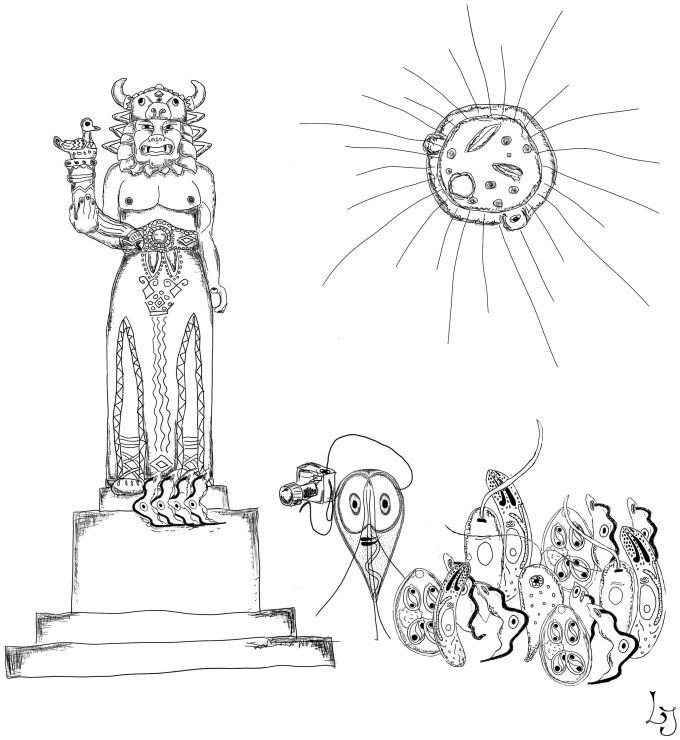
44th Jírovec's Protozoological Days

Conference Proceedings



Department of Biology and Ecology
University of Ostrava, Faculty of Science
Ostrava 2014

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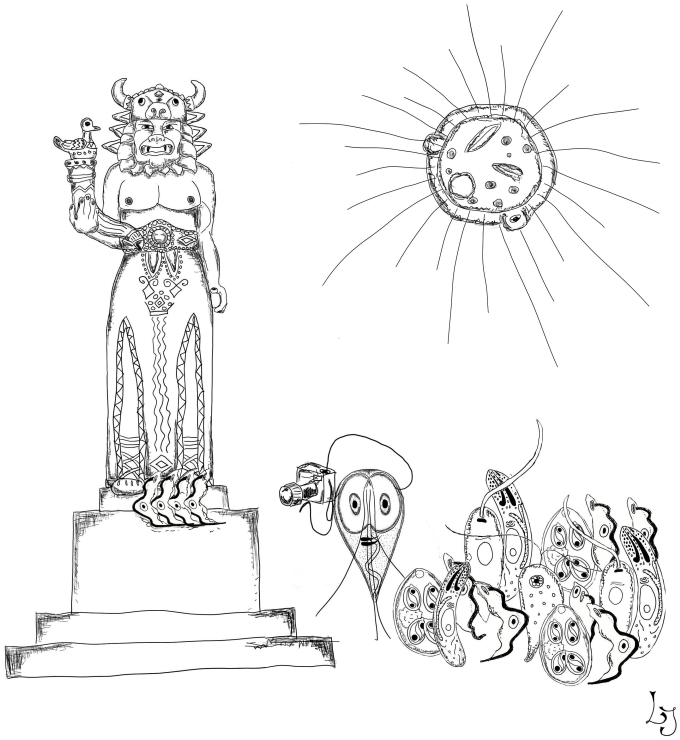
Conference Proceedings



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This publication did not undergone any language editing.

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Content

Foreword

Dear Friends of Czech Protozoology,

welcome to the 44th Jírovec's Protozoological Days of the Czech Society for Parasitology! Our meeting is organized by members of the Protozoological section. This year, our traditional Czech conference is taking place in the Visalaje recreation area, which belongs to the village Krásná situated in the Beskydy mountains in the Moravian-Silesian region. The conference venue is close to Lysá hora – the highest peak of Beskydy.

Thanks to the unity and cohesion of the Czech protozoological community, it was possible to keep many people interested in similar topics coming every year to very distant places in Czech Republic, discuss their research, but also make some trips and have fun together. Nonetheless, the number of 'foreign' experts and students working in the field of Protistology and Parasitology in the Czech Republic is increasing every year. In the end of the previous 43rd Protozoological Days, most of the members of the Protozoological section of the Czech Society for Parasitology voted for English as the preferred language of presentations to make the meeting understandable to our 'foreign' colleagues who work with us in the former Czechoslovakia. Some of our close or potential (or both) scientific collaborators (un)fortunately yet understand neither Czech nor Slovak language very well. According to the opinions and wishes of the majority of members of the Protozoological section of the Czech Society for Parasitology, the 44th Jírovec's Protozoological Days will for the first time use English as the only official language. Nevertheless, we all know that people will discuss their problems mainly in their own languages. Although the presentations and posters are more or less obliged to be in English, if someone would not understand, the others could help to translate to Czech, Slovak, Russian or other languages.

This protozoological meeting offers a great opportunity for students and young scientists to meet each other and to meet Czech as well as 'foreign' experts in the field of both Protistology and Parasitology. Master and PhD students can present their results and their ongoing research to a relatively broad audience interested in various taxa of unicellular eukaryotes and to discuss their ideas with both the experts and other students. On the other hand, students can also profit from the presentations of the experts.

The aim of switching to English for scientific program is not to transform Protodays to a conventional international conference. Most of participants will still be Czech or Slovak, but we can likely expect a nice cooperation with Polish and Russian speaking people who are excellent in the field of

Protistology and Parasitology. The switching to English is just an initial point to make the conference attractive for "our foreign scientific partners" working in our favorite field in the former Czechoslovakia, who would otherwise not come. Since English is currently without any doubt absolutely obligatory for all biologists for publishing research papers, attending foreign conferences and discussing scientific problems with foreign partners, presenting the results in English will be a good training for students and will be beneficial for their scientific careers. Nevertheless, besides of all scientific benefits, all of us can potentially improve our knowledge of, not only English, but also of Czech, Slovak, Russian and other languages.

Except for switching to English as the conference language at the scientific level, no other radical changes to the Protodays meeting are expected. The conference will be under way of a traditional relaxed atmosphere. Most participants have already known each other and many of them are good friends. New members are greatly welcome to our community.

Enjoy the conference and have fun!

Matej Vesteg

List of Participants

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Program Schedule

Monday May 12, 2014	
13:00	Registration
18:00	*Dinner*

Tuesday May 13, 2014	
8:00	*Breakfast*
Protist Molecular Biology and Genomics	
9:00	Marek Eliáš: **Two Small Stories about Two Small GTPases**
9:20	Romana Petrželková, Anna Karnkowska, Romain Derelle, Vladimír Hampl, B. Franz Lang, Čestmír Vlček & Marek Eliáš: **Exploring the Ras Superfamily of GTPases in Protist Genomes**
9:40	Kristína Záhonová, Vladimír Klimeš, Zoltán Füssy, Erik Birčák, Matej Vesteg, Miroslav Oborník, Juraj Krajčovič & Marek Eliáš: **Transcriptome Analysis Reveals a Highly Unusual Non-Photosynthetic Plastid in the Euglenoid Flagellate *Euglena longa***
10:00	Zoltán Füssy, Kristína Záhonová, Vladimír Klimeš, Erik Birčák, Eva Kotabová, Juraj Krajčovič, Miroslav Oborník & Marek Eliáš: **The Retainment of the Secondary Plastid of *Euglena longa* is Connected to the Functional Calvin Cycle Localized to this Compartment**
10:20	*Coffee Break*
10:40	Erik Birčák, Lucia Hadariová, Viktor Demko & Juraj Krajčovič: **Expansion of Calpain Family of Proteases in Euglenozoa**
11:00	Tereza Ševčíková, Aleš Horák, Vladimír Klimeš, Veronika Zbránková, Alexandra Z. Worden, Pavel Přibyl, Jan Fousek, Čestmír Vlček, B. Franz Lang, Miroslav Oborník & Marek Eliáš: **Updating the Algal Phylogeny with New Plastid Genome Sequences: Did the Plastids in Alveolates Emerge through an Endosymbiosis of an Ochrophyte Alga?**
11:20	Anzhelika Butenko, Vyacheslav Yurchenko, Jan Votýpka, Julius Lukeš & Pavel Flegontov: **Genome Assembly, Annotation and Gene Content Analysis of Insect Trypanosomatid *Blechomonas ayalai***
11:40	Vojtěch Žárský & Jan Tachezy: **Bluff with the BLUF**
12:00	The presentation of the sponsor Olympus
12:10	The presentation of the sponsor BioTech
12:20	*Lunch*

Pathogenic Protists	
13:20	Aygul Ishemgulova, Alexei Kostygov, Jan Votýpka, Pavel Flegontov, Petr Volf & Vyacheslav Yurchenko: **The Whole Transcriptome Analysis of *Leishmania* Major Virulence Factors**
13:40	Danyil Grybchuk, Pavel Plevka, Nicolas Fasel, Jan Votýpka, Julius Lukeš & Vyacheslav Yurchenko: **dsRNA Viruses in Trypanosomatidae**
14:00	Michaela Veselíková, Brian Pannicucci & Alena Zíková: **The Adaptability of the *T. brucei* Bloodstream Mitochondria upon the Indirect Knockdown of an Essential Mitochondrially Encoded Protein**
14:20	Ondřej Gahura, Hana Váchová, Brian Panicucci, John E. Walker & Alena Zíková: **Optimizing the Inhibition of a Uniquely Composed *Trypanosoma brucei* F_1-ATPase**
14:40	Zuzana Kotrbová, Dana Hocková & Alena Zíková: **Enzymes of Purine Salvage Pathway in *Trypanosoma brucei* and Trypanocidal Action of Acyclic Nucleoside Phosphonates**
15:00	*Coffee Break*
15:20	Hana Váchová, Ulrike Holzgrabe, Heike Bruhn & Alena Zíková: **Deciphering Mode of Action of JK11 and TG142 Compounds in the Mitochondrion of *T. brucei***
15:40	Zdeněk Paris, Alan Kessler & Juan D. Alfonzo: **Queuosine: The Role of an Essential tRNA Modification in Parasitic Protist *Trypanosoma brucei***
16:00	Jitka Štáfková, Jan Mach & Jan Tachezy: **Mitochondrial Pyruvate Transporter of *Trypanosoma brucei***
16:20	Natalya Kraeva, Jan Votýpka, Jana Hlaváčová, Alexei Kostygov, Anzhelika Butenko, Pavel Flegontov, Petr Volf, Julius Lukeš & Vyacheslav Yurchenko: **Insight into *Leishmania* – Leptomonas Co-infection**
16:40	Pavlína Vobořilová, Jaroslav Kulda & Jan Tachezy: **Trichomonads in Cats and Dogs**
17:00	Poster Session
18:00	*Dinner*

