticks and tickborne pathogens.** Grant Agency of the Academy of Sciences of the Czech Republic (IAA600960910; P.I.: P. Kopáček; 2009–2012)
- **Functional genomics of the complement-like molecules in the tick *Ixodes ricinus*.** Grant Agency of the Czech Republic (P506/10/2136; P.I.: P. Kopáček; 2010–2012)
- **Structural proteomics of proteolytic systems in ticks.** Grant Agency of the Czech Republic (P207/10/2183; Co-P.I.: P. Kopáček; 2010–2012)
- **Endocytosis of the host hemoglobin in tick gut cells.** Grant Agency of the Czech Republic (GPP502/11/P682; P.I.: D. Sojka; 2011–2013)
- **The role of hemoglobin in tick metabolism and transmission of tick-borne pathogens.** Grant Agency of the Czech Republic (13-110435S; P.I.: P. Kopáček; 2013–2017)
- **Development of a ferritin 2-based vaccine preventing against tick transmitted diseases for veterinary and human use.** Ministry of Industry and Trade of the Czech Republic (FR-TI3/156; Co-P.I.: P. Kopáček; 2011–2014)
- **Development of protocol for obtaining monospecifically infected ticks and their correct application to experimental animals.** Ministry of Industry and Trade of the Czech Republic (FR-TI4/2012; Co-P.I.: P. Kopáček; 2012–2014)

4.2. Laboratory of Genomics and Proteomics of Disease Vectors

Research scientists:	**Michalis KOTSYFAKIS**, MSc, PhD (Greece) (head) RNDr. **Jindřich Chmelař**, PhD (till December 2013); **Alexandra Schwarz**, MSc, PhD (Germany; till February 2014); **James J. Valdés**, MSc, PhD (USA; till January 2013)
PhD students:	Mgr. **Veronika Dorňáková** (till August 2013); Mgr. **Jan Kotál**
Administration associates:	RNDr. **Petra Rozkošná** (till December 2012); Mgr. **Markéta Kremlová** (since January 2013)
Undergraduate student:	**Lovelyna Eromonsele** (Austria)
Technicians:	Ing. **Martina Dědouchová**; **Luisa Pellarová** (January–February 2013)

Research priorities

Investigation of the immunomodulatory effects of tick saliva on the host and their role in tick-borne pathogen transmission. Characterisation of arthropod serine and cysteine protease inhibitors, mainly from the ticks *Ixodes ricinus*, the vector of Lyme disease in Europe, and *I. scapularis*, the vector of Lyme disease in the eastern and central USA.

Characterisation of the potential immunomodulatory function of different tick cysteine and serine protease inhibitors

The potential effects of various tick cysteine and serine protease inhibitors in macrophage, neutrophil and monocyte activation were investigated. Immune cells were activated either by LPS or PMA in the presence/absence of tick inhibitors. The transcription level of immune-related genes was estimated by RT-PCR.

Characterisation of the serine protease inhibitor tryptogalinin

Ticks use Kunitz peptides (among other salivary proteins) to combat host defence mechanisms and to obtain a blood meal. Most of these Kunitz peptides, however, remain functionally uncharacterized, thus limiting our knowledge about the molecular events upon host-tick interactions. During 2013, we published in *PLoS ONE* the biochemical characterization of Tryptogalinin, a peptide that inhibits several serine proteases with high affinity; we named this novel Kunitz as tryptogalinin since it inhibits human skin b-tryptase (HSTb). Tryptogalinin is phylogenetically related to TdPI, another Kunitz peptide from *Rhipicephalus appendiculatus*, also reported to have a high affinity for b-tryptase. Using homology-based modelling (and other protein prediction programs) we were able to explain the multifaceted function of tryptogalinin.

Characterisation of the serine protease inhibitor elastofilin

Elastofilin is a tick salivary serine protease inhibitor shown (in our preliminary results) to affect the establishment of *Borrelia burgdorferi* in the host (by using a murine model of the disease). Unfortunately, our additional experiments showed that the observed effect of elastofilin in

Borrelia transmission could be partially attributed to a contamination of the recombinant protein with LPS. Accordingly, research effort during 2013 was directed to the large scale production of a new batch of recombinant elastofilin in LPS-free formulation. The large scale production of a new batch of recombinant elastofilin in LPS-free formulation proved that the protein does not play a role in *B. burgdorferi* transmission. It also gave us the opportunity to test the protein in an animal model of OVA-induced allergic inflammation of mouse lungs. Elastofilin drastically inhibited the recruitment of immune cells in the inflamed lungs of the mice and thus we demonstrated its immunomodulatory function.

Characterisation of tick cysteine protease inhibitors

The recombinant polypeptides which are encoded by two newly discovered cystatin genes of *I. ricinus* (named as A and B due to the requirements of the patent law) were isolated from bacterial cultures after inducing gene overexpression with IPTG. Real Time PCR has previously confirmed the presence of transcripts encoding for both polypeptides in *I. ricinus* salivary glands and according to the disclosed experimental plan of the grant we performed animal vaccination experiments with both proteins to test the potential of both proteins for vaccine development that will confer animal protection against tick feeding. Unfortunately, none of the two proteins confered protection of the vaccinated animals.

Selected publications

- Horká H., Staudt V., Klein M., Taube C., Reuter S., Dehzad N., Andersen J.F., Kopecký J., Schild H., **Kotsyfakis M.**, Hoffmann M., Gerlitzki B., Stassen M., Bopp T., Schmitt E. 2012: The tick salivary protein sialostatin L inhibits the Th9-derived production of the asthma-promoting cytokine IL-9 and is effective in the prevention of experimental asthma. *Journal of Immunology* 188: 2669–2676. [IF=5.520]
- Ma D., Mizurini D.M., Assumpção T.C.F., Li Y., Qi Y., **Kotsyfakis M.**, Ribeiro J.M.C., Monteiro R.Q., Francischetti I.M.B. 2013: Desmolaris, a novel Factor XIa anticoagulant from the salivary gland of the vampire bat (*Desmodus rotundus*) inhibits inflammation and thrombosis. *Blood* 122: 4094–4106. [IF=9.775]
- **Schwarz A.**, von Reumont B.M., Erhart J., Chagas A.C., Ribeiro J.M.C., **Kotsyfakis M.** 2013: De novo *Ixodes ricinus* salivary transcriptome analysis using two different next generation sequencing methodologies. *FASEB Journal* 27: 4745–4756. [IF=5.480]
- Tsujimoto H., **Kotsyfakis M.**, Francischetti I.M., Eum J.H., Strand M.R., Champagne D.E. 2012: Simukunin from the salivary glands of the black fly *Simulium vittatum* inhibits enzymes that regulate clotting and inflammatory responses. *PLoS ONE* 7: e29964. [IF=3.730]
- Valdés J.J., **Schwarz A.**, Cabeza de Vaca I., Calvo E., Pedra J.H.F., Guallar V., **Kotsyfakis M.** 2013: Tryptogalinin is a tick Kunitz serine protease inhibitor with a unique intrinsic disorder. *PLoS ONE* 8: e62562. [IF=3.534]

Research projects

- **Exploring the salivary transcriptome of *Ixodes ricinus*, the Lyme disease vector in Europe, and the potential role of its cystatins in pathogen transmission.** Marie Curie EU FP7 Reintegration grant (PIRG07-GA-2010-268177; P.I.: M. Kotsyfakis; 2010–2014)
- **Rickettsial immunity during tick transmission.** National Institutes of Health, USA, R01 grant (1R01AI093653-01A1; P.I.: J. Pedra; 2011–2016)
- **Effect of tick saliva cystatins on Th9 cells and the development of experimental asthma.** Bilateral collaborative grant between German and Czech Research Institutions, Grant Agency of Czech Republic (P302/11/J029; P.I.: J. Kopecký, E. Smitt; 2011–2013)
- **The role of tick salivary protease inhibitors in tick-pathogen-host interactions.** Grant Agency of Czech Republic (P502/12/2409; P.I.: M. Kotsyfakis; 2012–2015)

4.3. Laboratory of Tick Transmitted Diseases

Research scientists:	RNDr. **Ondřej HAJDUŠEK**, PhD (head) RNDr. **Radek Šíma**, PhD*; RNDr. **Veronika Urbanová-Burešová**, PhD*
PhD student:	RNDr. **Marie Jalovecká***
Undergraduate students:	Bc. **Helena Mondeková**; Bc. **Jiří Tápal**; **Tereza Pospíšilová**; **Zuzana Zemanová**
Technicians:	Mgr. **Adéla Harcubová**; Ing. **Gabriela Loosová**; MVDr. **Gabriela Štěpánová**

* Also members of the research team of Petr Kopáček.

Research priorities

Laboratory of Tick Transmitted Diseases (founded in 2012) is focused on the molecular interactions between ticks (e.g. iron and heme metabolism pathway, tick immune molecules) and tick-transmitted pathogens and testing of anti-tick vaccines (improvement of the recent vaccine based on Ferritin 2) and vaccines interfering with the pathogen transmission. We have set-up in our laboratory (BSL2) complete transmission model for *Borrelia* infections, which we use for testing of the tick candidate genes implicated in the tick-parasite interactions using method of RNA interference (RNAi) and also vaccines blocking the pathogen transmission. Recently, we make an effort to set-up a system for testing infections of *Babesia* and *Anaplasma*. The laboratory works in a close collaboration with the Laboratory of Vector Immunology (head: P. Kopáček).

Antigens for a new vaccine against ticks and tick-transmitted diseases

Ticks are blood-feeding parasites and vectors of some of the most devastating viral, bacterial and protozoal diseases known to humans and animals. *Ixodes ricinus* is a common tick in the Czech Republic and Europe, transmitting tick-borne encephalitis (TBE), Lyme disease (borreliosis), anaplasmosis and babesiosis. Immunization of the hosts using recombinant tick proteins reduces tick feeding and, more importantly, blocks transmission of pathogens from the tick into the host. However, available tick antigens still do not reach sufficient efficacy. We use RNA interference (RNAi) to screen genes of *I. ricinus* potentially involved in the tick iron metabolism and heme acquisition in order to find suitable vaccine candidates affecting tick feeding and development. These candidates will be then tested for their potential to inhibit transmission of the pathogens. We believe that vaccination with these proteins may have a great potential as a control strategy to reduce tick feeding and transmission of pathogens.

Lyme disease and babesiosis transmission models

Lyme borreliosis is an emerging vector-borne disease of temperate climates with a concurrent distribution spanning North America and Eurasia. It is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex, which are transmitted through the *Ixodes* ticks. Although Lyme

borreliosis is one of the best studied tick-borne zoonosis, the annual incidence leads over other vector-borne diseases with a continuous increase. There is currently no vaccine available to prevent Lyme disease in humans. One of the promising strategies to break *Borrelia* transmission development is a vaccine affecting basic tick physiological processes. Development of a promising vaccine against Lyme borreliosis would be greatly facilitated by a reproducible vector host transmission model. Our aim is to implement such model to find a molecule with proven anti-borrelial effect.

