# 45<sup>th</sup> Jírovec's Protozoological Days

**Conference** Proceedings



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### Foreword

#### Dear protistologists, parasitologists, molecular kinetoplastidologists, all other -logists; friends!

During several past years, Protodny conference gradually transformed into a meeting with ever growing international attendance and its impact on protistology (in a broad sense) here in Czech Rep. (and beyond!) has increased accordingly. You will feel the impact yourselves while you're there! This year, we will hear more than 40 speakers present their research. It is a big number, actually. Circa 20 others will bring a poster. Twenty more will not present, only listen and watch in awe.

This year, we will meet in Dubovice village ("Váňův statek", N49° 26' 2.51'', E15° 10' 40.06") near Pelhřimov. On Wednesday, we will go to Červená Řečice (approx. 10 km) to see the castle there through. The castle nearly become a ruin, but thanks to a local activity is turning back to a better shape. (Not sure how it will look after our visit, though.) We will have a snack there, too. Don't be afraid of the way back, we will go by bus.

I look forward to see you in Dubovice! I hope you will like Protodny, make new connections and learn a lot! I want to thank Petr Soukal and the partners of the conference (a.k.a. sponsors) – go and buy something from them, they deserve it!

Enjoy the conference and have fun!

Martin Kostka

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## **Program Schedule**

	Monday May 11, 2015
16:00	Registration
18:00	Dinner

Tuesday May 12, 2015			
8:00	Breakfast		
	Molecular Biology of Protozoa		
9:00	Petr Soukal, Štěpánka Hrdá, Anna Karnkowska, Miluše Hroudová, Čestmír Vlček & Vladimír Hampl: Gene Transfer Accompanying the Secondary Endosymbiosis of Euglenid Plastid		
9:20	Anna Vanclová, Anna Karnkowska, Naoji Yubuki & Vladimír Hampl: In silico Evidence for Tic21 and Tic32 Subunits of TIC Translocase Complex in Euglenids		
9:40	$\underline{\mathrm{Erik}\ \mathrm{Birčák}}$ & Juraj Krajčovič: Expansion of Meiotic Genes in Euglenozoa		
10:00	Zdeněk Paris, Alan Kessler, Helmut Stanzl, Mary Anne T. Rubio &Juan D. Alfonzo: Peculiarities of Queuosine Biosynthesis in Trypanosomes		
10:20	Coffee break		
10:40	Vojtěch David, Pavel Flegontov, Evgeny Gerasimov, Goro Tanifuji, Hassan Hashimi, Maria D. Logacheva, Shinichiro, Maruyama, Naoko T. Onodera, Michael, W Gray, John M. Archibald & Julius Lukeš: Alterna- tive U-indel Editing in Kinetoplastid Endosymbiont Perkinsela		
11:00	Mary Anne T. Rubio, Kirk W. Gaston, Ian M.C. Fleming, Zdeněk Paris, Katherine M. Anderson, Patrick A. Limbach Rievesch, F. Nina Papa- vasiliou & Juan D. Alfonzo: Interdependence of tRNA Editing and Methylation at a Single Site: Keeping a Mutagenic Enzyme in Check		
11:20	Zdeněk Verner, Mary Anne Rubio, Juan D. Alfonso, Ivan Hrdý & Jan Tachezy: <b>Hydrogenosomes of</b> <i>Trichomonas vaginalis</i> and <b>tRNA</b>		
11:40	Vojtěch Vacek, Anna Karnkowska, Sebastian Treitli, Lukáš Novák, Zuzana Zubáčová & Vladimír Hampl: Iron Sulphur Cluster Assembly in Monocercomonoides		
12:00	Sponzor – SeqMe		
12:10	Lunch		