Wednesday May 14, 2014	
8:00	*Breakfast*
Protist Cell Biology and Biochemistry	
9:00	Anna Karnkowska, Zuzana Zubáčová, Lukáš Novák, Vojtěch Vacek, Miluše Hroudová, Čestmír Vlček & Vladimír Hampl: **Genomic Sequence of Oxymonad *Monocercomonoides* – a Eukaryote without Mitochondrion**
9:20	Sebastian Cristian Treitli, Anna Karnkowska & Vladimir Hampl: **Genetic Evidence of Meiosis in *Monocercomonoides***
9:40	Lukáš Novák, Zuzana Zubáčová, Ondřej Brzoň, Anna Karnkowska & Vladimír Hampl: **Hydrogenosome-Like Organelle of *Trimastix pyriformis***
10:00	Luboš Voleman, Eva Martincová, Pavla Tůmová, Ivan Hrdý, Jan Tachezy & Pavel Doležal: **Dynamics of *Giardia intestinalis* mitosomes**
10:20	*Coffee Break*
10:40	Anna Vanclová, Róbert Šuták & Vladimír Hampl: **Membrane Proteome of Euglenid Plastids**
11:00	Marie Glavanakovová & Róbert Šuták: **Ferritin in *Naegleria gruberi***
11:20	Vladimir Varga, Polly Hayes, Sofia Olego-Fernandez, Jack Sunter, Michael Ginger & Keith Gull: **Major Transitions in Trypanosome Cell Morphology Produced via Modulation of a Cytoskeletal Calpain-Like Protein**
11:40	Presentation of the sponsor Roche
12:00	*Lunch*
13:00	Trip to Hukvaldy castle, hiking in Beskydy mountain or free program
19:00	*Dinner (or banquet?)*

Thursday May 15, 2014	
8:00	*Breakfast*
Protist Cell Biology and Biochemistry	
9:00	Jan Michálek, Aleš Tomčala, Ivana Schneedorferová & Miroslav Oborník: **The Fat Cousin of Apicomplexans: From Genes to Fats**
9:20	Ivana Schneedorferová, Jan Michálek, Aleš Tomčala & Miroslav Oborník: **The Fat Cousin of Apicomplexans: From Fat to Genes**
9:40	Alena Košťálová & Ivan Hrdý: **Is there a Cytosolic Hydrogenase in *Trichomonas vaginalis*?**

10:00	Vladimíra Najdrová, Luboš Voleman, Eva Martincová & Pavel Doležal: **Evolution of Membrane Insertion of Tail-Anchored Proteins in Eukaryotes**
10:20	*Coffee Break*
	Protist Biodiversity, Phylogeny and Systematics
10:40	Alexei Yu. Kostygov, Anastasiia Grybchuk (Ieremenko), Marina N. Malysheva, Alexander O. Frolov & Vyacheslav Yurchenko: **Molecular Revision of the Genus *Wallaceina***
11:00	Tomáš Skalický, Pavel Flegontov, Jan Votýpka, Maria D. Logacheva, Alexey A. Penin, Goro Tanifuji, Naoko T. Onodera, Milena Svobodová, Alexey S. Kondrashov, Petr Volf, John M. Archibald & Julius Lukeš: ***Paratrypanosoma* – a Novel Early-Branching Trypanosomatid**
11:20	Vojtěch David, Pavel Flegontov, Evgeny Gerasimov, Goro Tanifuji, Naoko Onodera, Ivan Fiala, Maria Logacheva, Hassan Hashimi, John Archibald & Julius Lukeš: **Mitochondrial Genome and U-Insertion/Deletion Editing of an Endosymbiotic Kinetoplastid *Perkinsela***
11:40	Petr Táborský, Tomáš Pánek, Ivan Čepička: **Extensive Diversity of Free-living Preaxostyla**
12:00	Presentation of the sponsor Life Technologies
12:10	Presentation of the sponsor KRD
12:20	*Lunch*
13:20	Eliška Ptáčková, Giselle Walker & Ivan Čepička: **The Phylogenetic Evidence for the Paraphyly of the Genus *Mastigella* (Amoebozoa: Archamoebae)**
13:40	Jan Pyrih & Jan Tachezy: **Searching for Appropriate Amino-Acid Substitution Matrices to Understand Secondary Endosymbiosis of Plastid**
14:00	Jaromír Cihlář, Aleš Horák, Arnab Pain & Miroslav Oborník: **Heme Pathway of *Vitrella brassicaformis***
14:20	*Coffee Break*
14:40	Alena Kodádková, Pavla Bartošová-Sojková & Ivan Fiala: ***Bipteria* sp. – a Basal Marine Myxosporean Shed the Light on Myxosporean Evolution**
15:00	Tomáš Pánek, Alastair G. B. Simpson, Vladimír Hampl, Andrew J. Roger, Miluše Hroudová, Čestmír Vlček & Ivan Čepička: ***Creneis carolina* gen. et sp. nov. – Novel Anaerobic Lineage of Heterolobosea with Unique Cell Structure and Life Cycle**
15:20	Ivan Čepička, Petr Táborský, Tomáš Pánek: **'Anaeramoeba', a Novel Anaerobic Marine Amoeba with Uncertain Phylogenetic Position**

15:40	*Coffee Break*
16:00	Meeting of the Protozoological section of the Czech Society for Parasitology
17:00	Closing remarks and announcement of the best pregradual and postgradual students posters and presentations
18:00	*Dinner*

Friday May 16, 2014	
8:00	*Breakfast*
9:00	Departure of Participants (till 10 a. m.)

Speakers' names are underlined.

Poster Session

Heather Esson, Jitka Kručinská & Miroslav Oborník: **Characterization of the Chromerosome, a Mysterious Organelle in *Chromera velia***

Ivan Fiala & Pavla Bartošová-Sojková: **Adaptive Radiation and Evolution within the Myxozoa**

Lada Hofmannová, Lucie Štohanzlová, Dagmar Jirsová, Lucas A. Wauters, Maria Vittoria Mazzamuto, Claudia Romeo & David Modrý: **Comparison of *Eimeria* Species in Native and Introduced Squirrels**

Nikola Polanská, Tatiana Košťálová, Tereza Kratochvílová, Michaela Kindlová, Petra Sumová, Marina Gramiccia, Luigi Gradoni & Petr Volf: **Canine Antibodies against Recombinant Salivary Proteins of *Phlebotomus perniciosus***

Johana Rotterová, Ludmila Nováková & Ivan Čepička: **Revealing High Diversity of Anaerobic Ciliates**

Eva Roubalová, Zoltán Füssy, Chris Bowler & Miroslav Oborník: **Silencing of Heme Pathway Genes in Model Diatom *Phaeodactylum tricornutum***

Ivan Fiala & Pavla Bartošová-Sojková: **Adaptive Radiation and Evolution within the Myxozoa**

Lada Hofmannová, Lucie Štohanzlová, Dagmar Jirsová, Lucas A. Wauters, Maria Vittoria Mazzamuto, Claudia Romeo & David Modrý: **Comparison of *Eimeria* Species in Native and Introduced Squirrels**

Aleš Tomčala, Ivana Schneedorferová, Jan Michálek & Miroslav Oborník: **Coupled Scientific Methodology for Metabolic Pathway Investigation: The Fat Story**

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Katarína Krnáčová, Michaela Vinarčíková, Ivana Rýdlová & Anton Horváth: **Oxidative Phosphorylation of *Euglena gracilis***

Eva Martincová, Natalia Wandyszewska, Jan Pyrih, Martin Kolísko & Pavel Doležal: **Putative Protein Translocase(s) in the Mitosomes of *Giardia intestinalis***

Martina Zátopková, Pavel Škaloud, Tereza Ševčíková & Marek Eliáš: **Hunting for New Eustigmatophytes: Molecular Characterization of *Chlorobotrys polychloris***

The names of the presenters are underlined.

Abstracts

Expansion of Calpain Family of Proteases in Euglenozoa

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Calpains are Ca^{2+}-dependent cysteine proteases that participate in various cellular processes. Calpains are well characterized in many animal and plant model organisms, but there is still not enough information about their structure and function in many lower eukaryotes. Recent studies in this area revrealed many new types of protein domains combined with calpain-specific catalytic core domain CysPc. Therefore we studied the distribution of calpain genes in various genomes and transcriptomes of different genera of Euglenozoa. Our main goal was to find and characterize different architectures and structures of calpain genes in euglenoid flagellate *Euglena longa* and *Eutreptiella gymnastica*.

Genome Assembly, Annotation and Gene Content Analysis of Insect Trypanosomatid *Blechomonas ayalai*

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A new species-rich clade of insect trypanosomatids named *Blechomonas* has recently been discovered. Our study is aiming at comprehensive characterization of this newly described group of flea parasites and therefore we performed genome assembly, annotation and gene content analysis of *Blechomonas ayalai*. Genome sequencing of *B. ayalai* was conducted using Illumina HiSeq and MiSeq technologies and the genome was assembled *de novo* using Velvet 1.2.10. The best assembly with N50 of ≈ 95 kbp was chosen for analysis. *Ab initio* gene prediction was performed with Augustus 2.5.5 using a training set of 778 high-confidence gene models. Manual curation of the annotation included correcting frameshifts and uncertain start codons. Gene content analysis of *B. ayalai* was carried out using a reference dataset of 38 annotated proteomes or ORFs for unannotated genomes, representing 27 kinetoplastid species. The study indicates greater similarity of *B. ayalai* gene set to that of *Leishmania* spp. rather than that of *Trypanosoma* spp. (1362 genes that don't occur in *Trypanosoma* spp. are shared between *B. ayalai* and at least one *Leishmania* sp.; only 62 genes are exclusively shared between *B. ayalai* and at least one *Trypanosoma* sp.). 140 genes of *B. ayalai* are specifically shared with other monoxenous trypanosomatids and 13 genes with bodonids *Bodo saltans* and/or *Trypanoplasma borreli*. 16 common genes are present in *B. ayalai* and in both of basal trypanosomatid clades: *Trypanosoma* and *Paratrypanosoma*. Further study of *Blechomonas* sp. with respect to peculiarities of it's host lifestyle, possibly allowing *Blechomonas* to be occasionally transmitted to a vertebrate should provide an insight into the emergence of medically important dixenous trypanosomatid parasites.

