46th Jírovec's Protozoological Days

Conference Proceedings

Charles University in Prague
Faculty of Science, Department of Parasitology
Praha 2016

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Content

Foreword

Dear protistologists!

It is my honor and pleasure to welcome you, and especially our guest – Mark Field (University of Dundee, UK) and Romain Derelle (Université Paris-Sud, F), to the 46th Jírovec's Protozoological Days of Czech Society of Parasitology (May 2 – May 6, 2016). This year's meeting will be held at the westernmost part of South Moravian region — in pension Rumburak on the river Dyje (Thaya) close to the castle Bítov.

I wish you many unforgettable moments and a lot of new experiences!

Petr Soukal

Program Schedule

Monday May 2, 2016	
13:00	Registration
18:00	Dinner

Tuesday May 3, 2016	
8:00	Breakfast
	Molecular Biology of Protozoa
9:00	Tatiana Yurchenko, Tereza Ševčíková, Hynek Strnad, Anzhelika Butenko & Marek Eliáš: **Horizontal transfer of a novel six-gene operon from a bacterium into the plastid genome of eustigmatophyte algae**
9:20	Natalia Gumińska & Rafał Milanowski: **The physical form of released nonconventional introns in euglenids**
9:40	Romana Petrželková & Marek Eliáš: **Ras superfamily GTPases in Microsporidia: Extremely divergent genes in extremely divergent parasites**
10:00	Eva Doleželová, Brian Panicucci & Alena Panicucci Zíková: **Role of inhibitory factor IF1 during the differentiation of *T. brucei***
10:20	Tomáš Pánek, Martin Sokol, David Žihala, Romain Derelle, Eliška Zadrobílková, Ivan Čepička & Marek Eliáš: **Two new non-canonical nuclear genetic codes from a rhizarian and a fornicate with UAG, but not UAA, as a sense codon**
10:40	Coffee break
11:00	Aygul Ishemgulova, Pavel Flegontov, Jana Hlaváčová, Alexei Kostygov, Jan Votýpka, Julius Lukeš & Vyacheslav Yurchenko: **Comparison of classical and CRISPR/Cas9-mediated approaches for targeted genome modification in *Leishmania mexicana***
11:20	Lenka Horváthová, Vojtěch Žárský, Romain Derelle, Alžběta Krupičková, Veronika Klápšťová, Luboš Voleman, Markéta Petrů, Marek Eliáš, Tomáš Pánek, Ivan Čepička, Gerard Huysmans, Mohamed Chami, Olivera Francetic & Pavel Doležal: **Ancient mitochondrial protein secretion**
11:40	Binnypreet Kaur, Drahomíra Faktorová, Priscila Peña-Diaz & Julius Lukeš: **Genetic modifications and functional analyses of Diplonemids**
12:00	Heather Esson & Miroslav Obornik: **SOD it all: Apparent multiple origins of plastid superoxide dismutases in chromerid algae**
12:20	Danyil Grybchuk, Alexei Kostygov, Julius Lukeš & Vyacheslav Yurchenko: **Monoxenous trypanosomatids are infected by insect- and fungi-specific RNA viruses**

12:40	SeqMe – sponzor presentation
12:50	Lunch
	Biodiversity, Phylogeny and Systematics of Protozoa
14:00	Pavla Hanousková & Ivan Čepička: **Unexpected diversity of the peculiar genus *Creneis* (Excavata: Heterolobosea)**
14:20	Petr Táborský, Tomáš Pánek, Martin Kolísko & Ivan Čepička: **´Anaeramoeba´ - new deep amoeboid lineage of metamonads**
14:40	Michael Kotyk, Zuzana Varadínová & Ivan Čepička: **Minions of Great *Cthulhu* awakening – a new insight into the diversity of parabasalid symbionts of cockroaches**
15:00	Goro Tanifuji, Vojtěch David, Julius Lukeš & John M. Archibald: **Genome(s) of Kinetoplastid endosymbiont *Perkinsela* sp. and its host *Paramoeba pemaquidensis***
15:20	Inga Meyer-Wachsmuth, Astrid Holzer & Ivan Fiala: **Myxozoan diversity as revealed by eDNA: Data mining discovers potentially new lineages of Myxozoa**
15:40	Coffee break
16:00	Poster Session
18:00	Dinner
19:00	Demonstration of Protist
20:00	Meeting of Protistology Section of CSP

Wednesday May 4, 2016	
8:00	Breakfast
Protist Cell Biology and Biochemistry	
9:00	Mark C. Field: **Evolution of the trypanosome nucleus - gene expression mechanisms**
10:00	Lukáš Novák, Zuzana Zubáčová, Anna Karnkowska & Vladimír Hampl: **Preaxostyla: the evolution of anaerobic protists**
10:20	Vacek Vojtěch, Sebastian Treilti, Anna Karnkowska, Lukáš Novák, Zuzana Zubáčová, Ivan Čepička & Vladimír Hampl: **Iron Sulfur Cluster Assembly in amitochondriate oxymonad *Monocercomonoides***
10:40	Coffee break
Biodiversity, Phylogeny and Systematics of Protozoa	
11:00	Julius Lukes: **Never alone: human microbiome and eukaryome**
11:20	Maja Łukomska-Kowalczyk, Anna Karnkowska, Małgorzata Krupska, Rafał Milanowski & Bożena Zakryś: **Hypervariable regions of 18S rDNA as DNA barcodes for autotrophic euglenids**
11:40	Cihlář Jaromír, Füssy Zoltán, Horák Aleš, Kořený Luděk & Oborník Miroslav: **The evolution of heme pathway with regard to endosymbiotic gene transfer**
12:00	Jan Janouskovec & Juan F. Saldarriaga: **Dinoflagellate evolution**
12:20	Treitli Sebastian Cristian, Kotyk Michael, Vlasáková Jitka, Šrámová Eliška, Smejkalová Pavla, Naoji Yubuki, Novotná Kristýna Klára, Čepička Ivan & Hampl Vladimír: **Diversity of the genus *Monocercomonoides***
12:40	Bohemia Genetics – sponzor presentation
12:50	Lunch
14:30	Group photo creating
15:00	Trip
19:00	Raut

Thursday May 5, 2016	
8:00	Breakfast
Molecular Biology of Protozoa	
9:00	Romain Derelle, Franz Lang & Marek Elias: ***Malawimonas* enter the 'omic' era**
10:00	Vojtěch Žárský, Eva Nývltová & Jan Tachezy: ***Entamoeba*'s sophisticated non-evil cousin**
10:20	Anna Vanclová, Anna Karnkowska, Vladimír Hampl & Mark Field: ***Euglena gracilis* plastid proteome**
10:40	Coffee break
11:00	Kristína Záhonová, Alexei Kostygov, Tereza Ševčíková, Vyacheslav Yurchenko & Marek Eliáš: **An unprecedented non-canonical nuclear genetic code with all three termination codons reassigned as sense codons**
11:20	Natalya Kraeva, Drahomíra Faktorová, Eva Horáková, Luděk Kořený, Julius Lukeš & Vyacheslav Yurchenko: **Catalase in Leishmaniinae: with me or against me?**
11:40	Sneha Kulkarni, Helmut Stanzl, Alan Kessler, Veronika Běhálková, Juan D Alfonzo & Zdeněk Paris: **Queuosine: The role of an essential tRNA modification in parasitic protist *Trypanosoma brucei***
12:00	Jan Michálek, Aleš Tomčala, Ivana Schneedorferová & Miroslav Oborník: **Fatty acid biosynthesis in chromerid algae – A bioinformatic approach for identification of key enzymes**
12:20	Erik Birčák, Dominika Vešelényiová & Juraj Krajčovič: **How comparative analysis shows relationship of genes involved in meiosis in Euglenozoa**
12:40	KRD – sponzor presentation
12:50	Lunch
Cell Biology of Protozoa	
14:00	Miroslav Oborník & Zoltán Fussy: **Formation of zoospores by budding in *Vitrella brassicaformis***
14:20	Ivana Schneedorferová, Aleš Tomčala, Iva Opekarová, Jan Michálek & Miroslav Oborník: **Confirmation of *Chromera velia* mixotrophy by metabolomic studies**
14:40	Aleš Tomčala, Ivana Schneedorferová, Jan Michálek, Veronika Kyselová & Miroslav Oborník: **Description of *de novo* fatty acid biosynthesis of apicomplexan cousins *Chromera velia* and *Vitrella brassicaformis***

15:00	Jarmila Bíla, Márie Glavanakovová, Katarína Ženíšková & Róbert Šuťák: **Iron metabolism in *Naegleria gruberi***
15:20	Coffee break
15:40	Luboš Voleman, Vladimíra Najdrová, Pavla Tůmová, Zdeněk Švyndrych, Guy M. Hagen, Jan Tachezy & Pavel Doležal: **Synchronized and ER--associated division of *Giardia intestinalis* mitosomes**
16:00	Petr Rada, Abhijith Radhakrishna Makki, Ivan Hrdý & Jan Tachezy: **Targeting of C-tail anchored proteins into hydrogenosomes and endoplasmic reticulum of *Trichomonas vaginalis***
16:20	A. Hartigan, I. Estensoro, M. Vancová, T. Bílý, S. Patra, E. Eszterbauer & A. S. Holzer: **Myxozoan parasite adapts old dance moves into a new routine**
16:40	Eva Nývltová, Róbert Šuťák, Zdeněk Verner & Jan Tachezy: **Krezol production in *Mastigamoeba balamuthi***
17:00	Veronika Kyselová, Aleš Tomčala, Ivana Schneedorferová & Miroslav Oborník: **Feeding a fish model *Danio rerio* on *Chromera velia* causes severe rearing problems and prohibits maturation of the fish - a pilot study**
17:40	Closing remarks
18:00	Dinner

Friday May 6, 2016	
8:00	Breakfast
9:00	Departure of Participants (till 10 a. m.)

Speakers' names are underlined.

