50th Jírovec's Protozoological Days

Conference Proceedings

Biology Centre CAS, Institute of Parasitology,
České Budějovice 2021

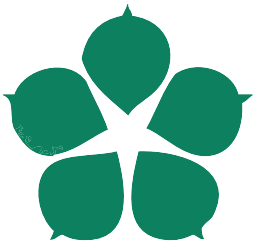
FIND ALL WORDS RELATED TO OUR PASSION!

(WORDS ARE HIDDEN → ↓ ↘)

ENJOY!

L 5 0 E U K A R Y O T ' G O L G I Z
E D I N O F L A G E L L A T E S P O
C P A R C H E A P L A S T I D A C O
A R B I K O N T S S T R O M A O R S
N U C L E U S R H O P T R I E S L P
D N A A L G A E V O L U T I O N U O
M E M B R A N E C Y T O S O M E M R
P O R W T A P I C O P L A S T O E E
D U N I K O N T S S C I E N C E N S
C I L I A T E S F L A G E L L U M A
R N A H A P T I S T A V A C U O L E
C R Y P T I S T A Y P L A S T I D S

Partners



Jihočeský kraj

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

This conference is supported by the Biological Centre CAS, Institute of Parasitology, Czech Society for Parasitology, University of South Bohemia, Faculty of Science, Region of South Bohemia (SDO/OEZI/750/21 - reg.n. 458-01-015/21) and by European grant CZ.02.2.69/0.0/0.0/16_028/0006247. The funding is very acknowledged.

Sponsors


eurofins
Genomics


BioTek
innovative


PHOTON
SYSTEMS
INSTRUMENTS


eppendorf


Schoeller
INSTRUMENTS


DYNEX


fisherscientific
by Thermo Fisher Scientific


VWR
We Enable Science


ALCHIMICA


OLYMPUS
Your Vision, Our Future

Content

Program

Monday

14:00	Registration start	
19:00	Welcome reception	

Tuesday

7:30	Breakfast	
9:00	**Lilach Sheiner**	mitochondrial enzymes in *Toxoplasma* - a complex story!
10:00	Petr Rada	Double stranded RNA viruses are released from *Trichomonas vaginalis* inside exososomes and affect the exosomal cargo.
10:20	Nadine Zimmann	*Trichomonas vaginalis*' inner Pac-Man: Composition and targeting of the lysosomal degradome
10:40	Abhijith Makki	Hydrogenosomal protein import machinery in *Trichomonas vaginalis*
11:00	Coffee break	
11:20	Jana Salamonová	Coevolution of the genetic code and release factors in mitochondria
11:40	Lucia Tomečková	En route to determine *Euglena gracilis* plastid translocons by CRISPR-Cas9-mediated Tic21 protein tagging
12:00	Jitka Kučerová	Where hydrogenosomes and ER meet

12:20	Justyna Zitek	Characterisation of mitochondrion-related organelle in *Paratrimastix pyriformis* using LOPIT proteomics
12:40	Vladimír Hampl	Mitochondrial targeting potential of proteins from amitochondriate protist *Monocercomonoides exilis*
13:00	Lunch	
14:20	Shaghayegh Sheikh	A dynamin-related protein from Nucleocytoviricota may shed light on the evolutionary history of mitochondria remodelling in opisthokonts
14:40	Zdeněk Verner	Anaerobic peroxisomes in *Entamoeba histolytica*: hints and proofs
15:00	Ansgar Gruber	Multi-class predictions of intracellular locations of proteins in organisms with complex plastids
15:20	Jiří Týč	SBEM analysis of *Trypanosoma brucei* life cycle progression from procyclic to metacyclic stages in 3D
15:40	Lawrence Rudy Cadena	Bacterial origin of mitochondrial cristae
16:00	Sponsor presentation	
16:15	Coffee break	
16:35	Poster session	
19:00	Dinner	
20:00	Demonstration of Protist	

	CSP Meeting	

Wednesday

7:30	Breakfast	
9:00	Michal Richtář	Viruses of the Nucleocytoviricota group in eustigmatophyte algae
9:20	Pavla Tůmová	Insight into the genome segregation machinery of Giardia intestinalis using FIB/SEM tomography
9:40	František Stejskal	Management of metronidazole-refractory giardiasis at the tertiary care hospital in Prague
10:00	Iva Hammerbauerová	Companion animals as a source of zoonotic giardiasis: molecular characterization of *Giardia intestinalis* populations from dogs, cats and chinchillas.
10:20	Group photo	
10:40	Coffee break	
11:20	**Gaelle Lentini**	Structural insights into an atypical secretory pathway kinase crucial for *Toxoplasma gondii* invasion
12:20	Minal Jain	Cleavage of *Trypanosoma brucei* FoF1-ATP synthase subunit alpha: A Unique and Rare attribute
12:40	Daria Tashyreva	Massive accumulation of Ba and Sr in marine diplonemids (Euglenozoa)
13:00	Sponsor presentation	

13:15	Lunch	
15:00	Passivity / Free time	
19:00	Reception	

Thursday

7:30	Breakfast	
9:00	Vladimir Varga	Expansion microscopy facilitates quantitative super-resolution studies of cytoskeletal structures in kinetoplastid parasites
9:20	Jana Pilátová	Paradigm shift in eukaryotic biocrystallization
9:40	Ivan Čepička	Anaeramoebae – not so boring amoebae
10:00	Dovile Barcyte	*Olisthodiscus* represents a new class of Ochrophyta
10:20	Marek Valt	Discovery of a novel deep-branching protist with an extraordinary morphology and energetic metabolism
10:40	Valéria Juricová	The molecular mechanism of predation of the diplonemid *Hemistasia phaeocysticola*
11:00	Coffee break	
11:20	Vijaya Geetha Gonepogu	Dynamics and Isolation of Guanine Crystals from *Chromera velia*
11:40	Shun-Min Yang	Using a diatom model to study an unusual histone protein in *Chromera velia*
12:00	Ayush Sharma	Agrobacterium-mediated transformation of *Chromera velia*
12:20	Pragya Tripathi	Tagging of pyruvate dehydrogenase (pdhD) E3 subunit in *Diplonema papillatum*

12:40	Pavla Šnebergerová	Plasmepsin IX/X analogues in *Babesia* and their validation as novel drug targets
13:00	Lunch	
14:20	**Robert Hirt**	Trichomonads, masters of mucosal surfaces
15:20	Daniel Sojka	Targeting proteasomes in ticks and tick-borne pathogens as a novel intervention strategy
15:40	Priscila Peña-Diaz	The *Monocercomonoides* Fe-S cluster assembly system
16:00	Michael Hammond	The distinctive flagellar proteome of *Euglena gracilis* illuminates the complexities of protistan flagella
16:20	Sponsor presentation	
16:35	Coffee break	
17:00	Vendula Rašková	Characterisation of the Pam related proteins in *Trypanosoma brucei*
17:20	Michaela Kunzová	The absence of the FoF1 ATP synthase inhibitory peptide IF1 is necessary for efficient and complete differentiation of the infectious metacyclic form of *Trypanosoma brucei*
17:40	Ondřej Gahura	Toolkit for biogenesis of divergent trypanosomal mitoribosome includes multitude of novel and conserved assembly factors
18:00	Julie Kovářová	Gluconeogenesis in the bloodstream form of *Trypanosoma brucei*

18:20		Concluding remarks
18:40	**Dinner**	

Friday

7:30	Breakfast	
	Departure	

List of Posters

Anna	Brotánková	Trypanosomes of the group of *Trypanosoma theileri*: Phylogeny and new potential vectors
Pia	Corre	Evolution of glycolytic enzymes in anaerobic eukaryotes
Magdaléna	Franková	Molecular mechanisms of RNA polyadenylation in *Euglena* plastid
Prashant	Chauhan	Role of lineage-specific mitoribosomal assembly factor mtSAF24 in *Trypanosoma brucei*
Natalia	Janowicz	Generation of membrane potential in *Trypanosoma brucei* mitochondria by putative ATP/ADP carriers from Fornicata
Anna	Kolešová	Characterization of type II secretion system in mitochondria of *Naegleria gruberi* - the unexpected evolutionary link between bacteria and mitochondria
Bankatesh	Kumar	The impact and mechanism of queuosine tRNA modification on differentiation and virulence of *Leishmania mexicana*
Lenka	Marková	Using CRISPR/Cas 9 to iluminate *Giardia intestinalis* encystation pathway
Daniel	Méndez-Sánchez	Morphological and molecular diversity of *Bothrostoma*, a neglected genus of Metopida (Ciliophora, Armophorea)
Seda	Mirzoyan	Investigating the Mitogenomes of Heterolobosea Representatives Using the Oxford Nanopore

		DNA Sequencing Technology
Monika	Novotná	Pentoses as energy sources of Mastigamoeba balamuthi
Aneta	Perglerová	Genotyping of *Giardia intestinalis* from patients with metronidazole-refractory giardiasis from the Bulovka University Hospital in Prague, Czech Republic.
Ondřej	Pomahač	Twilight of the *Metopus*, or How to Do Taxonomy With a Hammer
Jonathan	Wong	Identification of the Oxa1 related machinery for insertion of mitochondrially encoded membrane proteins in *Trypanosoma brucei*
Marie	Zelená	Expansion Microscopy (ExM) as a tool to study flagellar tip proteins in *Trypanosoma brucei*

Tuesday

fisher scientific
part of Thermo Fisher Scientific

Fisher Scientific, spol. s r. o.
Kosmonautů 324
530 09 Pardubice
Tel.: **466 798 230**, fax: **466 435 008**
e-mail: **info.cz@thermofisher.com**
e-shop: **www.thermofisher.cz**

Pro svou práci volte kvalitu!

Firma **Fisher Scientific, spol. s r. o.** je českou pobočkou mezinárodní společnosti **Thermo Fisher Scientific**, která je největším světovým dodavatelem laboratorní techniky a vybavení laboratoří

- **laboratorní přístroje a zařízení**
- **laboratorní sklo, plasty, spotřební materiál**
- **laboratorní chemikálie, rozpouštědla, speciální chemikálie**
- **přístroje a spotřební materiál pro molekulární biologii, biochemii, mikrobiologii**
- **pomůcky pro ochranu zdraví a bezpečnost práce v laboratoři**
- **laboratorní nábytek, digestoře**

KERN
heidolph
Julabo
Fisherbrand
thermo scientific
gibco
invitrogen
applied biosystems
Honeywell
GE Healthcare
HAMILTON
3M

www.thermofisher.cz

Lilach Sheiner

lilach.sheiner@glasgow.ac.uk

Wellcome Centre for Integrative Parasitology, University of Glasgow, Glasgow, United Kingdom

Mitochondrial enzymes in *Toxoplasma* - a complex story!

The mitochondrial electron transport chain (mETC) and F1Fo-ATP synthase are of central importance for energy and metabolism in eukaryotic cells. The Apicomplexa, important pathogens of humans causing diseases such as toxoplasmosis and malaria, depend on their mETC in every known stage of their complicated life cycles. Using a complexome profiling proteomic approach, we have characterised the *Toxoplasma* mETC complexes and F1Fo-ATP synthase. We identified and assigned 60 proteins to complexes II, IV and F1Fo-ATP synthase of *Toxoplasma*, of which 16 have not been identified previously. Notably, our complexome profile elucidates the composition of the *Toxoplasma* complex III, the target of clinically used drugs such as atovaquone. We identified two new homologous subunits and two new parasite-specific subunits, one of which is broadly conserved in myzozoans. We demonstrate all four proteins are essential for complex III stability and parasite growth, and show their depletion leads to decreased mitochondrial potential, supporting their assignment as complex III subunits. Our study highlights the divergent subunit composition of the

apicomplexan mETC and F1Fo-ATP synthase complexes and sets the stage for future structural and drug discovery studies.

Petr Rada[1], Ravi Kumar Narayanasamy[1], Alois Zdrha[1], Ivan Hrdý[1], Petrus Tang[2], Jan Tachezy[1]

radapetr81@gmail.com

[1] Department of Parasitology, Faculty of Science, Charles University, Biocev, Průmyslová 595, Vestec 25250

[2] Department of Parasitology, College of Medicine, Chang Gung University, Taoyuan City 33302, Taiwan

Double stranded RNA viruses are released from *Trichomonas vaginalis* inside exososomes and affect the exosomal cargo

Trichomonas vaginalis is a parasitic protist that infects the human urogenital tract, where it causes human trichomoniasis. Approximately 40% up to 70% of *T. vaginalis* strains harbor endosymbiotic double stranded RNA viruses called trichomonasviruses (TVV). During infection, trichomonads adhere to the host mucosa, acquire nutrients from the vaginal/prostate environment, interact with the host immune response and release small extracellular vesicles called exosomes that contribute to the trichomonad adherence. Moreover, trichomonads that harbor trichomonasviruses produce exosomes that have immunosuppressive effect to the host immune system. To investigate effect of TVV endosymbionts on the exosomal cargo we first derived isogenic *T. vaginalis* clones with and without TVV. TVV replication in *T. vaginalis* positive clone was inhibited with 2′-C-methylcytidine that

revealed strong selectivity for TVVs over trichomonad growth. Then exosomes rel eased from TVV positive and negative clone were isolated and used for analysis of small RNA and protein content. RNA analysis revealed that more than 90% of the reads are ribosomal-related transcripts. TVV negative clone had more ribosomal-related reads. The second highest cluster was tRNA-related transcripts, which constitute around 5% of the reads. The positive clone had a higher level of tRNA-related reads. Proteomic analyses revealed 1448 and 1391 in TVV positive and negative samples, respectively. 124 proteins revealed significantly higher quantity in the TVV positive clone including membrane associated adhesine BspA. Importantly, proteomic analysis revealed presence of TVV capsid protein and RdRp in exosomes of TVV positive strain. Moreover, up to 6029 RNA read mapped to TVV genome. These results suggested that TVV might be present in exosomes. Indeed, electron microscopy revealed presence of TVV virions in vesicles of about 125 nm corresponding to exosomes. Based on these res ults we hypothesize that TVVs module RNA and protein cargo of exosomes, and are released from *T. vaginalis* in these vesicles. Because exosomes can fuse with membranes of the host cells, TVV may have potential to modulate the host immune response.

Nadine Zimmann[1], Petr Rada[1], Ivan Hrdý[1], Jan Tachezy[1]

nadine.hohensee@natur.cuni.cz

[1] Charles University, Biocev, Vestec, Czech Republic

Trichomonas vaginalis' inner Pac-Man: Composition and targeting of the lysosomal degradome

Lysosomes play a central role in cell homeostasis and participate in a variety of cellular processes including phagocytosis, autophagy, and secretion, yet little is known about this organelle in the human parasite *Trichomonas vaginalis*. In this study, we used high-resolution label-free quantitative mass spectrometry to analyse the degradome of highly-purified phagolysosomes and lysosomes. We obtained the organelles by density gradient centrifugation in (i) Percoll and (ii) OptiPrep, and (iii) by phagocytosis of lactoferrin-covered magnetic Dynabeads. In total, 3,443 (Percoll), 4,099 (OptiPrep), and 1,351 (Dynabeads) proteins were identified. Based on that we established the lysosomal proteome of 462 proteins which were sorted into 23 functional classes. Hydrolases represent the largest functional class and include proteases, lipases, phosphatases, glycosidases and other hydrolases. Many of them, especially within the cysteine peptidases, were shown to be potent virulenc e factors towards host cells or bacteria. Therefore, we used the cysteine peptidases TvCP2 and CLCP as a model to investigate lysosomal targeting and secretion. Secretion of TvCP2 was inhibited by chloroquine and brefeldin A,

indicating its secretion through lysosomes rather than through the conventional endoplasmic reticulum-to-Golgi pathway. Wildtype CLCP is lysosome-resident, but it gets secreted to the cell environment once either of its two glycosylation sites Nx[ST] is mutated. Our studies are the first to show direct involvement of lysosomes in secretion and further demonstrate that the Nx[ST] motif plays an important role in lysosomal targeting in this organism. Overall, the lysosomal proteome that we established can be useful for future comparative studies.