Heme Pathway of *Vitrella brassicaformis*

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We have identified transcripts of heme biosynthesis genes within the transcriptome of *V. brassicaformis*. Our data suggest that the synthesis of heme in *V. brassicaformis* is homologous to that of *C. velia*. Similarly to *C. velia* (Kořený et al. 2011), we have identified ALA-synthase (ALAS) but neither Glutamyl-tRNA reductase nor Glutamate semialdehyde aminotransferase, suggesting that *V. brassicaformis* also uses C4 pathway to synthesize ALA. Interestingly, we have found only one copy of Uroporphyrinogen decarboxylase (UROD) comparing to *C. velia* which has three copies UROD. We have performed phylogenetic analyses of all genes of the pathway. In most of the phylogenetic trees *V. brassicaformis* enzymes form a sister group with those of *C. velia* with a single exception of Uroporphyrinogen synthase (UROS). *C. velia* does not branch with *V. brassicaformis* but shows rather sister position to diatom *Thalassiosira pseudonana*. We also tried to identify possible subcellular localization of all enzymes using *in silico* targeting predictions. According to our findings, ALAS is targeted to the mitochondria. Most of remaining enzymes show plastid localization, while in the case of one copy of ALA-dehydratase, Uroporphyrinogen synthase, Coproporphyrinogen oxidase and Ferrochelatase signal peptide was not identified. In spite of relatively close relationship between chromerid algae we have found several differences between their heme pathways.

'Anaeramoeba', a Novel Anaerobic Marine Amoeba with Uncertain Phylogenetic Position

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We have isolated and cultured seven strains of anaerobic amoebae ('Anaeramoeba') from anoxic/microoxic marine coastal sediments worldwide. The strains morphologically represent three distinct species that are highly reminiscent of the genera *Flamella* (Gracilipodida), *Flabellula* or *Paraflabellula* (Tubulinea) by having extremely flattened, fan-shaped cells with trailing uroidal filaments. On the other hand, 'Anaeramoeba' displays several unique ultrastructure features. It possesses acristate mitochondrion-related organelles, presumably hydrogenosomes, parietal nucleoli in the nucleus, and a large MTOC in the cytoplasm. Two non-conspecific strains are also able to produce bikont or tetrakont flagellates with isokont flagella that might be distributed into separate monokinetids. Phylogenetic analyses of SSU rDNA and five protein-coding genes did not resolve phylogenetic position of 'Anaeramoeba' suggesting that it forms a deep eukaryotic lineage.

Mitochondrial Genome and U-Insertion/Deletion Editing of an Endosymbiotic Kinetoplastid *Perkinsela*

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Perkinsela sp. is a basally-branching kinetoplastid and obligate intracellular symbiont of *Paramoeba* spp., the causative agent of Amoebic gill disease inflicting salmonids. Here we present a study based on the combination of Next-generation sequencing data from two strains of *Paramoeba pemaquidensis*. Our focus is on the genome of symbiont mitochondrion and U-insertion/deletion type of post-transcriptional RNA editing which is typical for kinetoplastid mitochondria. The genomes of the endosymbiont mitochondria were assembled into the sets of linear fragments with terminal repeats bearing transcribed regions. Interestingly, all mitochondrial and nuclear subunits of complex I of the electron transport chain were not found, suggesting its complete loss. High coverage of long Illumina reads allowed us to dissect U-insertion/deletion editing events in unprecedented detail. All of the transcripts undergo heavy U-insertion/deletion editing, usually at 5' and 3' ends of the transcript.

Two Small Stories about Two Small GTPases

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Small GTPases are one of the hallmarks of the complex molecular fabric of the eukaryotic cell. I will present two small stories concerning these GTPases that illuminate different aspects of the evolution of the eukaryotic cell and eukaryotes in general. One story concerns the ARF-related GTPase family functionally associated with the endomembrane system and the cytoskeleton. I argue that the previously suggested hypothesis about the origin of this family from prokaryotic MglA/RarD GTPases is unlikely, since rather than ancestral this is a derived GTPase subgroup characterized by a unique loss of a functionally critical and highly conserved Asp residue. Instead, I found an obvious ARF-related gene in the genome of *Candidatus* Korarchaeum cryptofilum, an archaeon representing a lineage thought to be related to archaeal ancestors of eukaryotes, which offers an alternative scenario for the emergence of the eukaryotic ARF-related GTPases. The main character of the second story is the Rheb GTPase, a widely conserved protein involved in sensing the nutrient status of the cell. While conventional Rhebs consist of a GTPase domain followed by a prenylated C-terminal tail, I found two eukaryotic clades with a novel Rheb structure. Whereas Euglenozoan Rhebs gained an N-terminal phosphoinositide-binding FYVE domain, Rhebs in the recently redefined Cryptista clade, comprising cryptomonads, katablepharids, and palpitomonads, gained an N-terminal PX domain also known to bind phosphoinositides. In addition to providing an interesting case of convergent evolution at the molecular level, the comparative analysis of Rheb proteins thus revealed a new synapomorphy of the Euglenozoa and the first known synapomorphy of the Cryptista clade.

Characterization of the Chromerosome, a Mysterious Organelle in *Chromera velia*

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Chromera velia is an algal symbiont of stony corals and is closely related to the exclusively parasitic Apicomplexa, a group that includes the causative agents of malaria (*Plasmodium falciparum*) and toxoplasmosis (*Toxoplasma gondii*). A multi-membrane organelle originally identified as the mitochondrion was later determined to be a novel organelle and renamed the "chromerosome". While electron micrographs indicate that the chromerosome is always present in a subset of cells within a given population, its morphology is variable and its cellular function during the life cycle of *C. velia* is unclear. Here we propose a research program for elucidating the role of the chromerosome based on comparative morphology and cell biology. We also present preliminary data based on these approaches, which suggest that the chromerosome is homologous to the digestive vacuole in *Plasmodium* trophozoites.

Adaptive Radiation and Evolution within the Myxozoa

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The evolutionary trajectories of endoparasites are greatly influenced by interactions with their hosts. Thus, endoparasite diversification can be expected to reflect processes such as co-speciation and host switching along with the evolution of host specificity. In addition, parasites must achieve transmission to new hosts, a process that typically requires persistence outside their hosts. Finally, parasites with complex life cycles, such as myxozoans, require the ability to exploit distinctly different hosts. Drivers of diversification and evolution within the Myxozoa will therefore include biotic factors associated with host exploitation and abiotic factors associated with the environment of their free-living spores.

We expanded on these themes by focusing on patterns of evolution within the Myxozoa. We compared diversification of the malacosporeans and myxosporeans. We considered more specific adaptations displayed particularly by the myxosporeans that have enabled this group to exploit a variety of hosts and tissues and to survive in the environment when switching hosts. We also include the evolution of parasitism of myxozoans, including discussion of the first myxozoan hosts and how myxozoan life cycles may have expanded to incorporate intermediate hosts.

The Retainment of the Secondary Plastid of *Euglena longa* is Connected to the Functional Calvin Cycle Localized to this Compartment

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Euglenida comprise species living in various environments. While *Euglena gracilis* lives autotrophically or mixotrophically, and tolerates loss of its green plastid upon stress, *E. longa* (syn. *Astasia longa*) is a naturally "bleached" osmotrophic relative of *E. gracilis*, intriguingly keeping its plastid as an essential organelle. *E. longa* plastidial DNA encodes only for ribulose-bisphosphate carboxylase (Rbc, EC: 4.1.1.39) large subunit (RbcL), proteins of the plastid transcriptional and translational systems, and non-coding RNAs (tRNAs and rRNAs). Our bioinformatic analyses of recently obtained transcriptome data from *E. longa* revealed that there are no predicted plastid-targeted enzymes of the heme, DOXP, or type II fatty acid biosynthesis. However, we were able to predict plastidial localization of a majority of Calvin cycle enzymes, including the small Rbc subunit (RbcS). We used a Calvin cycle inhibitor, glycolaldehyde (GLA), and observed a constant inhibitory effect of GLA on the growth of *E. longa* to an extent observed for streptomycin (SM)-treated cultures. Mixotrophic *E. gracilis* controls were tolerant to SM or GLA. Nevertheless, GLA also inhibited the growth of *Trypanosoma brucei* lacking the Calvin cycle, suggesting that a general side-effect may play a role in our observations. Rather than using another inhibitor, we tested the possibility of *E. longa* to utilize Rbc for CO_2 fixation. Preliminary data show that *E. longa* does fix CO_2 at a considerable pace: about 20 % of mixotrophic *E. gracilis* fixation rate. Interestingly, this fixation activity decreased in *E. longa* 2 h post SM application. We report that the RbcL protein localizes to the plastids of both studied *Euglena* species.
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Ferritin in *Naegleria gruberi*

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Ferritins are complex protein structures for iron storage and detoxification that play an important role in the metabolism of this crucial element. They are typically found in the cytoplasm of the cell; however, they were also identified in mitochondria and chloroplasts. Its function in these organelles is still not clear. They are widely distributed among living organisms, but uncommon in non-photosynthetic protists. Surprisingly, ferritin gene was found in the genome of free-living amoeboflagellate *Naegleria gruberi*. In this study we aimed to determine cellular localization of this protein and investigate its function in *N. gruberi*.

dsRNA Viruses in Trypanosomatidae

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Double-stranded RNA (dsRNA) viruses of trypanosomatids is an emerging field of research. These viruses belong to the *Totiviridae* family and were first described as parasites of *Saccharomyces cerevisiae*. Subsequently, they were discovered in several *Leishmania* species. The groundbreaking result came in 2011 when it was shown that presence of these viruses in *Leishmania* parasite influences the course and severity of leishmaniasis. Despite intense research, some of their important features, such as virus-host interaction patterns and diversity in different hosts, are not well understood. Our study is mainly directed toward broad survey of dsRNA viruses in various species within the family Trypanosomatidae. Our major goal is to uncover virus diversity based on nucleotide sequence of their dsRNA genomes. This is very important in terms of tracking their phylogenetic history and virus-host interaction and coevolution. Such a survey will also allow us to identify conservative sequences in different dsRNA viruses. Both aspects can provide deeper understanding of dsRNA viruses' role as pathogenicity factors in medically significant species of *Leishmania*. To date, we adopted the methodology of detecting dsRNA viruses in representatives of different genera of Trypanosomatidae. Using DNase/RNase digestion tests and anti-dsRNA antibody based detection we successfully identified dsRNA viruses in *Paratrypanosoma confusum*, *Leptomonas moramangonensis* and *Crithidia pragensis* which suggests the vast underexplored variety of dsRNA viruses in Trypanosomatidae. Thus, the search for trypanosomatid viruses should be continued including other representatives of this family.