Babesiosis is a tick-borne malaria-like disease of mammals. Because of the global environmental changes and continuous expansion of tick range, importance of babesiosis as an emerging zoonosis is increasing. Interplay between the parasite, tick, and vertebrate host represents a complex system of multiple molecular interactions. To date, only a limited number of molecules have been identified to play a role in this system. Our research is focused on the identification and characterization of molecular mechanisms of *Babesia* persistence within the tick vector and its transmission to the vertebrate host. We are currently working on the setting of *Babesia microti* transmission model in our laboratory and use of this model for testing of the tick immune genes in *Babesia* infection by RNA interference and vaccination.

Selected publications

- Alama-Bermejo G., **Šíma R.**, Raga J.A., Holzer A.S. 2013: Understanding myxozoan infection dynamics in the sea: seasonality and transmission of *Ceratomyxa puntazzi*. *International Journal for Parasitology* 43: 771–780. [IF=3.404]
- **Hajdušek O.**, **Šíma R.**, Ayllon N., **Jalovecká M.**, Perner J., de la Fuente J., Kopáček P. 2013: Interaction of the tick immune system with transmitted pathogens. *Frontiers in Cellular and Infection Microbiology* 3: 26. [IF=2.620]
- Kopáček P., **Hajdušek O.**, **Burešová V.** 2012: Tick as a model for the study of a primitive complement system. *Advances in Experimental Medicine and Biology* 710: 83–93. [IF=1.825]

Research projects

- **ANTIDotE – Anti-tick vaccines to prevent tick-borne diseases in Europe.** FP7 HEALTH project (P.I.: J. Hovius, 2014–2018)
- **Proteins of the tick iron metabolism pathway – antigens for a new vaccine against ticks and tick-transmitted diseases.** Grant Agency of the Czech Republic (13-27630P; P.I.: O. Hajdušek; 2013–2015)
- **Lyme disease transmission model: an essential tool to study vaccine candidates against human borreliosis.** Grant Agency of the Czech Republic (13-12816P; P.I.: R. Šíma; 2013–2015)

5. Fish parasitology

5.1. Laboratory of Helminthology

Research scientists:	Prof. RNDr. **Tomáš SCHOLZ**, CSc. (head) RNDr. **František Moravec**, DrSc. (researcher emeritus) RNDr. **Jan Brabec**, PhD; RNDr. **Anna Faltýnková**, PhD; **Aneta Kostadinova**, MSc, PhD (Bulgaria); RNDr. **Roman Kuchta**, PhD; Mgr. **Miroslava Soldánová**, PhD Temporary contracts from projects: RNDr. **Eva Bazsalovicsová**, PhD (Slovakia); **David González-Solís**, MSc, PhD (Mexico); RNDr. **Mikuláš Oros**, PhD; RNDr. **Martina Orosová**, PhD (both Slovakia); **Jesus Hernández-Orts**, MSc, PhD (Mexico); **Bjoern C. Schaeffner**, MSc, PhD (Germany); **Aneta Yoneva**, MSc, PhD (Bulgaria)
PhD students:	**Anirban Ash**, MSc (India); **Simona Georgieva**, MSc (Bulgaria); **Carlos A. Mendoza-Palmero**, MSc (Mexico)
Research assistants:	Ing. **Radmila Řepová** (part time); Ing. **Blanka Škoríková**
Technician:	**Martina Borovková**
Undergraduate students:	Bc. **Lenka Čapková**; Bc. **Kateřina Leštinová**; Bc. **Ivana Vlnová**; Bc. **Jana Zikmundová**; **Eliška Panáčková**; **Markéta Pilařová**

Research priorities

Morphology, systematics and phylogeny of tapeworms (Cestoda) and other endohelminths parasitic in freshwater and marine fish, including causative agents of fish-borne diseases (broad fish tapeworm – *Diphyllobothrium*), and life-cycles, ecology and molecular taxonomy of larval stages of trematodes (Digenea) in freshwater molluscs and fish.

Diversity of helminths parasitising teleost fish

Morphological and taxonomic evaluation of parasitic flatworms (Cestoda, Digenea and Monogenea) and nematodes (Nematoda), parasites of freshwater and marine fish, made it possible to provide new data on the species richness, systematics, host specificity and geographical distribution of numerous taxa, many of them having been described as new for science. Biodiversity studies have been focused on hot spots of teleost diversity and ecosystems under high anthropogenic pressure, such as Africa, Amazonia and South East Asia.

Systematics and evolution of basal tapeworms (Cestoda)

Using combination of morphological, electron microscopical and molecular methods, the evolutionary history of the basal groups of tapeworms (Cestoda) has been studied. Several species-rich genera of monozoic (nonsegmented) tapeworms (Caryophyllidea) have been revised and their phylogenetic relationships have been assessed based on comparative analyses of two nuclear and two mitochondrial genes.

Integrative taxonomy approaches to trematode diversity and life-cycles

A series of studies focused on species delimitation using integrated molecular, morphological and ecological evidence provided reliable estimates of the diversity and/or information on the life-histories of the digenean trematodes (families Diplostomidae and Echinostomatidae) in natural populations in Europe and Africa.

Selected publications

- **Ash A.**, **Scholz T.**, de Chambrier A., **Brabec J.**, **Oros M.**, Kar P.K., Chavan S.P., Mariaux J. 2012: Revision of *Gangesia* (Cestoda: Proteocephalidea) in the Indomalayan region: morphology, molecules and surface ultrastructure. *PLoS ONE* 7: e46421. [IF=3.730]
- **Brabec J.**, **Scholz T.**, Kraľová-Hromadová I., **Bazsalovicsová E.**, Olson P.D. 2012: Substitution saturation and nuclear paralogs of commonly employed phylogenetic markers in the Caryophyllidea, an unusual group of non-segmented tapeworms (Platyhelminthes). *International Journal for Parasitology* 42: 259–267. [IF=3.637]
- **Georgieva S.**, **Soldánová M.**, Pérez-del-Olmo A., Dangel R.D., Sitko J., Sures B., **Kostadinova A.** 2013: Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity. *International Journal for Parasitology* 43: 57–72. [IF=3.404]
- **Kuchta R.**, **Brabec J.**, Kubáčková P., **Scholz T.** 2013: Tapeworm *Diphyllobothrium dendriticum* (Cestoda) – neglected or emerging human parasite? A review. *PLoS Neglected Tropical Diseases* 7: e2535. [IF=4.489]
- **Moravec F.** 2013: Parasitic Nematodes of Freshwater Fishes of Europe. Revised second edition. Academia, Prague, 601 pp. ISBN: 978-80-200-2272-1

Research projects

- **A Survey of the Tapeworms (Cestoda: Platyhelminthes) from the Vertebrate Bowels of the Earth.** National Science Foundation, USA (Planetary Biodiversity Inventory, Co-P.I.: T. Scholz; P.I.: J.N. Caira, University of Connecticut, Storrs; 2008–2013)
- **ECIP – European Centre of Ichthyoparasitology.** Grant Agency of the Czech Republic – program of centres of excellence (P505/12/G112; Co-P.I.: T. Scholz; P.I.: M. Gelnar, Masaryk University, Brno; 2012–2018)
- **PARAPOPGENE – Comparative genetic patterns in parasite populations and species: the search for structuring forces.** FP7-PEOPLE-2009 IOF (M.I. Blasco Costa; Marie Curie Action; project No. GA 252124; P.I.: T. Scholz; 2010–2013)
- **Revision of monozoic tapeworms (Cestoda: Caryophyllidea): a key to understanding the evolution of cestodes?** Grant Agency of the Czech Republic (524/08/0885; P.I.: T. Scholz; 2008–2012)
- **Trematode communities in molluscs as a model system to forecast the impact of climate change in freshwater ecosystems in Central Europe.** Grant Agency of the Czech Republic (P505/10/1562; P.I.: A. Faltýnková and A. Kostadinova; 2010–2013)
- **From fish to man and from water to the earth: evolutionary history of tapeworms parasitizing tetrapodes (Cestoda: Diphyllobothriidea).** Grant Agency of the Czech Republic (P506/12/1632; P.I.: R. Kuchta; 2012–2016)
- **Host-parasite relationships and evolution of parasitism.** Grant Agency of the Czech Republic (206/09/H026 – project to support PhD studies; P.I.: T. Scholz; 2009–2012)
- **PARATUN: Parasites of Atlantic bluefin tuna (*Thunnus thynnus*) in natural populations and aquaculture.** Parasite communities and associated pathologies. Ministry of Science and Innovation of Spain (AGL2010-20892; Co-P.I. A. Kostadinova; P.I.: F.E. Montero, University of Valencia, Spain; 2011–2013)
- **BIOFRESH Database: Freshwater Parasites.** FP7 Biofresh Contingency Fund (226874; P.I. A. Kostadinova; 2012–2013)
- **Integrative taxonomy: a powerful tool to unravel hidden diversity of fish parasites in Brazil.** CAPES, program "Ciência sem fronteiras" – visitant researcher modality (No. 135/2012; P.I.: T. Scholz; 2013–2015)

5.2. Laboratory of Fish Protistology

Research scientists:	**Astrid HOLZER**, PhD (Austria) (head) **Gema Alama-Bermejo**, MSc, PhD (Spain); RNDr. **Pavla Sojková-Bartošová**, PhD (Slovakia); RNDr. **Ivan Fiala**, PhD; **Ashlie Hartigan**, MSc, PhD (Australia); RNDr. **Miloslav Jirků**, PhD; RNDr. **Martin Kostka**, PhD
PhD students:	RNDr. **Alena Kodádková**; **Sneha Patra** MSc (India); RNDr. **Tomáš Tyml**
Research assistant:	RNDr. **Hana Pecková**
Technician:	**Marie Fučíková** (part time)
Laboratory worker:	**Ivana Reitingerová**
Undergraduate students:	Bc. **Martina Cinková**; Bc. **Marie Hlavničková**; Bc. **Martina Hrabcová-Loudová**; **Martina Jedličková**; **Jiří Kyslík**; **Kamila Štauberová**; **Klára Zítková**

Research priorities

Our main interests are eukaryotic microorganisms infecting fish and amphibians, including all aspects of their structure, biology, life cycles, host-parasite relationships and especially their phylogeny and evolution. More recently, we have initiated a new line of functional research focusing on parasite motility and on the analysis of transcriptomic data. Our two main focuses are on myxozoans and amoeboid organisms. We furthermore carry out research into a range of parasitic protists that create economic and health consequences for the aquaculture industry, in collaboration with various academic and commercial partners worldwide.