Poster Session

Anzhelika Butenko, Fred R. Opperdoes, Dmitry Filatov, Marek Eliáš, Vyacheslav Yurchenko, Pavel Flegontov & Julius Lukeš: **A comparative study of metabolic and sex-related gene repertoires in kinetoplastids**

Cihlář Jaromír, Füssy Zoltán, Horák Aleš, Kořený Luděk & Oborník Miroslav: **The evolution of heme pathway with regard to endosymbiotic gene transfer**

Luboš Voleman, Zuzana Drašnarová, Ásgeir Ástvaldsson, Staffan G. Svärd & Pavel Doležal: **Introduction of APEX tag into anaerobic protist**

Carolina Hierro Yap, Ondřej Gahura, Brian Panicucci & Alena Zíková: **Role of OSCP protein in bloodstream form and dyskinetoplastic *Trypanosoma brucei***

Juráň Josef & Kaštovský Jan: **Do we need to protect microalgae and protists at all?**

Veronika Klápšťová, Alžběta Krupičková, Lenka Horváthová & Pavel Doležal: **Characterization of mitochondrial secretin complex**

Agata Kulczycka, Maja Łukomska-Kowalczyk & Bożena Zakryś: ***Euglena jirovecii* Fott (Euglenea) – unique autotrophic euglenid swimming backwards**

Vladimíra Najdrová & Pavel Doležal: **The guided entry of tail-anchored proteins pathway in *Giardia intestinalis***

Anna Nenarokova, Pavel Flegontov, Mark C. Field & Julius Lukeš: **Functional annotation of *Euglena gracilis* mitochondrial proteome**

Aygul Ishemgulova, Natalia Kraeva, Drahomíra Faktorová, Lucie Podešvová, Julius Lukeš & Vyacheslav Yurchenko: **Conditional gene expression systems and their potential limitations in developmental studies of *Leishmania***

Johana Rotterová, Ludmila Nováková & Ivan Čepička: **Diversity of Armophorea and new marine anaerobic ciliates hosting prokaryotic symbionts**

Miroslav Obornik & Abdoallah Sharaf: **Phylogenetic Affinities of a Norwegian Sarcinochrysidales isolate algea (Heterokonta) Based on Analysis of Molecular Data**

Tomáš Skalický, Eva Dobáková, Pavel Flegontov, Martina Tesařová, Dagmar Jirsová, Jan Votýpka, Vyacheslav Yurchenko & Julius Lukeš: ***Patrypanosoma*: from free living to a parasitic way of life**

Pavla Bartošová-Sojková, Ashlie Hartigan & Gema Alama-Bermejo: **Origin, diversity and evolution of cystatin superfamily in parasitic cnidarians**

Martin Sokol, Marek Eliáš & Tomáš Pánek: **Alternative genetic codes and their distribution in the tree of life**

Darja Stojanovová, Jan Pyrih & Jan Tachezy: **Cytosolic iron-sulfur cluster assembly machinery in *Trichomonas vaginalis***

Kristýna Uhrová, Eliška Zadrobílková, Tomáš Pánek & Ivan Čepička: **Great diversity of potentially uncultivable anaerobic heteroloboseans**

Natalia Wandyszewska, Luboš Voleman & Pavel Doležal: **Unconventional targeting of tail-anchored proteins in *Giardia intestinalis***

David Žihala, Vladimír Klimeš, Francisco Rivero & Marek Eliáš: **The intricate evolutionary history of RhoBTB proteins**

The names of the presenters are underlined.

Abstracts

Iron metabolism in *Naegleria gruberi*

Jarmila Bíla[1], Márie Glavanakovová[1], Katarína Ženíšková[1] & Róbert Šuťák[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Naegleria gruberi is a free-living heterotrophic amoeba well known for its ability to transform from an amoeba to a flagellate form. It is a close relative to *Naegleria fowleri*, an opportunistic pathogen that can cause fatal meningoencephalitis in humans, a disease currently without efficient treatment. Unlike most nonphotosynthetic unicellular eukaryotes, the genome of *N. gruberi* contains a gene encoding ferritin. *In silico* prediction indicated a mitochondrial presequence suggesting a localization of this ferritin in mitochondria. We confirmed its mitochondrial localization *in vivo* with a specific antibody against this protein and by using fluorescence microscopy. Moreover, using yeast mitochondrial processing peptidase we have cleaved the mitochondrial presequence of purified *N. gruberi* ferritin and identified the cleavage site by Edman degradation. Iron-dependence of ferritin expression, as revealed by immunoblotting, supports its role in iron storage in mitochondria. Iron availability often affects the metabolic activity and becomes a limiting factor for the pathogenity of microorganisms. We thus focused on the impact of the availability of iron on the metabolism in *N. gruberi*. We quantified the changes in the activities of different enzymes caused by the lack or excess of iron in the medium. The most significant changes were observed in the activities of two enzymes: hydrogenase and alcohol dehydrogenase.

How comparative analysis shows relationship of genes involved in meiosis in Euglenozoa

Erik Birčák[1], Dominika Vešelényiová[1] & Juraj Krajčovič[1]
[1]Comenius University, Faculty of Natural Sciences, Department of Genetics, Bratislava, Slovakia

Understanding the presence and structure of genes involved in meiosis and meiotic processes in phylum Euglenozoa became an ongoing task. Despite the fact that sexuality in eukaryotes is a widespread phenomenon, there are still several groups of organisms without observed meiosis or sexual reproduction. Phylum Euglenozoa was long considered as one of these groups, but recent research on the parasite *Trypanosoma brucei* showed the presence of sexual cycle, which was underlined by the presence of several key meiosis-specific genes. Our analysis indicates that these genes are spread across the entire phylum and their occurrence is not unique, which may indicate the presence of the meiotic cycle in greater number of organisms than just *T. brucei*. We used many genomic and transcriptomic assemblies from global databases and private sources as our main source of data supplemented by several raw data from sequence read archive. There we searched for homologues of 60 genes related to meiosis and meiotic process and their phylogenic relations. We present these results as an overview of homologs and their phylogenic relations.

A comparative study of metabolic and sex-related gene repertoires in kinetoplastids

Anzhelika Butenko[1], Fred R. Opperdoes[2], Dmitry Filatov[3], Marek Eliáš[1], Vyacheslav Yurchenko[1,4], Pavel Flegontov[1,4] & Julius Lukeš[4,5,6]
[1]University of Ostrava, Faculty of Science, Life Science Research Centre, Ostrava
[2]de Duve Institute, Université catholique de Louvain, Brussels, Belgium
[3]University of Oxford, Department of Plant Sciences, Oxford, UK
[4]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[5]University of South Bohemia, Faculty of Science, České Budějovice
[6]Canadian Institute for Advanced Research, Toronto, Ontario, Canada

Kinetoplastids (Euglenozoa, Kinetoplastea) is a widespread group of single-celled eukaryotes, which includes free-living kinetoplastids and parasitic trypanosomatids. Trypanosomatids can be restricted to one host (monoxenous) or have a life cycle involving two hosts (dixenous). The latter group contains *Trypanosoma* and *Leishmania* species pathogenic for vertebrates. Comparative analysis of the genome of a free-living kinetoplastid, *B. saltans* along with several trypanosomatid genomes can shed the light on the emergence of parasitism from a free-living lifestyle. Our analysis of 13 kinetoplastid genomes (including the genomes of *Leptomonas pyrrhocoris*, *Leptomonas seymouri*, *Blechomonas ayalai* and *Paratrypanosoma confusum* sequenced by our group) revealed that the adoption of the parasitic lifestyle led to the loss of 50 % of genes, resulting in the loss of complete metabolic pathways, such as lysine and histidine catabolism and aromatic amino-acid degradation. The acquisition of novel genes involved in pteridine reduction, threonine dehydration, the urea cycle, protection against ROS, and diaminopimelate metabolism is observed along with gene losses. *B. saltans* and trypanosomatids still share some metabolic traits: glycosomes, a unique set of the pyrimidine biosynthetic pathway genes, an ATP-phosphofructokinase, an alternative oxidase, synthesis of fatty acids via a set of elongases and a few others. We also searched for meiosis-associated genes and performed the analysis of recombination using the genomes of 6 *L. pyrrhocoris* isolates originating from Central America. The results indicate the presence of meiosis-related genes in *L. pyrrhocoris* along with the absence of recombination (very low levels of it were detected for several scaffolds).

The evolution of heme pathway with regard to endosymbiotic gene transfer

Cihlář Jaromír[1,2], Füssy Zoltán[2], Horák Aleš[1,2], Kořený Luděk[3] & Oborník Miroslav[1,2,4]

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

[3]University of Dundee, College of Life Sciences, Dundee, UK

[4]Institute of Microbiology CAS, Centre Algatech, v.v.i., Třeboň

Acquisition of organelles essentially leads to increased metabolic integration with the host cell. Gene transfer to nucleus and respective protein retargeting back to the nascent organelle are processes that reinforce this initially fragile host-symbiont relationship. We investigated the evolution of tetrapyrrole biosynthesis enzymes used for heme and chlorophyll production in algae with plastids of complex origins. These include microalgae with highly autonomous tertiary endosymbiont of diatom provenance (dinotoms), algae with plastids containing vestigial nucleomorph (*Bigelowiella* and *Guillardia*), and dinoflagellate possessing a chlorophyte-derived plastid that replaced the original organelle derived from a red alga (*Lepidodinium*). In line with previous results, we describe tetrapyrrole pathway as a mosaic of enzymes of various evolutionary origins. Further, the whole pathway can be relocated to newly acquired homologous organelle "as is", without the need for recruitment of additional genes, as in the case of *Lepidodinium*. We also discuss the peculiar duplicate pathway of *Bigelowiella natans* and, in *Guillardia theta*, a bifurcation of the pathway to supply mitochondria with heme. A novel paralogue of glutamate-1-semialdehyde aminomutase was found in dinoflagellates, possibly acquired by LGT from proteobacteria early in the evolution of the group. Similarly, a proteobacterial ferrochelatase resides in the genome of dinoflagellates branching together with rhodophytes and Apicomplexa, sister to green lineage/cyanobacteria and heterotrophic eukaryotes, suggesting an ancient prokaryote to eukaryote gene transfer.

Genome(s) of Kinetoplastid endosymbiont *Perkinsela* sp. and its host *Paramoeba pemaquidensis*

Goro Tanifuji[1,2,3], Vojtěch David[1,2,3], Julius Lukeš[3,4] & John M. Archibald[1,2,3]
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[3]Canadian Institute for Advanced Research, CIFAR Program in Integrated Microbial Biodiversity, Toronto, ON M5G 1Z8
[4]nstitute of Parasitology, Biology Centre, and University of South Bohemia, České Budějovice, Czech Republic

Perkinsela sp. is an early branching kinetoplastid protist living within *Paramoeba* (amoebozoa) as an obligate endosymbiont. To our knowledge, this relationship is the only example of eukayote-to-eukaryote endosymbiotic event without the involvement of plastid. We have sequenced *Paramoeba pemaquidensis* and assembled four genomes, corresponding to the nuclear and mitochondrial compartments of *Perkinsela* and its host. By combination of comparative genomics and high pressure freezing electron microscopy, we have uncovered significant functional reduction of the symbiont, namely diminished mitochondrial energy metabolism, reduced cytoplasmic volume and the absence of flagella. We have also demonstrated extensive endocytic activity of *Perkinsela* towards host cytoplasm, likely the main source of its nutrients. Other features, commonly associated with endosymbiosis, such as endosymbiotic genome rearrangement and horizontal gene transfer were also noticed.