Abhijith Makki[1], Sami Kereïche[2], Ivan Hrdý[1], Jan Tachezy[1]

abhijith.makki@natur.cuni.cz

[1] Department of Parasitology, Faculty of Science, Charles University, BIOCEV, 25250 Vestec, Prague, Czech Republic

[2] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic

Hydrogenosomal protein import machinery in *Trichomonas vaginalis*

Mitochondria are sites of important cellular functions such as energy metabolism, iron-sulfur cluster assembly, control of apoptosis etc. Nuclear-encoded mitochondrial proteins are synthesized on the cytosolic ribosomes and transported to the organelles by the cytosolic chaperones and mitochondrial protein import machinery based on specific targeting signals. Although, the basic principles of protein import have been explained, many questions remain unanswered, particularly for reductively evolved mitochondria such as hydrogenosomes. We have investigated the hydrogenosomal protein import machinery in *Trichomonas vaginalis* with a focus on the composition, function and structure. Adaptation to operate under anaerobic conditions has resulted in an enormous reduction of both mitochondrial functions as well as proteome of *T. vaginalis* hydrogenosomes. Our work points out that these adaptations, particularly the loss of respiratory

complexes that led to a low membrane potential, or its absence were seminal for the shaping of protein import into *T. vaginalis* hydrogenosomes.

Jana Salamonová[1], David Žihala[1], Marek Eliáš[1]

j.salamonova31@gmail.coml

[1] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00 Slezská Ostrava

Coevolution of the genetic code and release factors in mitochondria

Deviations from the standard genetic code are broadly distributed across the tree of life. Particularly prone to the modifications are the genetic codes of the mitochondrial translation systems, from which a number of various codon reassignments has been described. The most frequent alterations of the mitochondrial genetic code concern the stop codons. These types of deviations are inevitably associated with the changes in the mitochondrial release factors that mediate translation termination. However, until recently, the connection between the mitochondrial genetic code and release factors has not been at the centre of much attention. Employing bioinformatics approaches, we could identify several so far unreported codon reassignments in the mitochondria of the stramenopile group Labyrinthulea (Labyrinthulomycetes) and the supergroup Opisthokonta. We also pointed to unique mutations in the mitochondrial release factors that possibly enable the changes in the use of the terminati on codons. Altogether, these studies further enrich our understanding of the rules governing the evolution of one of the central molecular process in the cell.

Lucia Tomečková[1], Vladimír Hampl[1]

hadariol@natur.cuni.cz

[1] Faculty of Science, Charles University,BIOCEV, 25250 Vestec,Prague, Czech Republic

En route to determine *Euglena gracilis* plastid translocons by CRISPR-Cas9-mediated Tic21 protein tagging

Euglena gracilis is a photosynthetic flagellate protist possessing chlorophyte-derived secondary plastids enclosed by three envelopes. Additional membranes in the plastid envelope necessitate more sophisticated protein import that remains in the case of *E. gracilis* still unclear. Precursor proteins contain N-terminal bipartite targeting sequences consisting of a signal and a transit peptide directing their transport through endoplasmic reticulum, Golgi apparatus and finally into plastids. Golgi-derived vesicles carrying plastid proteins fuse with the outermost envelope membrane probably by a SNARE-independent mechanism and the cargo proteins are released beyond the membrane. It has been expected that majority of the plastid proteins pass across the middle and the inner membrane via protein complexes derived from translocons of outer and inner chloroplast membrane (TOC and TIC, respectively). However, this model has been questioned by transcriptomic and proteomic studies, which revealed only a highly reduced TIC complex and absolutely no TOC complex subunit. Alternative hypothesis for the transport across the middle membrane involving distant homologues of SELMA (symbiont-derived ER-associated degradation-like machinery) translocon was

suggested. We have decided to start with characterisation of the translocon complexes of *E. gracilis* using the only known component Tic21 as a bait. Utilizing a recently published protocol for the CRISPR-Cas9 gene editing technique, we have introduced an HA-tag into one of the three known copies of this gene. By PCR and Western blot screening of 60 clones we have selected two lineages that express the tagged version of this protein. These will be employed for determining of Tic21 interacting partners using co-immunoprecipitation.

Jitka Kučerová[1]

kucerji3@natur.cuni.cz

[1]Department of Parasitology, Faculty of Science, BIOCEV, Charles University, 25242 Vestec, Prague, Czech Republic

Where hydrogenosomes and ER meet

The presence of organelle interactions was for a long time hidden from the eyes of scientists. However, with time the importance of organelle contact sites rises. The interactions are widespread amongst organelles and organisms. The most studied interactions involve endoplasmic reticulum (ER) and mitochondria. Interactions between ER and mitochondria are referred to as mitochondria-associated membranes (MAM) in mammalian cells. In yeast, interactions between ER and mitochondria are executed through molecular tether ERMES. ERMES stands for ER-mitochondria encounter structure. It is a crucial complex which is involved in the transfer of lipids and proteins from ER to mitochondria, mitochondrial dynamics, mitochondrial DNA inheritance and mitophagy. Disrupted function of these proteins leads to impaired structure of mitochondria and eventually to cell death. In *Trichomonas vaginalis*, the human urogenital parasite, three genes for putative, highly divergent components of ER MES complex were predicted. Apart from text-book aerobic organisms, *T. vaginalis* possesses hydrogenosome instead of mitochondria. The close association between hydrogenosomes and ER is often

observable. Our work focuses on investigation of the ERMES complex in *T. vaginalis*, cellular localization, interactions between components and identification of their possible interacting partners.

Justyna Zítek[1], Sebastian C. Treitli[1], Zuzana Zubačová[1], Daryna Zavadska[1], Vladimír Hampl[1]

justyna.kopycinska@gmail.com

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

Characterisation of mitochondrion-related organelle in *Paratrimastix pyriformis* using LOPIT proteomics

Protist group Preaxostyla comprises of unicellular eukaryotic flagellates with reduced mitochondria that live in low oxygen environments. Notably, in one lineage (oxymonads) mitochondrion was lost completely as has been shown in the case of *Monocercomonoides exilis. Paratrimastix pyriformis* is a free-living bacterivorous flagellate representing a sister lineage to oxymonads. In previous studies it has been experimentally confirmed that mitochondrion-related organelle (MRO) is present in *Paratrimastix pyriformis* and contains complete glycine cleavage system. To characterize in more details this MRO we performed localization of organelle proteins by isotope tagging (LOPIT) analysis. LOPIT approach relies on assumption that proteins localized in the same organelle co-fractionate and therefore have similar abundance distribution profiles to known organellar marker proteins. This study revealed the presence of approximately 30 proteins in the organelle among which were neither enzymes involved in extended glycolysis

(pyruvate:ferredoxin oxidoreductase, hydrogenases) nor proteins functioning in FeS cluster assembly (SUF pathway) suggesting that this MRO is not involved in these basic processes. On the other hand, we have detected a pathway of one-carbon folate metabolism connected to glycine cleavage system and leading to the production of formate. We hypothesise that one-carbon metabolism and the formate production specifically may be the primary function of this MRO.

Yi-Kai Fang, Zuzana Vajtová, Vladimír Hampl

vlada@natur.cuni.cz

Department of Parasitology, Faculty of Science, BIOCEV, Charles University, Vestec, Czech Republic

Mitochondrial targeting potential of proteins from amitochondriate protist *Monocercomonoides exilis*

Monocercomonoides exilis, a protist thriving in chinchilla's intestines, lacks according to all available data any form of mitochondrion. This absence is believed to be the result of a relatively ancient secondary loss rather than an ancestral feature. We have decided to use this organism as a model to answer a question on how frequently proteins capable of mitochondrial targeting arise under neutral evolution, i. e. in the situation of no selective pressure for or against this feature. To address this problem, we are systematically detecting proteins from the complete *M. exilis* proteome, which are recognized and imported into two types of mitochondrial organelles differing in the presence of electrochemical potential – hydrogenosomes of *T. vaginalis* and mitochondria of *T. brucei*. For the former, we

have developed a bulk *in vitro* import assay of urea-denatured *M. exilis* cytosolic proteininto hydrogenosomes and tes tedit using HA-tagged *T. vaginalis* frataxin as a positive control. After incubation, hydrogenosomes were repurified, treated with proteinase K and washed to remove organelle associated contaminants. 38 *M. exilis* proteins were identified in the cleaned hydrogenosomes by mass spectrometry of which 24 showed an increased intensity with the time of incubation. These are now being validated by expressing in *T. vaginalis* under a native ferredoxin promotor with an HA tag. In the nine finished cases, the immunofluorescence showed a cytosolic signal with no observable accumulations in the organelles. Western blot, however, detected a minor hydrogenosomal signal in seven cases, and in four of them (aspartate aminotransferase, enolase, phosphoglycerate dehydrogenase and cyclophilin) it remained after proteinase K treatment suggesting these proteins are imported inside the vesicle. Our interim results suggest that amitochondriate cell of *M. exilis* does not con tain proteins preadapted for specific and efficient import into hydrogenosomes but some highly expressed mostly metabolic enzymes are able to leak in. We believe that this phenomenon might play a role in the establishing of the organelle during endosymbiosis. The experiments on aerobic electrochemical potential-containing mitochondrion of *T. brucei* are pending.

Shaghayegh Sheikh[1,2], Tomáš Pánek[3], Jiří Týč[1,2], Kristína Záhonová[4], Julius Lukeš[1], Marek Eliáš[5], Hassan Hashimi [1,2]

shaghayegh.sheikh@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic
[2] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
[3] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 43, Prague 2
[4] Faculty of Science, Charles University, BIOCEV, 25250 Vestec, Czech Republic
[5] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, Ostrava, Czech Republic

A dynamin-related protein from Nucleocytoviricota may shed light on the evolutionary history of mitochondria remodelling in opisthokonts

Optic Atrophy 1 (Opa1) and Mgm1 genes are found in metazoans and fungi, respectively, within the supergroup Opisthokonta. They are members of the dynamin-related GTPase family and are involved in mitochondrial fusion and cristae remodeling. Opa1 and Mgm1 affect cristae junction morphology in different ways. Opa1 and Mgm1 are considered to be closely related homologs in literature, although experimental data does not support that. To shed light on their

relationship, we reconstructed molecular phylogeny of dynamin-related proteins in eukaryotes. Our study has shown a new subgroup of the dynamin family that we call MidX, which we found in genomes of Nucleocytoviricota viruses as well as in several phylogenetically unrelated eukaryotes. Our phylogenetic analysis indicates that MidX is the closest relative of Mgm1. Also like Mgm1, MidX possesses a mitochondrial targeting presequence, although a transmembrane domain is absent. Our study aimed to understand how a Nucleocytoviricota viruses-encoded protein can impact mitochondria, which may also give us insight into the evolutionary history of Mgm1 and Opa1, since MidX is related to the former. To this end, we engineered a cell line that exogenously expresses in *Trypanosoma brucei* Hyperionvirus MidX, a lineage that completely lacks a dynamin-family remodeler of mitochondrial membranes. We show that MidX is targeted into the mitochondrial matrix and closely associates with the inner membrane. MidX expression massively affects mitochondrial morphology, altering the normally reticulated mitochondrion into a tubular-shape and eventually a fragmented appearance. Our results from transmission electron microscopy led us to analyse MidX-expressing cells by serial block-face scanning electron microscopy, which allowed us to reconstruct the organelle's 3D structure. This data shows that the massive remodeling results in pockets of cytoplasm being engulfed by the mitochondrion upon MidX expression. Our data indicates that MidX remodels mitochondria, presenting a possibility that the common ancestor of MidX and Mgm1 may have replaced Opa1 in Fungi, eventually giving rise to Mgm1.

Zdeněk Verner[1], Vojtěch Žárský[1,2], Tien Le[1], Marie Vancová[3], Daniel Rozbeský[4], Abhijith Makki[1], Corinna Lender[5], Iris Bruchhaus[5], Jan Tachezy[1]

zdenek.verner@natur.cuni.cz

[1] Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 25250 Vestec, Czech Republic
[2] Department of Botany, University of British Columbia, 3529-6270 University Boulevard, Vancouver, BC, V6T 1Z4, Canada
[3] Institute of Parasitology, Biology Centre CAS, Branišovská 31, 370 05 České Budějovice
[4] Department of Cell Biology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 25250 Vestec, Czech Republic
[5] Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Straße 74, D-20359 Hamburg, Germany

Anaerobic peroxisomes in *Entamoeba histolytica*: hints and proofs

E. histolytica is believed to be devoid of peroxisomes, like most anaerobic protists. In this work, we provided the first evidence that peroxisomes are present in *E. histolytica*, albeit proteins involved in peroxisome biogenesis was reduced to only seven members (Pex1, Pex6, Pex5, Pex11, Pex14, Pex16, and Pex19). Targeting matrix proteins to peroxisomes is reduced to the PTS1-dependent pathway mediated via the soluble Pex5 receptor. Immunofluorescence microscopy showed that peroxisomal markers (Pex5, Pex14, Pex16, Pex19) are present in vesicles distinct from mitosomes, the endoplasmic reticulum and the endosome/phagosome system, except Pex11, which has dual localization in peroxisomes and mitosomes.

Immunoelectron microscopy revealed that Pex14 localized to vesicles of approximately 90-100 nm in diameter. Proteomic analyses of affinity-purified peroxisomes and in silico PTS1 predictions provided datasets of 655 and 56 peroxisomal candidates, respectively; however, only six proteins were shared by both datasets, including myo-inositol dehydrogenase. The presence of peroxisomes in *E. histolytica* and other archamoebae breaks the paradigm of peroxisome absence in anaerobes and provides a new potential target for the development of antiparasitic drugs.

Ansgar Gruber[1]

ansgar.gruber@paru.cas.cz

[1] Institute of Parasitology, Biology Centre CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic

Multi-class predictions of intracellular locations of proteins in organisms with complex plastids

Diatom plastids evolved by eukaryote-eukaryote endosymbiosis. This process led to a complex plastid ultrastructure, with a total of four membranes surrounding the stroma. The two innermost membranes correspond to the outer and inner envelope of primary plastids found in Archaeplastida. The second membrane from the outside (third from the inside) is considered to correspond to the former plasma membrane of the endosymbiont. Hence, the space between this second and third plastid membranes, the periplastidic compartment (ppc), is a remnant of the cytosol of the former endosymbiont.

Cell biological processes as well as metabolic reactions have been shown to take place in this compartment, however, genome wide predictions of the proteins targeted to this compartment were so far based on manual annotation work exclusively.

With the increase of published experimental data, this situation has changed. At least a subset of the ppc proteins can be predicted from genome data with high specificity. This allows for the estimation that at least 81 proteins are targeted to the ppc in *Phaodactylum tricornutum* (Pt), and 180 proteins in *Thalassiosira pseudana* (Tp). The discrepancy can only partially be explained by genome size (10.814 predicted proteins in *P. tricornutum,* vs 13.344 predicted proteins in *T. pseudonana*), and is supported by previous experimental studies on selected proteins. In contrast to the discrepancy in the number of predicted ppc proteins, the numbers of predicted proteins for plastids (1315 in Pt, 1338 in Tp) and mitochondria (545 in Pt, 475 in Tp) are more similar between the two species.