Genomic Sequence of Oxymonad *Monocercomonoides* – a Eukaryote without Mitochondrion

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Metamonada is a subgroup of Excavata consisting of unicellular, multiflagellated, heterotrophic eukaryotes characterized by anaerobic lifestyle. Three distinct lineages can be found within Metamonada: Fornicata (i.e *Giardia*), Parabasalia (i.e. *Trichomonas*) and Preaxostyla (*Trimastix* and oxymonads). From the genomic perspective, metamonads are not very densely sampled. The only genomes of metamonads published so far belong to parasites – *T. vaginalis*, *G. intestinalis* and *S. salmonicida*. Genomic sequence from non-parasitic species may help to reveal the common features of all metamonads and clarify the evolution of this group. Our model organism, oxymonad *Monocercomonoides* sp. PA 203, was isolated from the faeces of *Chinchilla*. The sequenced genome of *Monocercomonoides* assembled into 2174 scaffolds that cover 75 Mb and contain 16,781 predicted protein-coding genes. Functional annotation of the genome is in progress, however, we have already documented components of energy metabolic map, which closely parallels that of *Giardia* and *Entamoeba*. Despite the intensive search for genes coding mitochondrial proteins, none have been identified indicating that *Monocercomonoides* represents the first known eukaryote lacking this compartment. We hypothesise that a preadaptation to this loss has been the replacement of mitochondrial pathway for the synthesis of FeS clusters ISC by a bacterial system SUF.

Comparison of *Eimeria* Species in Native and Introduced Squirrels

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The red squirrel (*Sciurus vulgaris*) is a species common throughout Eurasia. In Great Britain, Ireland and Italy, their numbers have decreased drastically in recent years, also in association with the introduction of alien tree squirrels, mainly the eastern grey squirrel (*Sciurus carolinensis*) from North America. Our study was focused on the squirrel populations in Italy to compare gastrointestinal parasites in *S. vulgaris*, *S. carolinensis* and also *Callosciurus erythraeus*, the Pallas's squirrel, originated from South-eastern Asia. *Eimeria* species were dominant coproscopical findings in all three squirrel species. Morphological determination of the eimerian oocysts revealed three morphotypes (I, II, III) in *S. vulgaris*, two (II, III) in *S. carolinensis* and slight infections with morphotype II in *C. erythraeus*. Similarities of morphotype II and III required deeper determination based on genetic analyses. So far, the sequences obtained do not support the hypothetical exchange of *Eimeria* spp. Comparison of the internal transcribed spacers (ITS1 and 2) of nuclear ribosomal DNA and partial cytochrome c oxidase subunit 1 (cox 1) gene of mtDNA of the morphotype II showed different *Eimeria* haplotypes/species, both in allopatric and sympatric population of native red and introduced grey squirrels.
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Molecular Revision of the Genus *Wallaceina*

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We performed molecular revision of the genus *Wallaceina*, which was established in the very twilight of the traditional morphotype-based approach to classification of the Trypanosomatidae. This genus was separated from *Crithidia* since it had a completely unique variant of endomastigotes. In molecular phylogenetic studies all four described species of *Wallaceina* were shown to be extremely close to each other and to some other undescribed isolates or even species attributed to the different genus (*Blastocrithidia*) clustered within Leishmaniinae clade. Recent inclusion of unrelated species from "*Leptomonas collosoma* clade" led to further confusion. To clarify this situation we performed molecular phylogenetic analysis of the available isolates of supposed *Wallaceina* species. Our results demonstrated that all Leishmaniinae-bound wallaceinas are just different isolates of the same species that we suggest to rename back to its original name of *Crithidia brevicula* Frolov, Malysheva, 1989. Here we also propose to discontinue using the generic name *Wallaceina* and give a new name to clade containing *Leptomonas collosoma*.

The Whole Transcriptome Analysis of *Leishmania* Major Virulence Factors

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Over the years many genes were implicated in *Leishmania* pathogenicity. In this work we compared the expression profiles of the virulent and avirulent strains of *L. major* using next-generation sequencing. Long-term cultivation of the *L. major* virulent strain LV561 (LRC-L137; MHOM/IL/1967/Jericho-II) yielded an avirulent line LV561/AV with attenuated infectivity for mice and sand fly vectors. Such reduced virulence was primarily explained by the differences in LPG and GP63 expression on the cell surface, but several other molecules (e.g. NADPH-diaphorase and peroxidase) were also considered to be involved. Whole transcriptome analysis of poly-A RNA revealed that 230 and 357 transcripts are upregulated in LV561/V (virulent) and LV561/AV (avirulent) strains, respectively ($p \leq 0.05$). Eleven out of the top 50 genes with elevated expression in LV561/V were localized to the chromosome 19. This disproportion is even more staggering in the case of the LV561/AV, where 29 out of the top 50 upregulated transcripts originated from the chromosome 35. Among differentially expressed genes we identified those involved in cell attachment, membrane dynamics, protection from the oxidative stress, glucose and folate transport, proteases and others. Interestingly, a significant proportion of genes with elevated expression in LV561/AV may be involved in chromatin remodeling and RNA processing suggesting that attenuation of pathogenicity is directly linked to gene expression.

Bipteria sp. – a Basal Marine Myxosporean Shed the Light on Myxosporean Evolution

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Myxosporeans form three main phylogenetic lineages: two of them include species clustering according to host habitat, i.e. marine and freshwater lineage, and a recently revised third sphaerosporid lineage. The phylogenetic position of these lineages is not clear and thus we cannot assess the evolutionary oldest myxosporean species. The marine lineage consists almost exclusively of marine myxosporeans parasitizing predominantly teleost fishes, however, a few myxosporeans infect cartilaginous fishes and cluster as the basal species of the first branching marine group – the *Ceratomyxa* clade. This may suggest the marine lineage to be the most basal myxosporean lineage with evolutionary old, shark-infecting species. We discovered a new myxosporean *Bipteria* sp. from the gall bladder of chimaera *Chimaera monstrosa* from North Atlantic. Based on the SSU rDNA phylogeny, *Bipteria* sp. is the most basal species within the marine lineage. Morphology of *Bipteria* sp. spore is characterized by crescent shape with wing-like appendages similar to the *Ceratomyxa* morphotype that has been supposed as the evolutionary primitive one. The most basal phylogenetic position and a parasitism in evolutionary old fish suggest *Bipteria* sp. as a possible ancestor of all myxosporeans. It is assumed that Myxozoa evolved within cnidarians as a sister lineage to the Medusozoa. Based on the molecular and fossil dating of jellyfishes, medusozoans evolved during Cambrian radiation (540–510 Ma), which does not correspond to the evolutionary origin of *C. monstrosa*, host of *Bipteria* sp., that evolved later in the Silurian era (420 Ma). Thus, we can assume that myxosporeans were single host parasites of annelids for about 100 million years which evolved in the Cambrian era.

Is there a Cytosolic Hydrogenase in *Trichomonas vaginalis*?

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In *T. vaginalis* genome, up to nine genes encoding Fe-dependent hydrogenase homologues have been identified, six of which have conserved cysteine residues known to form the essential H cluster, which is crucial for the proper hydrogenase activity. Five of those six hydrogenases have been found in the proteome of hydrogenosome, while the distribution of the other enzymes remains unknown. Hydrogenosomal hydrogenases produce molecular hydrogen as one of the end products of the pyruvate decarboxylation and are iconic, organelle-defining enzymes of anaerobic eukaryotes. Long after the discovery of hydrogenosomal hydrogenases of trichomonads, also low activity, cytosolic hydrogenases have been found in anaerobic amoebae and diplomonads. Cytosolic localization of hydrogenase in *T. vaginalis* was never proposed or rigorously tested, however, our recent metabolic studies indicated the possible presence of hydrogenase in *T. vaginalis* cytosol that could function in redox balance maintenance. Biochemical assays consistently detected relatively high cytosolic hydrogenase activity. The identification of the corresponding enzyme is under way.

Enzymes of Purine Salvage Pathway in *Trypanosoma brucei* and Trypanocidal Action of Acyclic Nucleoside Phosphonates

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Trypanosoma brucei is a medically and veterinary important protozoan parasite. Since commonly used drugs are toxic and inefficient against the diseases caused by this pathogen, it is necessary to search for new therapeutic alternatives. Unlike mammals, *T. brucei* cannot synthesize purines *de novo* and it depends strictly on the uptake of these essential molecules from its environment. To transform, interconvert and metabolize purines, complex and versatile purine salvage pathway (PSP) has evolved in these purine auxotrophs. Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and xanthine PRT (XPRT) are two key enzymes of the redundant PSP in *T. brucei*. RNAi silencing of the glycosomal XPRT led to a strong growth phenotype in the media containing only xanthine while no growth phenotype was observed when hypoxanthine was added. RNAi silencing of the cytosolic HGPRT resulted in slight phenotype in the media containing hypoxanthine suggesting that XPRT can also act on hypoxanthine when HGPRT is ablated. Moreover, the double-knock down of HGPRT/XPRT was lethal when 6-oxo purines were the only purine sources in the media implying that no other enzymes can circumvent the action of PRTs. In vitro studies confirmed affinity of the recombinant HGPRT to hypoxanthine or guanine and not to xanthine. Moreover, we screened nearly 100 acyclic nucleoside phosphonates (ANPs), which are potential inhibitors of HGPRT and XPRT. Some of the ANPs have the effective 50 % inhibitory concentration (IC50) in the single □M values. The mode of action of these drugs was studied using a cell line overexpressing the v5-tagged HGPRT suggesting that ANPs binds to and inhibit the activity of the HGPRT enzyme.

Insight into *Leishmania* – Leptomonas Co-infection

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The family Trypanosomatidae unites parasitic flagellates undergoing cyclical development in invertebrate (monoxenous) or invertebrate and vertebrate (dixenous) hosts. Dixenous parasites (*Leishmania* and *Trypanosoma*) are best known as agents of important diseases in humans. However, several cases of monoxenous species (in particular, *Leptomonas seymouri*) found co-infecting along *Leishmania* donovani were reported recently. In our study we confirmed the presence of *L. seymouri* in the previously analyzed clinical isolates of *L. donovani* Ld_39 and Ld_2001 from India. This posed two related questions: a) can *L. seymouri* withstand elevated temperature, and b) can it (co)-infect vertebrate hosts in laboratory conditions? Here we show that in contrast to other monoxenous species (*Blechomonas ayalai* and *Leptomonas pyrrhocoris*) *L. seymouri* can be cultivated at high temperature. Morphological studies revealed distinct morphotypes when trypanosomatids were grown in different experimental conditions. To identify genes responsible for the thermo stability of *L. seymouri* we analyzed whole-transcriptome profiles of these cultures. To answer the second question we experimentally co-infected *L. seymouri* and *L. major* into the Balb/c mice and followed infection by PCR analysis. Future studies should shed more light on this important medically relevant problem.