Malawimonas enter the 'omic' era

Romain Derelle[1], Franz Lang[2] & Marek Elias[3]
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[2]Université de Montréal, Département de Biochimie, Montreal, Canada
[3]University of Ostrava, Faculty of Science, Department of Biology and Ecology, Ostrava, Czech Republic

Role of inhibitory factor IF1 during the differentiation of *T. brucei*

Eva Doleželová[1], Brian Panicucci[1] & Alena Panicucci Zíková[1,2]
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[2]University of South Bohemia, Faculty of Science, Branisovska 31a, České Budějovice

Trypanosoma brucei undergoes a complex life cycle as it alternates between a mammalian host and the blood-feeding insect vector, a tsetse fly. Due to the different environments, the distinct life stages differ in their energy metabolism, i.e. insect stage (procyclic cells, PF) depends on mitochondrial oxidative phosphorylation (OXPHOS) for ATP production while the bloodstream stage (BF) gains energy by aerobic glycolysis. The dramatic switch from the OXPHOS to glycolysis happens during the complex development of the PF in the tsetse fly. The molecular mechanism behind this shift is still unknown. Importantly, an induced over--expression of a differentiation factor, RNA-binding protein 6 (RBP6), results in the appearance of epimastigotes and metacyclic trypanosome *in vitro* (Kolev, 2012). We have established this RBP6 overexpressing cell line and the presence of the distinct cell types was verified using DAPI staining to visualize position of the kinetoplast to nuclei and by an endocytosis test. Moreover, we checked for changes in expression of subunits of respiratory complexes III and V. Interestingly, the level of *T. brucei* inhibitory factor 1 (TbIF1), a specific natural inhibitor of complex V, was significantly increased in the RBP6-induced cells. At the same time, we detected elevated levels of radical oxygen species (ROS) and changes in mitochondrial membrane potential. This is similar to what is reported in cancer cells, where high levels of IF1 expression inhibits ATP synthesis and creates a ROS signal that triggers the metabolic switch from OXPHOS to aerobic glycolysis. Determining how TbIF1 is regulated and what is the signaling mechanism during the trypanosome differentiation are important aims of this project.

Introduction of APEX tag into anaerobic protist

Luboš Voleman[1], Zuzana Drašnarová[1], Ásgeir Ástvaldsson[2], Staffan G. Svärd[2] & Pavel Doležal[1]
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The intermembrane space (IMS) of aerobic mitochondria contains proteins of the respiratory chain and machineries for the protein import and assembly. However, we do not know anything about the IMS of hydrogenosomes and mitosomes. The reason is that it is impossible to isolate IMS with classical methods like cell fractionation. Recently, the new ascorbate peroxidase (APEX) tag has been developed. It is suitable for EM as well as fluorescence visualization and behaves like biotin ligase when exposed to biotin-phenol. Altogether, it allows compartment specific protein labeling and their subsequent isolation. We have managed to establish this technique for visualization of mitosomal matrix in *Giardia intestinalis*. Our further aim is to use APEX tag for characterization of IMSs of hydrogenosomes and mitosomes of *Trichomonas vaginalis* and *Giardia intestinalis*, respectively.

Horizontal transfer of a novel six-gene operon from a bacterium into the plastid genome of eustigmatophyte algae

Tatiana Yurchenko[1,2], Tereza Ševčíková[1], Hynek Strnad[3], Anzhelika Butenko[1] & Marek Eliáš[1,2]

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[2]University of Ostrava, Faculty of Science, Institute of Environmental Technologies, Ostrava, Czech Republic

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

Genes in plastid genomes (plastomes) have been primarily inherited from the cyanobacterial plastid ancestor, but cases of genes acquired by horizontal gene transfer (HGT) have been occassionally reported from plastomes of different algal lineages. Here we report an interesting case of HGT-mediated gene acquisition revealed by sequencing plastomes of the eustigmatophyte algae *Monodopsis* sp. MarTras21 and *Vischeria* sp. CAUP 202. While the gene complement of the newly and previously sequenced eustigmatophyte plastomes proved to be highly conserved, those of *Monodopsis* sp. and *Vischeria* sp. harbour a cluster of six genes not reported from any plastid genome sequenced so far. All six genes have homologs in various bacteria, where they are usually organized in the same six-gene cluster, i.e. a putative operon. Phylogenetic analyses showed that the cluster from eustigmatophyte plastomes is nested among sequences from the order Cytophagales (phylum Bacteroidetes), with the cluster from *Sporocytophaga myxococcoides* constituting a robustly resolved sister group to the eustigmatophyte clade. Sequence analyses using different homology-detection tools failed to detect functionally characterized homologs of the protein encoded by the first gene of the operon, whereas the remaining five proteins could be assigned only to broader enzyme superfamilies. Nevertheless, based on these analyses we speculate that the newly detected operon encodes enzymes of a pathway synthesizing a prenylated aromatic compound, possibly an antimicrobial or other protective substance. To our knowledge, this is the first report of an expansion of the metabolic capacity of a plastid mediated by HGT into the plastid genome.

SOD it all: Apparent multiple origins of plastid superoxide dismutases in chromerid algae

Heather Esson[1] & Miroslav Obornik[1,2]
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Chromera velia and *Vitrella brassicaformis* are unicellular algal symbionts of stony corals. Known informally as the "chromerids," they and the non-photosynthetic colpodellid flagellates form a monophyletic group which is closely related to the parasitic Apicomplexa. The plastids of *Chromera* and *Vitrella* are derived from a red algal ancestor and are the closest extant relatives of the relict, non-photosynthetic plastid, known as the "apicoplast," present in some apicomplexans; understanding photosynthesis in these algae, therefore, provides an opportunity to further understand the evolutionary loss of photosynthesis en route to a parasitic lifestyle. Biochemical analysis of Photosystem I (PSI) in *Chromera* has revealed the presence of two strongly associated, distinct superoxide dismutases (SODs) – enzymes that catalyse the transformation of superoxide radicals to hydrogen peroxide. In an effort to further understand the evolution of these proteins, we retrieved eleven SOD protein sequences from the genomes of *Chromera velia* and *Vitrella brassicaformis*. Based on analyses with SignalP and TransitP, two iron (Fe) SODs in *Chromera* and two in *Vitrella* possess bipartite plastid targeting sequences. Phylogenetic analyses of manganese (Mn) and FeSODs recover a clade largely consisting of chromerid, apicomplexan and stramenopile FeSODs. While support values are low to non-existant, *Chromera* FeSODs form a clade with an Oxyrrhis marina FeSOD possessing a bipartite transit sequence. *Vitrella* FeSODs group with apicomplexans or stramenopiles. While long-branch attraction cannot be eliminated as a factor in our analyses, these results suggest that chromerid plastid FeSODs have an unexpectedly complex evolutionary history.

Evolution of the trypanosome nucleus - gene expression mechanisms

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The Nuclear envelope (NE) is the defining structure of the eukaryotic cell. Prominent structures of the NE are the nuclear pore complexes (NPC) and the filamentous lamina underlying the nuclear membranes. Until recently, these structures were only characterised in model organisms that belong to a relatively narrow eukaryotic group. Here we provide more insight into the evolution of the NE from pan-eukaryotic homology searches and phylogenetic analyses of the individual NE components and recent experimental data on the composition of the NE from the diverged eukaryotic parasite *Trypanosoma brucei*. We found that an NPC very similar to that in humans was already present in the last eukaryotic common ancestor (LECA). Although lamins were assumed a derived feature of animal nucleus, we found lamin homologs with shared domain architecture and sequence motifs in diverse protists. The additional NE components facilitating connections between the nucleoskeleton and the NPC, cytoskeleton and chromatin were likely also integrated into the LECA lamina. Our data further suggest that different nucleoskeletal structures that support the nuclear membranes, organise chromatin and connect nucleus to the cytoskeleton operate at the nuclear periphery of trypanosomes. These findings contribute to the understanding of the origin and evolution of the eukaryotic cell.

The evolution of heme pathway with regard to endosymbiotic gene transfer

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Acquisition of organelles essentially leads to increased metabolic integration with the host cell. Gene transfer to nucleus and respective protein retargeting back to the nascent organelle are processes that reinforce this initially fragile host-symbiont relationship. We investigated the evolution of tetrapyrrole biosynthesis enzymes used for heme and chlorophyll production in algae with plastids of complex origins. These include microalgae with highly autonomous tertiary endosymbiont of diatom provenance (dinotoms), algae with plastids containing vestigial nucleomorph (*Bigelowiella* and *Guillardia*), and dinoflagellate possessing a chlorophyte-derived plastid that replaced the original organelle derived from a red alga (*Lepidodinium*). In line with previous results, we describe tetrapyrrole pathway as a mosaic of enzymes of various evolutionary origins. Further, the whole pathway can be relocated to newly acquired homologous organelle "as is", without the need for recruitment of additional genes, as in the case of *Lepidodinium*. We also discuss the peculiar duplicate pathway of *Bigelowiella natans* and, in *Guillardia theta*, a bifurcation of the pathway to supply mitochondria with heme. A novel paralogue of glutamate-1-semialdehyde aminomutase was found in dinoflagellates, possibly acquired by LGT from proteobacteria early in the evolution of the group. Similarly, a proteobacterial ferrochelatase resides in the genome of dinoflagellates branching together with rhodophytes and Apicomplexa, sister to green lineage/cyanobacteria and heterotrophic eukaryotes, suggesting an ancient prokaryote to eukaryote gene transfer.

The physical form of released nonconventional introns in euglenids

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Euglenids (Euglenida) are one of the marine and freshwater single-celled flagellates, which are Excavate eukaryotes. In spite of many years of study, knowledge about the organization of their genetic material remains unclear. It has been uncovered that nuclear genes of euglenids contain two major types of introns: conventional spliceosomal, typical for eukaryotes and non-conventional ones (containing non-canonical, variable borders, able to form RNA secondary structure bringing together intron ends and removed by an unknown mechanism). Some researchers also distinguish intermediate introns, which combine features of both types specified above (stable RNA secondary structure and one or both canonical borders). The species *Euglena gracilis* has been used extensively in the laboratory as a model organism. Its tubA gene contains three conventional introns, two nonconventional ones and one intermediate intron. We designed an experiment to study the physical forms of excised introns in order to find out if the intermediate one is excised as conventional or nonconventional type. After total genomic RNA isolation, purification and quality control, cDNA synthesis and an inverse PCR amplification were performed, followed by cloning, product sequencing and output analysis. Obtained data revealed, that conventional introns, as expected, were excised as lariats, while nonconventional as well as intermediate introns were released as circular RNA particles with full-length ends. We suggest that this new type of intronic circRNA might play a role in intron mobility due to frequent insertions of nonconventional introns at new positions of genes.

Monoxenous trypanosomatids are infected by insect- and fungi-specific RNA viruses

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Total 46 individual isolates of Leishmaniinae were screened for presence of viral RNA using DNase I + S1 nuclease treatment of total RNA extracts. Viruses were found in eight such cultures: *Crithidia otongatchiensis*, *Crithidia* sp. ZM, *Crithidia* sp. C4, Trypanosomatidae sp. G15, *Leptomonas seymouri*, *Leptomonas pyrrhocoris* (isolates F19, F165 and H10). Three types of viral RNA-dependent RNA polymerases (RDRPs) were detected. All *Crithidia* species contained a single 6 kb long viral RNA coding for Phlebovirus-like RDRP. Phleboviruses are negative strand RNA viruses transmitted by sand flies. *L. seymouri* virus possessed a RDRP closely related to Narnavirus, which are known to infect fungi and oomycetes, but it also contained an extra RNA segment not present in previously studied species. RDRP and overall genomic structure of the *L. pyrrhocoris* virus was similar to that of the unclassified insect viruses described from bees (Chronic bee paralysis virus) and *Drosophila* (Dansoman virus). Presence of insect-specific viruses in monoxenous trypanosomatids opens up new horizons in studying the ecology and biology of RNA viruses. It suggests a possible exchange of viruses between trypanosomatids and their insect hosts during their shared evolutionary history. On the other hand, a stable viral infection in *L. seymouri* together with the absence of RNAi machinery in this species implies a selection-driven retention of the virus. Previously, this phenomenon was documented only in *Leishmania* species capable of infecting mammals, suggesting that *L. seymouri* may be evolutionary preadapted for dixenous lifecycle.