Myxozoa

Phylogenetic relationships among a variety of taxa inhabiting freshwater and marine environments and using frogs and fish as intermediate hosts were studied on the basis of ribosomal (SSU and LSU rDNA) and protein-coding genes (EF-2). A large focus was given to the taxonomy, diversity, cryptic species and the evolution of *Sphaerospora* and *Polysporoplasma* spp. The results of the combination of morphological, biological and DNA sequence data led to the amendment of the genus *Sphaerospora*. The design of specific PCR assays and *in situ* hybridisation protocols contributed important information on the host specificity and localisation of two sphaerosporid pathogens in European pond-reared common carp. The first part of our motility studies focused on pre-sporogonic stages of the bile myxozoan *Ceratomyxa puntazzi* from Mediterranean sharpsnout seabream. We demonstrated that F-actin-rich cytoskeletal elements polarise at one end of the parasites and in the filopodia which are rapidly created *de novo* and re-absorbed. We discovered that the mechanism of budding is an active polarisation process of cytokinesis, which is independent from a contractile ring and thus differs from the mechanism generally observed in eukaryotic cells.

Amoebae

Research on Amoebae is focused on amphizoic species (*Naegleria*, *Acanthamoeba* and *Paramoeba* spp.) and their relation to fish pathology as well as on free-living species (*Hartmanella*, *Flabella*, *Vermistella*, etc.) and their phylogeography and biology. A large part of the culture collection of amoebae of the Laboratory of Fish Protistology was documented in detail with regard to (ultra)structure and molecular phylogeny in the comprehensive monograph "Illustrated Guide to Culture Collection of Free-living Amoebae".

A commercial project financed by and elaborated with the Skretting Aquaculture Research Centre is focused on the identification of amoebic gill disease (AGD) agents from various Atlantic salmon culture sites in Scotland and on testing of potential amoeboicidal substances in cell cultures of *Paramoeba* spp. as well as determining the survival of amoebae when exposed to mucus samples from salmon, which had received different in-feed treatments. The results form an important basis for developing anti-parasitic diets for Atlantic salmon.

Selected publications

- **Alama-Bermejo G.**, Bron J.E., Raga J.A., **Holzer A.S.** 2012: 3D morphology, ultrastructure and development of *Ceratomyxa puntazzi* stages: first insights into the mechanisms of motility and budding in the Myxozoa. *PLoS ONE* 7: e32679. [IF=3.730]
- **Alama-Bermejo G.**, Šíma R., Raga J.A., **Holzer A.S.** 2013: Understanding myxozoan infection dynamics in the sea: seasonality and transmission of *Ceratomyxa puntazzi*. *International Journal for Parasitology* 43: 771–780. [IF=3.404]
- **Bartošová P.**, **Fiala I.**, Cinková M., Jirků M., Caffara M., Fioravanti M.L., Atkinson S.D., Bartholomew J.L., **Holzer A.S.** 2013: *Sphaerospora* sensu stricto: taxonomy, diversity and evolution of a unique lineage of myxosporeans (Myxozoa). *Molecular Phylogenetics and Evolution* 68: 93–105. [IF=4.018]
- **Jirků M.**, Kvičerová J., Modrý D., Hypša V. 2013: Evolutionary plasticity in coccidia – striking morphological similarity of unrelated coccidia (Apicomplexa) from related hosts: *Eimeria* spp. from African and Asian pangolins (Mammalia: Pholidota). *Protist* 164: 470–481 [IF=3.558].
- Pawlowski J., Audic S., Adl S., Bass D., Belbahri L., Berney C., Bowser S.S., Čepička I., Decelle J., Dunthorn M., Fiore-Donno A.M., Gile G.H., Holzmann M., Jahn R., **Jirků M.**, Keeling P.J., **Kostka M.**, Kudryavtsev A., Lara E., Lukeš J., Mann D.G., Mitchell E.A.D., Nitsche F., Romeralo M., Saunders G.W., Simpson A.G.B., Smirnov A.V., Spouge J.L., Stern R.F., Stoeck T., Zimmermann J., Schindel D., de Vargas C. 2012 CBOL Protist Working Group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. *PLoS Biology* 10: e1001419 [IF=12.690].

Research projects

- **MODBIOLIN - Use of model organisms to resolve Crucial biological problems on the path to innovations.** European Commission (FP7-REGPOT-2012-2013-1; Coordinator: F. Sehnal, I. Co-I.: A.S. Holzer, II. Co-I.:I. Fiala; 2013–2015)
- **A new approach for the comparative study of the life cycle of Myxozoa - Identification of genes and cellular components Important for proliferation of parasites.** AS CR Program for international collaboration (M200961205; P.I.: A.S. Holzer; 2012–2014)
- **ECIP - European Centre Ichtyoparazitology.** Centre of Excellence, Grant Agency of the Czech Republic (505/12/G112; Coordinator: M. Gelnar, Masaryk University, Brno, I. Co-I.: A.S. Holzer, II. Co-I.:I. Fiala; 2012–2018)
- **Biology and phylogeny of sphaerosporid myxosporeans from economically important fish: Application of molecular tools.** Czech Science Foundation (506/11/P724; P.I.: P. Bartošová; 2011–2013)
- **Opening new chapter: diversity, biology and phylogeny of Myxozoa parasitizing Amphibia.** Czech Science Foundation (506/10/2330; P.I.: M. Jirků; 2010–2013)

6. Opportunistic diseases

6.1. Laboratory of Veterinary and Medical Protistology

Research scientists:	Doc. Ing. **Martin KVÁČ**, PhD (head) Prof. MVDr. **David Modrý**, PhD (part time) RNDr. **Bohumil Sak**, PhD
PhD students:	Ing. **Šárka Čondlová**; Ing. **Ivana Hájková**; Mgr. **Michaela Kotková**, DiS; Ing. **Karel Němějc**; MVDr. **Jitka Poláková**; Ing. **Veronika Prantlová**; Ing. **Pavla Wagnerová**
Research assistants:	Ing. **Lenka Hlásková**; RNDr. **Dana Květoňová**
Undergraduate students:	Bc. **Jana Anderlová-Muzikářová**; Bc. **Pavel Barvíř**; Bc. **Ondřej Grym**; Bc. **Dita Havrdová**; Bc. **Nikola Hromadová**; Bc. **Marie Jalovecká**; Bc. **Vladimír Kural**; Bc. **Anna Mynářová**; Bc. **Eva Myšková**; Bc. **Barbora I. Uhlířová**; **Martina Bicanová**; **Nikola Havrdová**; **Lucie Honsová**; **Anna Hořická**; **Kristýna Huclová**; **Alžběta Jarolímková**; **Jana Ježková**; **Michaela Kestřánová**; **Michaela Maroušová**; **Lucie Munzarová**; **Radek Pokorný**

Research priorities

The focus of this group is to determine the zoonotic sources of emerging parasitic diseases, especially the opportunistic nature of the occurrence of cryptosporidia and microsporidia in immunodeficient (e.g. AIDS) patients and animals.

Porcine cryptosporidiosis – *Cryptosporidium scrofarum*

Previous molecular epidemiology and experimental infection studies revealed the susceptibility of pigs to a number of species including *C. suis*, *C. parvum*, *C. hominis*, *C. felis*, *C. meleagridis* and pig genotype II. We described the morphological, biological and molecular characteristics of pig genotype II and proposed it as a new species, *Cryptosporidium scrofarum*. The age susceptibility of pigs to *C. scrofarum* was experimentally established on five weeks of age. Both *C. suis* and *C. scrofarum* are not associated with diarrhoea. Generally, *C. suis* is primarily detected among piglets up to five weeks old, whereas *C. scrofarum* is dominant species in older pigs.

Microsporidia and *Cryptosporidium* of wild great apes

To evaluate the impact of close contact with humans on the occurrence of potentially zoonotic protists in great apes, we conducted a monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in *Gorilla gorilla gorilla* at different stages of the habituation process in the Central African Republic, *Gorilla beringei beringei* habituated either for tourism or for research in Rwanda, *Pongo abelii* and *Pongo pygmaeus* from Indonesia. We detected *Enterocytozoon cuniculi* genotypes I, II and III, several genotypes of *E. bieneusi* including novel genotypes gorilla 1–8, *Giardia intestinalis* subgroup A II, *C. bovis*, *C. muris*, *C. meleagridis*, *C. parvum* and *C. tyzerri*-like.

Latent microsporidiosis in immunocompetent hosts

To evaluate the spreading of microsporidial infection in the body of host, efficacy of treatment, possibility of infection reactivation and immune response, we monitored the course of *E. cuniculi* infection in immunocompetent and immunodeficient mice and horses using molecular and immunological methods. Whereas *E. cuniculi* caused lethal microsporidiosis in immunodeficient mice, the infection in immunocompetent mice and horses remained asymptomatic despite parasite dissemination into many organs during the acute infection phase. Activation of immune response did not lead to infection clearance. Albendazole treatment did not lead to the elimination of microsporidia in BALB/c mice and following dexamethasone immunosupression resulted in a chronic microsporidia infection dissemination into most organs in mice.

Selected publications

- **Kotková M.**, **Sak B.**, **Květoňová D.**, **Kváč M.** 2013: Latent microsporidiosis caused by *Encephalitozoon cuniculi* in immunocompetent hosts: a murine model demonstrating the ineffectiveness of the immune system and treatment with albendazole. *PLOS One* 8: e60941 [IF=3.534]
- **Kváč M.**, Kestřánová M., **Pinková M.**, **Květoňová D.**, **Kalinová J.**, **Wagnerová P.**, **Kotková M.**, Vítovec J., Ditrich O., McEvoy J., Stenger B., **Sak B.** 2013: *Cryptosporidium scrofarum* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic pigs (*Sus scrofa*). *Veterinary Parasitology* 191: 218–227. [IF=2.545]
- **Kváč M.**, McEvoy J., Loudová M., Stenger B., **Sak B.**, **Květoňová D.**, **Ditrich O.**, **Rašková V.**, Moriarty E., Rost M., Macholán M., Piálek J. 2013: Coevolution of *Cryptosporidium tyzzeri* and the house mouse (*Mus musculus*). *International Journal for Parasitology* 46: 805–817. [IF=3.404]
- **Sak B.**, Petrželková K., **Květoňová D.**, Mynářová A., Shutt K.A., Pomajbíková K., Kalousová B., **Modrý D.**, Benavides J., Todd A., **Kváč M.** 2013: Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western lowland gorillas (*Gorilla gorilla gorilla*) at different stages of habituation in Dzanga Sangha protected areas, Central African Republic. *PLoS ONE* 8: e71840. [IF=3.534]
- Selman M., **Sak B.**, **Kváč M.**, Farinelli L., Weiss L.M., Corradi N. 2013: Extremely reduced levels of heterozygosity in the vertebrate pathogen *Encephalitozoon cuniculi*. *Eukaryotic Cell* 12: 496–502. [IF=3.179]