Mitochondrial Pyruvate Transporter of *Trypanosoma brucei*

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In most eukaryotes pyruvate, the end product of glycolysis is transported into the mitochondria for further oxidation leading to ATP formation and other important biosynthetic reactions. That makes pyruvate a keystone intermediate metabolite of cellular metabolism. Mitochondrial pyruvate carrier (MPC) of yeast, fruit fly and human has recently been identified. Heterodimeric MPC, consisting of small hydrophobic subunits MPC1 and MPC2/3, transports pyruvate across inner mitochondrial membrane. We are studying cellular metabolism in *Trypanosoma brucei*, an important pathogen of humans and cattle in sub-Saharan Africa. Its peculiar mitochondrion undergoes remarkable changes between two principal life stages. Consequently, cellular metabolism as well as pyruvate metabolism reflects these changes. We identified homologs of both MPC subunits in the genome of *T. brucei* and confirmed localization exclusively in the mitochondrial membrane. We created MPC1 knock-out cell lines in both procyclic and bloodstream stages and we discuss the changes in the activities of metabolic enzymes as well as metabolic end products regarding inability to transport pyruvate across mitochondrial membrane.

The Fat Cousin of Apicomplexans: From Genes to Fats

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Two species of algae – *Chromera velia* (Moore et al. 2008) and *Vitrella brassicaformis* (Oborník et al. 2012) are being intensively studied for their close relationship to apicomplexan parasites. The recent discovery of chromerids was followed by the sequencing of their genomes and transcriptomes. When combined with mass spectrometry the genomic and transcriptomic data give us a great opportunity to look inside the metabolism of chromerids. We performed the mass spectrometry of fatty acid and lipid content of both chromerids. In contrast to *V. brassicaformis*, *C. velia* was found to be highly specialized in storing a large amounts of triglycerids. By using homology search we managed to identify a set of genes responsible for basic fatty acid synthesis and subsequent functional modifications. Both species also posses numerous multienzymes related to type I fatty acid or polyketide synthases. The genome of *C. velia* also encodes the second largest known polypeptide in protists – a putative fatty acid or polyketide synthase in total length of 11,656 amino acids. The genetic equipment for fatty acid and lipid synthesis seem to be very similar in both known chromerids and thus we expect fundamental difference in transcription regulation.

Evolution of Membrane Insertion of Tail-Anchored Proteins in Eukaryotes

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Special class of membrane proteins, so called tail-anchored (TA) proteins, carry a single C-terminal transmembrane domain (TMD). The proteins lack N-terminal signal peptide and the TMD serves also as a targeting signal. This topology predisposes TA-proteins to mediate interactions among membrane bounded compartments by their N-terminal domains such as vesicular transport, regulation of apoptosis and protein translocation. In some eukaryotes, specific GET pathway controls precise insertion of TA-proteins into the endoplasmic reticulum membrane. Our bioinformatic analyses revealed the absence of key GET proteins in most eukaryotic lineages except opisthokonts. We use *Giardia intestinalis* in order to characterize putative distinct GET machinery common to all other lineages.

Hydrogenosome-Like Organelle of *Trimastix pyriformis*

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Preaxostyla are the third group of protists, besides parabasalids and fornicates, constituting the clade Metamonada. Unlike their medically and economically important relatives, the cell biology of Preaxostyla is still understudied, which has been effectively preventing us from inferring the ancestral state of the metamonad cell. Even more importantly, oxymonads, the crown-group of Preaxostyla, are very serious candidates for being secondarily amitochondrial. In order to understand the reductive evolution of their mitochondrion, we study the closest known taxon to oxymonads, genus *Trimastix*, particularly *T. pyriformis*. We have performed multiple experiments to learn about cellular localization of various proteins putatively associated with the mitochondrion. Results showing that *T. pyriformis* does indeed possess a mitochondrion have been already presented and published. Recently, we have gathered experimental evidence also for the presence of all paralogues of [FeFe]-hydrogenase, maturases of [FeFe]-hydrogenase, MCF carrier and mitochondrial processing peptidase within the organelle. On the other hand, our experimental data do not support organellar localization of pyruvate:ferredoxin oxidoreductase, aconitase, malic enzyme, ornithine transcarbamoylase and serine hydroxymethyl transferase. These results are consistent with hypothesis that *T. pyriformis* contains a reduced mitochondrion functionally similar to a hydrogenosome.

Creneis carolina gen. et sp. nov. – Novel Anaerobic Lineage of Heterolobosea with Unique Cell Structure and Life Cycle

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We report the light-microscopic morphology and ultrastructure of a novel free-living, heterotrophic protist, *Creneis carolina* gen. et sp. nov., isolated from marine anoxic sediments. *C. carolina* is a heterotrophic, obligatory anaerobic amoeboid flagellate and superficially resembles *Mastigamoeba* (Amoebozoa: Archamoebae) or *Breviata* (Obazoa: Breviatea) by possessing a single anterior flagellum closely associated with the nucleus and by the anaerobic lifestyle. However, its life cycle contains multiflagellate cells with unusual morphology. The structure of the mastigont of *C. carolina* is unique and not readily comparable with any eukaryotic group. Unexpectedly, phylogenetic analyses of SSU rDNA and concatenate of α- and β-tubulin genes with SSU rDNA convincingly showed that *C. carolina* is a member of Heterolobosea and specifically belongs to Tetramitia. We present also multigene phylogenetic analysis that indicates an independent origin of the anaerobiosis in Psalteriomonadidae Cavalier-Smith, 1993 and Creneidae fam. nov.

Queuosine: The Role of an Essential tRNA Modification in Parasitic Protist *Trypanosoma brucei*

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A general feature of tRNAs is a high number of modified nucleotides that are introduced post-trancriptionally. Queuosine (Q) is one of the most complex tRNA modifications. Despite its omnipresence in bacteria and eukaryotes, a role of Q-tRNA modification is not clear. A comprehensive analysis of tRNA guanine transglycosylase (TGT), the enzyme responsible for Q-tRNA formation in eukaryotes, is crucial for our general understanding of this tRNA modification. Yeast *Saccharomyces cerevisiae*, the universal model for tRNA modification studies, cannot be used in this very particular case because it lacks the Q-modified tRNAs due to the absence of the TGT gene in its genome. This calls for employing of another suitable model system, *Trypanosoma brucei* being our first choice. This is further justified by the fact that trypanosomes cause considerable human and animal mortality; hence, the understanding of the Q-tRNA modification is likely to contribute to the understanding of a fundamental molecular process but also to the development of new drugs against diseases caused by trypanosoma and related flagellates.

Exploring the Ras Superfamily of GTPases in Protist Genomes

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GTPases of the Ras superfamily constitute a large group of proteins involved in many eukaryote-specific cellular processes. Since protists represent a major portion of the eukaryote diversity and may exhibit lineage-specific elaborations as well as ancestral characters lost in conventional model species, their studies are crucial for proper understanding of the evolution of both the Ras superfamily and the eukaryotic cell itself. We will present some of our findings stemming from analyses of the Ras superfamily in several protist groups. First, we examined genomes of 18 microsporidian species and found 16 different Ras superfamily genes, most of them represented in each species; for the first time we could identify a highly divergent microsporidian candidate for the beta subunit of the signal recognition particle receptor. Our results attest to a highly divergent yet stable core of genes in microsporidia. Second, we annotated Ras superfamily GTPases in the genome of the oxymonad *Monocercomonoides* sp., which revealed 107 genes and 6 putative pseudogenes. Families of multiple lineage-specific paralogs were found for many genes, some of them bearing signs of pseudogenezation, pointing to a previously unsuspected importance of gene turnover in the oxymonad lineage. Third, the genome of the enigmatic excavate *Malawimonas jakobiformis* includes nearly 100 different Ras superfamily GTPases, each represented by two nearly identical allelic variants (or recently emerged paralogs). A salient feature of this GTPase complement is retention of a high number of sporadically occurring yet apparently ancestral paralogs. We believe that further investigations of malawimonads may reveal a lot about last common eukaryote common ancestor.

Canine Antibodies against Recombinant Salivary Proteins of *Phlebotomus perniciosus*

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Leishmaniasis is caused by protozoan of genus *Leishmania* and transmitted by the bites of Phlebotominae sand flies. During blood feeding sand fly females inject saliva into the host. Repeatedly bitten hosts response to the saliva by production of the species-specific antibodies that persist for weeks and are known to positively correlate with the number of the sand fly bites. These facts make the anti-saliva antibodies an interesting research tool in epidemiological studies. Unfortunately, the availability of the saliva for the large scale studies has many technical limitations which might be overcome by using the recombinant salivary proteins (rSPs). We focused on the antigenic properties of rSPs of *Phlebotomus perniciosus*, important vector of visceral leishmaniasis in Europe. We tested the feasibility of *P. perniciosus* yellow protein (ABA43050.1) and apyrase (ABB00906.1) as the markers of sand fly exposure. Sera of Italian dogs originating from the longitudinal and cross-sectional studies on seroprevalence of canine leishmaniasis were tested for IgG antibodies against rSPs by ELISA and the antigenicity of the rSPs was compared with the whole *P. perniciosus* saliva. We found the strong positive correlation between saliva and the rSPs, thus the rSPs can be used as the antigens for field epidemiological studies.

The Phylogenetic Evidence for the Paraphyly of the Genus *Mastigella* (Amoebozoa: Archamoebae)

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The group of Archamoebae comprises free-living and endobiotic amoeboflagellates and amoebae that live in anoxic or microoxic habitats. The group splits into four major lineages, Rhizomastixidae, Entamoebidae, Pelomyxidae, and Mastigamoebidae, whose interrelationships have not been completely resolved. Up to now, there are several key members of the archamoebae (e. g. *Mastigella*) from which sequence data are still missing. We have successfully isolated and cultured several strains of genera *Mastigella* and *Pelomyxa*. We determined actin gene sequences of most strains and SSU rRNA gene sequences of two *Mastigella* strains. Phylogenetic analyses showed that *Pelomyxa* formed an internal branch of *Mastigella*. In addition, both genera shared several morphologic features that also suggest their common evolutionary history.