Unexpected diversity of the peculiar genus *Creneis* (Excavata: Heterolobosea)

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Creneis is a recently (2014) discovered genus of marine anaerobic heteroloboseids. Its only species, *C. carolina*, displays several uncommon features including amoeboid flagellates with a single flagellum, a multiflagellate form with ca. 14 flagella and the unique structure of its flagellar apparatus. Therefore, its affiliation to Heterolobosea is recognizable only thanks to the results of molecular-phylogenetic analyses. *Creneis* was described on the basis of a single isolate and has never been observed again. We have established six marine *Creneis* strains in culture. According to the morphology and SSU rRNA gene sequences, our strains represent five novel species of *Creneis*. The species morphologically differ from each other as well as from *C. carolina*; the diagnostic features include the cell size, character of the flagellum, type and arrangement of pseudopodia, and character of the uroid and uroidal adhesive filaments. At least three new species are able to form the fast-swimming "multiflagellate" form, which, however, possesses only four or five flagella. Our results show that *Creneis* is a widespread and diverse lineage of anaerobic protists. Because six known species (including *C. carolina*) are represented by only seven strains, its true diversity is certainly much higher.

Myxozoan parasite adapts old dance moves into a new routine

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Cellular motility is essential for microscopic parasites, it is used to reach the host, migrate through tissues, or evade host immune reactions. Many cells employ an evolutionary conserved motor protein – actin, to crawl or glide along a substrate. We describe the peculiar movement of *Sphaerospora molnari*, a myxozoan parasite with proliferating stages circulating in the blood of its host, the common carp. Myxozoa are highly adapted parasitic cnidarians alternately infecting vertebrates and invertebrates. *S. molnari* blood stages have developed a unique "dancing" behaviour, using the external membrane as a motility effector to rotate and move the cell. *S. molnari*'s blood stage movement is exceptionally fast, non-directional and constant. It is based on two cytoplasmic actins that are highly divergent from those of other metazoans while retaining the same tertiary structure and biological function. Since common actin staining and immunolabelling of *S. molnari*'s actin fibres was unsuccessful, a specific polyclonal antibody was produced. We show the in situ localization of this actin in the parasite blood stages and prove the importance of this type of motility for evasion from the cellular host immune response. This new type of motility holds key insights into the evolution of cellular motility and its functionality.

Role of OSCP protein in bloodstream form and dyskinetoplastic *Trypanosoma brucei*

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Mitochondrial (mt) ATP synthase is responsible of ATP generation in most eukaryotic cells. While the procyclic form (PF) of *T. brucei* uses the conventional function of the enzyme, the bloodstream form (BF) exploits the reversed ATP hydrolytic activity coupled to the proton translocation across the inner mt membrane in order to maintain the mt membrane potential ($\Delta\Psi$). Despite ATP synthase overall structure and mechanism have remained conserved throughout evolution, composition of *T. brucei*'s peripheral stalk, which docks F_1 moiety to the membrane, differs remarkably, being OSCP the only conserved subunit thereof. In other species OSCP constitutes the only physical link of the peripheral stalk to F_1-ATPase via an interaction with α subunit. However, critical residues for the interaction are found neither in OSCP nor in α subunit. Consequently, OSCP role in F_1-ATPase immobilization remains hypothetical in *T. brucei*. OSCP is required for ATP synthase function in PF, as its silencing by RNAi resulted in an evident growth phenotype. In contrast, OSCP knock-down in BF or dyskinetoplastic (Dk) cell lines did not affect growth rate. ATP synthase of Dk cells cannot translocate protons due to the loss of the mt encoded proton pore subunit a. Nevertheless, ATP hydrolysis by F_1-ATPase remains essential for $\Delta\Psi$ by supplying ADP3-/ATP4- exchange across the mt membrane. OSCP double knockout in BF and in Dk cell lines will enable us to determine whether the F_1-peripheral stalk interaction is OSCP-mediated, or involves other subunits, e.g. kinetoplastid-specific ATPaseTb2 (peripheral stalk) or p18 (F_1 sector), and whether the interaction network is preserved in the reduced ATP synthase of Dk cells.

Comparison of classical and CRISPR/Cas9-mediated approaches for targeted genome modification in *Leishmania mexicana*

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Leishmania is a protozoan parasites causing leishmaniasis in tropical and sub-tropical regions. There is no satisfactory treatment against leishmaniasis, thus understanding biology of this parasite and studies of its virulence factors are important. Through comparative bioinformatics approach we have identified several novel putative virulence factors in *Leishmania* infection. To investigate their roles in *Leishmania* infectivity, we have generated several knock-out lines ablating these genes. Classical knock-out strategies are based on replacement of a target gene by the antibiotic resistance cassette through homological recombination. *Leishmania* is aneuploid organism which causes difficulties in targeted genome modification using classical strategies. The CRISPR/Cas9-mediated knock-out strategy allows overcoming this hurdle. In this work we compared advantages and disadvantages of classical and CRISPR/Cas9-mediated approaches for targeted genome modification in *Leishmania mexicana*.

Dinoflagellate evolution

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Dinoflagellates have numerous morphological, genetic and biochemical properties that make them distinctly odd among eukaryotes. By using transcriptomes we have greatly increased the amount of available protein sequence data from the group in order to improve the resolution of dinoflagellate relationships. In doing so we were able to map the evolutionary history of many dinoflagellate features and could reassess their fossil and geochemical record. We show that the dinoflagellate theca originated only once, through a process that probably involved changes in the metabolism of cellulose, and describe a revised model of how thecal plate numbers subsequently increased or decreased in different thecate lineages. Many dinoflagellates that lost photosynthesis have retained non-photosynthetic plastids with vital metabolic functions, and this metabolism may have been important in the evolution of bioluminescence. The dinosterols used by paleontologists to posit the existence of dinoflagellates in the Proterozoic originate late in the evolution of the group, making that hypothesis unlikely. We also identified new forms of histone-like proteins, rhodopsins and other proteins, and observe that many proteins responsible for important changes in the biology of dinoflagellates have closest homologs in bacteria or viruses.

Do we need to protect microalgae and protists at all?

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Many states use red lists of nearly all groups of organisms to protect their natural biodiversity. Nevertheless, the practical protection of protist organisms, including microalgae, is still almost completely missing. Compiling red lists of microorganisms is a quite challenging task requiring knowledge of ecology, distribution, and endangering factors of this group of organisms. This set of information is much less comprehensive in comparison with the same set available for vascular plants, invertebrates, vertebrates or macroscopic fungi. It is primarily due to problematic taxonomic determination and our limited understanding of protists' distribution. This poster represents main problems of compiling a red list of microscopic organisms. It makes an effort to address some important topics, e.g. endemism vs. rarity, selection of a good morphologically determined species, or a role of ecological preferences. The goal is to provide practical red list that could be used not only by specialist from algology or protistology, but mainly by people from nature conservation institutions (environmentalist, restoration ecologist, etc.). The method of assembling an algal red list is introduced on the case study of Euglenophytes in the Czech Republic. This red list combines data from literature with floristic data collected from more than 200 localities across the state. It includes species typical for oligotrophic and mesotrophic habitats (e.g. *Trachelomonas conica*, *Phacus moniliatus* var. *suecicus* etc.) as well as for endangered habitats such as wetlands or peat bogs (e.g. *Phacus similis*, *Euglena adhaerens*, etc.). In addition, taxa with rare occurrence or decreasing range of their distribution (e.g. *Colacium vesiculosum* or *Lepocinclis fusiformis* etc.) were added. The aim of this case study was to prepare a methodology for compilation of red lists reflecting recent and historical data on distribution, ecological preferences, and typical habitats of algae, with great emphasis on the list's applicability. This method can be combined with the idea of 'flagship species'. These species are characterized by clear morphology and ecology and play the role of key species for endangered biotopes. This methodology should then be applicable to all protist organisms.

Genetic modifications and functional analyses of Diplonemids

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Diplonemids are the unicellular, heterotrophic, biflagellated, eukaryotic protist either free-living or parasitic in nature. Diplonemids (Diplonemea, Euglenozoa) are the sister group of medically important kinetoplastids and ecologically relevant euglenids. Until 2009, Diplonemids were considered as an obscure and rare sister group of kinetoplastids and euglenids that are known for decades to be virtually omnipresent and very species-rich; but recently, diplonemids appeared as abundant protists with novel lineages in the world photic layer; present from surface layer to deep sea, but still nothing is know about their sudden abundance and role in marine ecosystem. The International Tara Oceans expedition (2009-2012) further revealed that diplonemids are the 3rd most diverse and 7th most abundant among marine eukaryotes. The goal of our project is to turn diplonemids into genetically tractable system and establish it as a model organism to study the function of diplonemid genes. Our model species is *Diplonema papillatum* that can be easily cultivated axenically in the laboratory, reaches high density and grows in large volumes. We measured the cell viability and found out the *D. papillatum* is sensitive to multiple drugs that can be used as a selectable markers. Recently, we are preparing a range of constructs that will be used to deliver DNA into this protist, so far not subjected to genetic manipulations.

Characterization of mitochondrial secretin complex

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Type II secretion system is one of seven secretion systems found in gram negative bacteria, that provides transport of folded proteins through their outer membrane. Passage through this membrane is mediated by a pore assembled from subunits of protein called GspD or secretin. GspD was together with several other components of this secretion system discovered in the mitochondria of several protist sepcies including *Naegleria gruberi*, *Andalucia godoyi* and Malawimonads. We are trying to illuminate the import, assembly and the structure of the mitochondrial secretin complex. Our second aim is to characterize the interactions of the mitochondrial GspD with other components of the secretion system. Finally, we are interested in finding whether GspD interacts with the putative substrate of the mitochondrial type II secretion system.

Minions of Great *Cthulhu* awakening – a new insight into the diversity of parabasalid symbionts of cockroaches

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Parabasalian symbiots of xylophagous cockroaches from families Cryptocercidae and Isoptera (termites) have been attracting the interest of biologists for several decades; they often possess large, complex, and visually attractive cells and are of great ecological significance. Phylogenetic analyses showed that the complex forms of parabasalians (= hypermastigotes) have arisen several times independently. On the other hand, only a little attention has been paid to endosymbionts of "normal" cockroaches, although these insects show a great diversity in morphology and lifestyles. We have examined the intestines of 250 cockroaches belonging to 100 species and 20 subfamilies (out of 33), established 50 stable cultures of trichomonads, sequenced their SSU rRNA gene, and studied the morphology of some of them. Approximately one half of the obtained trichomonads formed a considerably diversified clade that contained the recently discovered hypermastigote *Cthulhu* with approximately 20 flagella and trichomonad genera *Hexamastix* and *Cthylla* with 6 flagella. By contrast, our strains have cells equipped with three or four. Thus, *Cthulhu* and *Cthylla* are not orphans anymore, but are surrounded by a cloud of lesser trichomonads. Most of the remaining strains belong to the understudied genus *Hypotrichomonas*, where they represent several novel species.

Catalase in Leishmaniinae: with me or against me?