Research projects

- **Prevalence, genotypic characterization and clinical effects caused by *Blastocystis hominis* in patients with HIV and AID.** Polish Society for AIDS Research. (P.I.: M. Kicia; Contractor: M. Kváč; 2013–2014)
- **Clinical, immunological and molecular profile of microsporidiosis and cryptosporidiosis in patients living with HIV in the population of Lower Silesia.** Polish Society for AIDS Research (P.I.: M. Kicia; Contractor: M. Kváč; 2013–2014)
- **The application of molecular methods to identify and characterize microsporidia in immunocompetent and immunosuppressed patients with kidney disease and evaluating the impact of selected drugs on the process of microsporidia invasion in *in vitro* research.** National Science Centre, Poland (P.I. Kicia; Contractor: M. Kváč; 2013–2017)
- **Development of scientific team and laboratory for infectious diseases common to humans and great apes.** Ministry of Education, Youth and Sports (CZ.1.07/2.3.00/20.0300, P.I.: D. Modrý, Co-P.I.: M. Kváč; 2012–2015)
- **Diversity, biology and phylogeny of *Cryptosporidium* spp.** parasiting in rodents. Ministry of Education, Youth and Sports (KONTAKT LH 11061, P.I.: M. Kváč; 2011–2014)
- **Anti-inflammatory activity of extracts isolated from selected Indonesian plants and their effect on opportunistic parasitoses.** Grant Agency of the Czech Republic (505/11/1163; PI: K. Doležal, Co-PI: I. Fojtová, Co-PI: B. Sak; 2011–2015).

6.2. Laboratory of Parasitic Therapy

Research scientists: MVDr. **Kateřina JIRKŮ-POMAJBÍKOVÁ**, PhD (head)
Technician: **Jana Vášová**
Undergraduate students: **Olinka Hložková**; **Zuzana Lhotská**; **Jiřina Růžková**

Research priorities

This laboratory was established in October 2013 and, thus, its research is at the very beginning. Main lines of this laboratory are focused on investigation of an impact of the commensal gut eukaryotes on some immune-mediated diseases (IMD) mainly inflammatory bowel diseases, identification of additional organisms and novel therapeutic approaches. Why is this kind of research important? Since there has been a sharp increase in incidence of these IMD in industrialized countries over the last decades. The reason that many people suffer from various IMD is our modern life styles, which have reduced our contact with microbes through the adoption of highly hygienic habits, access to clean food and water and over-use of antibiotics. Therefore, our organisms have limited contact with antigens required for proper immune system development and immunoregulation. In recent year, the research has shown that helminths, gut bacterial communities and even commensal protists inhabiting gut may positively influence the health status of individuals suffering from some IMD.

Selected publications (including those of K. Pomajbíková before her lab was established)

- Jirků M.*, **Pomajbíková K.***, Petrželková K.J., Hůzová Z., Modrý D., Lukeš J. 2012: Detection of *Plasmodium* spp. in human feces. *Emerging Infectious Diseases* 18: 634–636. (*equal contribution). [IF = 5.993]
- **Pomajbíková K.**, Oborník M., Horák A., Petrželková K.J., Grim J.N., Levecke B., Todd A., Mulama M., Kiyang J., Modrý D. 2013: Novel insights into the genetic diversity of *Balantidium* and *Balantidium*-like cyst-forming ciliates. *PLoS Neglected Tropical Diseases* 7: e2140. [IF = 4.489]
- **Pomajbíková K.**, Petrželková K.J., Petrášová J., Profousová I., Kalousová B., Jirků M., Sá R.M., Modrý D. 2012: Distribution of the entodiniomorphid ciliate *Troglocorys cava* Tokiwa, Modrý, Ito, Pomajbíková, Petrželková & Imai, 2010 (Entodiniomorphida: Blepharocorythidae) in wild and captive chimpanzees. *Journal of Eukaryotic Microbiology* 59: 97–99. [IF = 2.162]
- Sak B., Petrželková K.J., Květoňová D., Mynářová A., Shutt K.A., **Pomajbíková K.**, Kalousová B., Modrý D. 2013: Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western lowland gorillas (*Gorilla gorilla gorilla*) in different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic. *PLoS ONE* 8: e71840. [IF = 3.534]
- Schovancová K.*, **Pomajbíková K.***, Procházka P., Modrý D., Bolechová P., Petrželková K.J. 2013: Impact of high dietary starch on the ciliate, *Neobalantidium coli*, in captive chimpanzees. *PLoS ONE* 8: e81374. (*equal contribution) [IF = 3.534]

Research projects

This laboratory has been established and involved within the frame of two European projects:

- **European project for development of research infrastructure: use of model organisms to resolve crucial biological problems on the path to innovations.** 7th FP–EU, specific program Capacities (Biology Centre of ASCR, 2007–2015; lab has been included in September 2013).
- **European project for the promotion and popularization of research, development and innovation.** Operational Programme (P.I.: University and Academic Technology Transfer Centre, 2012–2015; lab has been included in March 2014).

Laboratory of Molecular Helminthology

(closed in December 2013)

Research scientist:	RNDr. **Jan DVOŘÁK**, PhD (head)
Technician:	**Irena Husáková**
Undergraduate students:	Bc. **David Opavský**; **Iveta Červenková**; **Marie Kropšová**; **Mirka Kříhová**

Research priorities

Schistosomiasis caused by trematode parasites, *Schistosoma* blood flukes, represents one of the most serious chronic infection in the developing world with more than 200 million people infected and many more at risk. The ability of schistosomes to survive in the mammalian hosts for decades is due to their various modulatory mechanisms. Our interest is in their protein molecules that can actively influence host physiology. Disrupting of these mechanisms by specific drug/vaccine treatment targeting parasite molecules may lead to potential disease treatment. Such modulatory factors of schistosomes can serve as instrumental molecules for pharmacology. Our research is focused on the studies of proteolytic enzymes and macromolecular protease inhibitors from *Schistosoma mansoni*. Proteases (proteolytic enzymes, peptidases, peptide hydrolases) provide essential functions in all life forms including parasitic organism. So far, very little is known about many groups of proteolytic enzymes that can be found in the *S. mansoni* genome. Besides relatively well characterised enzymes associated with skin invasion and blood digestion, there are groups of proteases that were surprisingly neglected. Many of these enzymes share significant homology with various mammalian proteolytic regulatory factors. Main goal of our project is to identify and describe proteases and macromolecular inhibitors from *S. mansoni* expressed during infection of their mammalian hosts. We hypothesise that these molecules play significant role in host-parasite interactions. Our work includes various laboratory techniques in molecular biology, biochemistry and immunochemistry, and confocal microscopy.

Research projects

- **Trypsin proteases of blood fluke *Schistosoma mansoni*.** Ministry of Education, Youth and Sports (KONTAKT ME grant – American Science Information Center; ME10011, P.I.: Jan Dvořák; 2010 –2012).

Supporting facility

Laboratory of Electron Microscopy

Research scientists:	Ing. **Jana NEBESÁŘOVÁ**, CSc. (head) RNDr. **Marie Vancová**, PhD
PhD students:	Mgr. **Tomáš Bílý**; Mgr. **Martin Strnad**
Technicians:	Mgr. **Jan Langhans**; **Petra Masařová**; Mgr. **Martina Tesařová**; **Jiří Vaněček**
Undergraduate students:	Bc. **Denisa Martykánová**; **Antti Kettunen** (Austria)

Electron microscopy is used to image the structure of molecules, cells and tissues at sub-nanometer resolution. Transmission electron microscopy (TEM) is dedicated for the examination of samples cut into ultrathin sections with the thickness 80–100 nm so that the electron beam can pass through the sample and form an image on the detector. In scanning electron microscopy (SEM), the electron beam is scanned over the small sample area to produce secondary signals carrying information about the specimen surface topography or composition.

The team of the Laboratory of Electron Microscopy (LEM) works closely with several research groups of the Biology Centre but also from other institutions to plan, optimise and implement experiments, producing images that allow scientists to understand their samples at the subcellular level. Members of LEM are experts in preparing, imaging and interpreting a wide range of biological samples. They use a broad spectrum of traditional and novel preparation techniques for optimum preservation of sample morphology and localisation of proteins.

Technical equipment

- **Transmission electron microscopes**
  - JEOL 2100F (2012) equipped for electron tomography, STEM and image recording with CCD camera Orius SC1000 (Gatan)
  - JEOL 1010 (1996) equipped with SSC camera MegaView 3
  - Low voltage electron microscope LV EM 5 (2002), Delong Instruments, Inc.
- **Scanning electron microscopes**
  - JEOL 7401F (2005) with cryo-attachment ALTO 2500 GATAN
  - JEOL 6300 (1993)
- **Ultramicrotomes Leica** with and without cryo-chamber
- **High Pressure Freezer Leica EM Pact2** – a system for vitrifying samples up to 200 μm in thickness without the artifacts of chemical fixation
- **Automatic freeze substitution system Leica EM AFS** for substitution and low temperature embedding after cryofixation and for the PLT technique

Selected publications

- Bruňanská M., Drobníková P., Mackiewicz J.S., **Nebesářová J.** 2013: Cytocomposition of the vitellarium in *Khawia sinensis* Hsü, 1935 (Cestoda, Caryophyllidea, Lytocestidae): another caryophyllidean species with lamellar bodies and lipids. *Parasitology Research* 112: 2703–2711. [IF=2.327]
- **Nebesářová J.**, **Langhans J.**, Šlouf M., Pavlová E., **Vancová M.** 2013: Is it possible to measure diameters of metal nanoparticles using BSE imaging in FESEM? *Micron* 44: 159–166. [IF=2.062]
- Šlouf M., Hrubý M., Bakaeva Z., Vlková H., **Nebesářová J.**, Philimonenko A.A., Hozák P. 2012: Preparation of stable Pd nanocubes and their use in biological labeling. *Colloids and Surfaces B-Biointerfaces* 100: 205–208. [IF=3.554]
- **Vancová M.**, Štěrba J., Dupejová J., Simonová Z., **Nebesářová J.**, Novotný M.V., Grubhoffer L. 2012: Uptake and incorporation of sialic acid by the tick *Ixodes ricinus*. *Journal of Insect Physiology* 58: 1277–1287. [IF=2.379]

Patent

- Hozak P., Krivjanska M., Mosa M., **Nebesářová J.**, Šlouf M. 2012: Set of mutually recognizable nanoparticles for e.g. immunocytochemical analysis of antigens in biological structures, comprise gold, silver, palladium, platinum and cobalt oxide. Patent numbers: CZ201000647-A3; CZ304250-B6

Research projects

- **Electron Microscopy.** Programme of the Technology Agency of the Czech Republic to support the development of long-term collaboration of the public and private sectors on research, development and innovations. The project is managed by a consortium of representatives of eight participating organisations – FEI Czech Republic, Delong Instruments, Crytour, Institute of Macromolecular Chemistry of ASCR, Institute of Molecular Genetics of ASCR, Institute of Scientific Instruments of ASCR, Biology Centre of ASCR (LEM), Research and Testing Institute Plzeň; 2012–2019.
- **Collaboration within projects not related directly to the Institute's research plan.**
- **Contractual services.**

Special activities

Collections of parasitic organisms

A collection of cryopreserved cultures of blood flagellates and amphizoic amoebae is maintained at the Laboratory of Fish Protistology. An extensive collection of helminths, curator of which was František Moravec (till December 2013), is available for comparative studies. It comprises more than 3 000 species from around the world, including numerous type specimens.