Jiří Týč[1], František Kitzberger[1,2], Eva Doleželová[1], Alena Zíková[1], Jana Nebesářová[1], Marie Vancová[1]

jiri.tyc@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Budweis, Czech Republic

[2] Faculty of Science, University of South Bohemia, Budweis, Czech Republic

SBEM analysis of *Trypanosoma brucei* life cycle progression from procyclic to metacyclic stages in 3D

Trypanosoma brucei, the causative agent of Human and Animal Trypanosomiases, undergoes a striking cellular transformation during its digenetic life cycle. The transition between the insect forms found in various tse-tse tissues to the mammalian life cycle stages are accompanied by extensive organelles remodelling, including mitochondria regression and repositioning of mitochondrial DNA and flagellum. We use serial block-face scanning electron microscopy (SBEM) to reconstruct three distinct insect life cycle forms generated in vitro, the procyclic trypomastigotes, the epimastigotes and the infective **metacyclic trypomastigotes.** We aim to describe the changes among the three insect stages in high quantifiable detail including the volume, number and spatial organization of the organelles and other ultrastructural features of the cells. SBEM technology images regions of interest with dimensions up to several hundred s of µm yielding

tens of whole cells for an analysis, therefore allowing for statistical evaluation of the obtained data. The final resolution at 6,5 nm (in X, Y axes) and 100nm (Z) **or higher facilitated** the distinction of fine ultrastructural features within the cell. Cells were segmented and visualized in 3D using Microscopy Image Browser and Amira software. This approach provided quantifiable results (volumes, lengths) and ultrastructural details of all cellular organelles and allowed us to compare the three insect forms to the published reconstruction of *T. brucei* mammalian bloodstream form (Hughes et al., J. Cell Sci. 2017). Our study provides a deeper understanding of how organelles are remodelled during the complex life cycle of these medically and veterinary important parasites.

Acknowledgement: This work was supported by TACR (TE01000008) and Czech BioImaging (LM2018129).

Muñoz-Gómez S.A.[1,2], Cadena L.R.[3,4], Sheikh S.[3,4], Leger M.M.[5], Bílý T.[3,4], Gardiner A.[6], Koblizek M.[6], Roger A.J.[7,8], Slamovits C.H.[8], Lukeš J.[3,4], Hashimi H.[3,4]

rudy.cadena@paru.cas.cz

[1] Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique Evolution, Orsay, France

[2] School of Life Sciences, Biodesign Center for Mechanisms of Evolution, Arizona State University, Tempe, AZ, USA

[3] Institute of Parasitology, Biology Center, Czech Academy of Sciences

[4] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

[5] Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain

[6] Centre Algatech, Institute of Microbiology CAS, Třeboň, Czechia.

[7] Centre for Comparative Genomics and Evolutionary Bioinformatics, Dalhousie University, Halifax, Canada

[8] Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Canada

Bacterial origin of mitochondrial cristae

The mitochondrial inner membrane is distinctly folded into electron transport chain enriched invaginations termed cristae, the morphological hallmark of the organelle in aerobic eukaryotes. These highly dynamic structures are likely homologous to the intracytoplastmic membranes (ICMs) found in alpha-proteobacteria for energy production. The biogenesis of cristae is dependent on the multi-protein complex MICOS (MItochondrial Contact site and Cristae Organizing System), as disruption of MICOS results in the detachment of cristae from the inner boundary membrane and the accumulation of internal stacked membranes throughout the matrix.

Phylogenetic analysis of MICOS subunits reveals that MICOS is an ancient eukaryotic complex, correlated with ubiquitous distribution across aerobic eukaryotes. Mic60, one of the core MICOS subunits, was found in various ICM bearing alpha-proteobacteria, the progenitors of mitochondria. The presence of alpha-Mic60 in these organisms implies that MICOS predates the evolution of mitochondria and therefore is of pre-endosymbiotic origin. This credits the hypothesis that both mitochondrial cristae and alpha-proteobacteria ICMs evolved from the same common ancestor, however, to the extent of which alpha-Mic60 is involved in ICM formation is still relatively obscure.

Here, we reveal that alpha-Mic60 is indeed involved in the development of the alpha-proteobacterial envelop in the non-sulfur purple bacterium *Rhodobacter sphaeroides*. Strains lacking or overexpressing alpha-Mic60 result in deformities to ICMs. This is consistent with the idea that alpha-Mic60 plays a role in the development of alpha-proteobacterial envelops into functional, mature ICMs, comparable to that of MICOS role in cristae biogenesis. Alpha-Mic60 is also shown to form part of a larger complex, apparently creating contact sites with outer membrane proteins. The parallels between eukaryotic Mic60 and prokaryotic alpha-Mic60 shows cogent evidence that mitochondria inherited a pre-existing ultrastructure modified for efficient energy production from their alpha-proteobacterial ancestors.

Brotánková Anna[1], Svobodová Milena[1], Fialová Magdaléna[1], Čepička Ivan[2], Brzoňová Jana[1]

brotankova.a@gmail.com

[1] Department of Parasitology, Faculty of Science, Charles University
[2] Department of Zoology, Faculty of Science, Charles University

Trypanosomes of the group of *Trypanosoma theileri*: Phylogeny and new potential vectors

Trypanosomes from *Trypanosoma theileri* group were detected in mosquitoes, tabanids, deer and sheep keds from Czechia by dissections and PCR examinations in 2017-2019. In mosquitoes, the highest prevalence was observed in *Aedes excrucians* (21.7 %), *Ae. Punctor* (21.1 %) and *Ae. vexans* (9.5 %). *T. theileri* was also detected in tabanids with prevalence 44 %, in species *Tabanus bromius* and *Hybomitra ciureai*. *Lipoptena fortisetosa* was PCR positive for *T. theileri* (1.3 %) while *L. cervi* was negative. Sheep trypanosome *T. melophagium* was found in sheep keds (*Melophagus ovinus*) in one from six investigated herds (16.7 %).

Selected trypanosome cultures from mosquitoes, tabanids and sheep keds were experimentally fed to mosquitoes *Culex pipiens quinquefasciatus*, *Cx. p. molestus*, and *Ae. aegypti* and *Phlebotomus perniciosus* to test their vectoral capacity. While *Culex* mosquitoes were rarely infected, *Ae. aegypti* (with prevalence 46.5 % - 90.8 %) and *Ph. perniciosus* (60.0 % - 67.9 %) were discovered to be susceptible for trypanosomes isolated from mosquitoes and in Giemsa stained samples, the infectious stages of *T. theileri* were observed in dissected *Ae. aegypti* hindgut.

While most of our *T. theileri* SSU rRNA sequences belong to the TthII lineage, only few clustered within the TthI lineage. In phylogenetic analysis, some mosquito isolates formed individual branches.

In our study, trypanosomes from the group *T. theileri* were detected in various Diptera families such as mosquitoes, tabanids, sheep and deer keds. High prevalence in mammalophilic mosquitoes, and successful development after experimental feeding, suggests that mosquitoes may have a role in transmission. Phylogenetic analysis revealed high diversity of *T. theileri*.

Pia Corre[1], Pavla Hanouskova[1], Alexander K. Tice[2], Matthew W. Brown[2], Marek Elias[3], Ivan Cepicka[1], Tomas Panek[1]

correp@natur.cuni.cz

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7 128 44 Prague Prague, Czech Republic
[2] Department of Biological Sciences, Mississippi State University, Starkville, USA
[3] Department of Biology and Ecology, Ostrava University, Chittussiho 10, 710 00 Slezská Ostrava, Czech Republic

Evolution of glycolytic enzymes in anaerobic eukaryotes

Glycolysis is a central metabolic pathway in eukaryotic and prokaryotic cells and it consists of ten steps, each operated by a different enzyme. During glycolysis, glucose is converted into pyruvate while producing ATP. Embden-Meyerhof-Parnas glycolysis is the starting point of the core carbon metabolism in eukaryotes. In aerobic lineages, glycolysis is actually just a minor source of energy, since the vast majority of ATP production comes from subsequent Krebs cycle and oxidative phosphorylation, both located in mitochondria. However, several lineages of protists are known to lack those mitochondrial pathways, relying instead on

substrate-level phosphorylation. Therefore in these lineages, glycolysis and subsequent fermentation play a central role in energy metabolism. Optimizing the energy output from glycolysis can thus be an important adaptation on the evolutionary path to anaerobiosis. It has been shown that the origins of some of these enzymes in eukaryotic lineages differ and involve horizontal gene transfer from prokaryotes. A significant variation of the standard glycolytic pathway found in some anaerobic protists (e.g. trichomonads, diplomonads, or oxymonads) is pyrophosphate-dependent glycolysis, which uses pyrophosphate (PPi) dependent enzymes such as PPi-phosphofructokinase (PFP) and pyruvate-phosphate dikinase (PPDK). Some anaerobes use alternative enzymes also for other glycolytic steps (e.g. class II fructose-bisphosphate aldolase). Tremendous success in cultivation and transcriptomics/genomics of diverse aerobic and anaerobic eukaryotes in the last 10 years provides an excellent source of publicly available data allowing for powerful comparisons. These data are used in combination with data from aerobic and anaerobic Heterolobosea generated here to build global phylogenies of all glycolytic enzymes present in eukaryotes to evaluate the role of non-canonical variants of glycolytic enzymes in the evolution of anaerobiosis.

Jiří Pergner[1], Magdaléna Franková[1], Anna Hrčková[2], Karolína Vavroušková[2], Štěpánka Vaňáčová[2], Sara Zimmer[3], Vyacheslav Yurchenko[1], Marek Eliáš[1]

jiri.pergner@gmail.com

[1] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, Ostrava, 710 00 Czech Republic
[2] CEITEC-Central European Institute of Technology, Masaryk University, Kamenice 5/A35, Brno, 625 00, Czech Republic
[3] University of Minnesota, 1303 Ordean Ct, Duluth, MN, 55812, USA

Molecular mechanisms of RNA polyadenylation in *Euglena* plastid

Euglenophytes plastids evolved via a complex process of secondary endosymbiosis and are thus in some attributes different then primary plastids. Previous study has documented polyadenylation of several plastid transcripts in *Euglena gracilis*. While polyadenylation of plastid transcripts was documented in primary plastids, the aforementioned study was first description of RNA polyadenylation in secondary plastid. In our study we focus on unravelling the molecular mechanism of RNA polyadenylation of transcripts in *Euglena* plastid. We identified several proteins putatively involved in RNA metabolism in recently published *Euglena* plastid proteome. We aim on determinating the nature of

Euglena plastid transcripts polyA tails and in vitro and in vivo characterization of function of particular RNA processing proteins in *Euglena* plastid.

Prashant Chauhan[1,2], Laura Zellner[1,2], Patrick Beauchamp[1], Alena Zíková[1,2], Ondřej Gahura[1]

pra751990@gmail.com

[1] Institute of Parasitology , Biology Centre, Czech Academy of Sciences
[2] Faculty of Science, University of South Bohemia

Role of lineage-specific mitoribosomal assembly factor mtSAF24 in *Trypanosoma brucei*

Translation in mitochondria, organelles with vestigial genomes, is catalyzed by mitochondrial ribosomes, or mitoribosomes, which diverged considerably from their endosymbiotic bacterial ancestors and between eukaryotic lineages. In *T. brucei*, conserved functional cores of large and small mitoribosomal subunits (mtLSU and mtSSU) are surrounded by distinct shells, provided by an extended repertoire of proteins. Proteins also stabilize the highly reduced ribosomal RNA (rRNA) and replace its missing structural elements. CryoEM structures of precursors of mtSSU and mtLSU suggested that biogenesis of the divergent *T. brucei* mitoribosomes involves both conserved and derived mechanisms. An mtSSU precursor, termed

mtSSU assemblosome, contains a subset of ribosomal proteins and 34 assembly factors complexed with immature rRNA. The structural hallmark of the assemblosome is a protrusion, located on the immature intersubunit side, capped with a low-resolution disc-shaped density. The disc contacts exclusively a lineage-specific homopentameric assembly factor mtSAF24. While the C-terminal domain (CTD) of mtSAF24 is buried and keeps the decoding center in an immature conformation, its NTD constitutes the proximal part of the protrusion and interacts only with the unknown peripheral disc. We hypothesize that the disc is a piece of a phospholipid bilayer and that the assemblosome is attached to the inner mitochondrial membrane. We will generat e a mtSAF24 RNAi cell line to confirm the essential role of the factor in the mtSSU assembly and mt translation by sucrose gradient fractionation and translation assay, respectively. To test, whether the binding to the unknown disc is required for the mtSAF24 function, we will attempt to rescue the mtSAF24 knock-down phenotypes by the expression of tagged CTD. To directly show, that mtSAF24 interacts with phospholipids, we will perform a liposome floatation assay with recombinant NTD and CTD. Strikingly, an ortholog of mtSAF24 in T. cruzi was reported as a flagellum-associated cation channel, suggesting that mtSAF24 act as a dual function protein. We will use a custom anti-mtSAF24 antibody and endogenous tagging to dissect the subcellular localizatio n of mtSAF24 and to identify possible extra-mitochondrial interactors. We aim to shed light on the function of mtSAF24 in *T. brucei* and determine, whether the mtSSU assembly occurs in the association with the inner mitochondrial membrane, which has not been observed in any organism.

Natalia Ewa Janowicz[1], Michaela Husova[2,3], Carolina Hierro-Yap[2,3], Alena Zíková-Panicucci[2,3], Pavel Doležal[1]

natalia.wandyszewska@gmail.com

[1] Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic
[2] Biology Center, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic
[3] Faculty of Science University of South Bohemia, České Budějovice, Czech Republic

Generation of membrane potential in *Trypanosoma brucei* mitochondria by putative ATP/ADP carriers from Fornicata

ADP/ATP carrier (AAC) plays a crucial role in the energy metabolism of the eukaryotic cell by exporting ATP produced in the mitochondrion to cytosol in exchange for ADP. In non-ATP producing mitochondrion-related organelles (MROs), AAC works in reverse importing ATP into the MROs to provide energy for protein import and chaperones such as HSP70. Therefore, AAC has been identified

even in highly reduced mitosomes of *Entamoeba histolytica*. Several anaerobic protists of Fornicata group were thought to lack AAC in their MROs, but we have recently identified putative AAC genes in four species of fornicates: *Ergobibamus cyprinoides*, *Chilomastix caulleryi*, *Dysnectes brevis* and *Giardia intestinalis*. These species represent both free-living and parasitic anaerobic protists and possess MROs at different stage of metabolic reduction. To investigate their activity in ATP/ADP exchange, we heterologously expressed these putative carriers in *Trypanosoma brucei* cells lacking their own canonical AAC. We confirmed the mitochondrial localization of the four fornicate AACs by immunofluorescence and/or digitonin fractionation. The ATP/ADP exchange activity of the four fornicate AACs was determined in digitonin-permeabilized cells after ATP addition by measuring the polarization of the mitochondrial inner membrane using a fluorescent indicator. Our preliminary data suggest that none of the fornicate AACs studied is capable of ATP/ADP exchange.

Anna Kolešová[1], Martin Benda[1], Markéta Petrů[1], Pavel Doležal[1],

kolesovan@natur.cuni.cz

[1] Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic

Characterization of type II secretion system in mitochondria of *Naegleria gruberi* - the unexpected evolutionary link between bacteria and mitochondria

Type II protein secretion systems (T2SS) are molecular machines that promote specific transport of folded periplasmic proteins in Gram-negative bacteria across a dedicated channel in the outer membrane. Secreted substrates, released to the milieu or displayed on the cell surface, contribute to bacterial adaptation to a range of habitats, from deep-sea waters to animal and plant tissues. Our group succeeded in identifying homologues of core components of bacterial type 2 secretion system in mitochondria of several lineages of unicellular eukaryotes (1). Until this discovery mitochondria were thought to be devoid of protein secretion pathways as these have not yet been identified in any other cellular system.