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The enzyme catalase is widely distributed in the variety of life form. It decomposes hydrogen peroxide (H_2O_2) to water and molecular oxygen, thereby protecting cells from the reactive oxygen species (ROS). The recently established subfamily Leishmaniinae is divided into two clades comprising monoxenous (one-host) trypanosomatids species of the genera *Crithidia*, *Lotmaria*, *Leptomonas* and dixenous (two-hosts) *Leishmania* causing clinically diverse disease leishmaniases. In contrast to theirs' monoxenous ancestors, *Leishmania* species do not encode for catalase gene. Recently, several lines of evidence suggested that generation of diffusible ROS H_2O_2 could play a role in *Leishmania* differentiation from the extracellular procyclic promastigotes to the infective metacyclics and amastigotes. To shed more light on the gain/loss of a catalase gene in Leishmaniinae and potential involvement of H_2O_2 in parasites' differentiation and survival within macrophages, we overexpressed a monoxenous *Leptomonas pyrrhocoris*-derived catalase in the dixenous *Leishmania mexicana*. We have also generated a catalase-null *Leptomonas seymouri* mutant. As expected, the catalase overexpressing *Leishmania* cells showed significant increase in their resistant to hydrogen peroxide compared to wild type counterparts with EC50 values 0.33 and 1.68 mM H_2O_2, respectively. In order to follow the *L. seymouri* infection, we also established a catalase-null cell line expressing mCherry fluorescent protein. We believe such multi-dimensional approach will help to understand molecular details of the *Leishmania* oxidative defense mechanisms and evolution of dixeny in general.

Ancient mitochondrial protein secretion

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The bacterial origin of mitochondria has been evidenced by a number of shared features with current bacteria, including some of the protein transport components. However, most of the original bacterial protein transport pathways have been lost from the mitochondria and replaced by the protein import apparatus. To some detail, mitochondria of Discoba and Malawimonads represent an evolutionary intermediate stage as they carry the largest mitochondrial genomes encoding bacterial SecY and Tat translocases. By a multi-phylome approach we have analysed eukaryotic proteomes for nuclear encoded genes, which are exclusive to Discoba and Malawimonads. We show that their nuclei encode for about forty genes not found in other eukaryotic lineages. These include eight components of bacterial type II secretion system (T2SS), which are expressed in mitochondria and possibly mediate the secretion of the proteins from the organelle.

Euglena jirovecii Fott (Euglenea) – unique autotrophic euglenid swimming backwards

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We report and re-describe very rare and unique photosynthetic euglenid - *Euglena jirovecii* Fott. It is the only known euglenoid which can swim backwards – flagellum pushes the cell, instead of pulling it. *Euglena jirovecii* was described from a fish pond Kaprova in Lnáře in Bohemia (Czech Republic) by the Czech phycologist Bohuslav Fott in 1953. Since then, it has been found only once in the world - in the USA in 1994. The discovery of this species in small, eutrophic bodies of water in Poland creates a chance for its better knowledge through morphological and molecular studies. It seems that *E. jirovecii* can be confused with *Euglena agilis*, which is similar in terms of some of the morphological features. Phylogenetic analysis based on the nuclear SSU and LSU rDNA sequence data confirmed the close relationship of both species.

Queuosine: The role of an essential tRNA modification in parasitic protist *Trypanosoma brucei*

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Transfer RNAs (tRNAs) are a type of non-coding RNAs, which are extensively modified post-transcriptionally, in order to increase their structural stability or fidelity. In particular, the modifications present in the anti-codon loop, have a crucial role in accurate codon selection and translational frameshifting prevention. Queuosine (Q), a hyper modified guanosine, is one such modification, that is found at the wobble position 34 of tRNAs that contain a 5'-GUN-3' anticodon sequence (His, Asp, Asn, Tyr). Although Q is present in nearly all forms of life, its exact physiological role remains unclear. We aim to study the enzyme responsible for incorporation of Q into the tRNAs, known as tRNA guanine transglycosylase (TGT), in *Trypanosoma brucei*, a protozoan parasite that causes sleeping sickness in humans and nagana in livestock. We have identified two TGT homologs in *T. brucei*, TbTGT1 and TbTGT2, using bioinformatics approaches. Since preliminary data shows that TGT is uniquely essential for the growth of the parasite, while literature so far suggests that its absence has no noticeable phenotype in the mammalian hosts of *T. brucei*, TGT becomes an ideal target for drug development against the disease. We utilize techniques, such as RNA interference (RNAi), in situ tagging of proteins, and other methods of molecular biology, to comprehensively study the TGT enzyme system, including its structure, kinetics, localization and its relation to the pathogenicity of the parasite in its mammalian host.

Feeding a fish model *Danio rerio* on *Chromera velia* causes severe rearing problems and prohibits maturation of the fish - a pilot study

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Recently found alveolate *Chromera velia* was proved as the closes known photosynthetic relative of apicomplexan parasites represented by genus *Plasmodium* that causes severe health and economic losses. *C. velia* is becoming an excellent model for study biological processes in apicomplexans due to cheap and easy cultivation. Lipidomic studies revealed surprisingly high level of essential fatty acid namely eicosapentaenoic acid (EPA) present mostly in galactolipids. *Danio rerio* belonging to Cypriniformes is an important and widely used vertebrate model organism. In addition, *D. rerio* is a popular and easy reared freshwater aquarium fish also known as zebrafish. Aim of study was to investigate which lipid molecule is most suitable for yield of EPA and transfer this fatty acid to the *D. rerio* muscles. Experiment with different types of feed mixture provides EPA in three different types of lipid classes, namely phospholipids in cod muscle, triacyglycerols in linseed granule and galactolipids in *C. velia*. Changing of EPA content was investigated by means of gas chromatography. However all tested groups thrive well, in group fed on mixture enriched with algae arise severe problems with fish surviving and condition. The malnutrition and/or unknown diseases are occurred and dissection of surviving fishes showed no visible traces for gonads. Furthermore experiment with salinity indicates that *Chromera velia* can survive in salt free medium, almost distilled water.

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Never alone: human microbiome and eukaryome

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Humans host in their bodies as many as 1,000 different species of bacteria, collectively called the microbiome. Thanks to very affordable and high-throughput sequencing, within the last few years there is an explosion of knowledge about how microbiome influences human health and disease. The microbiome is being implicated with a growing list of diseases and disorders but surprisingly little attention is given to eukaryotes living in humans. These are called the eukaryome, and are mostly considered to be parasites with negative influence on human health. I will argue that many of them are rather commensals, potentially beneficial for our health and positively influencing the diversity of human microbiome.

Hypervariable regions of 18S rDNA as DNA barcodes for autotrophic euglenids

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Autotrophic euglenids (Euglenea) are common and abundant protists in freshwater habitats. Based on morphology they are easy to recognize as a group, but species identification is more problematic, because as other eukaryotic microorganisms they have limited number of features which might be observed under light microscope and for many species there are virtually no good diagnostic morphological characters. On the other hand there is no universal DNA barcode which could be effective in molecular identification for all protist groups. For autotrophic euglenids it is known, that ITS sequences are extremely variable, and thus not suitable. The COI gene was also rejected due to impossibility of universal euglenid primers design. Therefore using plethora of available methods we verified three fragments of 18S rDNA (containing hypervariable regions V2-V3, V4 and V9) as a potential DNA barcodes. We found that variable regions V2-V3 and V4 of 18S rDNA have a proper length and are very efficient in identification of autotrophic euglenid species. We also designed primers that enable efficient PCR amplification of both fragments and we tested the performance of proposed DNA barcodes in species assignment using sequences of autotrophic euglenids isolated from environmental samples and using mixtures of cultures.

Myxozoan diversity as revealed by eDNA: Data mining discovers potentially new lineages of Myxozoa

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Myxozoa are microscopic cnidarian parasites, with a two-host life cycle including vertebrates, mainly fish, as intermediate host, and invertebrates as definitive hosts. Geographic sampling is still patchy and new species and host taxa are frequently being described. While it is inferred that Myxozoa split off their marine cnidarian ancestors, the earliest myxozoan lineage is currently known only from freshwater habitats. The biodiversity of this group is still largely undiscovered, especially in marine environments. Large scale projects using DNA extracted from environmental samples (eDNA) in order to characterise the taxonomic make-up of their biological content has become possible with the rise of high throughput sequencing. de Vargas et al. (2015) published around 350 18S amplicon datasets targeted on eukaryotes from marine samples taken all around the world. Currently we are mining these datasets for myxozoan sequences by stand-alone blasting against custom built myxozoan queries. Several sequences have been identified to date as possibly belonging to up to four yet unknown lineages of Myxozoa. In our analysis two sequences cluster with Malacosporea, potentially indicating a first marine malacosporean lineage. We designed primers to capture these new lineages and get longer sequences to be able to verify their taxonomic placement. The use of eDNA for detecting Myxozoa can prove useful not only in biodiversity studies but also e.g. in aquaculture, where certain species of Myxozoa can lead to significant economic losses. Its power to detect Myxozoa in freshwater will be studied by testing water samples from a reservoir during one year, examining its potential as analytical method for monitoring Myxozoa.

Fatty acid biosynthesis in chromerid algae – A bioinformatic approach for identification of key enzymes

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Chromera velia (Moore et al. 2008) and *Vitrella brassicaformis* (Oborník et al. 2012) are a missing links between free-living phototrophs and apicomplexan parasites. Their genomes and transcriptomes were recently sequenced. With available data we are searching for metabolic similarities between the two species and related protists. One of the most improtant metabolic feature is synthesis of fatty acids. Molecular phylogeny and targeting prediction of particular enzymes is giving us an image of responsible metabolic processes. The basal synthesis of fatty acids in both algae is secured by plastid-localized FAS II pathway which is similar to the most apicomplexans. The role of putative cytosolic FAS I multienzymes is probably primary elongation. Subsequential elongation and desaturation is done by the sets of elongases and desaturases. Despite the fact that qualitative spectra of fatty acids detected using mass spectrometry are almost identical, equipments of enzymes and their localization is different.

The guided entry of tail-anchored proteins pathway in *Giardia intestinalis*

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The special class of membrane proteins, so called tail-anchored (TA) proteins, carry a single C-terminal transmembrane domain that anchors them organellar membranes. TA proteins mediate interactions among membrane bounded compartments by their N-terminal domains during various processes such as vesicular transport, regulation of apoptosis or protein translocation. In some eukaryotes, the specific pathway controls precise post-translational insertion of tail-anchored proteins into the endoplasmic reticulum membrane – Guided Entry of Tail-anchored proteins (GET) pathway. Our bioinformatics analyses revealed the absence of most of the GET proteins in majority of the eukaryotic lineages except opisthokonts. However, one of the components of GET pathway (Get3) is conserved in all eukaryotic groups excavates included. We are using *Giardia intestinalis* in order to characterize its GET machinery. We have shown that giardia Get3 is a cytoplasmic protein with affinity to the endoplasmic reticulum. Using chemical cross-linking followed by affinity purification of biotinylated Get3, the specific set of interacting proteins has been identified. In addition to giardia-specific information, our general aim is to define the evolution of GET pathway in eukaryotes.