A collection of holotypes and paratypes of about 300 species of parasitic arthropods, on 430 microscopic slides, is deposited at the Institute, as well as a large collection of several thousand specimens of parasitic mites and fleas from mammals, birds and reptiles, and a small collection of ticks in alcohol. The Institute maintains laboratory colonies of ticks (8 species), mosquitoes (4 species, 5 lines) and arboviruses (33 species and strains).

More information can be found at http://www.paru.cas.cz/en/collections/.

Publishing and editorial activities

FOLIA PARASITOLOGICA – an international journal

Editor-in-Chief: **Tomáš Scholz**

Assistant Editors: **Ivan Fiala** (parasitic protists & myxozoans; molecular phylogenetics)
Aneta Kostadinova (ecological parasitology & helminths)
Tomáš Scholz (helminths & parasitic arthropods)

Editorial Assistant: **Petra Rozkošná**

Folia Parasitologica is an international journal for parasitology, publishing articles written in English. It was founded in 1953 as an annual edition. Since 1966, it has been published four times a year. The Editor-in-Chief and three Assistant Editors from the Institute of Parasitology are aided by an international Board of Editorial Advisors, consisting of 26 highly regarded scientists, overwhelming majority of them being foreign parasitologists. Each manuscript is rigorously reviewed by at least two referees who are known for excellence in their field of expertise. As a result of this demanding review process, the rejection rate is about 60%. Folia has a wide international authorship: in 2012–2013, around 90% of senior authors of published papers were from abroad.

The Impact Factor of Folia was 2.515 in 2012 and 1.211 in 2013, reflecting the journal's good position among international parasitology periodicals. Folia is desk-top edited, figure reproduction being the only operation entrusted to a commercial printer. Final printing is on high-quality glossy paper of A4 size, allowing for the excellent reproduction of line drawings and B&W/colour photographs. However, the journal will move to an Open Access mode since January 2015, without any hard copies published. Folia is widely indexed/abstracted in key biological databases such as BIOSIS (Biological Abstracts, Biological Abstracts/RRM, Abstracts of Entomology, Zoological Record), CAB (Helminthological and Protozoological Abstracts, Review of Medical and Veterinary Entomology, Tropical Diseases Bulletin), ISI (Current Contents/Agriculture, Biology & Environmental Sciences, Science Citation Index Expanded, Web of Knowledge) and NLM (MEDLINE). Full text of articles can be downloaded from the Folia website, ProQuest Biology/Medicine Journals or CABI Full Text databases.

EMBO Practical Course on Electron Microscopy and Stereology in Cell Biology, České Budějovice, 12–22 June 2012

This prestigious international course funded by the European Molecular Biology Organization (EMBO) was organised in České Budějovice for the fourth time. The aim of the course is teaching of cutting-edge electron microscopy techniques that can be used to study cellular processes at the ultrastructural level. Team of 20 expert teachers from around the world was explaining all difficulties in techniques of electron microscopy to 24 foreign students selected on the basis of their projects. The majority of the course was dedicated to the practical training to teach students the best methods of preserving, visualising and localising molecules of interest on cellular structures.

Based on feedback from participants, the course was very successful. It brought many new contacts, international collaboration and friendships and a number of keen electron microscopists.

Electron Microscopy in Cell Biology, České Budějovice, 13 June 2012

A one-day international seminar, which was dedicated to the presentation of newest electron microscopy methods, opened the EMBO Practical Course. Top-class foreign experts who came to teach at the EMBO Practical Course (Gareth Griffiths – University of Oslo, Heinz Schwarz – Max-Planck Institute for Entwicklungsbiologie, Tübingen, John Lucoq – University of Dundee, Yannick Schwab – IGBMC Strasbourg, Herb Hagler – University of Texas Southwestern Medical School), presented six lectures, which showed possibilities of current electron microscopy in biological and biomedical research. The seminar was attended by almost 100 participants mainly from the Czech scientific community.

ANTIGONE semi-annual workshop, Český Krumlov, Czech Republic, 26–29 May 2013

A total of 11 participants from 4 member countries (Czech Republic, Greece, Spain and UK) of work-packages WP3 and WP8 of FP7 EU project ANTIGONE, participated in semi-annual meeting organised by the Laboratory of Molecular Ecology of Vectors and Pathogens in Český Krumlov to evaluate the progress of the joint EU project, to discuss the future strategy and milestone delivery.

Ticks meet mosquitoes, First traditional retreat workshop, Lipka, Šumava, 20–23 June 2013

Twenty five participants from the *Anopheles* group INSERM U963/CNRS UPR9022 – Strasbourg, France (Stephanie Blandin), Max-Planck Institute for Infection Biology, Berlin, Germany (team of Elena Levashina) and the Institute of Parasitology, BC ASCR (teams of O. Hajdušek and P. Kopáček) discussed research problems and future collaboration.

List of the Institute's employees by professional classification

(as of 30 June 2014)

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Publication activities

2012

Chapters in monographs

1. **FLEGONTOV P.**, **LUKEŠ J.** 2012: Chapter 6. Mitochondrial genomes of photosynthetic euglenids and alveolates. In: L. Marechal Drouard (Ed.), Advances in Botanical Research 63. Elsevier Inc., London, pp. 127–153.
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International activities

Cooperation with foreign research institutions

Research area: Molecular biology of parasitic protists and nematodes & Molecular taxonomy and phylogeny of parasites

- CNRS, Ecole Normale Supérieure, Paris, France (C. Bowler)
- Comenius University, Bratislava, Slovakia (A. Horváth)
- Edinburgh Napier University, Edinburgh, UK (S. Rueckert)
- FDA, Center for Biologics Evaluation and Research, Bethesda, Maryland, USA (R. Duncan)
- Kansas State University, Manhattan, Kansas, USA (M. Herman)
- McGill University, Québec, Canada (R. Salavati)
- Natural History Museum, London, UK (V. Smith)
- Ohio State University, Columbus, Ohio, USA (J. Alfonzo)
- Station Biologique de Roscoff, Roscoff, France (C. de Vargas, S. Audic)
- The State University of New York at Buffalo, Buffalo, New York, USA (L. Read)
- University of British Columbia, Vancouver, Canada (P.J. Keeling, B.R. Green)
- University of California, Riverside, California, USA (D.A. Maslov)
- University of Edinburgh, UK (A. Schnaufer)
- University of Glasgow, Glasgow, UK (H. de Koning)
- University of Lancaster, Lancaster, UK (M.L. Ginger)
- University of Montreal, Québec, Canada (G. Burger)
- University of Tsukuba, Tsukuba, Japan (R. Niwa)
- University of Zurich, Zurich, Switzerland (L. Keller)

Research area: Biology of disease vectors

- Academic Medical Center, Amsterdam, The Netherlands (J.W.R. Hovius)
- Animal Health and Veterinary Laboratories Agency, UK (A. Fooks)
- Barcelona Supercomputing Center, Barcelona, Spain (V. Guallar)
- Catholic University Leuven, Belgium (E. de Clercq)
- Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (N. Zeidner)
- Dresden University of Technology & University Clinic Carl Gustav Carus, Dresden, Germany (T. Chavakis)
- Georgia Southern University, Statesboro, Georgia, USA (J.H. Oliver, Jr.)
- Hokkaido University, Sapporo, Hokkaido, Japan (K. Yoshii)
- Indiana University, National Centre of Glycomics and Glycoproteomics, Bloomington, Indiana, USA (M.V. Novotný)
- Institute of Bioorganic Chemistry and Fundamental Medicine, Novosibirsk, Russia (S. Tkachev)
- Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia (B. Klempa)
- Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia (M. Kazimírová)
- Johannes Gutenberg University of Mainz, Mainz, Germany (E. Schmitt, S. Tenzer)
- Kagoshima University, Korimoto, Kagoshima, Japan (K. Fujisaki)
- Max Planck Institute for Infection Biology, Berlin, Germany (E.A. Levashina)
- Muhlenberg College, Allentown, Pennsylvania, USA (M.J. Edwards)
- National Institutes of Health, Rockville, Maryland USA (J. Valenzuela, J. Anderson, J. Ribeiro)

- NRC Institute for Biological Sciences, Ottawa, Canada (R. Mackenzie, S. Logan)
- Oklahoma State University, Stillwater, Oklahoma, USA & Instituto de Investigación en Recursos Cinégeticos, Ciudad Real, Spain (J. de la Fuente)
- ONIRIS, Biologie, Epidémiologie et Analyse de Risque en Santé Animale, Nantes, France (L. Malandrin)
- Parasitological Institute, Slovak Academy of Sciences, Košice, Slovakia (B. Peťko)
- Robert Koch-Institut, Berlin, Germany (M. Niedrig)
- Russian Academy of Medical Sciences, Irkutsk, Russia (V. Zlobin)
- State University of New York, Stony Brook, USA (B.J. Luft)
- Pirbright Institute, Woking, UK (J.K. Fazakerley, L. Bell)
- University of Aberdeen, Aberdeen, UK (A. Bowman)
- University of Arizona, Tucson, Arizona, USA (J.H. Law, R. Miesfeld, C. Bender, J. Winzerling)
- University of California, Berkeley, California, USA (R.S. Lane)
- University of California, San Francisco, California, USA (C.R. Caffrey, J.H. McKerrow)
- University of Glasgow, Glasgow, UK (A. Kohl, B. Shields)
- University of Granada, Granada, Spain (M. Hackenberg)
- University of Liège, Liège, Belgium (A. Vanderplasschen)
- University of Leipzig, Leipzig, Germany (M. Pfeffer)
- University of Maryland School of Medicine, Baltimore, Maryland, USA (J.H. Pedra)
- University of Neuchâtel, Neuchâtel, USA (P. Guerin, L. Gern)
- University of North Florida, Jacksonville, USA (K. Clark)
- University of Reading, UK (E. Gould, T. Gritsun)
- University of Rome La Sapienza, Roma, Italy (B. Arca)
- University of Southern Mississippi, Hattiesburg, Mississippi (S. Karim)
- University of Stirling, Stirling, UK (M. Weidmann)
- University of Strasbourg, Illkirch, France (N. Boulanger)
- University of Tampa, Tampa, Florida, USA (N. Belfiore)
- University of Utrecht, The Netherlands (F. Jongejan)
- Vienna University of Veterinary Medicine, Austria (N. Nowotny)