Accordingly, the discovery of key T2SS genes in eukaryotic organisms is surprising, and if T2SS function similarly to bacterial membranes, it would be interesting to see what proteins it carries. To demonstrate this theory in practice, we work on the identification of proteins and metabolites exported in vitro from isolated mitochondria of *Naegleria gruberi* and identification of the protein-protein interactions among the components of mitochondrial T2SS. This project includes two parallel strategies: (i) we characterize the proteome of *N. gruberi* in the context of other cellular compartments and (ii) we develop methods of reverse genetics for N. gruberi to functionally characterize individual components of this newly discovered but ancestral pathway.

1. L. Horváthová, Nat. Commun. 12, 2947 (2021).

Bankatesh Kumar[1], Michaela Boudová[2], Sneha Kulkarni[1,2], Monika Švábová[2], Zdeněk Paris[1,2]

bankatesh.kumar@paru.cas.cz

[1] Biology Center, Institute of Parasitology, Czech Academy of Sciences

[2] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

The impact and mechanism of queuosine tRNA modification on differentiation and virulence of *Leishmania mexicana*

Leishmania mexicana is a eukaryote, unicellular, digenetic parasite. The life cycle of *L. mexicana* begins in the midgut of sandflies, where procyclic promastigotes proliferate and mature into the infectious metacyclic forms and are finally phagocytosed by macrophages. After infection of the second host, cells proliferate and differentiate into the amastigote stage. The progression of the *L. mexicana* life cycle entails a series of differentiation processes that include remodeling of cellular architecture and change in differentially expressed transcripts. This requires rapid translational adaptation to enable survival under dynamically changing environments. Remarkably, regulation of gene expression occurs mainly via post-transcriptional mechanisms, making these organisms excellent models for studying these processes in cell development.

One possible way to fine-tune gene expression at the post-transcriptional level may involve effects on wobble base pairing, facilitated by tRNA modifications, particularly those found at positions 34 and 37 of the anticodon loop. One such modified nucleoside is queuosine (Q), a hyper modified analogue of guanosine (G), found at the wobble position 34 of tRNAs containing GUN anticodon sequence (AsnGUU, AspGUC, HisGUG, TyrGUA). In eukaryotes, the formation of Q-tRNAs entails replacing G34 for Q, which is catalyzed by a highly conserved enzyme called tRNA-guanine transglycosylase (TGT). The position of Q at the wobble base of the anticodon of tRNAs strongly implicates its role in translation via codon usage-based mechanism. Here we employ CRISPR/Cas 9 gene knockout of LmTGT2 to study whether the presence or absence of the Q-tRNAs influences global translation and subsequent cellular functions. Based on our preliminary results, we conclude that Q modification is necessary during experimental infection of *L. mexicana*, both in vivo and in vitro. However, the detailed regulatory loops of queuosine tRNAs during the life cycle of this protist remain to be elucidated.

Lenka Marková[1], Vendula Horáčková[1], Aneta Pastyříková[1], Pavel Doležal[1]

markova.lenka.cz@seznam.cz

[1] Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 252 50 Vestec

Using CRISPR/Cas 9 to iluminate *Giardia intestinalis* encystation pathway

The process of encystation is an important part of life cycle of many pathogenic organisms. *Giardia intestinalis* is an intestinal parasite of various vertebrates including humans from the supergroup of Excavata. The infectious agent of *Giardia* is the cyst, that leaves body of the host through feces and then remains in the environment. Encystation of *Giardia* occurs in the lower parts of gastrointestinal tract and is induced by increased pH and higher concentration of bile. During the encystation, specific endomembrane organelles, called the encystation-specific vesicles (ESVs) are formed. These vesicles originate in ER and they are used for the accumulation and transport of the cyst wall material (CWM) to the surface of the cell and its posttranslational modifications. CWM

is composed of a fibrous matrix, containing three paralogous cyst wall proteins (CWP 1-3) and a *Giardia*-specific β-1,3-GalNAc homopolymer.

Using the versatile CRISPR/Cas9 system introduced to *G. intestinalis* in our laboratory, we study two different aspects of encystation:

1) The carbohydrate component of *Giardia* cyst wall and the pathway of its synthesis. We are preparing knock-out of genes coding glucosamine-6-P isomerase and UDP-glucose 4-epimerase – two important enzymes involved in formation pathway of main cyst polysaccharide β-1,3-GalNAc or Giardan. Our aim is to assess the role of the carbohydrates in the formation of encystation vesicles and the biogenesis of the cyst wall.

2) Detailed characterization of – CWP1. We already generated a cell line lacking *cwp1* gene. These cells provide excellent background to dissect the actual of CWP1 by the re-introduction of its truncated forms. We will also introduce CWP homologues from other organisms of Metamonada group in order to study the evolution of this unique and highly resistant cyst component.

This work aims to provide new insight into the biology of giardia cysts as well as to refine CRISPR/Cas9 methodology in *Giardia.*

Daniel Méndez-Sánchez[1], Johana Rotterová[1,2], Ondřej Pomahač[1], William Bourland[1], Ivan Čepička[1]

mendezsd@natur.cuni.cz

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic

[2] Current address: Coastal Institute, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI,USA

Morphological and molecular diversity of *Bothrostoma*, a neglected genus of Metopida (Ciliophora, Armophorea)

Order Metopida includes a diverse group of free-living ciliates thriving in anoxic/hypoxic environments. It currently comprises three families, Tropidoatractidae, Apometopidae, and Metopidae. Of these, Metopidae is the most diverse, including 14 genera. However, in phylogenetic analyses, it is also non-monophyletic. *Bothrostoma* is a poorly known metopid genus, with four species described solely on observations of living cells. In contrast to other metopids, cells of *Bothrostoma* are not twisted anteriorly, have a straight preoral dome, possess a long paroral membrane, and an adoral zone confined to the ventral side. *Bothrostoma* spp., like other metopids, harbor prokaryotic methanogenic symbionts; however, nothing is known about their identity or diversity. To study the diversity and phylogenetic position of *Bothrostoma* and their prokaryotic

methanogens, we collected freshwater sediment samples from a variety of remote localities. We examined mo rphology and 18S rRNA gene sequences from a total of 21 populations, additionally, the identity of the methanogenic symbionts for some populations was achieved through 16S rRNA gene sequences. Our results show that the genus *Bothrostoma* comprises at least nine species and further diversity likely remains to be discovered. Phylogenetically, it forms a moderately supported clade with *Planometopus*, not closely related to *Metopus*. Species delimitation is challenging due to intraspecific morphologic and 18S rRNA gene sequence variability. Studied species of *Bothrostoma*, including strains from geographically distant localities, seem to harbor a single species of methanogenic endosymbiont, *Methanobacterium* sp., suggesting a degree of host-symbiont specificity.

Seda Mirzoyan[1], Pavla Hanousková[1], Eliška Zadrobílková[1], Yana Eglit[2], Alastair GB Simpson[2], Marek Eliáš[3], Ivan Čepička[1], Tomáš Pánek[1,3]

seda.mirzoyan@natur.cuni.cz

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic

[2] Department of Biology, Dalhousie University, Halifax, Canada

[3] Department of Biology and Ecology, Ostrava University, Chittussiho 10, 710 00 Slezská Ostrava, Czech Republic

Investigating the Mitogenomes of Heterolobosea Representatives Using the Oxford Nanopore DNA Sequencing Technology

Third-generation long-read sequencing technologies are becoming an essential tool for the reconstruction of complete genomes of non-model prokaryotic and eukaryotic organisms. Although the ultra-long reads produced by the Oxford Nanopore technology have demonstrated their power in assembly contiguity through large and complex repeat regions, their assembly accuracy (~ 90-95%) has been criticized when compared to other next-generation sequencing

technologies e.g. Illumina sequencing. Therefore, generating hybrid assemblies based on long Nanopore reads polished by more accurate short reads produced by Illumina is an optimal approach for recovering high-quality genomes that possess both satisfactory contiguity and accuracy. However, novel sequencing chemistries, advanced flow cell technologies and basecalling algorithms as well as new approaches in read-based polishing software significantly increased the accuracy of nanopore-based genome assembl ies. Thus, it is possible to produce high-quality genomes based solely on the Nanopore data. Here we present the results of our pilot study that examines the usage of the Oxford Nanopore DNA sequencing technology for whole-genome sequencing of Heterolobosea representatives that often possess AT-rich nuclear and mitochondrial genomes. The quality of the Nanopore-based genome assemblies is demonstrated on the mitochondrial genomes of two deep-branching heteroloboseans, complemented by mitogenome sequences of two other related species sequenced by the Illumina technology. The advantages and disadvantages of both methods as well as the comparative analyses of the four mitochondrial genomes are discussed herein.

Monika Novotná[1], Zdeněk Verner[1], Jan Tachezy[1]

novotnamon@natur.cuni.cz

[1] Dept. Parasitology, Fac. Science, Charles University, BIOCEV, Průmyslová 595, 252 50 VESTEC

Pentoses as energy sources of *Mastigamoeba balamuthi*

Mastigamoeba balamuthi is an anaerobic free-living amoeba, a sister species of a parasitic *Entamoeba histolytica*. Genome of *M. balamuthi* suggests that it can use pentose saccharides arabinose and xylose as well as aminoacid arginin as energy sources. To evaluate this capacity, we tested cultivation with various energy sources: glucose, arabinose, xylose or without saccharides, forcing them to utilize aminoacids. In a pilot experiment, we assessed growth on different energy sources. Results show that arabinose and xylose stimulated the growth of *Mastigamoeba* more than glucose or arginin, suggesting pentoses to be a preferred source of energy. Besides growth curves, we evaluated size of the cells and their morphology. We observed that *M. balamuthi* is very polymorphic, their form is frequently asymmetrical or irregular. The cells have lobosa, pseudopodoia and subpseudopodia. The subpseudopodia are continually produced and resorbed. Locom otion is relatively slow and occurs by the extension of single broad pseudopodium bearing conical or finger-like subpseudopodia. Cells grown on

glucose and arabinose were very similar in both size and shape, but cells on glucose formed more pseudopodia than those grown with arabinose. Cells in medium without saccharides were smaller than others and formed less pseudopodia. Finally, cells with xylose were the largest ones with elongated lobopodia with numerous subpseudopodia. In next steps, we plan to develop a defined medium, where we could manipulate amount of arginine to assess its effect on overall energy metabolism. Also, we plan to compare metabolic end-products from different media, numbers of nuclei, motility and encystation capability in response to different energy sources.

Aneta Perglerová[1], Lenka Lecová[1], Ivana Zicklerová[2], Lenka Richterová[2.3.4], Eva Nohýnková[1], František Stejskal[1,5,6], Pavla Tůmová[1]

perglerovaa@natur.cuni.cz

[1] Ústav imunologie a mikrobiologie, 1. lékařská fakulta, Univerzita Karlova, Studničkova 7, Praha 2

[2] Oddělení klinické mikrobiologie, Fakultní nemocnice Bulovka, Budínova 2, Praha 8

[3] Ústav mikrobiologie 3. LF UK, FNKV a SZÚ

[4] NNB, Klinika infekčních a tropických nemocí 1.LF UK

[5] Klinika infekčních nemocí, 2. LF UK a Fakultní nemocnice Bulovka, Budínova 2, Praha 8

[6] Infekční oddělení Krajské nemocnice Liberec, Husova 10, Liberec

Genotyping of *Giardia intestinalis* from patients with metronidazole-refractory giardiasis from the Bulovka University Hospital in Prague, Czech Republic.

Giardiasis is the most common enteric infection of parasitic origin. Its manifestation varies from asymptomatic to acute or chronic diarrhoea. It is treated with

metronidazole (MTZ) or other 5-nitroimidazoles, but an increased incidence of MTZ-refractory infections has been reported in recent years. In this study performed since September 2019 to May 2021, *Giardia*-positive stool samples were collected from 23 patients treated at the Department of Infectious Diseases of the Bulovka University Hospital in Prague in order to find out whether there are some common sequence characteristics in parasites from MTZ-resistant cases. The patient set consisted both of travellers, including cases with treatment difficulties. The majority of cases were imported (22/23; 96%), mostly from India (27%) and Egypt (27%). The failure of treatment with a standard MTZ dosing (≥ 500mg MTZ tid for ≥10 days) occurred in 39% (9/23) of the patients. All of these patients suffere d from diarrhoea and most of them (5/9; 56%) acquired infection in India, where five out of six travellers returning from India (83%) were infected with MTZ-refractory giardiasis. Interestingly, all giardiasis cases in patients returning from Egypt were sensitive to routine MTZ treatment. For genotyping, genomic DNA was extracted from *Giardia* cysts isolated from stool samples of 15 patients and analysed by nested PCR and sequencing using three genes established for *Giardia* genotyping, namely *β-giardin*, *glutamate dehydrogenase* and *triosephosphate isomerase*. It revealed that *Giardia* parasites from the treatment failure cases all belonged to the same genetic group - assemblage B, whereas parasites from cases sensitive to MTZ belonged to various genetic groups. Despite the limited set of patient samples, it seems that the MTZ treatment failure could be associated with a particular genetic profile and geographical o rigin of the *Giardia* parasites.

This study was supported by institutional financial support by Charles University to P.T. (PRIMUS/20/MED/008) and SVV 260520 (Studium infekčních chorob a jejich příčinných agens).

Ondřej Pomahač[1], Daniel Méndez-Sánchez[1], Johana Rotterová[1,2], William Bourland[1], Ivan Čepička[1]

yceev@seznam.cz

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic

[2] Current address: Coastal Institute, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA

Twilight of the *Metopus*, or How to Do Taxonomy with a Hammer

Metopida and Clevelandellida are anaerobic ciliates, each of the two groups being morphologically and ecologically distinct. Despite that fact, the latter group forms an internal brach of the former. Metopids are free-living, while Clevelandellids are intestinal symbionts of various animals. Besides Clevelandellids, one more lineage of endobiotic metopids has been discovered, being represented by a single species, *Parametopidium circumlabens*, from sea urchins. Our research is focused on the closest known free-living relatives of Clevelandellids and *Parametopidium*, i.e. metopid members of so-called IAC clade, which are potentially important for understanding the origin and evolution of the endobiotic life style in ciliates. A significant fraction of the described IAC metopids is formally assigned to the genus *Metopus*. Up to now this genus has served as a collective taxon for almost every organism recognized as a metopid, i.e. superficially similar to the type species *M.*

es. During more than 200 years, over 100 morphospecies have been mentioned in literature. Even newly discovered morphospecies, as well as species with molecular sequences not closely related to *M. es*, are still often assigned to *Metopus*. Nevertheless, phylogenetic analysis showed that this approach is no longer justified and suggested there is the time to end this tradition. Within Metopida, the IAC clade, with considerable overlooked diversity, represents one of the most understudied subgroups. Using a culture-based approach, molecular methods and modern microscopy techniques, may allow resolution of the problematic taxonomy of metopids. Here we describe a newly discovered diversity of IAC metopids (at least four lineages on the genus level, each with several sub-lineages) based on the 18S rRNA gene phylogeny and accompanied by basic morphological and ecological characterization. Interestingly, numerous IAC strains reported from different parts of the world apparently occur in the Czech Republic as well. On the other hand, many of our strains do not fall into any described morphospecies. Our findings stress the necessity to reserve the genus name *Metopus* only for its type species (*M. es*) and its closest relatives, and to reassess the taxonomy of the entire order Metopida.