Functional annotation of *Euglena gracilis* mitochondrial proteome

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Euglena gracilis is a secondary green alga belonging to the phylum Euglenozoa, which also contains kinetoplastid parasites *Trypanosoma* and *Leishmania*. Despite the fact that *E. gracilis* is a well-studied model organism, little is known about its single reticulated mitochondrion. Few previous studies have focused primarily on individual functional groups of mitochondrial proteins, making no attempt to examine mitochondrial proteome as a whole. In this study, we have made an *in silico* prediction of the entire *E. gracilis* mitoproteome based on *de novo* transcriptome sequencing as a part of the *Euglena* genome project. For prediction, we analyzed N-terminal targeting signals (TargetP), similarity to known mitochondrial proteins (BlastP and Blast2GO), and orthologous group information (OrthoMCL) of proteins. The resulting set contains more than 1000 proteins, including subunits of respiratory chain complexes I-V, other components of OxPhos and tricarboxylic acid cycle, mitoribosomal proteins, etc. There are also homologues of *Trypanosoma* editosome components, although RNA editing is not known to occur in *Euglena* mitochondrion; probably these proteins acquired other functions, such as those in mitochondrial RNA processing.

Preaxostyla: the evolution of anaerobic protists

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Preraxostyla, the least studied major branch of metamonads (Excavata), are becoming an increasingly important model system for understanding the evolution of anaerobic lifestyle and reduction of mitochondria. The clade can be divided into two distinct groups: a basal paraphyletic assemblage of free-living trimastigids with typical excavate morphology and a morphologically very diversified monophyletic crown group of oxymonads exclusively inhabiting guts of various metazoans. We focus on genomics, transcriptomics and cell biology of two representatives of preaxostyla, a freshwater trimastigid *Paratrimastix pyriformis* containing a hydrogenosome-like mitochondrial organelle and a simple-celled oxymonad *Monocerecomonoides* sp. which has been recently shown to be the first known example of a completely amitochondrial eukaryote. We will present recent advancements in the characterization of *P. pyriformis* mitochondrial organelle.

Formation of zoospores by budding in *Vitrella brassicaformis*

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Chromerids are coral associated algae, which are closely related to apicomplexan parasites. Two species of chromerids have been described so far: *Chromera velia* and *Vitrella brassicaformis*. Only an asexual life cycle is known for both algae. In such life cycle, zoospores are formed in zoosporangia: in *C. velia*, the number of spores in zoosporangium is always even and the number does not exceed 10 spores per sporangium, while zoosporangia of *V. brassicaformis* contain dozens of spores. *C. velia* zoospores are formed in a process resembling schizogony in apicomplexan parasites. We show here that in *V. brassicaformis*, zoospores are formed differently: transmission electron microscopy pictures demonstrate that budding is a process standing behind a high number of zoospores in *V. brassicaformis*. This way of a spore formation is quite rare in algae; we believe that homologous process in apicomplexan parasites is ectomerogony known from the genus *Eimeria*. It appears that ancestor of chromerids, colpodellids and apicomplexans possessed complex life cycle, which was fragmented after the split of chromerid–colpodellid branch from an apicomplexan ancestor.

Two new non-canonical nuclear genetic codes from a rhizarian and a fornicate with UAG, but not UAA, as a sense codon

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The original presumption that all organisms use the same (standard) genetic code for translation of mRNA sequences into proteins has been challenged by discoveries of deviations of this universal language in both prokaryotes and eukaryotes. In eukaryotes the nuclear genetic code has proven to be much more conservative than that of mitochondria, and plastids; just a few its variants are known. Generally, we can sort them into 3 groups: (1) UGA serves as a sense codon; (2) UAA and UAG simultaneously serve as sense codons; (3) CUG encodes serine or alanine (rather than leucine). We analyzed transcriptomic data from two unrelated protists and found out that these organisms, as only eukaryotes known so far, use UAG as a sense codon in nuclear genetic code while retaining UAA as a termination codon. One of these organisms uses UAG as codon for leucine, similarly to a code variant described from certain mitochondria. The other one instead uses UAG to encode glutamine, resembling thus the non-canonical genetic code of several eukaryotic groups including many ciliates, hexamitin diplomonads, some oxymonads, and some ulvophytes; however, all these taxa have at the same time reassigned also the UAA codon. Phylogenetic analyses place the first organism into the rhizarian lineage Sainouroidea, whereas the second one represents an undescribed lineage of "Carpediemonas-like organisms" in Fornicata (Metamonada). Our findings thus once again show protists as an inexhaustible resource of peculiar departures from the "standard" biology

Ras superfamily GTPases in Microsporidia: Extremely divergent genes in extremely divergent parasites

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Microsporidia are intracellular parasites of economically important animals and even humans. However, essentially nothing is known about Ras superfamily proteins in microsporidia, despite the importance of these proteins in eukaryote-specific cellular processes. To investigate the evolutionary history of the Ras superfamily in microsporidia, we scrutinised genome sequences from 24 core microsporidian taxa and detected 16 different groups of orthologs. Some of them could be assigned to conserved eukaryotic orthologs unequivocally by BLAST searches, which was confirmed by phylogenetic analyses. However, genes from the remaining groups were so divergent that their relationship to Ras superfamily members from other eukaryotes proved difficult to discern. The recently sequenced genome of the least divergent and basal microsporidial species, *Mitosporidium daphniae*, provided us a bridge between the core microsporidia and other eukaryotes. Including sequences from this moderately divergent species and employing more sensitive (profile HMM-based) homology search methods, the microsporidial Ras superfamily members could be matched with 14 orthologous groups from other eukaryotes. In spite of this extreme reduction, we detected some gene duplications and innovations as well. Most notably, almost all studied core microsporidia possess an extremely divergent Arf/Arl/Sar1 family protein with two unusual conserved cysteine residues in the N-terminal extension, which we speculate are modified by lipidic moieties to facilitate membrane attachment of the protein. The function of this protein remains unknown, but the respective gene belongs among the most highly expressed genes in *Spraguea lophii*, suggesting an important, microsporidia-specific role.

Conditional gene expression systems and their potential limitations in developmental studies of *Leishmania*

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Leishmania species are protozoan parasites of the family Trypanosomatidae. They are spread by insect vectors and cause a spectrum of diseases known as leishmaniases. The clinical manifestations vary from spontaneously healing skin lesions to progressive and potentially fatal visceral infections. According to the World Health Organization, there are 300,000 estimated cases of visceral leishmaniasis with over 20,000 deaths annually and about 1 million cases of cutaneous leishmaniasis reported in the last 5 years. Conventional and conditional systems allow for a controlled activation or repression of gene expression in time and space. Such systems are nowadays widely used to analyse a variety of cellular processes in numerous parasites including *Leishmania*. A T7-driven, tetracycline-inducible system for protein expression was established in a human pathogen *Leishmania mexicana*. The gene expression in this strain is strongly regulated and dose- and time dependent. We believe that it can be widely used by the parasitology community to analyse effects of genes of interest on biology, physiology and virulence of parasites causing cutaneous leishmaniases. This system was used to analyse gene expression profiles in *L. mexicana* developmental stages. The transcription/translation of the gene of interest was significantly decreased upon *Leishmania* differentiation into metacyclics and amastigotes. However, the same expression profile was documented for the T7 polymerase. The expression was demonstrated to be not locus-specific but dependent on untranslated regions flanking open reading frames of studied genes. We concluded that the previously established conventional gene expression systems might have certain limitations in their common applications.

Targeting of C-tail anchored proteins into hydrogenosomes and endoplasmic reticulum of *Trichomonas vaginalis*

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The α-helical C-tail anchored proteins represent a heterogenous group of membrane proteins with a large functional N-terminal domain exposed to the cytosol and a short membrane insertion at their C-terminus. They are components of the outer membrane of organelle such as mitochondria and their relatives, namely hydrogenosomes and mitosomes, as well as they serve as membrane proteins of the endoplasmatic reticulum (ER) or plasma membrane. In general, targeting signals consist of short transmembrane domain (TMD) and positively charged flanking regions. Targeting signals that discriminate the insertion of C-tail anchored proteins between organelles and ER are poorly understood. Proteomic analysis of *Trichomonas* hydrogenosomes revealed the presence of twelve C-tail anchored proteins. First, we confirmed the topology of six representative C-tail-anchored proteins in the outer hydrogenosomal membrane by protein protection assay. Further we investigated character of targeting signals, which are responsible for delivery of C-tail anchored proteins into the hydrogenosome or ER. We subsequently replaced various motifs and patterns between C-terminal domain of protein disulfide isomerase (PDI), which is present in the outer membrane of ER and C-terminal domain of the hydrogenosomal protein TVAG_272350. Expression of some recombinant versions of PDI and TVAG_272350 in trichomonads resulted in delivery of protein disulfide isomerase into hydrogenosome and targeting of TVAG_272350 into ER. Our data indicates that structure of the whole C-terminal domain is critical for specific delivery of C-tail anchored proteins into hydrogenosomes and ER.

Diversity of Armophorea and new marine anaerobic ciliates hosting prokaryotic symbionts

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Ciliates are famous for their ability to thrive in various extreme habitats, including microaerophilic and anaerobic environments. Anaerobiosis has independently arisen in at least eight main lineages of ciliates out of total 11 known classes. We have mapped the diversity of the free-living representatives of class Armophorea, resulting in a long-term cultivation of over 180 strains from fresh water, brackish, and marine anoxic sediments worldwide. We cultivated and identified over 35 species of the families Metopidae and Caenomorphidae, including current describing 15 novel species, using molecular and morphological techniques, such as determining their SSU rDNA sequences, performing protargol staining techniques, studying light-microscopic and scanning electron microscopy morphology. Interestingly, according to our analysis the second free-living family of Armophorida, Caenomorphidae, forms an independent deep ciliate lineage. Additionally, we used transmission electron microscopy to assess the ultrastructure of some of the strains and confirmed the presence of endosymbiotic methanogenic Archaea by fluorescence microscopy. Using scanning electron microscopy, we observed various ectobionts of the surface of many marine species. Importantly, we present discovery of several new species of the deep lineage of marine anaerobic ciliates (Muranea) that host prokaryotic endo- and ectosymbionts and whose mitochondrion related organelles do not possess cristae.

Phylogenetic Affinities of a Norwegian Sarcinochrysidales isolate algea (Heterokonta) Based on Analysis of Molecular Data

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The photosynthetic stramenopiles (heterokont algae or stramenochromes) are one of the most actively studied groups of protists, and 14 new taxonomic classes have been described since 1972. Molecular phylogenetic analyses show that the heterokont algae are a monophyletic group that is either derived from or sister to, a clade of entirely nonphotosynthetic stramenopiles. We report the small-subunit ribosomal RNA nucleotide sequences data of an unknown Norwegian marine algal isolate which were inferred for *Sarcinochrysis* sp., as well as for Bicosoecida sp.; a small unicellular flagellates Protist, included among the heterokonts. *Sarcinochrysis* forms a clade which with species belonging to the order Sarcinochrysidales. The closest relatives of the studied isolate were members of *Sarcinochrysis*. The results of this study will improve the knowledge of monophyletic classes for the heterokont algae and will be useful for future heterokonts evolution studies.