Searching for Appropriate Amino-Acid Substitution Matrices to Understand Secondary Endosymbiosis of Plastid

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Acquiring the plastid from cyanobacteria by plant ancestor was unique event in the evolution of eukaryotes. This plastid enabled development of plants into various forms from brown algae to sequoias. Moreover, this very same plastid together with the host cell was then secondarily acquired by members of other eukaryotic clades. We know that plastid in Euglenozoa originates in green plants and than plastid in members of cryptophytes, haptophytes, stramenopiles and alveolates is derived from red plants. Because secondary endosymbiosis in Euglenozoa is relatively new, we can more easily track the evolution by phylogenetics using usual set of substitution matrices. In contrary, more ancient, red secondary endosymbiosis was source of many scientific discussions and so far there is no agreement in, if the plastid was acquired several times independently or just once by common ancestor of all these lineages. Here we present substitution matrices which we believe are more appropriate to track the evolution of this extremely divergent group of eukaryotes.

Revealing High Diversity of Anaerobic Ciliates

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It is a well-known fact that several lineages of ciliates have independently adapted to anoxic environments. So far, anaerobes have been found in six lineages – Armophorea, Litostomatea, Plagiopylea, Oligohymenophorea, Prostomatea, and Cariacotrichea. Although endobiotic anaerobic ciliates (e.g. *Balantidium*, *Nyctotherus*, and rumen ciliates) are relatively well studied, the free-living ones almost lack the attention of protistologists. To assess the diversity of free-living anaerobic ciliates, we have isolated and cultivated more than 50 strains from fresh water, brackish, and marine anoxic sediments worldwide. We determined their SSU rDNA sequences and studied light-microscopic morphology of some of them. Several novel clades of metopids were found, one of them with distribution limited to South America whereas at least some of others are cosmopolites. In addition, we cultivate anaerobic plagiopylids and scuticociliates, and phylogenetic analysis suggests that some of them represent novel lineages on the genus level. Importantly, two new deep lineages of marine ciliates were discovered. The first one is related to Lamellicorticata, Spirotrichea, and Cariacotrichea and may form a new class. The second one clusters within Prostomatea and Plagiopylea. From our further observations, we conclude that marine anaerobic sediments harbor a high diversity of undescribed ciliates.

Silencing of Heme Pathway Genes in Model Diatom *Phaeodactylum tricornutum*

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Diatoms' evolution involving secondary endosymbiosis has resulted in complex metabolism. Thus in some cases multiple variants of a gene are present in the genome, each of them having different origin. This is also the case for some heme pathway enzymes-encoding genes. Heme biosynthesis represents one of the most essential pathways in living organisms. This work aims to elucidate the functions of individual isoenzymes in this mosaic pathway. We have revised genes of heme pathway using available genomic and transcriptomic data and RACE. We have revealed that there are three different gene variants coding for uroporphyrinogen decarboxylase and coproporphyrinogen oxidase present in the genome, each of them with different origin. We have focused on these two triplets of enzymes and we have prepared constructs containing anti-sense sequences of all isoenzymes. Both single and double knockdown lines were prepared by introducing these silencing vectors into *P. tricornutum* cells by microparticle bombardment. Maintenance of three different gene variants during evolution presumably means that they will somehow vary in their functions. Therefore we want to investigated phenotypes of knockdowns in different conditions and to assessed expression of remaining two genes in single knockdown lines to determine whether silencing of one gene enhances expression of the remaining variants. This will help to evaluate to what extent do functions of individual enzymes overlap and to review if the corresponding gene products mutually complement their functions.
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The Fat Cousin of Apicomplexans: From Fat to Genes

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Chromera velia and *Vitrella brassicaformis* are photoautotrophic alveolates recently found in Australian corals. The ultrastructure, photosynthetic pigment profiles and phylogenetic analyses together with some other molecular characters revealed that chromerids are the closest known photosynthetic relative of apicomplexan parasites. However, the intensive studies of *C. velia* physiology and metabolomics exposed its significantly high ability, in comparison with *V. brassicaformis*, to produce fatty acids. High performance liquid chromatography coupled with mass spectrometry shows that the concentration of storage triacylglyceroles is approximately one hundred times higher than abundance of structural galactolipids. Additionally, it has relatively rapid growth rate and large cells with the high tolerance to shear force. Furthermore, astonishing tolerance to the broad range of cultivation conditions including temperature and salinity has been observed. The genomic approach using homology search identifies a set of genes responsible for basic fatty acid synthesis and demonstrates similar genetic characteristic in lipid pathways of common oleaginous algae such as *Nannochloropsis oculata*. The mass chromatographic results indicate superior abundance of storage triacylglyceroles in lipid extracts of *C. velia* in comparison to *N. oculata*. All data confirm, that *C. velia* is prospective oleaginous alga.

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Paratrypanosoma – a Novel Early-Branching Trypanosomatid

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The kinetoplastids are widespread and important unicellular eukaryotes, many of which are devastating parasites. In the gut of *Culex pipiens*, we have discovered a new insect trypanosomatid named *Paratrypanosoma confusum* which GAPDH and SSU rRNA-based phylogenetic analyses place into a separate branch between free-living *Bodo saltans* and parasitic *Trypanosoma* species. From draft genome sequence data we have identified 114 protein genes shared between the new isolate, 15 trypanosomatid species, *B. saltans*, and *Naegleria gruberi*, as well as 129 protein genes shared with the early-branching kinetoplastid *Perkinsela*. Protein-by-protein phylogenies together with analysis of concatenated alignments show that the new isolate branches at the base of the family Trypanosomatidae. *P. confusum* forms two different life stages in axenic cell culture; a "swimmer" (promastigote-like) stage and sessile stage with entirely different and surprising morphology. This newly identified insect flagellate represents a missing link between free-living bodonids and obligatory parasitic trypanosomatids, further analysis of which should provide insight into the emergence of parasitism in this medically important group.

Adaptive Radiation and Evolution within the Myxozoa

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The evolutionary trajectories of endoparasites are greatly influenced by interactions with their hosts. Thus, endoparasite diversification can be expected to reflect processes such as co-speciation and host switching along with the evolution of host specificity. In addition, parasites must achieve transmission to new hosts, a process that typically requires persistence outside their hosts. Finally, parasites with complex life cycles, such as myxozoans, require the ability to exploit distinctly different hosts. Drivers of diversification and evolution within the Myxozoa will therefore include biotic factors associated with host exploitation and abiotic factors associated with the environment of their free-living spores. We expanded on these themes by focusing on patterns of evolution within the Myxozoa. We compared diversification of the malacosporeans and myxosporeans. We considered more specific adaptations displayed particularly by the myxosporeans that have enabled this group to exploit a variety of hosts and tissues and to survive in the environment when switching hosts. We also include the evolution of parasitism of myxozoans, including discussion of the first myxozoan hosts and how myxozoan life cycles may have expanded to incorporate intermediate hosts.

Updating the Algal Phylogeny with New Plastid Genome Sequences: Did the Plastids in Alveolates Emerge through an Endosymbiosis of an Ochrophyte Alga?

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Algae with secondary plastids of a rhodophyte origin constitute a diverse and enormously important group of eukaryotes, yet their evolutionary history remains a controversial topic. To address some of these issues we sequenced and analysed plastid genomes of two ochrophyte (heterokontophyte) algae, *Ochromonas* sp. CCMP1393 (Chrysophyceae) and *Trachydiscus minutus* (Eustigmatophyceae). The two new genomes provided strong support for the existence of the clade Limnista (constituted by chrysophytes and eustigmatophytes) and revealed an unexpectedly elevated substitution rate in this lineage. The expanded sampling of plastid genome sequences prompted us to revisit the phylogenetic relationship between the plastids of ochrophytes and alveolates, the latter represented by their least divergent member known so far, *Vitrella brassicaformis*. Phylogenomic analyses using site-homogeneous models placed *Vitrella* with maximal support within the Limnista group. Analyses using site-heterogeneous CAT models strongly supported the monophyly of Limnista to the exclusion of *Vitrella*, but still showed *Vitrella* as a lineage of the ochrophyte radiation rather than as a representative of a separate lineage of alveolates that is thought to had diverged hundreds of millions of years before the existence of the last ochrophyte common ancestor. We suggest that the plastid in *Vitrella* (and perhaps plastids of other alveolates including, e.g., apicomplexans) may have originated by an endosymbiosis of an early ochrophyte, or even a Limnista-related, alga in an alveolate host.

Comparison of *Eimeria* Species in Native and Introduced Squirrels

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The red squirrel (*Sciurus vulgaris*) is a species common throughout Eurasia. In Great Britain, Ireland and Italy, their numbers have decreased drastically in recent years, also in association with the introduction of alien tree squirrels, mainly the eastern grey squirrel (*Sciurus carolinensis*) from North America. Our study was focused on the squirrel populations in Italy to compare gastrointestinal parasites in *S. vulgaris*, *S. carolinensis* and also *Callosciurus erythraeus*, the Pallas's squirrel, originated from South-eastern Asia. *Eimeria* species were dominant coproscopical findings in all three squirrel species. Morphological determination of the eimerian oocysts revealed three morphotypes (I, II, III) in *S. vulgaris*, two (II, III) in *S. carolinensis* and slight infections with morphotype II in *C. erythraeus*. Similarities of morphotype II and III required deeper determination based on genetic analyses. So far, the sequences obtained do not support the hypothetical exchange of *Eimeria* spp. Comparison of the internal transcribed spacers (ITS1 and 2) of nuclear ribosomal DNA and partial cytochrome c oxidase subunit 1 (cox 1) gene of mtDNA of the morphotype II showed different *Eimeria* haplotypes/species, both in allopatric and sympatric population of native red and introduced grey squirrels.
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Extensive Diversity of Free-living Preaxostyla

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Preaxostyla (Excavata: Metamonada) is one of the least studied eukaryotic lineages. All Preaxostyla are anaerobic and can be divided into free-living, morphologically relatively uniform *Trimastix* (3 species), and endobiotic, morphologically diverse oxymonads (more than 100 species living mostly in termites). In order to examine the diversity of free-living Preaxostyla more deeply, we isolated and cultured 35 freshwater and two marine strains morphologically consistent with *Trimastix*, and determined their SSU rDNA sequences. Results of phylogenetic analyses showed that the strains are extensively diversified. The two marine strains form either a clade or two paraphyletic basal lineages of Preaxostyla. The freshwater strains constitute several lineages that form a robust clade with oxymonads. Although the precise phylogenetic position of the oxymonads is not completely resolved, it seems that they are closely related to a novel free-living clade represented by four strains. The strains were examined also by means of light microscopy. Morphology of both marine and several freshwater strains roughly corresponds to *Trimastix marina* suggesting that this species is polyphyletic. Most freshwater strains are similar to *T. pyriformis*, but are phylogenetically far too diverse to represent a single species. Three strains forming a clade within the *T. pyriformis* complex possess tiny cells in comparison with the other strains. In conclusion, the phylogenetic diversity of free-living Preaxostyla is much higher than that of the endobiotic oxymonads, despite they are much less specious and morphologically diverse.