Jonathan Wong[1,2], Vendula Rašková[1,2], Alena Zíková[1,2], Julius Lukeš[1,2], Ignacio Durante[1], Ondřej Gahura[1]

jonathan.wong@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Branišovská 31, 370 05 Ceske Budejovice, Czech Republic
[2] Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 Ceske Budejovice, Czech Republic

Identification of the Oxa1 related machinery for insertion of mitochondrially encoded membrane proteins in *Trypanosoma brucei*

Mitochondria, organelles of endosymbiotic origin, house considerably reduced mitochondrial (mt) genomes that encode mostly core transmembrane subunits of the oxidative phosphorylation (OXPHOS) complexes found in the inner mitochondrial membrane (IMM). These mitochondrially synthesized proteins are co-translationally integrated into the IMM by members of the ubiquitous YidC/Oxa1/Alb3 family of insertases, as was experimentally documented in yeasts and mammals. Translating mitochondrial ribosomes (mitoribosomes) contact

insertases directly, but the process requires additional proteins, such as Mba1, an integral component of mitoribosomes in some lineages.

Although homologs of Oxa1 and Mba1 were found in most eukaryotic groups, the insertion machinery has not been studied outside Opisthokonta. We aim to characterize the machinery in *Trypanosoma brucei*, bearing highly divergent mitoribosomes featuring a non-canonical exit from the polypeptide tunnel. Three putative homologs of Oxa1 (TbOxa1) and one putative TbMba1 were identified in the *T. brucei* genome. Structure homology modelling of TbOxa1 and TbMba1 is compatible with conserved functions of these proteins.

To identify components of the insertion toolkit by co-immunoprecipitation and/or proximity labeling, we endogenously v5- and/or BioID-tagged all three individual TbOxa1 homologs, TbMba1 and the mitoribosomal proteins uL23m and mL78, located at the tunnel exit. Endogenous tagging confirmed mitochondrial localization for all three identified TbOxa1 in procyclic forms (PCF) of *T. brucei*. To address the importance of TbOxa1 homologs in the biogenesis of individual OXPHOS complexes and mitochondrial physiology, we generated RNAi cell lines in PCF and bloodstream form (BSF) trypanosomes. While single RNAi knock downs of two TbOxa1 homologs in PCF does not associate with a strong growth phenotype, knock down of one of the homologs in BSF affected the assembly of the ATP synthase, suggesting compromised insertion of the mt-encoded a-subunit. We will employ the CRISPR/Cas9 strategy to generate single, double and triple knock-out of TbOxa1 homologs to dissect their insertion substrate specificity or document their functional redundancy. We expect to define conserved and divergent traits of the trypanosomal insertion machinery of mt encoded proteins and thereby contribute to the understanding of evolution of protein targeting mechanisms.

Marie Zelená[1], Hana Váchová[1], Vladimír Varga[1]

marie.zelena12@gmail.com

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Expansion Microscopy (ExM) as a tool to study flagellar tip proteins in *Trypanosoma brucei*

Eukaryotic flagella play essential roles in cell motility, sensing, and signaling. The structure of the flagellum is well conserved. Although most parts of the flagellum were subjects of numerous studies in the past, very little is known about the flagellar tip. Yet, the tip is the sole site of the assembly of the microtubule-based cytoskeleton of the flagellum, the axoneme. Using the kinetoplastid parasite *Trypanosoma brucei* we have previously identified over 50 proteins localizing to the flagellar tip. These proteins show distinct fluorescent patterns in widefield microscopy, such as a dot, a horseshoe, or a comet tail. Further sub-localization of the flagellar tip proteins is a challenging task because such a small area is hard to resolve using conventional microscopic techniques. Therefore, we decided to utilize a new technique for super-resolution microscopy, called ultrastructure expansion microscopy (ExM). ExM enables us to resolve structures below the diffraction limit of a confocal microscope by embedding cells in a swellable polymer, which is then

expanded 4,5-fold in all directions. Here we demonstrate the use of ExM on *Trypanosoma brucei* cell lines expressing selected flagellar tip proteins endogenously tagged with a small epitote tag. This technique helped us determine the position of individual tip proteins in respect to the microtubular axoneme. Qualities such as fast sample preparation, fast imaging, and the ability to image cells rare in population make expansion microscopy a great tool to study the flagellar tip.

Wednesday

ALCHIMICA s.r.o. has been operating in the market for 25 years. ALCHIMICA s.r.o., Research & Development Division, represents leading suppliers of laboratory chemicals in the Czech market: **PANREAC APPLICHEM, GLENTHAM LIFE SCIENCES, CARLO ERBA REAGENTS, REAGENTIA** and **CHEMFACES**. These suppliers cover a wide range of chemicals for biochemistry, DNA analysis, organic and analytical chemistry, microscopy and histology, natural substance standards etc. Our online store shop.alchimica.cz currently contains more than 296,000 items.

In addition to products we offer from our above mentioned suppliers we also represent **NeoFroxx**, a German company fully specializing in biochemistry.

In case you are looking for a chemical that is not placed in our offer, contact us at the below-given e-mail address. Thanks to our membership in INDIS GROUP, the global network of chemical traders, we are able to find and deliver even less usual substances.

We provide transport for orders in the Czech Republic free of charge.

Contact information:

ALCHIMICA s.r.o.
Národní obrany 45
160 00 Praha 6

220 515 541
233 310 866
labchem@alchimica.cz
www.shop.alchimica.cz
www.alchimica.cz

Michal Richtář[1], Tereza Ševčíková[1], Dovilė Barcytė[1], Tatiana Yurchenko[1], Marek Eliáš[1]

michal.richtar@osu.cz

[1] Department of Biology and Ecology, Faculty of Science, University of Ostrava

Viruses of the Nucleocytoviricota group in eustigmatophyte algae

Eustigmatophyceae is a small class of ochrophyte algae. The model genus of this group – *Nannochloropsis* – is a microalga playing an important role in a biofuel industry and polyunsaturated fatty acid production. Recently described new species and their novel biological aspects gained attention in the eustigmatophyte community and beyond. The discovery of endosymbiotic bacteria Ca. *Phycorickettsia trachydisci* in *Trachydiscus minutus* or the integration of *ebo* operon into the plastid genomes of *Vischeria* and *Monodopsis* is surely just the beginning of a story about eustigmatophyte biology mysteries. After the discovery of interactions between eustigmatophytes and bacteria, we turned our attention to other

entities – viruses. Viruses are a part of life of probably every living organism, but one group of these viruses is extremely peculiar. *Nucleocytoviricota* (NCLDV – nucleocytoplasmic large dsDNA v iruses) is a group of viruses with some extreme features including a big genome (up to several Gb) or large virion size (comparable to some bacteria and even to picoeukaryotes). Members of this viral group infect mainly unicellular eukaryotes (amoebae and algae) but some of them (*Poxviridae*) infects also metazoans. We have discovered that representatives of this viral group also integrate into the genomes of eustigmatophytes. Our analyses comprised over 35 eustigmatophyte genomes and included 8 viral marker proteins. The results revealed sequences related to *Phycodnaviridae* and *Mimiviridae* families of NCLDV viruses. The only taxa with scarce presence of any NCLDV sequences were marine *Nannochloropsis* and *Microchloropsis* species, and a few members of Eustigmatales lacked *Mimiviridae* genes. Ranges of the genome sizes and the presence/absence of RNA polymerase genes correlated with typical characteristics of each NCLDV group, furthe r supporting the inferred origin of viral insertions. Strikingly, the genome of the eustig *Characiopsis acuta* additionally harbors an insertion of a NCLDV with an alternative genetic code (UAG=Ser), which we demonstrate to belong to a NCLDV lineage normally infecting the green algal genus *Scotinosphaera* sharing the same genetic code deviation. Our analyses can bring a crucial view into the evolution and diversity of viral genes integrated into the eustigmatophyte genomes and potentially into the *Nucleocytoviricota*-Eustigmatophyceae relationships in general.

Pavla Tůmová[1], Gerhard Wanner[2], Eva Nohýnková[1]

ptumo@lf1.cuni.cz

1 Ústav imunologie a mikrobiologie, 1. lékařská fakulta, Univerzita Karlova, Praha
2 Biozentrum of Ludwig-Maximilian University, Munich, Germany

Insight into the genome segregation machinery of *Giardia intestinalis* using FIB/SEM tomography

The conserved features in eukaryotic genome segregation are less understood and their study requires to investigate variety of mitotic mechanisms in different eukaryotic groups. A binucleated flagellate *Giardia intestinalis* is a representative of ancient Metamonads. It is a challenging model due to its rapidly dividing nuclei and a minimal set of homologues to mammalian or yeast mitotic multiprotein assemblies which are necessary for fidelity and performance of this important step in the parasite multiplication. Moreover, *Giardia* genome exhibits chromosome- and gene- copy number variations, likely due to missing factors to stabilize genome and prevent aneuploidy. The details of chromatin/spindle interface are unknown in *Giardia* from both ultrastructural and biochemical points of view. By using FIB/SEM imaging, we investigated *Giardia* mitotic architecture, including kinetochores. Despite repertoire simplification for structural establishment of kinetochore, a simple trilaminar kinetochore (45 nm in diameter) attached to just one microtubule was observed as the chromatin segregating device in *Giardia*. Such one-microtubule/kinetochore attachment might represent a basal eukaryotic situation.
This study was supported the Grant Agency of the Czech Republic, Project Nr. 20-06498S to E.N.; institutional support from Charles University in Prague

(PRIMUS/20/MED008) and by the Ministry of Education, Youth and Sports of the Czech Republic MSMT-10251/2019, project Nr.: LTAB19004 both to P.T.

Frantisek Stejskal[1,2,3], Milan Trojanek[1], Vyacheslav Grebenyuk[1], Aneta Perglerova[3], Pavla Tumova[3], Lenka Richterova[4], Ivana Zicklerova[4], Eva Nohynkova[3]

fstej@lf1.cuni.cz

[1] Department of Infectious Diseases, 2nd Faculty of Medicine, Charles University and University Hospital Na Bulovce, Prague
[2] Department of Infectious Diseases, Regional Hospital Liberec
[3] Department of Immunology and Microbiology, 1st Faculty of Medicine, Charles University, Prague
[4] Department of Microbiology, University Hospital Na Bulovce, Prague

Management of metronidazole-refractory giardiasis at the tertiary care hospital in Prague

Giardiasis represents one of the most common imported enteric infections from tropical regions. Resistance to metronidazole (MTZ) which is drug of choice for giardiasis is an increasing problem. We are presenting a retrospective analysis of epidemiological and clinical characteristics of patients with laboratory (microscopy or PCR) confirmed giardiasis treated at the Department of Infectious Diseases of University Hospital Na Bulovce in Prague during 1/2004 and 12/2020. Giardiasis was diagnosed in 327 persons (131 female and 196 male). The majority of

infections were imported from Indian Subcontinent (212 cases; 64.8%), followed by Sub-Saharan Africa (36 cases), Southeast Asia (29) and Latin America (22). The most common health problems were diarrhea and bloating. The response to treatment with MTZ was evaluated in 155 patients during 2004 and 2014. Out of 32 patients treated with the lower dose (250 mg tid for 7-10 days) only 3 (9%) responded, 14 (44%) failed and 15 did not com e for the follow up. Out of 123 treated with the higher dose (≥500 mg tid for ≥10 days) 42 (34%) responded, 36 (29%) failed and 45 did not come for the follow up. During 2015 and 2020, the first-line treatment was higher dose of MTZ. Out of 80 patients 31 (39%) were treated successfully, 28 (35%) failed and 21 did not come for the follow up. The second-line treatment was combination of tinidazole (500 mg bid) plus albendazole (400 mg bid) for 14-16 days. Out of 28 patients 19 (68%) were treated successfully, 6 did not come for the follow up and 3 (11%) failed. But these three patients were successfully treated with the third-line treatment with paromomycine (500-1000 mg tid for 10-15 days). Our study revealed a low effectiveness of MTZ especially in patients returning from South Asia in which prolong administration of drug combination should be recommended as the first-line treatment strategy.

Iva Hammerbauerová[1], Lenka Lecová[1], Eva Nohýnková[1], Pavla Tůmová[1]

iva.hammerbauerova@gmail.com

[1] Institute of Immunology and Microbiology, First Factulty of Medicine, Charles University

Companion animals as a source of zoonotic giardiasis: molecular characterization of *Giardia intestinalis* populations from dogs, cats and chinchillas.

Giardia intestinalis is a parasitic protist from the order Diplomonadida that can cause an intestinal disease of both humans and animals. There are eight known genetic groups (assemblages) of *G. intestinalis* differing in host specificity. Two of them, assemblages A and B, can infect humans. To evaluate the potential of zoonotic transmission of *Giardia* from pets to humans, we carried out a screening of dogs, cats and chinchillas from Czech households, shelters and breeders. The animals' stool was examined by microscopy and by nested PCR in a multilocus genotyping scheme using the beta-giardin, glutamate dehydrogenase and triose-phosphate isomerase loci to determine whether pets carry the zoonotic assemblages. 99 dogs, 61 cats and 21 chinchillas were examined. The results point to a more serious risk of zoonotic transmission only from chinchillas, where the zoonotic assemblage B was found in 16 of 18 *Giardia*-positive samples, i.e. in

76.2% o f all samples. Indeed, we recently documented a direct zoonotic transmission of *G. intestinalis* infection from a chinchilla to a child. On the other hand, dogs and cats seem to present a rather small risk of zoonotic transmission of *G. intestinalis*, as zoonotic assemblages were found only in 4% (dogs) and 1.6% (cats) of animals tested.

This study was supported by institutional financial support from Charles University in Prague to P.T. (PRIMUS/20/MED008) and SVV 260 520 (Studium infekčních chorob a jejich příčinných agens).

Gaelle Lentini[1], Rouaa Ben Chaabene[1], Oscar Vadas[1], Chandra Ramakrishnan[2], Adrian B. Hehl[2], Budhaditya Mukherjee[1], Ved Mehta[3,4], Volodymyr M. Korkhov[3,4], Matteo Lunghi[1], Jonas Grossmann[5,6], Bohumil Maco[1], Rémy Visentin[1], Dominique Soldati-Favre[1]

gaelle.lentini@unige.ch

[1] Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland

[2] Institue of Parasitology, University of Zurich, Zurich, Switzerland

[3] Institute of Biochemistry, ETH Zürich, Zurich, Switzerland

[4] Paul Scherrer Institute, Villigen, Switzerland

[5] Functional Genomic Center Zurich, ETH Zurich and University of Zurich, Zurich, Switzerland

[6] The Swiss Institute of Bioinformatics, SIB, Lausanne, Switzerland

---

Structural insights into an atypical secretory pathway kinase crucial for *Toxoplasma gondii* invasion

Active host cell invasion by the obligate intracellular apicomplexan parasites relies on the formation of a moving junction, which connects parasite and host cell plasma membranes during entry. Invading *Toxoplasma gondii* tachyzoites secrete their rhoptry content and insert a complex of RON proteins on the cytoplasmic side of the host cell membrane providing an anchor to which the parasite tethers. We ideintified a rhoptry-resident kinase RON13 as a key virulence factor that plays a crucial role in host cell entry. Cryo-EM, kinase assays, phosphoproteomics and cellular analyses reveal that RON13 is a secretory pathway kinase of atypical structure that phosphorylates rhoptry proteins including the components of the RON complex. Ultimately, RON13 kinase activity controls host cell invasion by anchoring the moving junction at the parasite-host cell interface.