Confirmation of *Chromera velia* mixotrophy by metabolomic studies

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Recently discovered alga *Chromera velia* has unique phylogenetic position. Together with *Vitrella brassicaformis* is the most related photosynthetic organism to phylum Apicomplexa. Members of the apicomplexans are responsible for deadly diseases of humans and animals. The easy and rapid cultivation of the *C. velia* makes this alga great model for studying elementary biochemical principles and helps to understand evolutionary shift from photosynthesis to parasitism. Gas and high performance liquid chromatography with mass spectrometry were used to reveal essence of mixotrophy in *Chromera velia*. Nitrogen is limiting nutrient for the marine ecosystems. The level of organic and inorganic nitrogen influences quality and quantity of lipids in different way. Thus labeled glycine was added to the culture medium as a source of organic nitrogen and subsequent analysis showed that this molecule was utilized by the *C. velia* as a precursor for chlorophyll and pigment biosynthetic pathways. The labeled carbon from gain of glycine was also occurred in high amount in storage lipids and proteins in very short period. Rapid utilization of glycine for lipid metabolism is direct proof of mixotrophy. This feature of photosynthetic ancestor of apicomlexans could be a first metabolic step towards parasitism.

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Patrypanosoma: from free living to a parasitic way of life

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Paratrypanosoma confusum is a novel uniflagellar monoxenous kinetoplastid discovered in the gut of mosquito *Culex pipiens*. As the most basal trypanosomatid lineage, it represents a possible evolutionary link between free-living bodonids and obligatory parasitic trypanosomatids. In axenic culture, *P. confusum* creates three morphologically distinct stages. A promastigote-like motile stage, equipped with a long flagellum, changes under certain conditions into a sessile stage. During this transformation, the paraflagellar rod is dismantled and the external part of the flagellum creates a sticky pad that is used for cell attachment to surfaces. Time-lapse videos proved the flagellum transformation process in sessile stage to be reversible. Furthermore, *P. confusum* forms an oval, amastigote-like stage with a very short external flagellum when grown on semi-solid agar plates. *P. confusum* exhibits social motility behavior known from genus *Trypanosoma*. Preliminary analyzes suggest that one of signaling molecules moderating this behavior could be biopterin. Moreover, differential expression analyzes performed on transcriptomes of promastigote-like stage and sessile stage revealed over-expression of pteridine transporter genes in the sessile stage. Presence of RNA interference machinery core gene homologs (Ago1, Piwi1 and DCL1) both in genome and transcriptome together with confirmed presence of dsRNA viruses within *P. confusum* cells suggests that both dsRNA viruses and RNA interference coexists in ancient species which later evolved to retain only one. Further analyzes of the genome of the monoxenous *Paratrypanosoma* should provide insight into the emergence of dixenous parasitism of the medically important trypanosomatids.

Origin, diversity and evolution of cystatin superfamily in parasitic cnidarians

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Myxozoans are a major group of cnidarian parasites with some species significantly impacting aquaculture and fisheries. Our research seeks to understand the origin, evolution and diversity of gene architecture of I25 cysteine protease inhibitors in cnidarians with special interest on parasitic taxa. These proteins are key elements involved in host-parasite interactions. We identified cystatin superfamily homologs in available databases of cnidarians and performed comprehensive phylogenetic analyses in order to infer their position within other metazoans. We additionally compared the architecture and number of genes encoding each I25 cysteine protease inhibitor identified in free-living and parasitic cnidarians and traced these features within the cnidarian phylogeny. Our findings provided deeper understanding of the functional diversification and lineage-specific adaptations of studied proteins potentially related to parasitic lifestyle. Moreover, implementing cnidarians as a primitive metazoan group within the phylogeny shed light on the origin and evolution of these inhibitors in the Metazoa.

Alternative genetic codes and their distribution in the tree of life

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The genetic code determines how genes are translated into proteins. It follows a very conservative set of rules that had been established before the three domains of life (Archaea, Eubacteria, and Eukaryota) diverged. The diverse assortment of its deviations present in organellar and nuclear genomes of distinct lineages has 'thawed' the concept of a single universal, entirely frozen code. Several types of deviations have been described from all domains of life, however, code deviations in Archaea and Eubacteria are far less frequent than in Eukaryotes. Interestingly, most types of code deviations have been reported in their mitochondria. The nature of the non-standard codes consists in various codon reassignments induced by mutations of tRNA molecules or aminoacyl-tRNA synthetases. Nevertheless, several examples of codon ambiguity, i.e. use of one codon to code for multiple amino acids, have been also reported. Here, we summarize non-canonical genetic codes described so far and their distribution in the tree of life with the special focus on those in eukaryotic, nuclear, and organellar genomes, taking into account some new instances of non-canonical genetic codes in eukaryotic nuclear and mitochondrial genomes discovered recently in our laboratory. We also describe some of the most plausible mechanisms responsible for the origin of non-standard genetic codes.

Cytosolic iron-sulfur cluster assembly machinery in *Trichomonas vaginalis*

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Cytosolic Iron-sulfur (FeS) cluster assembly (CIA) pathway is essential for most eukaryotic cells to facilitate maturation of cytosolic and nuclear FeS-proteins. CIA pathway in the cytosol is functionally linked with ISC machinery that is present in mitochondria. Since now, 15 components of ISC assembly and 9 key components of CIA pathway have been characterized in *Saccharomyces cerevisiae*. We focused on the synthesis of FeS clusters in the human pathogen *Trichomonas vaginalis*, which is adapted to live in oxygen-poor environment of the urogenital tract. We identified homologous proteins for most of CIA components in this parasite. Nar1, Cia2 are encoded by single copy genes, whereas Cia1, Nbp35 and Cfd1 are each present as two paralogs. Interestingly, we did not find any gene for Tah18, Dre2, Mms19 and Grx3/4. The absence of Dre2 and Tah18 seems to be a general trend in Metamonades clade. We expressed all CIA components with hemagglutinin tag in *T. vaginalis* under control of both strong artificial and native promotors. The localization of these proteins was investigated by immunofluorescent microscopy and protein fractionation, which confirm their presence in cytosol and partially in the proximity of hydrogenosomes. Further, we immunoprecipitated Cfd1A, Cfd1B, Nbp35A and Nbp35B to find their interacting partners. Initial experiments using crosslinker DSP revealed significant enrichment of the hydrogenosomal membrane proteins in the samples with immunoprecipetated Cfd1A and Cfd1B, which suggested link between the organel and proteins involved in cytosolic cluster assembly. However, immunoprecipitation under native conditions did not confirm these results. Moreover, we observed rather poor reproducibility of protein composition in immunoprecipitated samples, thus further optimalization of this approach is required.

´Anaeramoeba´ - new deep amoeboid lineage of metamonads

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We have isolated and cultured nine strains of anaerobic amoeba (´Anaeramoeba´) from marine coastal hypoxic sediments worldwide. ´Anaeramoeba´ cells were fan-shaped, with extremely flattened pseudopodia and trailing uroidal filaments. We were able to distinguish six morphospecies that differed in size, nuclear morphology, and character of pseudopodia, but all shared a unique combination of morphological features. We have examined three strains using transmission electron microscopy; they possessed double membrane-bound organelles with no cristae (presumably hydrogenosomes) associated with prokaryotes (probably methanogens). The cytoplasm contained a large acentriolar centrosome, and no basal bodies/centrioles were observed. Strains representing different species formed peculiar isokont fla gellates with two or four, characteristically thickened flagella. SSU rDNA and five gene analyses showed that ´Anaeramoeba´ clade is monophyletic, but were unable to determine its phylogenetic position. We analyzed transcriptome data of two species and performed y phylogenomic analysis based on 160 protein-coding genes. Surprisingly, the analysis robustly showed that ´Anaeramoeba´ clade represents a novel lineage within Metamonada (Eukaryota: Excavata) instead of belonging to Amoebozoa.

Description of *de novo* fatty acid biosynthesis of apicomplexan cousins *Chromera velia* and *Vitrella brassicaformis*

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Chromera velia and *Vitrella brassicaformis* are photoautotrophic alveolates recently found in Australian corals and shown to be the closest known photosynthetic relatives of apicomplexans. Parasites from the phylum Apicomplexa, such as *Plasmodium*, cause malaria and other deadly diseases in humans and animals. *Chromera velia* and *V. brassicaformis* present extraordinary model organisms to study the evolution of parasitism in apicomplexans. Fatty acid biosynthesis is one of the most important biosynthetic pathways and provides building blocks for membranes. Combined genomic studies and analytical biochemistry techniques represent powerful tools that allow for detailed study of *de novo* synthesis of fatty acids. Both algae utilize type II fatty acid biosynthesis localized in the plastid to produce myristic, palmitic, and stearic acid. Subsequent modifications of saturated fatty acids are performed by elongases and desaturases localized in the lumen or membrane of the endoplasmic reticulum. Moreover, extensive lipidomic studies of fast growing *C. velia* revealed a surprisingly high ability to produce and accumulate fatty acids in the form of triacylglycerols. This feature of an apicomplexan photosynthetic cousin opens up the possibility of biotechnological applications.
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Diversity of the genus *Monocercomonoides*

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Monocercomonoides sp. is a genus of small flagellates belonging to the order Oxymonadida. They live as intestinal endosymbionts of insects, but some of them can be found also in the intestine of vertebrates. In this work we sequenced the SSU rRNA of 25 different strains of *Monocercomonoies* isolated from various insect and vertebrate hosts and from unused cesspit. We have performed phylogenetic analysis in order to understand the diversity of this genus. Our preliminary results indicate large variation among strains at the genetic level (up to 53.7 % of nucleotide differences). The strains formed two well supported clades, potentially distinct genera, however the position of these clades in the oxymonad trees is unclear and we have so far not found any good diagnostic feature discriminating between them. Morphological analysis of selected strains from the major clade, which we consider as the genus *Monocercomonoides*, failed to find suitable characters for species delineation and it is also very difficult to assign lineages to described species. Based on the morphology and host origin, we assume that the lineage containing *Chinchilla* isolate PA203, and isolates from guinea pig and *Chameleo cristatus* represent species *M. exilis*.

Great diversity of potentially uncultivable anaerobic heteroloboseans

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Heterolobosea is a group of amoeboflagellates, amoebae, and flagellates belonging to the supergroup Excavata. Approximately 140 species of Heterolobosea have been described, from which less than 20 are obligately anaerobic. Most anaerobic species (15) belong to the family Psalteriomonadidae. All known psalteriomonadids except for a single species and an environmental lineage have been established in stable laboratory cultures. The aim of this study was to examine the diversity of potentially uncultivable anaerobic heteroloboseids. We obtained multiple new isolates, mainly from freshwater hypoxic sediments, isolated DNA from fresh cultures (usually from 1st to 5th passage) and determined their SSU rDNA sequences using Psalteriomonadidae-specific primers. In total, we have analyzed sequences of 77 new strains, which represented six already described species of Psalteriomonadidae as well as 13 new ones. However, most of the new species died during the early stages of culture. Our results suggest that a great diversity of uncultivable psalteriomonadids exist in the nature.