Research area: Parasites of fish

- College of Charleston, Charleston, South Carolina, USA (I. de Buron)
- ECOSUR, Chetumal, Mexico (D. González-Solís)
- Fish and Wildlife Research Institute, St. Petersburg, Florida, USA (M. Bakenhaster)
- Hungarian Academy of Sciences, Budapest, Hungary (E. Eszterbauer)
- Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Russia (L.G. Poddubnaya)
- Mote Marine Laboratory, Sarasota, Florida, USA (K. Main, C. Yanes-Roca)
- Muséum d'Histoire Naturelle, Genève, Switzerland (A. de Chambrier)
- Muséum National d'Histoire Naturelle, Paris, France (J.-L. Justine)
- Natural History Museum, London, UK (D.I. Gibson, D.T.J. Littlewood)
- Oregon State University, Corvallis, Oregon, USA (J. Bartholomew, S. Atkinson)
- Parasitological Institute, Slovak Academy of Sciences, Košice, Slovakia (V. Hanzelová)
- Skretting Aquaculture Research Centre, Stavanger, Norway (C. McGurk)
- University of Bologna, Bologna, Italy (M.-L. Fioravanti, A. Gustinelli)
- University of Cape Town, Cape Town, South Africa (C.C. Reed)
- University of Connecticut, Storrs, Connecticut, USA (J.N. Caira)
- University of Iceland, Reykjavik, Iceland (K. Skirnisson)

- University of Malaya, Kuala Lumpur, Malaysia (M. Freeman)
- University of Sydney, Sydney, Australia (J. Šlapeta)
- University of Tasmania, School of Aquaculture, Launceston, Tasmania, Australia (B. Nowak)
- University of Valencia, Valencia, Spain (A. Pérez-del-Olmo, F. Montero)

Research area: Parasitic protists of man and animals with special reference to opportunistic parasites

- Canadian Institute for Advanced Research, University of Ottawa, Ottawa, Ontario, Canada (N. Corradi)
- CDC, Division of Parasitic Diseases, Atlanta, Georgia, USA (L. Xiao, V. Cama, E.W. Secor)
- Center for Food Safety, University of Georgia, Griffin, Georgia, USA (Y. Ortega)
- Christchurch Science Centre, Christchurch, New Zealand (E. Moriarty)
- Higher National School of Veterinary, EL Harrach, Algiers, Algeria (A.E. Laatamna, M. Aissi)
- North Dakota State University, Fargo, North Dakota, USA (J. McEvoy)
- Parasitological Institute of Slovak Academy of Sciences, Košice, Slovakia (M. Stanko)
- Staten Serum Institute, Microbiology and Infection Control, Copenhagen, Denmark (S.R. Stensvold)
- University of British Columbia, Department of Botany, Vancouver, Canada (L. Wegener-Parfrey)
- Wroclaw Medical University, Wrocław, Poland (M. Wesolowska, M. Kicia)
- Wroclaw University, Institute of Genetics and Microbiology, Wrocław, Poland (A. Perec-Matysiak)

Membership in international organisations

Masako Asahina-Jindrová

- Member of the American Society for Cell Biology
- Member of the Genetics Society of America

Maryna Golovchenko

- Adjunct member of the James Oliver, Jr. Institute of Arthropodology and Parasitology at the Georgia Southern University

Libor Grubhoffer

- Member of the Organizing Committee for the EMBO Workshops on the Molecular and Population Biology of Mosquito and other Disease Vectors
- Adjunct member of the James Oliver, Jr. Institute of Arthropodology and Parasitology at the Georgia Southern University
- President of the National Committee of the International Union of Biological Sciences (IUBS)

Astrid Holzer

- Member of the British Society for Parasitology
- Member of the Fisheries Society of the British Isles

Michail Kotsyfakis

- Member of the American Society of Biochemistry and Molecular Biology

Julius Lukeš

- Member of the Faculty of 1000
- President of the International Society for Evolutionary Protistology
- Vice-President of the International Society of Protistologists
- Senior Fellow of the Canadian Institute for Advanced Research

František Moravec

- Honorary Member of the American Society of Parasitologists
- Honorary Member of the Society of Slovak Parasitologists

Jana Nebesářová

- Member of the European Microscopy Society

Miroslav Oborník

- Member of the International Society for Evolutionary Protistology

Nataliia Rudenko

- Adjunct member of the James Oliver, Jr. Institute of Arthropodology and Parasitology at the Georgia Southern University

Daniel Růžek

- Member of the International Scientific Working Group on Tick-Borne Encephalitis

Tomáš Scholz

- Corresponding member of the Natural History Museum, Geneva, Switzerland

Jan Štefka

- Member of the International Society of Phthirapterists

Jiří Vávra

- Member of the International Society of Protistologists

Membership on editorial boards

Acta Parasitologica (Poland): ***F. Moravec***, ***T. Scholz***

Acta Protozoologica (Poland): ***J. Vávra***

American Journal of Blood Research (USA): ***M. Kotsyfakis*** Associate Editor)

American Journal of Infectious Diseases and Microbiology (USA): ***D. Růžek***

Epidemiology and Vaccinal Prevention – Scientific and Practical Journal (Russia): ***D. Růžek***

Folia Parasitologica (Czech Republic): ***I. Dyková***, ***I. Fiala*** (Associate Editor), ***A. Kostadinova*** (Associate Editor), ***F. Moravec***, ***T. Scholz*** (Editor-in-Chief), ***J. Vávra***

Helminthologia (Slovakia): ***F. Moravec***

Journal of Agrobiology (Czech Republic): ***M. Kváč***

Journal of Eukaryotic Microbiology (USA): ***J. Lukeš***, ***J. Vávra*** (board of reviewers)

Journal of Fish Diseases (UK): ***A. Holzer***

Kinetoplastid Biology and Disease (UK): ***J. Lukeš***

Medical Virology (Russia): ***D. Růžek***

Parasite (France): ***F. Moravec***, ***T. Scholz***

Parasite & Vectors (UK): ***A. Kostadinova***

Protistology (Russia): ***J. Lukeš***

Systematic Parasitology (UK): ***A. Kostadinova*** (Editor-in-Chief), ***F. Moravec***, ***T. Scholz***

The Scientific World Journal (UK, USA, Egypt): ***D. Růžek***

Ticks and Tick-borne Diseases (Germany): ***J. Kopecký***

Veterinary Medicine and Animal Sciences (UK): ***A.S. Holzer***

World Journal of Virology (China): ***D. Růžek***

Teaching activities

The principal mission of the Institute of Parasitology is to perform basic research. However, participation of the staff in teaching is an integral part of their activities and is essential for further development of the Institute. Therefore, most of the key scientists participate in teaching, both by giving lectures and supervising graduate and undergraduate students.

The students actively participate in research projects of the Institute and all graduate students and selected undergraduates have part-time contracts at the Institute. Most students are from the University of South Bohemia in České Budějovice, especially its Faculty of Science (FS), but also from other faculties (Faculty of Agriculture – FA; Faculty of Health and Social Studies – FHS) and universities, such as Charles University in Prague, Masaryk University in Brno and the University of Veterinary and Pharmaceutical Sciences in Brno.

To facilitate scientific cooperation and participation of students in the research performed at the Institute, the Laboratory of Molecular Ecology of Vectors and Pathogens (head *L. Grubhoffer*) and the Laboratory of Evolutionary Protistology (head *M. Oborník*) have been established jointly with the University of South Bohemia.

List of PhD theses

(Faculty of Science, unless otherwise stated)

2012

- **Anirban Ash** (India): Diversity of tapeworms (Cestoda) in freshwater fish of India
  Supervisor: *T. Scholz*; *consultants*: *M. Oros* (Slovakia) and *P.K. Kar* (India)
- **Jan Brabec**: Molecular systematics and evolution of basal cestode lineages
  Supervisor: *T. Scholz*; *consultant*: *M. Oborník*
- **Zdeněk Franta**: Blood meal digestion in the hard tick *Ixodes ricinus*
  Supervisor: *P. Kopáček*
- **Jana Kvičerová**: Phylogeny of coccidia and coevolution with their hosts
  Supervisor: *V. Hypša*
- **Eva Nováková**: Molecular phylogeny and genome evolution of insect symbiotic bacteria
  Supervisor: *V. Hypša*
- **Jana Plchová (maiden name Horáčková)**: Differential expression of tick *Ixodes ricinus* genes induced by blood feeding or infection: genetic analysis of ML domain containing proteins
  Supervisor: *L. Grubhoffer*; *consultant*: *N. Rudenko*
- **Ján Štěrba**: Glycobiology of ticks and tick-borne pathogens. Glycans, glycoproteins and glycan-binding protein
  Supervisor: *L. Grubhoffer*

2013

- **Piya Changmai** (Thailand): Fe-S cluster synthesis in trypanosomes
  Supevisor: *J. Lukeš*
- **Karel Němejc**: Cryptosporidial and microsporidial infections of domestic pigs and wild boars. Faculty of Agriculture (ZF)
  Supervisor: M. Kváč; consultant: J. Vítovec (ZF)