Minal Jain[1,2], Karolina Subrtova[3], Brian Panicucci[1], Achim Schnaufer[4], Alena Zikova[1,2],

minaljain888@gmail.com

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Branisovska 31, Ceske Budejovice, Czech Republic
[2] Faculty of Science, University of South Bohemia, Branisovska 31, Ceske Budejovice, Czech Republic
[3] School of Biological Sciences, University of Edinburgh, West Mains Road, EH9 3JT, Edinburgh, UK
[4] School of Biological Sciences, University of Edinburgh, West Mains Road, EH9 3JT, Edinburgh, UK

Cleavage of *Trypanosoma brucei* FoF1-ATP synthase subunit alpha: A Unique and Rare attribute

In most eukaryotes, the mitochondrial FoF1-ATP synthase utilizes the proton motive force to generate chemical energy in the form of ATP to drive numerous cellular processes. While the structure and composition of the catalytic F1 domain

of the ATP synthase was long thought to be highly conserved across all domains of life, the recent crystal structure of the enzyme from the early diverging protist *T. brucei* revealed several unique attributes. Not only does the F1 domain contain three copies of an additional subunit, but the alpha subunit has two internal proteolytic cleavage sites that expel an octapeptide. This generates mature N- and C-terminal alpha peptides that are incorporated into the functional FoF1-ATP synthase. Here we aim to decipher the biological significance of this proteolytic cleavage. Surprisingly, the in vivo expression of just the mature C-terminal alpha is strictly localized to the mitochondrion. Furthermore, in vitro assays analysing the fluorescence of FRET peptides generated for each alpha proteolytic site demonstrate significantly more robust activity in *T. brucei* cytosolic fractions compared to mitochondrial material. Combined, this data suggests that the alpha proteolytic cleavage events may occur already in the cytosol. In addition, the in vivo expression of a proteolytic resistant alpha subunit reveals that this alpha mutant is inefficiently incorporated into the assembled FoF1-ATP synthase. Furthermore, in vivo expression of the alpha mutant results in the incomplete rescue of an alpha depleted *T. brucei* cell line. Finally, utilizing the in vitro FRET assay, we will biochemically enrich for proteolytically active protein fractions that can be analysed by mass spectrophotometry to identify the protease responsible for the excision of the alpha octapeptide.

Daria Tashyreva[1], Jana Pilátová[1,2,5], Jiří Týč[1], Marie Vancová[1], Bokhari Syed Nadeem Hussain[3], Radim Skoupý[4], Mariana Klementová[5], Hendrik Küpper[6], Peter Mojzeš[5], Julius Lukeš[1]

tashyreva@paru.cas.czl

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic
[2] Faculty of Science, Charles University, Prague, Czech Republic
[3] Institute of Plant Molecular Biology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic
[4] Institute of Scientific Instruments, Czech Academy of Science, Brno, Czech Republic
[5.] Institute of Physics, Czech Academy of Sciences, Prague, Czech Republic
[6] Institute of Plant Molecular Biology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

Massive accumulation of Ba and Sr in marine diplonemids (Euglenozoa)

Diplonemids (Excavata, Euglenozoa) are a group of small (~20 μm) flagellated heterotrophic protists, recognized as perhaps one of the most diverse and abundant groups of eukaryotes in the world ocean. Although diplonemids lack mineral exoskeletons and cell walls, we found that they accumulate copious

amounts of strontium and barium in form of intracellular celestite ($SrSO_4$) and barite ($BaSO_4$) crystals. Both elements are present in trace amounts in natural seawater, yet certain diplonemid species are capable of concentrating Sr and Ba up to 10 000x and 17 000x, respectively, resulting in more than an order of magnitude higher intracellular concentration than previously reported from any other unicellular organisms. The significance of Sr and Ba accumulation is still to be investigated, although we hypothesize that heavy barite and celestite crystals are involved in graviperception and maintenance of buoyancy.

Thursday

PHOTON SYSTEMS INSTRUMENTS

Professional Instruments for Plant Science and Algae Biotechnology

www.psi.cz

PSI PRODUCTS

- Growth Chambers • Incubated Shakers • Photobioreactors
- LED Light Sources • Gas Analyzer • Fluorometers • FluorCams
- PlanTherm • Thermoluminescence • Handheld Devices
- PlantScreen™ Phenotyping Systems

Peter Gorilak[1,2], Martina Pružincová[1], Hana Vachova[1], Marie Olšinová[3], Marketa Schmidt Cernohorska[4,5], Vladimir Varga[1]

varga@img.cas.cz

[1] Laboratory of Cell Motility, Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, Prague, 14220, Czech Republic
[2] Charles University, Faculty of Science, Albertov 6, Prague, 128 00, Czech Republic
[3] IMCF at BIOCEV, Faculty of Science, Charles University, Průmyslová 595, 252 50 Vestec, Czech Republic
[4] Laboratory of Adaptive Immunity, Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, Prague, 14220, Czech Republic
[5] Max Perutz Labs, University of Vienna, Vienna Biocenter (VBC), Dr Bohr-Gasse 9, 1030 Vienna, Austria

Expansion microscopy facilitates quantitative super-resolution studies of cytoskeletal structures in kinetoplastid parasites

Expansion microscopy (ExM) has become a powerful super-resolution method in cell biology. It is a simple, yet robust approach, which requires only a simple

instrumentation and reagents. We applied ExM to important kinetoplastid parasites *Trypanosoma brucei* and *Leishmania major*, which possess a well-defined cytoskeleton. We demonstrate that ExM recapitulates faithfully morphology of cytoskeletal structures known from previous electron-microscopy studies. Importantly, due to its rapidness of image acquisition and 3D reconstruction of cellular volumes ExM complements electron-microscopy approaches by providing highly quantitative data. We demonstrate this on examples of poorly understood microtubule structures, such as the neck microtubule of *T. brucei* or the pocket, cytosolic, and multivesicular tubule-associated microtubules of *L. major*. Finally, we show that established antibody markers of cytoskeletal structures function well in ExM, which will facilitate studies of biology of the kinetoplastid cytoskeleton.

Jana Pilátová[1,2,4], Miroslav Oborník[2], Tomáš Pánek[3], Ivan Čepička[3], Peter Mojzeš[4]

jana.pilatova@natur.cuni.cz

[1] Department of Experimental Plant Biology, Faculty of Science, Charles University, Viničná 5, CZ-12844 Prague 2, Czech Republic

[2] Institute of Parasitology, Czech Academy of Sciences, Branišovská 31, 37005 České Budějovice, Czech Republic

[3] Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ-12844 Prague 2, Czech Republic

[4] Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, CZ-12116 Prague 2, Czech Republic

Paradigm shift in eukaryotic biocrystallization

Crystalline inclusions noticeable in unicellular eukaryotes are mostly of unknown composition and functioning. Compared to well-known calcite scales, silicate frustules, and celestite skeletons, the intracellular crystals are considered to be oxalate, sometimes proteins and rarely purines. Herein we provide a great revision of the cellular microcrystals across the broad diversity of protists. The emergence of Raman microscopy enabled us to address various types of cell inclusions directly

in vivo and *in situ*. We have found that the prevailing chemical nature of intracellular biocrystals corresponds with purines (guanine, uric acid, and xanthine). These are generally present across the vast eukaryotic diversity, and based on our phylogenetic analysis, we infer the parallel independent emergence in evolution. Further understanding of purine crystals metabolism may bring important insights spanning from cellular biology to global ecology.

Ivan Čepička[1], Courtney W. Stairs[2], Petr Táborský[1], Martin Kolisko[3], Tomáš Pánek[1], Magdalena Pavlátová[1], Pavla Hanousková[1], Laura Eme[4], Erik Salomaki[3], Miluše Hradilová[5], Čestmír Vlček[5], Jon Jerlström-Hultqvist[6,7], Andrew J. Roger[7]

10p email

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic
[2] Department of Biology, Lund University, Sölvegatan 35, 22 362, Lund, Sweden
[3] Institute of Parasitology, Biology Centre, Czech Acad. Sci., České Budějovice, 370 05, Czech Republic
[4] Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique Evolution, Orsay, France
[5] Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic
[6] Current address: Department of Cell and Molecular Biology, BMC, Box 596, Uppsala Universitet, Sweden, SE-751 24 Uppsala, Sweden
[7] Centre for Comparative Genomics and Bioinformatics, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Canada

Anaeramoebae – not so boring amoebae

Anaeramoeba is a genus of marine amoebae and amoeboflagellates discovered in 2017. Six *Anaeramoeba* species with clearly distinct morphologies have been described; the true diversity, however, has to be much higher, since all but one

species were reported on the basis of a single isolate. Although the gross morphology of *Anaeramoeba* is highly reminiscent of several unrelated genera within Amoebozoa, its phylogenetic position is not resolved by single- or several-gene phylogenetic analyses. In addition, the cells of *Anaeramoeba* species show a unique combination of unusual features, such as anaerobiosis coupled with a conspicuous aggregate of acristate mitochondria and presumably syntrophic prokaryotic symbionts, an acentriolar centrosome, and extremely thick flagella in amoeboflagellate species. Our phylogenomic analyses surprisingly (or not so surprisingly) show that free-living anaeramoebae represent a novel lineage of Metamonada and are close relatives of predominantly endobiotic Parabasalia. Metabolic reconstructions of the *Anaeramoeba* MROs revealed several mitochondrial features previously not seen in metamonads. In addition, two newly isolated species of *Anaeramoeba* show a morphologically different mode of symbiosis with prokaryotes than the previously described ones.

Dovilė Barcytė[1], Wenche Eikrem[2,3,4], Anette Engesmo[2,4], Sergio Seoane[5], Jens Wohlmann[4], Aleš Horák[6,7], Tatiana Yurchenko[8], Marek Eliáš[9]

dovile.barcyte@gmail.com

[1] Department of Biology and Ecology, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic

[2] Norwegian Institute for Water Research, Gaustadallèen 21, 0349 Oslo, Norway

[3] Natural history Museum, University of Oslo, P.O. Box 1172 Blindern, 0318 Oslo, Norway

[4] Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo, Norway

[5] Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain

[6] Biology Centre, Czech Academy of Sciences, Institute of Parasitology, Branišovská 31, 37005 České Budějovice, Czech Republic

[7] Department of Molecular Biology, Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic

[8] Department of Biology and Ecology, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic

[9]Department of Biology and Ecology, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic

Olisthodiscus represents a new class of Ochrophyta

The phylogenetic diversity of Ochrophyta, a diverse and ecologically important radiation of algae, is still incompletely understood even at the level of the principal lineages. One taxon that has eluded simple classification is the marine flagellate genus *Olisthodiscus*. We investigated *Olisthodiscus luteus* K-0444 and documented its morphological and genetic differences from the NIES-15 strain, which we described as *Olisthodiscus tomasii* sp. nov. Phylogenetic analyses of combined 18S and 28S rRNA sequences confirmed that *Olisthodiscus* constitutes a separate, deep, ochrophyte lineage, but its position could not be resolved. To overcome this problem, we sequenced the plastid genome of *O. luteus* K-0444 and used the new data in multigene phylogenetic analyses, which suggested that *Olisthodiscus* is a sister lineage of the class Pinguiophyceae within a broader clade additionally including Chrysophyceae, Synchromophyceae, and Eustigmatophyceae. Surprisingly, the *Olisthodiscus* plastid genome contained three genes, *ycf80*, *cysT*, and *cysW*, inherited from the rhodophyte ancestor of the ochrophyte plastid yet lost from all other ochrophyte groups studied so far. Combined with nuclear genes for CysA and Sbp proteins, *Olisthodiscus* is the only known ochrophyte possessing a plastidial sulfate transporter SulT. In addition, the finding of a *cemA* gene in the *Olisthodiscus* plastid genome and an updated phylogenetic analysis ruled out the previously proposed hypothesis invoking horizontal *cemA* transfer from a green algal plastid into Synurales. A ltogether, *Olisthodiscus* clearly represents a novel phylogenetically distinct ochrophyte lineage, which we have proposed as a new class, Olisthodiscophyceae.

Marek Valt[1], Tomáš Pánek[1], Pavla Hanousková[1], Johana Rotterová[1], Matthew Brown[2], Ivan Čepička[1],

valtm@natur.cuni.cz

[1] Faculty of Science, Department of Zoology, Charles University, Prague 2, Czech Republic

[2] Department of Biological Sciences, Mississippi State University, Starkville, MS, USA

Discovery of a novel deep-branching protist with an extraordinary morphology and energetic metabolism

Protists represent a vast majority of main eukaryotic evolutionary lineages and constitute a critically important part of microbial communities in broad variety of habitats. With the advent of the genomic era, new deep-branching protist lineages with major impacts on our understanding of eukaryotic evolution and supergroup-level diversity are emerging and other groups are being redefined or further resolved. Currently, there are at least 9 eukaryotic supergroups recognized by the majority of protistologists. Two of them, CRuMs and Hemimastigophora, have been recognized only within the last three years. Here, we present another novel, putatively deep-branching organism, referred to as SUM-K. Based on morphological or ultrastructural characteristics, SUM-K cannot be readily classified

into any recognized protist lineage. A phylogenetic analysis, based on the 18S rRNA gene, completely failed to resolve its evolutionary position. Furthermore, a phylogenomic analysis based on transcriptomic data failed to affiliate SUM-K with any traditional eukaryotic supergroup, placing it as a novel deep lineage of Diaphoretickes. The energetic metabolism of SUM-K is also compelling. We routinely cultivate it under anoxic and microoxic conditions, but transcriptomic data also show the presence of complete pathways of aerobic metabolism as well as pathways typical for anaerobes.

Valéria Juricová[1,2], Aleš Horák[1,2], Tomáš Knobloch[2]

valeria.juricova@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Branišovská 31, 370 05 Ceske Budejovice, Czech Republic

[2] Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 Ceske Budejovice, Czech Republic

The molecular mechanism of predation of the diplonemid *Hemistasia phaeocysticola*

The Tara Oceans study has recently shown that a significant proportion of marine plankton is represented by a hitherto poorly studied group of heterotrophic flagellates, the diplonemids. Despite their cosmopolitan distribution and vast abundance, the feeding strategies of these protists have not yet been fully elucidated. This prompted us to investigate bacterivory as one of the ways in which they obtain energy. Bacterivorous behavior was detected and confirmed via

microscopy, fluorescence in situ hybridization, and growth experiments. We also designed a transcriptomic study of the diplonemid *Hemistastia phaeocysticola*, fed on bacteria species *Paracoccus sp.* We compared gene expression between the three cultivation conditions. *H. phaeocysticola* was fed either with nutrient-rich medium, bacteria, or starved. The medium-fed culture invested more in the expression of genes related to structure, transcription, translation, and the cytoskeleton. Genes of hungry diplonemid cells displayed highly increased expression in metabolism, motility, cell adhesion, and transcriptional repressors that are involved in the cellular response to starvation. We identified highly expressed genes when the cells were actively grazing on bacteria. These upregulated genes were related to protein remodeling, signaling, cell response, and phagocytosis. Among the most interesting phagocytosis genes were digestive enzymes, represented by several peptidases. The expression profile of *H. phaeocysticola* suggests that although this diplonemid is able to utilize bacteria, they are probably not its primary source of energy in the ocean. This observation was also supported by the finding that *H. phaeocysticola* thrives when fed on certain bacterial species but is unable to grow on a mixture of bacteria. Overall, in this study we confirm bacterivory as one of the feeding strategies used by H. phaeocysticola and we believe, that identified phagocytosis genes could be used as markers to detect and study bacterivory in the aquatic environment.

Vijaya Geetha Gonepogu[1,2], Jana Pilátová[1,3,5], Aleš Tomčala[4], Peter Mojzeš[5,6], Miroslav Oborník[1,2] , Ansgar Gruber[1]

geetha.gonepogu@paru.cas.cz

[1] Laboratory of Evolutionary Protistology, Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic

[2] Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic.

[3] Department of Experimental Plant Biology, Faculty of Science, Charles University, CZ-12844 Prague 2, Czech Republic.

[4] Faculty of Fisheries and Protection of Waters, CENAKVA, Institute of Aquaculture and Protection of Waters, University of South Bohemia, Husova 458/102, 370 05 České Budějovice, Czech Republic.

[5] Institute of Physics, Faculty of Mathematics and Physics, Charles University, CZ-12116 Prague 2, Czech Republic.

[6] Institute of Bio- and Geosciences/Plant Sciences (IBG-2), Forschungszentrum Jülich, D-52428 Jülich, Germany.