Coupled Scientific Methodology for Metabolic Pathway Investigation: The Fat Story

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Genomic, mass spectrometric, microscopy and cultivation experiments together provide sufficient data for metabolic pathway reconstruction. The closest photosynthetic alveolates *Chromera velia* and *Vitrella brassicaformis* were treated by mentioned methods to reconstruct the lipid pathway. BLAST homology search, high performance liquid chromatography coupled with gas chromatography mass spectrometry, staining the lipidic droplets with BODIPY®marker and followed by confocal microscopy and cultivation with specific lipid enzymes blocators were particularly used. The genomic approach revealed set of genes responsible for basic fatty acid synthesis and concluded that both algae have very similar genetic equipment for fatty acid and lipid synthesis. However the mass spectrometric approach significant differences in lipid distribution. It was found that *C. velia* contains approximately 100 times more storage lipids than *Vitrella brassicaformis*. These results were corroborated by BODIPY®marker staining. The importance of lipid metabolism in *C. velia* was illustrated by selective inhibitor of fatty acid synthesis II – triclosan. The incubation shows that triclosan administration is lethal for *C. velia* therefore no other source of fatty acids than FAS II is present. Coupled genomic and mass spectrometry approaches point to difference in transcription regulation in particular chromerid.

Genetic Evidence of Meiosis in *Monocercomonoides*

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Meiosis is a specific cell division essential for the sexual cycle. While it is almost certain than meiosis have evolved from mitosis, when this process occurred is a subject of debate. Meiosis has been observed in plants, animals, fungi, and numerous protists. In oxymonads reports of one-step meiosis have been made for Saccinobaculus, but so far no genetic approach has been made to identify meiosis in this group of protists. We surveyed the ongoing *Monocercomonoides* genomic project data for a previously described set of 29 conserved meiotic genes and additional 6 genes which were recently proven to be involved in meiosis and are conserved among eukaryotes. From these genes, 9 are meiosis-specific, being expressed just during meiosis. We were able to find orthologs for 29 of 35 genes including 8 of 9 genes are meiosis specific in model organism. These results could indicate that *Monocercomonoides* is capable of meiosis and sexual reproduction, even if so far no meiosis has been observed in this organism.

Iron-Sulphur Cluster Assembly in *Monocercomonoides*

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Oxymonads are group of anaerobic or microaerophilic protists living in guts of insects and vertebrates (especially reptiles and mammals). Today they are considered to be the last large group of eukaryotes without confirmed mitochondrion-like organelle. In their closest free-living relatives – *Trimastix* – were found mitochondrion-like organelles resembling hydrogenosomes. In the transcriptome and genomic projects of *Monocercomonoides* and *Trimastix* we were not able to identify any enzymes of ISC iron-sulfur cluster assembly machinery, which is ubiquitous in eukaryotic organisms. Instead we found subunits of SUF machinery, Suf B, C, and S, which is in eukaryotes known only in plastids and in *Blastocystis hominis*, where it is localized in cytosol. In the phylogenetic tree of concatenated Suf B, C, and S *Monocercomonoides* and *Trimastix* formed a single clade that indicates that transcripts are eukaryotic in origin and that they are not bacterial contamination. This was also proven by fluorescent in situ hybridization of subunits of SUF system in *Monocercomonoides*. Localization of these enzymes in the cell of *Monocercomonoides* is still unknown but their presence and lack of ISC pathway enzymes suggests that *Monocercomonoides* could completely lost mitochondrion because its main function was replaced by different pathway.

Deciphering Mode of Action of JK11 and TG142 Compounds in the Mitochondrion of *T. brucei*

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Causative agents of tropical neglected diseases bring enormous disease burden to the developing countries. Importantly, chemical compounds belonging to 4-oxopiperidine-3,5-dicarboxylates strongly inhibit the *in vitro* and *in vivo* growth of medically important parasites such as *Trypanosoma brucei*, *Plasmodium falciparum*, *Leishmania* major and bacteria as Staphylococcus epidermis and Pseudomona aeruginosa. Two compounds affecting the cell viability of the insect procyclic stage (PS) and the bloodstream stage (BS) in uM concentration were chosen for a detailed characterization of their mode of action. Interestingly, the mitochondrial membrane potential (um) of the BF cells is strongly decreased upon the treatment by these chemicals. Since FoF1 ATPase is responsible for maintaining the um, the activity of this complex was tested *in vitro*. Both compounds decreased the total ATPase activity at similar level as azide and oligomycin, known inhibitors of FoF1 ATPase. Moreover the synthetic activity of this complex was also strongly affected upon the treatment suggesting that these potential chemotherapeutics affects both directions of the FoF1 ATPsynthase/ATPase activity. In vitro activity of the purified F1ATPase was unchanged upon the treatment implying that these drugs may act only on fully assembled complex. However both compounds were active against the cells lacking mitochondrial genome suggesting that the subunit a is not involved in the binding of the inhibitors. Considering the effective concentration (EC50) of these compounds and the concentration used for *in vitro* assays to exert the phenotype, these drugs get concentrated in the mitochondria or more cellular targets are involved in their cytotoxic mode of action.

Membrane Proteome of Euglenid Plastids

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Euglenophyta is a group of photosynthetic euglenids belonging to the supergroup Excavata. These organisms harbour secondary plastids which had been obtained via endosymbiosis with green alga. As a result, these plastids are surrounded by three membranes instead of two; inner two membranes are homological to primary plastid membranes, origin of the third one is uncertain. Because of the additional membrane, mechanism of transport of nuclear-encoded plastid proteins differs from that of a primary plastid. It has been observed that proteins cross the outermost membrane in vesicles but the recognition and fusion mechanism is not fully understood. Transport across the two inner membranes may be mediated by TIC and TOC translocases homologues but no component of these complexes has been discovered yet. The aim of our work is to obtain and analyze chloroplast membrane proteome of the two distantly related euglenophytes *Euglena gracilis* and *Eutreptiella gymnastica* while focusing on identifying proteins that might be involved in protein transport. We isolated chloroplast membrane fraction from *E. gracilis* and using mass spectrometry and incomplete transcriptomic data we have detected approximately 400 proteins, some of them representing obvious contamination from the mitochondrion. Only three of those proteins are significant candidates for beta-barrel transport channels, typical part of the TOC complex. The number of detected proteins will probably increase after obtaining complete transcriptome. Isolation and sequencing of membrane fraction from *E. gymnastica* is currently in progress.

Major Transitions in Trypanosome Cell Morphology Produced via Modulation of a Cytoskeletal Calpain-Like Protein

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Eukaryotic microbes have a defined size, shape and form. The kinetoplastid parasite, *Trypanosoma brucei*, has many individual life cycle stages, each having a distinct morphology and very little is known about the processes involved in these transitions. We have identified a large calpain-like protein that contains numerous GM6 repeats (ClpGM6) involved in determining *T. brucei* cell shape, size and form. ClpGM6 is a cytoskeletal protein located within the flagellum along the flagellar attachment zone (FAZ). Reduction of ClpGM6 in trypomastigote forms has no effect on cell proliferation but resulting cells possess long free flagella and a shorter FAZ; accompanied by repositioning of the basal body, the kinetoplast, Golgi and flagellar pocket, reflecting an epimastigote-like morphology. Our observations highlight the role of the FAZ as the key structure for determining cell morphology in kinetoplastids. Dramatic changes of morphology can be achieved by the modulation of expression of a single gene, which affects FAZ length but not its integrity.

Localization of the Enzyme Uroporphyrinogen III synthase (Heme Pathway) in *Phaeodactylum tricornutum*

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Uroporphyrinogen III synthase (UROS) belongs to the enzymes of the heme pathway and is responsible for conversion of hydroxymethyl bilane into uroporphyrinogen III. Although the biosynthesis of heme seems to be essential in living cells, the subcellular localization of UROS and other heme pathway enzymes in *P. tricornutum* has not been experimentally determined yet. We obtained the UROS gene sequence by analyzing available genomic and RNA-seq data. We verified completeness of the 5' end of the transcript by rapid amplification of cDNA 5' ends (5' RACE), and we consequently predicted the signal peptide and transit peptide at the N-terminus of the deduced protein sequence. However, the subcellular target of the UROS is not clear. Although *in silico* prediction suggests the enzyme localization in plastid, our finding of tryptophan in the UROS signal peptide doubts such localization. Therefore we decided to validate the location of UROS by transfection of *P. tricornutum* by the N-terminal domain of the gene fused to yellow fluorescent protein (YFP). The visualization of UROS expression in the diatom cell is planned via confocal microscopy.

Does Mitochondria Reflect Diversity of Trypanosomatid Parasites?

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Trypanosomatids is a diverse group of parasites belonging to Euglenozoa-Kinetoplastida. Recently, we biochemically characterized and compared mitochondrial activities of the four most notorious members of trypanosomatids, namely *Crithidia fasciculata*, *Leishmania tarentolae*, *Phytomonas serpens* and procyclic stage of *Trypanosoma brucei*. In this follow-up study, we extended the sampling and included five more monoxenous species (*Blechomonas* sp., *Herpetomonas muscarum*, *Herpetomonas samuelpessoai*, *Leptomonas pyrrhocoris* and *Sergeia podlipaevi*) in which we performed the detection of subunits of respiratory chain complexes and measurements of their activities as well as evaluation of overall respiration. We detected differences in measured parameters of mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase and complexes II-V. Trypanosomal alternative oxidase known as TAO was detected only in *T. brucei* and *P. serpens*, a feature suggesting independent acquisition of the gene during the course of evolution. Also, all the monoxenous parasites – the five species and *C. fasciculata* – were difficult to stain using mitochondrial membrane potential-dependent probes. We speculate that this feature might be connected with overall surface charge likely connected with a specific life style of the parasites.