List of Master of Science theses

2012

- **Martina Cinková**: The phylogenetic relationships of Myxosporea infecting amphibians
  Supervisor: *I. Fiala*
- **Petr Franta**: Experimental vaccinations of rabbits with recombinant digestive peptidases of the tick *Ixodes ricinus*
  Supervisor: *P. Kopáček*; *consultant*: *Z. Franta*
- **Adéla Harcubová**: Vaccine potentiality of cystatin from tick *Ixodes ricinus*
  Supervisor: *J. Kopecký*; *consultant*: *J. Salát*
- **Radka Hobizalová**: Functional analysis of Salp25D, a homologue of peroxiredoxin, from castor bean tick *Ixodes ricinus*
  Supervisors: *N. Rudenko* and *M. Golovchenko*
- **Nikola Hromadová**: Endoparasites of cattle under various breeding management
  Supervisor: *M. Kváč* (ZF)
- **Filip Husník**: Evolutionary origin of intracellular symbionts in arthropods
  Supervisor: *V. Hypša*
- **Marie Jalovecká**: Development of protective immune response in gastric mucosa of mice infected with *Cryptosporidium muris* and *Cryptosporidium andersoni*
  Supervisor: *M. Kváč*; *consultants*: *B. Sak* and *J. Salát*
- **Martina Jonáková**: Circadian rhythm of secondary alga *Chromera velia*
  Supervisor: *M. Oborník*; *consultant*: *T. Doležal* (Faculty of Science – PřF)
- **Jana Kadlecová**: Characterization and function of the transferrin from the tick *Ixodes ricinus*
  Supervisor: *P. Kopáček*
- **Lucie Kafková (Hanzálková)**: Functional characterization of two paralogs that are novel RNA binding proteins influencing mitochondrial transcripts of *Trypanosoma brucei*
  Supervisor: *H. Hashimi*
- **Julie Kovářová**: Localization of the Fe-S cluster biosynthesis in the bloodstream stage of *Trypanosoma brucei*
  Supervisor: *J. Lukeš*
- **Martin Palus**: The role of genetic background of the host on the pathogenesis of tick-borne encephalitis
  Supervisor: *D. Růžek*
- **Šárka Pospíšilová**: The effect of tick's serpin IRS-2 on dendritic cells activated by TLR4 ligand
  Supervisor: *J. Lieskovská*
- **Zuzana Šimonová**: Determination of N-glycome of the tick *Ixodes ricinus* and *Dermacentor marginatus*: analysis of N-glykan in tick tissues and their comparison
  Supervisor: *L. Grubhoffer*; *consultant*: *J. Štěrba*
- **Vendula Vetišková**: Comparison of detection methods of tick-borne pathogens (*Borrelia burgdorferi* s. l. and tick-borne encephalitis virus) in *Ixodes ricinus* tick
  Supervisor: *L. Grubhoffer*; *consultant*: *H. Tykalová*

2013

- **Jana Anderlová (Muzikářová)**: Occurrence and prevalence of *Nosema* spp. in European honey bee (*Apis mellifera*) (ZF)
  Supervisor: M. Kváč
- **Alejandro Cabezas-Cruz** (Cuba): Cellular and molecular characterization of *Ehrlichia mineirensis*, a new organism isolated from *Rhipicephalus* (*Boophilus*) *microplus* ticks
  Supervisor: L. Grubhoffer
- **Šárka Čondlová**: Diversity of *Cryptosporidium* spp. infecting rodents from the genus *Apodemus* in the Czech Republic (ZF)

Supervisor: *M. Kváč*

- **Ivana Hájková**: Diversity of *Cryptosporidium* spp. infecting rodents from the subfamily Arvicolinae in the Czech Republic (ZF)
  Supervisor: *M. Kváč*
- **Jan Hladký**: Occurrence of intestinal parasites in various cattle herds
  Supervisor: *M. Kostka*
- **Marie Hlavničková**: Phylogenetic relationships of the genus *Ceratomyxa* (Myxozoa) infecting the gall bladder and the intestine of fish, based on multigene analyses
  Supervisor: *I. Fiala*
- **Jan Kotál**: Production and functional characterization of tick salivary protease inhibitors
  Supervisor: *M. Kotsyfakis*
- **Šárka Koudelková**: The utilization of mast cells for exploration of immunomodulatory effects of tick salivary proteins
  Supervisor: *H. Langhansová* (*Horká*)
- **Lucie Lakatosová**: *Blastocystis* subtypes in pigs
  Supervisor: *M. Kostka*
- **Lucie Ouředníková**: Identification and characterization of newly found antimicrobial peptide (IRAMP) from hard tick *Ixodes ricinus*
  Supervisor: *N. Rudenko*
- **Jana Páleníková**: Interactions of innate immunity cells with Lyme disease spirochetes and the effect of tick saliva molecules on these interactions
  Supervisor: *H. Langhansová* (*Horká*)
- **Martin Strnad**: Localization of Lyme disease spirochetes during infection of ticks *Ixodes ricinus*
  Supervisor: *M. Vancová*
- **Jana Širmarová**: The influence of tick saliva on the replication of tick-borne encephalitis virus in vivo and the influence of tick cystatins on gene expression of interferon regulated factors
  Supervisor: *J. Kopecký*; *consultants*: *J. Lieskovská* and *D. Růžek*
- **Barbora I. Uhlířová**: Endoparasite infections in sheep and goats in diverse farming systems (ZF)
  Supervisor: *M. Kváč*
- **Pavla Urbánková**: Occurrence of intestinal parasites in poultry
  Supervisor: *M. Kostka*
- **Zuzana Vavrušková**: Vector – patogen – host interaction on the example of spirochetes Lyme disease (and tick-borne encephalistis virus)
  Supervisor: *L. Grubhoffer*

List of Bachelor of Science theses

2012

- **Martina Aistleitner** (Austria): Cytotoxicity screen of the acyclic nucleoside phosphonates against bloodstream stage of *Trypanosoma brucei* and validation of their putative target hypoxanthine/xanthine/guanine phosphoribosyltransferase
  Supervisor: *A. Zíková*
- **Pavel Barvíř**: Endoparasitosis of feathered game (ZF)
  Supervisor: *M. Kváč*
- **Iveta Červenková**: Production of *Schistosoma mansoni* recombinant proteases
  Supervisor: *J. Dvořák*
- **Hana Chytráčková**: Antihemostatic effects of tick saliva
  Supervisor: *J. Kopecký*
- **Monika Fraňková**: A putative homolog of the eukaryotic inhibiting peptide in *Trypanosoma brucei*: its localization and function

Supervisor: *A. Zíková*

- **Ondřej Grym**: The role of free living birds and poultry in the environmental dissemination of human pathogenic *Cryptosporidium* spp. and microsporidia (ZF)
  Supervisor: *M. Kváč*
- **Dita Havrdová**: The role of Canidae in the environmental dissemination of human pathogenic *Cryptosporidium* spp. (ZF)
  Supervisor: *M. Kváč*
- **Lucie Honsová**: The incidence of potential agents of parasitic zoonoses in Svalbard (ZSF)
  Supervisor: *O. Ditrich* (PřF)
- **Alžběta Jarolímková**: Intestinal nematodes of horses (ZF)
  Supervisor: *M. Kváč*
- **Zuzana Kotrbová**: Validation of acyclic nucleoside phosphanates as inhibitors of 6-oxo purine phosphorybosyltransferases in *Trypanosoma brucei*
  Supervisor: *A. Zíková*
- **Marie Kropšová**: Host-parasite interaction between fluke *Schistosoma mansoni* and vasculature of definitive host
  Supervisor: *J. Dvořák*
- **Matěj Kučera**: Molecular characterization of NADPH oxidase in the gut of the tick *Ixodes ricinus*
  Supervisor: *P. Kopáček*; *consultant*: *J. Perner*
- **Vladimír Kural**: Poultry cryptosporidiosis (ZF)
  Supervisor: *M. Kváč*
- **Michaela Maroušová**: Cryptosporidial infections of pets (ZF)
  Supervisor: *M. Kváč*
- **Michaela Matějková**: Coevolution of rodents and their ectoparasites on a population level
  Supervisor: *J. Štefka*
- **Lucie Munzarová**: Detection of cryptosporidia by means of molecular methods in clinical samples: infection or the transit of oocysts through the host gastrointestinal tract? (ZSF)
  Supervisor: *M. Kváč*
- **Anna Mynářová**: The effect of clome human-apes contact on cryptosporidial and microsporidial infections
  Supervisor: *M. Kváč*
- **Ivana Pokorná**: Preparation of recombinant inhibitor of serine proteases from the tick Ixodes ricinus
  Supervisor: *J. Kopecký*; *consultant*: *J. Salát*
- **Pavla Šedivá**: Comparisons of methods for fixation and preparation of samples for tapeworms by scanning electron microscopy
  Supervisor: R. Kuchta

2013

- **Matthias Guggenberger (Rakousko)**: Interactions of *Trypanosoma brucei* FoF1 ATP synthase subunits – an application of yeast two
  Supervisor: *A. Zíková*
- **Paula Helebrandová**: Tick-borne encephalitis recombinant protein NS1
  Supervisor: *L. Grubhoffer*; *consultant*: *J. Černý*
- **Kateřina Hrubá**: Diversity, host specifity and phylogenetic relationships of tapeworm of the genus *Monobothrioides* (Cestoda: Caryophyllidea), parasites of catfish in Africa
  Supervisor: *T. Scholz*
- **Michaela Kestřánová**: Susceptibility of pigs to various *Cryptosporidium* species and genotypes (ZF)
  Supervisor: *M. Kváč*
- **Jitka Konvičková**: Dynamics of digestive enzymes in the gut of ticks *Ixodes ricinus* during blood feeding on the host

Supervisor: *P. Kopáček*

- **Helena Mondeková**: Analysis of the lipoprotein domain from the heme lipoglycoprotein of the tick *Dermacentor marginatus*
  Supervisor: *L. Grubhoffer*; *consultant*: *J. Štěrba*
- **Hana Váchová**: Inhibition of F1-ATPase from *Trypanosoma brucei* by its regulatory protein inhibitor TbIF1
  Supervisor: *A. Zíková*
- **Rita Urbanová**: Purification of hemelipoglycoprotein from hemolymph of the tick *Dermacentor marginatus*
  Supervisor: *J. Štěrba*