Dynamics and Isolation of Guanine Crystals from *Chromera velia*

Nitrogen availability is often limiting the growth rate of photosynthetic eukaryotes. The cytosol of the alga *Chromera velia*, the closest known photosynthetic relative

of apicomplexan parasites, contains a high abundance of crystals, which we have shown to consist of the nitrogen rich compound guanine. Upon nitrogen starvation, the crystals decrease in size and numbers. When nitrogen is added to nitrogen-starved cells, crystals are re-formed. The guanine crystals might therefore be storage compartments, which allow the accumulation of nitrogen beyond the immediate demand.

Counting of the crystals via polarizing light microscopy showed, that in the nitrogen replete cultures, the number of crystals did not change throughout the experiment, while in the nitrogen depleted culture, the number of crystals decreased, until no crystals could be observed at day five. In the transmission electron microscope, also in the cells from the nitrogen depleted cultures, crystals could still be observed, these crystals were smaller than the ones in cells from the nitrogen replete cultures. When the cells were transferred back to normal f/2 medium with nitrate, crystals could be detected with the light microscope again, and after 3 days, numbers and sizes of the crystals were the same between previously nitrogen-starved cells and cells that had been maintained in nitrogen replete medium throughout the experiment. From the results of this experiment, we conclude that the nitrogen stored in the guanine can be re-mobilized by the cells when new nitrogen cannot be assimilated, and that nitrogen starved cells react to the addition of nitrogen by forming guanine crystals.

Shun-Min Yang[1,2], Ansgar Gruber[1], Kateřina Jiroutová[1], Miroslav Oborník[1,2]

shun-min.yang@paru.cas.cz

[1] Institute of Parasitology, Biology Centre CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic
[2] Faculty of Sciences, University of South Bohemia, Branišovská 31a, 370 05 České Budějovice

Using a diatom model to study an unusual histone protein in *Chromera velia*

Chromera velia is a photosynthetic alveolate, known as the most closely related phototroph to apicomplexan parasites. It has rhodophyte-derived complex plastids and mitochondria with tubular cristae. The mitochondrial and plastid genomes of *C. velia* are present in an unusual linear form, with so far unknown organization of the DNA. After the whole genome sequence of *C. velia* was published in 2015, the histone genes were annotated, and several histone variants were identified. One of the histones H2A variants in *C. velia* harbors an N-terminal extension resembling a mitochondrial transit peptide. Due to the lack of a genetic transformation system in *C. velia*, we used the diatom *Phaeodactylum tricornutum*, to localize the histone variants in the cell. We generated genetically transformed cell lines of *P. tricornutum*, for ectopic expression of the *C. velia* histone H2A variants fused to the

green fluorescent protein (GFP). The resulting clones were selected and examined by confocal microscopy. Compared to the control, the histone H2A variant without pre-sequence, showed nuclear localization; while the variant with the pre-sequence was found in association with the plastids, and co-localized with DNA. This result suggests that the pre-sequence of the *C. velia* histone H2A variant likely leads the protein to the plastid where it might interact with the organellar DNA. Since histone proteins are rarely found in organelles and are absent from their bacterial ancestors, our finding underlines the unusual properties of organelle genomes in *C. velia*.

Ayush Sharma[1,2], Iva Mozgová[2,3], Miroslav Oborník[1,2]

ayush.sharma@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic
[2] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
[3] Institute of Plant Molecular Biology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

Agrobacterium-mediated transformation of *Chromera velia*

The discovery of *Chromera velia* was a breakthrough for understanding the evolution of parasitic apicomplexans, particularly the transition of the apicomplexan parasite from a photosynthetic or photoparasitic ancestor. The group Apicomonada (also chrompodellids), which contains colpodellids and chromerids (*C. velia* and *Vitrella brassicaformis*), branches on the root of parasitic apicomplexans. Apicomonads are considered one of the most understudied groups due to difficulties with cultivation of most species. To better understand their biology, a model organism representing the group is required. Chromerid *C. velia* has all the essential features necessary to make it the model organism for apicomonads: short life cycle, easy cultivation, genome sequence availability; however, a (stable) genetic transformation system has been missing so far. *Agrobacterium tumefaciens* Mediated Transformation (ATMT) method is a widely used transformation strategy due to its wide host range, from higher plants to single-cell algae. We used modified

pCAMBIA1304 as a vector-carrying genes coding for β-glucuronidase (*uidA*), green fluorescent protein (*gfp*), and chloramphenicol acetyltransferase (*cat*) to transform the *C. velia*. Using the ATMT we obtained chloramphenicol resistant transformants of *C. velia*. By PCR, we confirmed the presence of three different genes (*cat*, *gfp*, and *uidA*) in the transformed cells. In addition, the expression of GFP was verified by Western blot. The simplicity of Agrobacterium-mediated gene transfer and easy uptake of relatively large segments of DNA (up to 150 kb) will take research of *C. velia* to a substantially different level.

Pragya Tripathi[1,2], Corinna Benz[1,2], Julius Lukes[1,2]

pragya.tripathi@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic
[2] Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic.

Tagging of pyruvate dehydrogenase (pdhD) E3 subunit in *Diplonema papillatum*

Diplonemids are a group of highly abundant and diverse marine microeukaryotes that belong to the phylum Euglenozoa and a sister clade to the well-studied, mostly parasitic kinetoplastids. Not much is known about the biology of diplonemids, as few species have been formally described and just one, *Diplonema papillatum*, has been studied at the molecular level. *Diplonema papillatum*, the type species of Diplonemids, was recently established as a model organism, which can be genetically modified with ease. Homologous recombination is achieved by using long overlaps (about 1.5 kb) and can be utilised to endogenous tagging or knocking out specific genes.

Pyruvate dehydrogenase (PDH) is a multienzyme complex which catalyses the decarboxylation of pyruvate and generates acyl-coenzyme A (CoA) and NADH.

Since diplonemids have a fully active aerobic mitochondrion with a complete respiratory chain, the presence of a PDH activity to convert pyruvate into acetyl-CoA would be advantageous. At the genome level it seems that all diplonemids have lost the genes for the PDH complex E1 and E2 components (pdhA, pdhB and pdhC), but have retained pdhD E3 subunit (dihydrolipoamide dehydrogenase or E3). The E3 subunit of PDH is however also shared with other α-keto acid dehydrogenase (KADH) complexes, so it might function exclusively as a subunit of these other complexes in diplonemids. An alternative hypothesis proposes that a bacterial pyruvate decarboxylase (aceE), which is also present, may replace the missing E1 enzyme in a novel PDH complex in these organisms.

Using endogenous gene tagging of pdhD E3 subunit and aceE and proteomics, we aimed to answer the following question: Is the diplonemid mitochondrial pyruvate dehydrogenase (PDH) complex homologous to the canonical complex found in mitochondria of other eukaryotes, or is it a hybrid enzyme also consisting of a bacterial pyruvate decarboxylase (aceE)? Results of these experiments will be presented.

Pavla Šnebergerová[1], Dominika Reichensdörferová[1], Marie Jalovecká[1,2], Daniel Sojka[2]

pavlasnebergerova@seznam.cz

[1] Faculty of Science, University of South Bohemia, Branišovská 1760c, 37005 České Budějovice, Czech Republic
[2] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Branišovská 1160/31, 37005 České Budějovice, Czech Republic

Plasmepsin IX/X analogues in *Babesia* and their validation as novel drug targets

Cathepsin-D like aspartyl proteases (ASPs) of apicomplexan parasites represents a strikingly multifaceted group of enzymes crucial for parasitic lifestyle. Most apicomplexan species encode multiple ASP isoenzymes. For example, *Toxoplasma gondii* encodes for 7 isoenzymes (TgASP1-7) whereas *Plasmodium falciparum* expresses 10 ASPs, termed Plasmepsins (PfPMI-X). All apicomplexan ASPs underwent function-driven evolution from the ancestral protease gene that resulted in a cluster of six independent clades, tagged A to F. These clades reflect

a need for specific proteolytic roles of ASPs during the complex parasitic lifecycle that range from degradation of host haemoglobin in digestive vacuoles, through export of parasite produced effector proteins to host cell cytoplasm during intracellular parasitism, to regulation of egress and invasion. Clade C members - *T. gondii* ASP3 (TgASP3) and *P. falciparum* Plasmepsins IX/X (PfPMIX, PfPMX) have been recently validated as master proteases regulating proteolytic cascade that is associated with apical complex organelles and drives parasite invasion and egress of host cells. Bd(m)ASP3a and Bd(m)ASP3b are two parallelly expressed orthologues of clade C ASPs in *Babesia divergens / Babesia microti*, respectively. Our efforts include recombinant expression of active ASP3s from *B. divergens* and their biochemical characterization. Since TgASP3 and PfPMIX/PfPMX are not disposable enzymes that could be studied by conventional gene knock-out, we are currently establishing CRE/lox *B. divergens* strains to knock-out these enzymes conditionally and validate their indispensable roles during *Babesia* infection. Simultaneously, we went for an alternative strategy of trans-genera gene complementation where BdASP3a/b CDSs are integrated into the genome of TgASP3 conditionally knocked-out *T. gondii* strains. The latest results on transiently transfected Toxoplasma tachyzoites with plasmid constructs confirmed expression of BdASP3a/b to the secretory pathway resembling the one of TgASP3. Similarly, to TgASP3 and PMIX/X, the C clade ASP specific peptidomimetic inhibitor 49c has a strong effect on *B. divergens* in RBC cultures which is also confirmed in vivo in B. microti infected mice. Overall, based on our results and homology with other apicomplexan parasites, *Babesia* ASP3 enzymes appear to be promising proteolytic targets worthy of further examination with regards to the yet missing specific treatment of babesiosis.

Robert P. Hirt

Robert.Hirt@ncl.ac.uk[1]

[1] Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH UK.

Trichomonads, masters of mucosal surfaces

Trichomonads, including *Trichomonas* species, are common obligate symbionts of a broad range of birds and various mammals including humans with pathogenic potentials. In humans *T. vaginalis* and *T. tenax* are associated with respectively the urogenital tracts and the oral cavity. Although *T. vaginalis* has a relatively well-established pathobiology, this is less clear for *T. tenax*, although there is an increasing body of data indicating it is associated with periodontitis. It is also increasingly appreciated that *Trichomonas* species are associated with modified microbiota of the mucosal surfaces they are colonising and that complex network of *Trichomonas*-bacteria-virus interactions contribute in concert to their pathobiology through exacerbating the inflammatory tone on mucosal surfaces leading to various pathologies. Furthermore, there is also evidence for interactions between *Trichomonas* and fungi and in the context of acute respiratory distress syndrome (ARDS) and HIV-AIDS patients *Trichomonas* species can thrive in the lungs and were shown to be strongly associated with *Pneumocystis jiroveci*, a relationship that would have most certainly greatly interested Prof. Otto Jiroveci

who discovered the latter and studied the former too. Here I will present some of our comparative genomics works relating to genes acquired though lateral gene transfers from bacterial sources and how functional and bioinformatic data support a model where these genes have contributed to the capacity of *Trichomonas* species to target the wall of bacteria and fungi.
We hypothesise that the capacity to target members of the microbiota has contributed to facilitate the remarkable capacity of *Trichomonas* species to thrive at various mucosal surfaces across a very broad range of hosts.

Daniel Sojka[1], Luïse Robbertse[1], Marie Jalovecká[1,2], Pavla Fajtová[3], Anthony J. O'Donoghue[3]

sojkadan@gmail.com

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences
Branišovská 1160/31, 37005 Ceské Budejovice, Czech Republic
[2] Faculty of Science, University of South Bohemia, Branišovská 1760c,
37005 Ceské Budejovice, Czech Republic
[3] Center for Discovery and Innovation in Parasitic Diseases, Skaggs School
of Pharmacy and Pharmaceutical Sciences,
University of California San Diego, La Jolla, CA, USA

Targeting proteasomes in ticks and tick-borne pathogens as a novel intervention strategy

Proteasomes are large multi-component protein complexes present in the cytoplasm and nuclei of all eukaryotic cells. Their principal function is to degrade poly -ubiquitinated proteins in the cytosol and nucleus via the ubiquitin-proteasome system. Selective inhibition of the β1 “caspase-like”, β2 “trypsin-like” and β5

"chymotrypsin-like" proteasome proteolytic subunits has become a therapeutic strategy for some types of oncologic disorders. Only in the past few years this idea has been further developed into selective inhibition of parasite over host proteasomes and acquired reputation as an effective novel strategy for the treatment of infectious diseases including malaria, leishmaniasis, schistosomiasis and Chagas disease. Our long-term goal is to validate selective proteasome inhibition as a novel intervention strategy to reduce the burden of tick-borne diseases. We have primarily focused on tick-borne apicomplexan parasites of the genus *Babesia*. Our experimental data validate proteasomes in the two zoonotic species *Babesia divergens* and *Babesia microti* as novel targets for the development of specific therapy. We are aiming our current efforts to increase the selectivity index of *Babesia* specific proteasome inhibitors. This is done by de novo synthesized peptidomimetic inhibitors reflecting the examined substrate preferences of the *Babesia* proteasome catalytic subunits. In line with this approach, we have determined selective inhibitors among novel compounds primarily developed to target the proteasome of the malaria parasite *P. falciparum*, such as the derivates of carmaphycin B. Additionally, some proteasome inhibitors have been also found effective against the tick-borne spirochetes causing Lyme borreliosis. Most surprisingly, the selectivity of proteasome inhibitors for tick over host proteasome is also determined when the tick vectors are exposed to proteasome inhibitors via in vitro (membrane) feeding. Our current results thus uncover a novel concept of dual targeting of proteasomes in tick-borne pathogens and their tick vectors with an enormous potential for development of novel intervention strategies.

Priscila Peña-Diaz[1], Vojtěch Vacek[1], Marie Zelená[1], Ivan Hrdý[1], Joseph Braymer[2], Roland Lill[2,3], Beatrice Py[4], Vladimír Hampl[1]

penadiac@natur.cuni.cz

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

[2] Institut für Zytobiologie und Zytopathologie, Philipps-Universität Marburg, Robert-Koch-Strasse 6, 35032 Marburg

[3] LOEWE Zentrum für Synthetische Mikrobiologie SynMikro, Hans-Meerwein-Strasse, 35043 Marburg, Germany

[4] Laboratoire de Chimie Bactérienne, UMR7243 Aix-Marseille Université CNRS, 31 Chemin Joseph Aiguier, 13009, Marseille, France

The *Monocercomonoides* Fe-S cluster assembly system

One of the hallmark pathways of mitochondria is the assembly of Fe-S clusters, essential cofactors for certain enzymes. These cofactors in most eukaryotes are assembled by the Iron-Sulphur Cluster assembly pathway (ISC). In bacteria, the ISC may be functioning alongside the Sulphur assimilation system (SUF), performing the same tasks when under different physiological conditions. Both systems consist of a cysteine desulfurase that provides the sulphur, a scaffold protein on which the Fe-S cluster is assembled, aided by an ATPase that

hydrolyses ATP for this purpose, a ferredoxin that provides redox power for the reaction and transfer proteins that locate the freshly assembled cluster into their respective apoproteins. *Monocercomonoides exilis* is a protist without mitochondrion that bears a cytosolic SUF system composed basically of an unusually large desulfurase (SufDSU), a scaffold protein SufB, a ferredoxin and an ATPase SufC. In the following study we aimed to characterise the scaffold complex of the system. Predicted tertiary structures of both SufB and SufC, align well with the *E. coli* homologues and contain all residues known to be required for their function.Co-expression of SufB with SufC yields a soluble and stable complex after purification of one of the proteins. Size exclusion chromatography verifies that the proteins co-elute even in the presence of high ionic strength. However, a stoichiometric change is observed when in presence of ATP or GTP. The presence of the nucleotides also changes the specific activity of the ATPase SufC, suggesting that dimerization of the complex takes place after ATP hydrolysis and very possibly on different sites of the protein. We have characterised the SufC activity in the presence and absence of the scaffold protein SufB and suggest a model for their function and assembly.