The Adaptability of the *T. brucei* Bloodstream Mitochondria upon the Indirect Knockdown of an Essential Mitochondrially Encoded Protein

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The infective bloodstream stage of *Trypanosoma brucei* possesses a single mitochondrion that lacks cytochrome-containing respiratory complexes III and IV and thus employs the F_oF_1-ATPase to hydrolyze matrix ATP to maintain the essential membrane potential. Due to this reduced organellar function, it is currently presumed that out of the 18 mitochondrially (mt) encoded proteins, only A6 (a subunit of F_oF_1-ATPase) and possibly RPS12 (a ribosomal subunit) are essential in BF trypanosomes. We have identified the hypothetical mt matrix protein TbMT420 as a homologue to a yeast methyltransferase (Mtq1) that methylates a conserved GGQ motif of the peptide release factor 1 (Mrf1). Therefore, we generated viable knock-out cell lines to explore these components of the mt translation machinery in *T. brucei*. The ΔTbMrf1 cell line possesses a decreased mt membrane potential, an increased sensitivity to F_oF_1 inhibitors, and a decreased abundance of the F_oF_1-ATPase complex. However, the Î"TbMT420 cells exhibit milder phenotypes, suggesting that the methylation is not as influential as the loss of TbMrf1. We conclude that the loss of TbMrf1 leads to significantly less functional A6 and possibly RPS12, but enough F_oF_1-ATPase is still assembled to maintain a sufficient membrane potential. Interestingly, over time the phenotypes dissipate, possibly as the cells try to compensate for the loss of A6. We are currently exploring this possibility and determining if the ΔTbMrf1 cell line is still infectious in the animal host.

Oxidative Phosphorylation of *Euglena gracilis*

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Euglena gracilis is a non-parasitic secondary green alga belonging to Class Euglenoidea and (together with Kinetoplastea and Diplonemea) to Phylum Euglenozoa. In presented work we studied oxidative phosphorylation in *E. gracilis* strain Z and its stable bleached mutant WgmZOflL both cultivated either on the light or in the dark. Lysates of isolated mitochondria were analyzed by 2D (native vs. denaturation) PAGE stained by Coomassie blue. Position of particular enzymes were determined either by Western blot analysis of 2D gels with antibodies against subunits of different OXPHOS enzyme complexes or by histochemical detection of enzyme activities in native gels. Activity of OXPHOS enzymes were characterized also spectrophotometrically. Suitability of different substrates for respiration was determined by measurement of oxygen utilization by isolated mitochondria. Relative exploitation of lactate and succinate by different branches of respiratory chain (RC) were studied by using different specific inhibitors of RC enzymes. Cytochrome C reductase (complex III of RC) was isolated by chromatography and its enzyme and spectral characteristics were given.

Trichomonads in Cats and Dogs

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Four trichomonad species has been described in dogs and cats: *Tetratrichomonas canistomae* and *Tetratrichomonas felistomae* that are commensals of the host oral cavity; *Pentatrichomonas hominis*, a commensal of intestinal tract that could be found also in other mammals; and pathogenic *Tritrichomonas foetus* that causes, in addition to cattle infection, feline intestinal trichomonosis. Although, these trichomonads are probably of cosmopolitan distribution we have no information about their presence in Czech Republic. The aim of this study was to get information about presence of trichomonads of oral cavity and intestine in dogs and cats in the Czech Republic, and associations between the presence of trichomonads and potential host management, health and demographic risk factors. Cultivation and nested PCR were used to determine the presence of trichomonads. The prevalence of trichomonads from oral cavity was 45 % (n = 126) among dogs and 19 % (n = 135) among cats. By phylogenetic analysis of ITS1-5.8rRNA-ITS2 sequences we found that both dogs and cats harbor two distinct groups of organisms. The first group (12 isolates) displayed 100 % sequence identity with *Trichomonas tenax*, human mouth commensal. The second type (34 isolates) displayed 95 % sequence identity with *T. tenax* and possibly represent a new trichomonad species. None of isolated strains belong to *Tetratrichomonas* genera. *T. foetus* was found in the intestine of 36 % of dogs (n = 11) and 26 % of cats (n = 86). Furthermore *P. hominis* was detected in 4 out of 86 cats, of which two were also positive for *T. foetus*. In dogs infected by trichomonads we observed that dogs living separately were less frequently infected with oral trichomonads in comparison when more dogs were harboured together.

Dynamics of *Giardia intestinalis* mitosomes

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Typical aerobic mitochondria are very dynamic in structure, undergoing constant cycles of fusion and fission events. By contrast, mitosomes of *Giardia intestinalis* are the simplest known mitochondria among all eukaryotes. Giardia mitosomes do not produce any ATP and their only function is the assembly of iron-sulfur clusters. So far almost nothing is known about the dynamics of the mitosomes during the cell cycle of giardia parasites and under iron-dependent metabolic stress. Using Halo Tag technology, we show that giardia mitosomes are steady organelles, which do not fuse or divide during interphase. We attempt to identify the moment of organelle division and segregation during mitosis as well as the compensation for the reduced organelle number after cytokinesis. Compared to tiny mitosomes, the endoplasmic reticulum is the most dominant membrane-bound structure in giardia. The absence of the ERMES complex, which is responsible for the interactions between mitochondria and the ER suggests the presence of an unique mode of interaction between these organelles. We are using *in vivo* enzymatic tagging by bacterial biotin ligase anchored to the outer mitosomal membrane to find if mitosomes come into contact with other compartments. Iron is an indispensable factor for the cell growth and also the pathogenesis. Iron-restricted conditions often lead to massive morphological and metabolic changes in classical mitochondria and hydrogenosomes including the remodeling of the organellar proteome. Therefore, we grew giardia cells in iron-rich and iron-deficient media and followed the impact of the iron stress on the activity of FeS cluster containing enzymes, the levels of FeS cluster biosynthetic machinery and the morphology of the mitosomes.

Putative Protein Translocase(s) in the Mitosomes of *Giardia intestinalis*

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Mitosomes are the smallest forms of mitochondria among all eukaryotes. They are devoid of most of the typical mitochondrial functions such as respiration and ATP synthesis. In addition, they seem to also lack the protein translocase of the inner membrane. By the bioinformatic searches we selected two hypothetical proteins from *Giardia intestinalis* which could represent mitosomal Tim17 family proteins. These are GL50803_10182 and GL50803_10452 proteins. While we have confirmed their mitosomal localization, our current goal is to isolate these two proteins *in vitro* and analyze their capability to form the membrane channels.

Transcriptome Analysis Reveals a Highly Unusual Non-Photosynthetic Plastid in the Euglenoid Flagellate *Euglena longa*

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Euglenophyte algae are a subgroup of euglenoid flagellates (phylum Euglenozoa) that emerged by incorporating a secondary plastid from a green alga. Interestingly, some euglenophytes have lost the photosynthetic ability while retaining a colourless plastid. The best-known example is *Euglena longa* (previously called *Astasia longa*), which is closely related to the model euglenophyte *Euglena gracilis*. Although plastid genome of *E. longa* was sequenced many years ago, the actual metabolic capacity and biological function of the *E. longa* plastid could not be deduced due to the lack of nuclear genome sequence data. We recently sequenced the transcriptome of *E. longa* using the Illumina technology. Analyses of the transcriptome sequences did not reveal any signs of the light-phase photosynthetic machinery or of other biosynthetic pathways typical for plastids (heme biosynthesis, MEP isoprenoid pathway, fatty acid II pathway), except almost all enzymes of the Calvin cycle (including both large and small subunit of RuBisCO), raising an interesting question about the function of this putative pathway in a non-photosynthetic plastid. Furthermore, we found genes involved in the synthesis of galactolipids (both MGDG and DGDG), which are normally associated with thylakoid membranes. Thus, the *E. longa* plastid seems to be radically different from the apicoplast, a model non-photosynthetic plastid of apicomplexan parasites.

Hunting for New Eustigmatophytes: Molecular Characterization of *Chlorobotrys polychloris*

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Eustigmatophyceae is a separate lineage of ochrophyte algae that was considered unattractive and unimportant until recently, but nowadays the eustigmatophyte genus *Nannochloropsis* belongs among the most extensively studied groups of microalgae due to its biotechnological potential. However, there is much more to Eustigmatophyceae than *Nannochloropsis* and the phylogenetic breadth of this group has grown ever since its recognition as a distinct taxon in 1970's. Apart description of new taxa, many potential eustigmatophytes probably remain to be identified by reinvestigating species considered by traditional taxonomy as members of a different class, Xanthophyceae. We isolated one such alga, provisionally identified as *Chlorobotrys polychloris*, from the "Černohorské rašeliniště" peat-bog (The Krkonoše Mountains National Park) and establish its slowly-growing cultures in sterilized peat-bog water as a medium. This allowed us to obtain by PCR the first molecular data from this species, specifically a partial sequence of the nuclear 18S rRNA gene. The sequence confirmed that our alga belongs to eustigmatophytes and probably represents a new lineage in this group. The updated results of this work-in-progress will be presented.

Optimizing the Inhibition of a Uniquely Composed *Trypanosoma brucei* F_1-ATPase

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The transition of the parasitic *Trypanosoma brucei* between its invertebrate and vertebrate hosts is associated with substantial bioenergetic pathway changes. While substrate and oxidative phosphorylation (OXPHOS) provide the main source of ATP in the procyclic stage (PS), increased glycolysis of abundant glucose in the bloodstream form (BS) compensates for the absence of OXPHOS - requiring the F_1F_o-ATPase to maintain the mitochondrial membrane potential at the expense of ATP. A widespread natural protein inhibitor of F_1F_o-ATPase activity (TbIF1) is expressed in PS, while its ectopic expression is lethal in BS. To characterize TbIF1 inhibition, we isolated the F1-ATPase from PS by two-step chromatography. Besides the conserved eukaryotic components ($\alpha_3\beta_3\gamma\delta\epsilon$), the complex contains an additional trypanosomatid-specific protein, p18. The previously reported α subunit cleavage was confirmed and modeled to a region presumed to form a loop between the crown and NTP-binding domains, a unique feature of F_1-ATPase in Kinetoplastids. Furthermore, several recombinant TbIF1 mutants were characterized to determine their dissociation constants and oligomerization properties. While the C-terminal deletion of TbIF1 prevents homodimerization, it does not disrupt the pH sensitivity as it does in bovine IF1. Importantly, TbIF1 cannot inhibit bovine F_1-ATPase and *vice versa*, strengthening the differences between the parasite and mammals. The established purification of a uniquely composed F_1-ATPase is suitable for structure resolution by X-ray crystallography. Given the non-conventional function of F_1-ATPase in BS and the F_1-TbIF1 binding data, we propose that the structure could be exploited to design specific inhibitors for potential use in therapeutics.

Bluff with the BLUF

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Cells utilize sophisticated systems to sense their environment. The bacterial BLUF (sensor of Blue Light using FAD) is an FAD-binding domain that senses blue light and transmits the signal to other signaling domains. We discovered a novel conserved eukaryotic protein Ebluf (Eukaryotic BLUF) that is distantly related to the bacterial BLUF proteins. Interestingly, this protein is found only in organisms with flagellated cells, indicating that this protein might be involved in the function of flagella, centrioles or subsequently the cell division.

Partners of Conference

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