Stays of foreign students

- **Pilar Alberdi** and **Nieves Ayllón**: Instituto de Investigación en Recursos Cinegéticos, Spain (17.–29. 5. 2013; *Grubhoffer* lab)
- **Rym Antar**: University of Tunis El Manar, Tunisia (11. 3.–7. 6. 2013; *A. Kostadinova*)
- **Francesca Barbieri** and **Eleonora Bernardoni**: University of Bologna, Bologna, Italy (7. 2.–9. 3. 2013; *A. Kostadinova* and *R. Kuchta*)
- **Moges Beletew Shenkute**: Addis Ababa University, Addis Ababa, Ethiopia (17. 9.–16. 11. 2012; *T. Scholz* and *C.A. Mendoza-Palmero*)
- **Ana Born Torrijos**: Universidad de Valencia, Spain (1. 2.–30. 4. 2012 + 23. 5.–20. 6. 2013; *A. Holzer* and *A. Kostadinova*)
- **Martha Brandão**: Universidade Rural de Rio de Janeiro, Seopédica, Brazil (6. 3.–27. 4. 2012; *A. Kostadinova* and *T. Scholz*)
- **Sara Dallares Vilar**: Autonomous University of Barcelona, Spain (2.–16. 5. 2013; *A. Kostadinova*)
- **Tina Flemming**: Leonardo program student, Germany (September 2012–February 2013; *Kopáček* lab)
- **Turkan Gurbanova**: Baku University, Baku, Azerbajdzan (December 2012; *J. Kvičerová*)
- **Božena Haklová** and **Lucie Pangrácová**: Parasitological Institute, Košice, Slovakia (1.–5. 6. 2012; *N. Rudenko*)
- **Antónia Hasajová**: University of Veterinary Medicine and Pharmacy, Košice, Slovakia (29. 6.–2. 8. 2013; *M. Kváč* and *B. Sak*)
- **Laura Jeacock**: University of Edinburgh, Edinburgh, UK (5.–30. 8. 2012; *A. Zíková*)
- **Sayef Laamiri**: University of Tunis, Tunis, Tunisia (12. 6.–10. 8. 2013; *A. Holzer*)
- **Abd el Karim Lataamna**: École Nationale Supériure Vétérinaire, El Harrach, Alger, Algeria (27. 1.–26. 2. 2012 + 27. 5.–9. 6. 2013; *M. Kváč* and *B. Sak*)
- **Kinga Leśniańska**: Wroclaw University, Wroclaw, Poland (1. 6.–30. 9. 2013; *M. Kváč* and *B. Sak*)
- **Sara Madache**: University of Annaba, Annaba, Algeria (15. 11.–20. 12. 2012; *A. Kostadinova*)
- **Hannah Mainstone**: University of Glasgow, Glasgow, UK (1. 8. 2012–31. 5. 2013; *J. Kopecký*)
- **Salvatore Mele** and **Maria Cristina Piras**: University of Sassari, Sassari, Italy (20. 9.–20. 12. 2012; *A. Kostadinova* and *T. Scholz*)
- **Aline Rojas-Sánchez**: Universidad Nacional Autónoma de México, Mexico City, Mexico (6.–21. 8. 2013; *T. Scholz*)
- **Chris Selbach**: University of Essen, Duisburg-Essen, Germany (17.–24. 8. 2012 + 29. 10.–10. 11. 2012; *A. Kostadinova* and *M. Soldánová*)
- **Jelena Spisic**: Osijek University, Osijek, Croatia (April–June, 2012; *V. Hypša* and *J. Kvičerová*)

Stays of foreign researchers

- **Juan Alfonzo**: The Ohio State University, Columbus, USA (September 2012; *Lukeš* lab)
- **Klaus Brehm**: University of Würzburg, Germany; **Alain de Chambrier** and **Jean Mariaux**: Natural History Museum, Geneva, Switzerland; **Anindo Choudhury**: St. Norbert's College, De Pere, USA; **Pradip Kumar Kar**: Jhargram College, West Bengal, India; **John S. Mackiewicz**: State University of New York at Albany, USA; **Mikuláš Oros**: Parasitological Institute, Košice, Slovakia (defences of PhD theses of *A. Ash* and *J. Brabec*, June 2012; *Scholz* lab)
- **Magdaléna Bruňanská**: Parasitological Institute, Košice, Slovakia (15. 10.–6. 11. 2012 + 14.–25. 10.

2013; LEM – *Nebesářová* lab)

- **Alain de Chambrier**: Natural History Museum, Geneva, Switzerland (10.–15. 7. 2013; *Scholz* lab)
- **Triantafyllos Chavakis**: Dresden University of Technology, Germany (15. 1. 2013; *Kotsyfakis* lab)
- **Erik De Clercq**: Rega Institute for Medical Research, Leuwen, Belgie (18. 12. 2013; *Grubhoffer* lab)
- **Sirlei Daffre**: Universidade de São Paulo, Brazil (30. 9.–2. 10. 2013; *Kopáček* lab)
- **Edit Eszterbauer**: Institute for Veterinary Medical Research, Budapest, Hungary (20.–23. 2. 2013; *Holzer* lab)
- **Mark Fast**: Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada (13.–14. 11. 2013; *Holzer* lab)
- **José de la Fuente**: Instituto de Investigación en Recursos Cinegéticos, Ciudad Real, Spain & Oklahoma State University, Stillwater, USA (11.–15. 11. 2012; *Kopáček* and *Kotsyfakis* labs)
- **José de la Fuente**, **Pilar Alberdi** and **Nieves Ayllón**: Instituto de Investigación en Recursos Cinegéticos, Ciudad Real, Spain (26.–29. 5. 2013; *Grubhoffer* lab)
- **John McEvoy**: North Dakota State University, Fargo, USA (12.–17. 6. 2013; *Kváč* lab)
- **John McEvoy** and **Mark Clark**: North Dakota State University, Fargo, USA (21. 9.–1. 10. 2013; *Kváč* lab)
- **Charles McGurk**: Skretting R & D, Norway (8.–10. 12. 2012; *Holzer* lab)
- **Robert Glaser**: Ben Gurion University of the Negev, Israel (26.–29. 3. 2013; *Grubhoffer* lab)
- **Anna Gnipov**: Comenius University, Bratislava, Slovakia (September–December 2013; *Zíková* lab)
- **Joanna Hildebrand** and **Agnieszka Perec-Matysiak**: Institute of Genetics and Microbiology, Wroclaw University, Poland (27.–31. 5. 2013; *Kváč* lab)
- **Jiří (Art) Janata**: Georgia Institute of Technology, Atlanta, GA, USA (18. 11. 2013; *Grubhoffer* lab)
- **Nick Johnson**, **Karen Mansfield** and **Claire Jeffries**: Animal Health and Veterinary Laboratories Agency, United Kingdom (26.–29. 5. 2013; *Grubhoffer* lab)
- **Marta Kicia** and **Maria Weselowska**: Wroclaw Medical University, Poland (11.–25. 4. 2012 + 3.–18. 4. 2013; *Kváč* lab)
- **Armand Kuris**: University of California at Santa Barbara, USA (17.–24. 8. + 30. 8.–5. 9. 2012; *Scholz* lab)
- **Dounia Marzoug**: University of Oran, Algeria (15.–25. 11. 2012 + 24. 10.–1. 11. 2013; *Scholz* lab)
- **Nora Medrano-Mercado**: Universidad Mayor de San Simón, Cochabamba, Bolivia (22.–25. 5. 2012; *Kotsyfakis* lab)
- **Francisco Montero**: Instituto Cavanilles, Valencia, Spain (7.–29. 8. 2013; *Scholz* lab)
- **Anna Papa-Konidari** and **Aikaterini Tsioka**: Aristotle University of Thessaloniki, Grece (26.–29. 5. 2013; *Grubhoffer* lab)
- **Ingrid Papajová** and **Jana Pipiková**: Parasitological Institute, Slovak Academy of Science, Košice, Slovak Republic (4.–8. 3. 2013; *Kváč* lab)
- **Agnieszka Perec-Matysiak**: Wroclaw University, Wroclaw, Poland (25.–27. 6. 2012; *Kváč* lab)
- **Ana Pérez del Olmo**: University of Barcelona, Spain (20. 8.–3. 9. 2012; *Scholz* lab)
- **Branislav Peťko** and **Bronislava Víchová**: Parasitological Institute, Košice, Slovakia (1.–5. 6. 2012; *Grubhoffer* lab)
- **Bernd Sures**: University of Duisburg-Essen, Germany (19.–21. 8. 2012; *Scholz* lab)

Survey of lectures and courses (2012–2013) (hours/year)[1]

Name	Course	2012	2013
I. Fiala	Field parasitology	-	36
L. Grubhoffer	Biochemistry	60	60
L. Grubhoffer	Biochemistry 1 (CB + Linz)*	65	65
L. Grubhoffer	Biochemistry 2 (CB + Linz)	-	45
L. Grubhoffer	Glycobiochemistry (CB+Linz)	30	30
H. Hashimi	Cell regulation and signalling	-	39
V. Hypša	Biology of parasitism	26	26
V. Hypša	Biology of marine invertebrates	26	26
V. Hypša	Molecular phylogenetics	26	26
V. Hypša	Biology of parasitic arthropods	-	39
J. Kopecký	Immunology	40	40
J. Kopecký	Immunology (CB + Linz)	-	40
J. Kopecký	Parasite immunology	20	20
J. Kopecký	Cell and tissue cultures	20	20
J. Kopecký	Cell and tissue cultures (CB + Linz)	-	20
J. Kopecký	Parasite immunology	-	10[5]
M. Kostka	Protistology	26	-
M. Kostka	Biology of parasitic protozoa	39	-
B. Koudela	Biology of parasitic protozoa	-	80[3]
R. Kuchta	Special zoology of invertebrates	-	10
M. Kváč	Zoohygiene and prevention of diseases of farm animals	28[6]	28[6]
M. Kváč	Veterinary medicine	46	46
M. Kváč	Animal health	56[6]	56[6]
M. Kváč	Veterinary parasitology	42[6]	42[6]
J. Lukeš	Biology of parasitic protists	-	78
J. Lukeš	Biochemistry and molecular biology of parasites	30	-
J. Nebesářová	Electron microscopy for biologists I	40	40
J. Nebesářová	Electron microscopy	12[2]	12[2]
J. Nebesářová	Electron microscopy	12[3]	-
J. Nebesářová	Electron microscopy	36[5]	
J. Nebesářová	Biological electron microscopy I and II	18*	18*
M. Oborník	Bioinformatics	-	24
M. Oborník	Molecular taxonomy	-	56[6]
D. Růžek	Medical virology	48	48
B. Sak	Cell biology methods	28[2]	282
J. Salát	Parasite immunology	20[3]	-
T. Scholz	Biology of helminths	78	-
T. Scholz	Special zoology of invertebrates	-	3
J. Štefka	Conservation genetics	52[1]	-
J. Štěrba	Advanced biochemistry laboratory	42	42
J. Štěrba	Biochemistry laboratory 2 (CB + Linz)	42	42
J. Štěrba	Xenobiochemistry and toxicology (CB + Linz)	56	56
J. Štěrba	Chemistry seminar for 2 and 3 year	40	40
J. Štěrba	Biochemistry laboratory	56	72
J. Štěrba	Biochemistry laboratory (CB + Linz)	42	42
J. Štěrba	Instrumental methods in biochemistry and biophysics	-	12
J. Štěrba	Introduction to toxicology	36	24
M. Vancová	Electron microscopy for biologists I	20	20
M. Vancová	Electron microscopy	12[2]	12[2]

M. Vancová	Biological electron microscopy I and II	6*	6*
J. Vávra	Biology of parasitic protists	-	39
J. Vávra	Biology of parasitic protists	-	78
A. Zíková	Molecular biology of cell	-	26[1]

[1] Faculty of Science, University of South Bohemia, České Budějovice, unless otherwise stated; [2] Faculty of Health and Social Studies, University of South Bohemia, České Budějovice; [3] Faculty of Science, Masaryk University, Brno; [4] Faculty of Education, University of South Bohemia, České Budějovice; [5] Faculty of Science, Charles University, Prague; [6] Faculty of Agriculture, University of South Bohemia, České Budějovice; * (CB + Linz) – crossborder curriculum of Biological Chemistry (University of South Bohemia, České Budějovice & Johannes Kepler University in Linz, Austria)