Michael Hammond[1,#], Martin Zoltner[2,3,#], Jack Garrigan[2], Erin Butterfield[2], Vladimir Varga[4], Julius Lukeš[1,5] and Mark C. Field[1,2,*]

michael.hammond@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic
[2] School of Life Sciences, University of Dundee, Dundee, United Kingdom
[3] Charles University, Faculty of Science, Department of Parasitology, BIOCEV, Vestec, Czech Republic
[4] Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[5] Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic

The distinctive flagellar proteome of *Euglena gracilis* illuminates the complexities of protistan flagella

The eukaryotic flagellum is a prominent organelle with conserved structure and diverse functions. Here we present a proteomic study of the flagella and pellicle of *Euglena gracilis*, a photosynthetic and highly adaptable protist, which employs its flagella for both locomotion and environmental sensing. The biochemically distinct flagella of this euglenozoan yields 1,684 protein groups, which challenges previous

estimates on the protein composition of motile flagella across the eukaryotes. We have identified a range of unexpected similarities shared with mammalian flagella, such as an entire glycolytic pathway and a proteasome complex. Moreover, we have also documented a vast array of flagella-based signal transduction components, which coordinate gravity and light responsive motility. The *E. gracillis* pellicle was found to consist of 937 protein groups, from which we identify additional structural and signaling proteins present within this unique Euglenid trait. The pure flagella yield we report here recommends *E. gracilis* as an amenable organism for further flagella studies.

Vendula Rašková[1,2], Corinne von Känel[3], Ignacio Durante[1], Ingrid Škodová-Sveráková[4], Andre Schneider[3], Julius Lukeš[1,2]

vendula.raskova@paru.cas.cz

[1] Institute of Parasitology, Biology Centre, České Budějovice, Czech Republic

[2] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

[3] Department of Chemistry and Biochemistry, University of Bern, Switzerland

[4] Faculty of Sciences, Comenius University, Bratislava, Slovakia

Characterisation of the Pam related proteins in *Trypanosoma brucei*

Protein import into the mitochondria is controlled by several protein complexes. Translocation of mitochondrial proteins across the inner membrane (IM) is provided by the pre-sequence translocase-associated motor (PAM), specifically Pam18 and Pam16. How these translocase complexes originally evolved is important for understanding early eukaryotic evolution. However, since the acquisition of the mitochondrion occurred already prior the Last Eukaryotic Common Ancestor, tracing the origin and diversification of translocases is a challenging task.

Trypanosoma brucei is a parasitic protist that seves as an important model organism, not only due to the possession of unique features such as kinetoplas DNA, trans-splicing and RNA editing, but also for the study of eukaryogenesis. Recently identified TbPam16 and TbPam18 orthologs in *T. brucei* were, despite their conservation, shown not to participate in protein transport across the IM, being replaced by the non-orthologous TbPam27.

Here we explored the effects of RNAi knock-down of Pam orthologues in the procyclic stage of *T. brucei*. We show that TbPam16 and TbPam18 show a growth defect and that their downregulation affects mitochondrial membrane potential at late time points after induction. Moreover, TbPam16 and TbPam18 RNAi cell lines showed impaired mitochondrial translation and altered respiratory complexes activity. Finally, ablation of these proteins led to the reduction of kinetoplast DNA, with a selective elimination of the maxicircles.

Ondřej Gahura[1], Shintaro Aibara[2], Victor Tobiasson[2], Laura Zellner[1,3,4], Anna Huber[1,3,4], Alena Zíková[1,3], Alexey Amunts[2]

gahura@paru.cas.cz

[1] Institute of Parasitology, Biology Centre Czech Academy of Sciences

[2] Science for Life Laboratory, Stockholm University, Stockholm, Sweden

[3] Faculty of Science, University of South Bohemia, České Budějovice

[4] Johannes Kepler University, Linz, Austria

Toolkit for biogenesis of divergent trypanosomal mitoribosome includes multitude of novel and conserved assembly factors

Mitochondrial ribosomes, or mitoribosomes, diverged considerably not only from their bacterial ancestors, but also between eukaryotic lineages. Mitoribosomes of *Trypanosoma brucei* feature exceptionally reduced RNA content and expanded repertoire of proteins. Using cryoEM, we determined structures of several native trypanosomal mitoribosomal complexes, including abundant assembly intermediates of the large and small subunits (mtSSU and mtLSU). The mtSSU and mtLSU precursors contain 35 and 17 assembly factors, respectively, and rRNA in

largely immature conformation, and lack several proteins found in the mature mitoribosome. The decoding centre of mtSSU and peptidyl transferase centre of mtLSU, key functional parts of all ribosomes, are not formed in the respective precursors. Their maturation requires dissociation of several factors and remodelling of rRNA. The identified assembly factors include lineage-specific p roteins, as well as homologs of factors involved in biogenesis of bacterial ribosomes and/or mitoribosomes in other eukaryotic groups, such as GTPases, methyltransferases, pseudouridine transferases or a DEAD-box RNA helicase, documenting partial conservancy of assembly machineries of structurally and compositionally divergent trypanosomal ribosomes. Using biochemical approaches, we complement the structural data to get more detailed insight into both conserved and divergent mechanisms of mitoribosome biogenesis.

Julie Kovářová[1], Martin Moos[2], Brian Panicucci[1], Petr Šimek[2], Alena Zíková[1], David Horn[3]

julie.kovarova@paru.cas.cz

[1] Institute of Parasitology, Biology Centre CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic

[2] Laboratory of Analytical Biochemistry and Metabolomics, Biology Centre CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic

[3] Wellcome Centre for Anti-Infectives, University of Dundee, UK10p affiliation

Gluconeogenesis in the bloodstream form of *Trypanosoma brucei*

The infectious stage of *Trypanosoma brucei* resides primarily in the bloodstream and cerebrospinal fluid of its mammalian host, but it can also inhabit skin, adipose and other tissues. Until recently, these parasites were considered fully dependent on glucose uptake and metabolism, while gluconeogenesis was believed to be absent. Depletion of glucose transporters is lethal, but surprisingly it can be rescued by addition of glycerol in the culture medium. Indeed, glycerol is utilised in gluconeogenesis and fed into subsequent pathways, as was demonstrated by targeted LC-MS metabolomics. Deletion of the canonical gluconeogenetic gene, fructose-1,6-bisphosphatase as well as sedoheptulose-1,7-bisphosphatase does not abolish gluconeogenetic flux, suggesting that the dephosphorylation of fructose 1,6-bisphosphate can be also conveyed by another enzyme. The best candidate is phosphofructokinase, a glycolytic enzyme considered to be strictly irreversible

operating only in the di rection of fructose 6-phosphate phosphorylation, however the trypanosome homolog lacks the canonical regulation. Another unresolved and intriguing question is apparent lack of regulation between glycolysis and gluconeogenesis. Due to their opposing activities, the two pathways should be strictly controlled in a mutually exclusive manner. In *T. brucei*, the glycolytic and gluconeogenetic enzymes are localized inside glycosomes, peroxisome-derived organelles. Hence, the subcellular compartmentalisation may provide means for regulation. To decipher localisation of these two enzymes, we employ *in situ* tagging and specific antibodies for immunofluorescence microscopy at super-resolution in *T. brucei* bloodstream cells grown under various conditions. We expect that our data will shed light on the parasite's ability to adapt to various environmental niches and will enhance our understanding of the flexible and adaptable central carbon metabolism of *T. brucei*.

List of Participants

Amanda	Albanz	University of Ostrava	amandatabitaalbanaz@gmail.com
Dovile	Barcyte	University of Ostrava	dovile.barcyte@gmail.com
Claretta	Bianchi	University of Ostrava	clarettab85@gmail.com
William	Bourland	Faculty of Science,Charles University, Prague	willbour@me.com
Anna	Brotánková	Faculty of Science,Charles University, Prague,	brotankova.a@gmail.com
Lawrence Rudy	Cadena	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	rudy.cadena@paru.cas.cz
Pia	Corre	Faculty of Science, Charles University, Prague	piasantoscorre@gmail.com
Ivan	Čepička	Faculty of Science, Charles University, Prague	ivan.cepicka@centrum.cz
Eva	Doleželová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	dolezelova@paru.cas.cz
Dorsaf	Ennaceur	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	ennacerdorsaf@gmail.com
Diego Henrique	Fagundes Macedo	University of Ostrava	diegohqm@gmail.com
Martina	Foučková	Faculty of Science,Charles University, Prague	martina.fouckova@gmail.com
Magdaléna	Franková	University of Ostrava	Magdalena.Frankova@osu.cz
Ondřej	Gahura	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	gahura@paru.cas.cz
Vijaya Geetha	Gonepogu	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	geetha.gonepogu@paru.cas.cz
Ansgar	Gruber	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	ansgar.gruber@paru.cas.cz
Iva	Hammerbauerová	First Faculty of Medicine, Charles University, Prague	iva.hammerbauerova@gmail.com
Michael	Hammond	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	michael.hammond@paru.cas.cz
Vladimír	Hampl	Faculty of Science,Charles University, Prague	vlada@natur.cuni.cz

Robert	Hirt	Biosciences Institute, Faculty of Medical Sciences, Newcastle University	robert.hirt@newcastle.ac.uk
Aleš	Horák	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	stredniogar@gmail.com
Arzuv	Charyyeva	University of Ostrava, Department Biology and Ecology	arzuvc@gmail.com
Prashant	Chauhan	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	pra751990@gmail.com
Ľubomíra	Chmelová	University of Ostrava	lubomira.chmelova@gmail.com
Minal	Jain	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	minaljain888@gmail.com
Natalia	Janowicz	Faculty of Science, Charles University	natalia.wandyszewska@gmail.com
Kateřina	Jiroutová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	katcha@paru.cas.cz
Valéria	Juricová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	valeria.juricova@paru.cas.cz
Iosif	Kaurov	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	josef@paru.cas.cz
Martin	Kolísko	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	kolisko@paru.cas.cz
Anna	Kolešová	Faculty of Science, Charles University, Prague	annucha1987@gmail.com
Alexei	Kostygov	University of Ostrava	kostygov@gmail.com
Julie	Kovářová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	julie.kovarova@paru.cas.cz
Aneta	Kubánková	Faculty of Science, Charles University, Prague	aneta.kuban@gmail.com
Jitka	Kučerová	Faculty of Science, Charles University, Prague	kucerji3@natur.cuni.cz
Bankatesh	Kumar	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	bankatesh.kumar@paru.cas.cz
Michaela	Kunzová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	kunzova@paru.cas.cz

Gaelle	Lentini	University of Geneva	gaelle.lentini@unige.ch
Jan	Mach	Faculty of Science, Charles University, Prague	machjan1@gmail.com
Abhijith	Makki	Faculty of Science, Charles University, Prague	abhijith.makki@natur.cuni.cz
Lenka	Marková	Faculty of Science, Charles University, Prague	markova.lenka.cz@seznam.cz
Daniel	Méndez-Sánchez	Faculty of Science, Charles University, Prague	mendezsd@natur.cuni.cz
Seda	Mirzoyan	Faculty of Science, Charles University, Prague	seda.mirzoyan@natur.cuni.cz
Monika	Novotná	Faculty of Science, Charles University, Prague	novotnamon@natur.cuni.cz
Miroslav	Oborník	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	obornik@paru.cas.cz
Tomáš	Pánek	Faculty of Science, Charles University, Prague	panek3@natur.cuni.cz
Zdeněk	Paris	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	parda@paru.cas.cz
Magdaléna	Pavlátová	Charles University, Prague	majda.pavlatova@gmail.com
Priscila	Peña-Diaz	Faculty of Science, Charles University, Prague	penadiac@natur.cuni.cz
Aneta	Perglerová	First Faculty of Medicine, Charles University, Prague	perglerovaa@natur.cuni.cz
Jana	Pilátová	Faculty of Science, Charles University, Prague	jana.pilatova@natur.cuni.cz
Ondřej	Pomahač	Faculty of Science, Charles University, Prague	yceev@seznam.cz
Petr	Rada	Faculty of Science, Charles University, Prague	radapetr81@gmail.com
Vendula	Rašková	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	vendula.raskova@paru.cas.cz
Jana	Režnarová	University of Ostrava	janna.krallova@gmail.com
Michal	Richtář	University of Ostrava	m.richtar@volny.cz

Jitka	Richtová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	jitka.richtova@paru.cas.cz
Jana	Salamonová	University of Ostrava	j.salamonova31@gmail.com
Andreu	Saura	University of Ostrava	andr.saura@gmail.com
Ayush	Sharma	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	ayush.sharma@paru.cas.cz
Shaghayegh	Sheikh	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	sheikh.laboratory@gmail.com
Lilach	Sheiner	Wellcome Centre for Integrative Parasitology, University of Glasgow	lilach.sheiner@glasgow.ac.uk
Tomáš	Skalický	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	tomas.skalicky@paru.cas.cz
Daniel	Sojka	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	sojkadan@gmail.com
Matyáš	Soviš	Faculty of Science, Charles University, Prague	nasut@centrum.cz
František	Stejskal	Department of Infectious Diseases, Regional Hospital Liberec	fstej@lf1.cuni.cz
Pavla	Šnebergerová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	Pavlasnebergerova@seznam.cz
Jan	Tachezy	Faculty of Science, Charles University, Prague	tachezy@natur.cuni.cz
Daria	Tashyreva	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	tashyreva@paru.cas.cz
Lucia	Tomečková	Faculty of Science, Charles University, Prague	hadariol@natur.cuni.cz
Pragya	Tripathi	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	pragya.tripathi@paru.cas.cz
Pavla	Tůmová	First Faculty of Medicine, Charles University, Prague	ptumo@lf1.cuni.cz
Jiří	Týč	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	jiri.tyc@paru.cas.cz
Hana	Váchová	Institute of Molecular Genetics, CAS, Prague	hana.vachova@img.cas.cz

Marek	Valt	Faculty of Science, Charles University, Prague	valtm@natur.cuni.cz
Vladimir	Varga	Institute of Molecular Genetics, CAS, Prague	vladimir.varga@img.cas.cz
Zdeněk	Verner	Faculty of Science, Charles University, Prague	zdenek.verner@natur.cuni.cz
Jonathan	Wong	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	jonathan.wong@paru.cas.cz
Shun-Min	Yang	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	shun-min.yang@paru.cas.cz
Kristína	Záhonová	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	kika.zahonova@gmail.com
Alexandra	Zakharova	University of Ostrava	alexandraz.6946@gmail.com
Marie	Zelená	Institute of Molecular Genetics, CAS, Prague	marie.zelena12@gmail.com
Alena	Zíková	Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice	azikova@paru.cas.cz
Nadine	Zimmann	Faculty of Science, Charles University, Prague	nadine.hohensee@natur.cuni.cz
Justyna	Zitek	Faculty of Science, Charles University, Prague	justyna.kopycinska@gmail.com

Title: 50th Jírovec´s Protozoological Days
Subtitle: Conference Proceedings
Redaction: Tomáš Skalický (Institute of Parasitology, Biological Centre CAS)
Editor: Tomáš Skalický (Institute of Parasitology, Biological Centre CAS), Jitka Richtová (Institute of Parasitology, Biological Centre CAS), Katka Jiroutová (Institute of Parasitology, Biological Centre CAS)
Publisher: Biological Centre CAS, v.v.i.
Place and Year of Publication: České Budějovice, 2021
First Edition
This publication did not undergo any language (nor misspelling) editing.