Iron Sulfur Cluster Assembly in amitochondriate oxymonad *Monocercomonoides*

Vacek Vojtěch[1], Sebastian Treilti[1], Anna Karnkowska[2], Lukáš Novák[1], Zuzana Zubáčová[1], Ivan Čepička[3] & Vladimír Hampl[1]
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Oxymonads are a group of anaerobic or microaerophilic protists living in guts of insects and vertebrates. They are the only group of eukaryotes without mitochondrion, however in their closest free-living relative *Paratrimastix pyriformis* have been found organelles which are morphologically ntsimilar to hydrogenosomes. Concomitantly with the absence of mitochondrion, *Monocercomonoides* lacks classical mitochondrial ISC system for synthesis of Fe-S clusters. Instead, subunits of SUF system were found in genome and transcriptome of *Monocercomonoides*: SufB, SufC, SufS and SufU. All these proteins contain well conserved catalytic sites which are needed for their function in FeS cluster assembly. Heterologous localization of SufB and SufC in *Trichomonas vaginalis* expression system showed cytosolic localization. We have also found subunits of SUF system in transcriptomic data from *Paratrimastix pyriformis* and two other members of Preaxostyla – oxymonad strain NAU3 distantly related to *Monocercomonoides* and isolate MORAITICA, the deepest branching lineage of Preaxostyla available at the moment. Phylogenetic analyses of SUF subunits showed that all preaxostyla SUFs forms single clade, which is clearly distinct from clades of other eukaryotes – proving that common ancestor of all known Preaxostyla acquired SUF system by horizontal gene transfer independently from other eukaryotes. To prove that SUF subunits are indeed functionally active in *Monocercomonoides* we have performed several complementation experiments in *E. coli*. Preliminary experiments with complementation proved that SufB of *Monocercomonoides* can substitute SufB of *E. coli* in synthesis of Fe-S cluster and therefore SUF system is functionally active in Fe-S cluster assembly. Heterologous localization of SufB and SufC in *Trichomonas vaginalis* expression system showed cytosolic localization. Our results indicate that *Monocercomonoides* is the first known organism, which assemble Fe-S clusters in the cytosol by concerted action of SUF and CIA pathways.

Euglena gracilis plastid proteome

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Euglenids are marine or freshwater flagellates with wide variety of nutritional strategies including phototrophy which is the case of euglenophytes, an inner monophyletic group with green secondary plastids. *Euglena gracilis* is a well-studied member of this group and a model organism with potential applications in biotechnology. However, the genome of this organism is large and unusually organized - mainly due to the abundance of multiple types of introns - and remained unsequenced for a long time. Sequencing and assembly of *E. gracilis* genome conducted at the University of Dundee and led by prof. Mark Field is currently in progress and as a part of this project we focus on prediction and annotation of plastid-targeted genes. The plastid-targeting signal detection method, plastid proteome predicted *in silico* from preliminary data as well as its congruence with the data from mass spectrometry performed on protein sample from isolated *E. gracilis* plastids will be presented at the conference.

Krezol production in *Mastigamoeba balamuthi*

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Various volatile products of bacterial metabolism including p-cresol and indole are released to the environment where they play a key role for communication within the microbial ecosystems. Indole is a multi functional signaling molecule that is generated from tyrosine by many bacterial species, whereas p-cresol, a product of tyrosine degradation, is formed by restricted set of anaerobic bacteria that are capable to tolerate this toxin. Indole and p-cresol are typically produced in animal intestines, but they are also produced in soil, and various sediments. Unlike bacteria, eukaryotic microorganisms were never observed to produce p-cresol and the production of indole is rare. In our study we investigated production of the volatile compounds by free living *Mastigamoeba balamuthi* and its parasitic relative *Entamoeba histolytica*. We found that both amoebae produce significant amount of indole that is catalyzed by tryptophanase. Phylogenetic analysis revealed that tryptophanse gene was likely acquired from bacteria by a common free-living ancestor before diversification of the parasitic lineage. Surprisingly, *M. balamuthi* also produces p-cresol at bacteriostatic concentrations. To produce p-cresol, *M. balamuthi* acquired gene for 4-hydroxyphenylacetate decarboxylase (HPAD). In bacteria, genes for HPAD and S-adenosylmethionine-dependent activating enzyme (AE) are present in a common operon. In *M. balamuthi*, HPAD displayed a unique fusion with AE that suggests operon-mediated transfer of genes from bacterial donor. We also clarified that tyrosine-to-4-hydroxyphenylacetate conversion proceed via the Ehrlich pathway. The acquisition of the bacterial HPAD gene may provide *M. balamuthi* with a competitive advantage over the other microflora in its native habitat.

Synchronized and ER-associated division of *Giardia intestinalis* mitosomes

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Mitosomes are the smallest evolutionary forms of mitochondria that evolved in eukaryotes adapted to anaerobic environments. This adaptation manifests as the absence of the mitochondrial genome and vast majority of the mitochondrial proteome, including the components of the mitochondrial division machinery. Here, we studied the dynamics of mitosomes in the human parasite *Giardia intestinalis* during interphase and mitosis and during differentiation into the cyst stage. We found that mitosomal division is restricted to mitosis, when both central and peripheral organelles divide in a unique and synchronized manner. Surprisingly, despite the absence of the ERMES components, the division involves the association of mitosomes with the endoplasmic reticulum, a relationship commonly seen during the division of mammalian and fungal mitochondria. The mitosomes also divide during the encystation of the trophozoite. Thus, together with the duplicated nuclei the two sets of the organelles preconfigure the cyst for rapid excystation of two daughter trophozoites in a new host.

Unconventional targeting of tail-anchored proteins in *Giardia intestinalis*

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Sec20 is a common SNARE protein that can be found in endomembrane system of various organisms. Typically, it is involved in vesicle trafficking between Golgi apparatus and endoplasmic reticulum. In a paper published by Elias et al, 2008, the protein was shown to be partially localized in the mitosomes of *Giardia intestinalis*. In our experiments we have confirmed that result. However, when an antibody against the protein was raised, we revealed exlusive ER localization. We have analyzed the sequence of Sec20 transmembrane domain which ought to be responsible for localisation of this protein. According to the analysis, the TMD contains strong mitochondrial targeting signal, which we have confirmed experimentally in *Saccharomyces cerevisiae*. Furthermore, when isolated TMD was fused with GFP, the reporter protein was send to the mitosomes. Therefore, what is the reason for Sec20 localisation in the ER under physiological conditions? In order to exclude experimental artefacts, we have determined the influence of both HA-tag and the expression level on the protein localisation. None of these seem to contribute to the puzzling targeting of Sec20. Currently, we are investigating possible role of 3'UTR region of Sec20 gene in targeting of the protein.

An unprecedented non-canonical nuclear genetic code with all three termination codons reassigned as sense codons

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A limited number of non-canonical genetic codes have been described for eukaryotic nuclear genomes, most involving reassignment of one or two termination codons as sense codons. Here we describe an unprecedented genetic code variant that we discovered in a clade of trypanosomatids classified as the genus *Blastocrithidia*. In these protists all three standard termination codons have been reassigned, with UGA encoding tryptophan while UAG and UAA (UAR) specifying glutamate. Furthermore, UAA and less frequently UAG at the same time serve as bona fide termination codons. Surprisingly, the changed genetic code has not incurred modifications of the release factor eRF1 that mediate termination codon reassignments in other eukaryotes, indicating a unique molecular mechanism behind the code change in *Blastocrithidia*. Our results thus expand in an unexpected direction the space of a biologically possible variation in an essential molecular mechanism.

Entamoeba's sophisticated non-evil cousin

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The anaerobic amoeba *Mastigamoeba balamuthi* has a unique evolutionary position being a free-living relative of the pathogenic *Entamoeba histolytica*. We employ the draft genome sequence of *M. balamuthi* and of other amoebozoans to model and study major transitions during the evolution of anaerobic metabolism and parasitic life style. We further show how lateral gene transfer shaped the genome and metabolic capabilities of *M. balamuthi*. Interestingly peroxisomes - organelles thought to be lost in anaerobic organisms are described (not only) in anaerobic amoebozoans.

The intricate evolutionary history of RhoBTB proteins

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The Rho family is one of the major subgroups of the huge superfamily of Ras-like GTPases. Rho proteins are known primarily as regulators of various pathways that are connected to the actin cytoskeleton (cell movement, polarization, morphogenesis etc.). These proteins usually contain only the Rho-type GTPase domain, but some of them possess also a tandem of two so-called BTB domains and are called RhoBTB proteins. The BTB domain is a protein-protein interaction domain and is often a part of proteins that are connected to protein ubiquitination through Cullin3-dependend E3 ligases. Proteins with the domain architecture characteristic for RhoBTB proteins have been reported only from metazoans and dictiosteliid slime moulds, but this distribution was deduced from a phylognetically very limited survey. We have utilized the currently available wealth of genomic and transcritpomic data from diverse eukaryotes, including a wide coverage of protist taxa, and found out that RhoBTB proteins occur in many additional eukaryotic lineages. Although scattered, the phyletic pattern of RhoBTB genes is compatible with a hypothesis that a primordial RhoBTB gene was present already in the last eukaryotic common ancestor. Interestingly, RhoBTB proteins from some taxa (Amoebozoa, Apusomonadida, and Cryptomonadida) proved to possess a RING/U-box domain inserted into the first BTB domain. RING and U-box are related domains that constitute a class of E3 enzymes, so our findings further support the idea that RhoBTB3 proteins ancestrally served as components of ubiquitin-mediated regulation. These and other findings of our evolutionary analyses of RhoBTB proteins will be presented and discussed.

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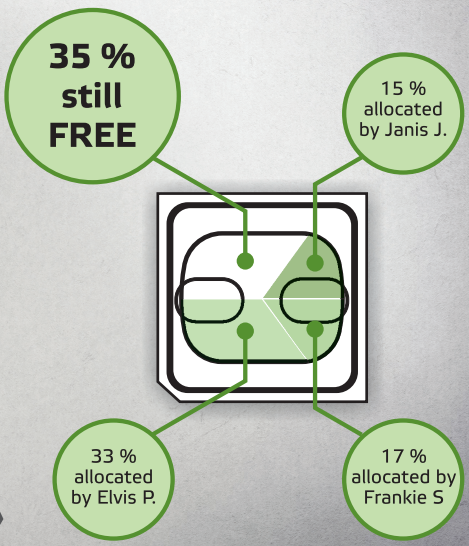
» Use only the sequencing capacity you really need

What amount of reads do I really need?



High Capacity Sequencing Chip

- All kinds of DNA fragments
- Scalable sequencing capacity
- High-scale multiplexing
- Ordering and results on-line

SPECIFICATIONS:

Samples	PCR fragments, Amplicon pools (AmpliSeq), Sheared DNA
Read Length	Up to 200-base reads, single-end
NGS platform	Ion Proton (Thermo Fisher Scientific)
Throughput	1 – 70 millions of filtered reads
Allocation	Customer-defined: min. 1 million of reads
Mean accuracy	≥99 %
Data analysis	Basecalling, Adapter clipping, Quality trimming and filtering
Special services	Efficient amplicon multiplexing; DNA shearing and size selection; AmpliSeq libraries; Individual bioinformatic pipelines
Frequency	Operated monthly

Example project costs:

1x library
1.000.000 reads

240 Euro / project

~1,20 Euro / Megabase!!!

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SEQme s.r.o. is an independent consulting and training company and a service provider in the field of DNA sequencing and Real-Time PCR.

More information about our services, trainings in Real-Time PCR, DNA sequencing and other techniques can be obtained at www.seqme.eu.

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Notes

Anna Vanclová draw the picture on the title page according to the ideas of Lukáš Novák.

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