Veterinary and Human Protozoology		
13:10	Anna Mynářová, Ivona Foitová, Martin Kváč, Dana Květoňová, Michael Rost, Helen Morrogh-Bernard, Wisnu Nurcahyo, Cathleen Nguyen & Bo- humil Sak: Do Cryptosporidium spp., Encephalitozoon spp., Ente- rocytozoon bieneusi and Giardia spp. Pose Any Risk to Orangu- tans?	
13:30	Jitka Prediger, Bohumil Sak, Pavel Prediger & Martin Kváč: Diversity of <i>Cryptosporidium</i> of Wild Rodents Genus <i>Rattus</i> in the Czech Republic, Slovakia and Kenya	
13:50	Veronika Prantlová, Bohumil Sak, Dana Květoňová, John McEvoy, Ynés Ortega, Tomáš Albrecht, Jaroslav Piálek, Michael Rost & Martin Kváč: Multilocus Genotyping of Cryptosporidium in Two European House Mice (Mus musculus musculus and Mus m. domesticus): Preliminary Results	
14:10	<u>Marie Jalovecká</u> , Jiří Ťápal, Laurence Malandrin, Ondřej Hajdušek & Petr Kopáček: Implementation of the <i>Babesia microti</i> Transmis- sion Model: an Essential Tool to Study <i>Babesia</i> -tick molecular interactions	
14:30	Sponzor – Life Technologies	
14:40	Coffee break	
15:00	<u>Šárka Čondlová</u> , Michalea Horčičková, Martin Kváč, Bohumil Sak & Dana Květoňová: <b>Diversity</b> , <b>Phylogeny and Biology of</b> <i>Cryptosporidium</i> <b>spp. Infecting Rodents of Genus</b> <i>Apodemus</i>	
15:20	Michaela Kotková, Bohumil Sak & Martin Kváč: Latent Encephalito- zoon cuniculi Infection is Reactivated after Factitious Immuno- deficiency in Mice	
15:40	Barbora Mitková, Dagmar Jirsová, Andrei D. Mihalca, Hans-Peter Fue- hrer, Georg Duscher, Jana Juránková, Lucia Frgelecová, Moneeb A. Qa- blan & David Modrý: <i>Hepatozoon</i> spp. in Carnivores from Czech Republic with Focus on Canidae	
16:00	Pavla Wagnerová, Abd Elkarim Laatamna, Bohumil Sak & Martin Kváč: Equine Cryptosporidiosis and Microsporidiosis: Minireview	
16:20	Poster Session	
18:00	Dinner	
19:00	Demonstration of Protist	
20:00	Meeting of Protistology Section of ČPS	

Wednesday May 13, 2015			
7:30	Breakfast		
	Myxozoans		
8:30	Jiří Kyslík, Ashlie Hartigan, Astrid Holzer & Ivan Fiala: <b>New Markers</b> for Resolving Myxozoan Phylogenetic Relationships		
8:50	<u>Tereza Tomková</u> , Alena Kodádková, Pavla Sojková, Cecile Reed & Ivan Fiala: <b>The Morphological and Molecular Characterisation of</b> <i>Ce-</i> <i>ratomyxa</i> <b>Species from Clinid Fish from the Tip of Africa</b>		
9:10	Astrid S. Holzer, Ashlie Hartigan, Sneha Patra & Edit Eszterbauer: Highly Motile and Proliferating Quickly in Carp Blood: Is the Myxozoan Sphaerospora molnari an emerging pathogen?		
	General Protistology		
9:30	Lukáš Novák, Zuzana Zubáčová, Anna Karnkowska, Martin Kolisko, Mi- luše Hroudová, Courtney W. Stairs, Alastair G. B. Simpson, Patrick J. Ke- eling, Andrew J. Roger, Ivan Čepička & Vladimír Hampl: Evolutionary History of the Arginine Deiminase Pathway among Eukaryotes		
9:50	Emily Seward & Steve Kelly: You Are What You Eat: Metabolic Specialisation Drives Genome Evolution in Parasitic Microor- ganisms		
10:10	Coffee break		
	Biodiversity, Phylogeny and Systematics of Protozoa		
10:30	Alexei Yu. Kostygov, Anastasiia Grybchuk-Ieremenko, Eva Dobáková, Dalibor Váhala, Jan Votýpka, Julius Lukeš, Dmitri A. Maslov & Vya- cheslav Yurchenko: The Closest Monoxenous Relative of <i>Leishma- nia</i> Is an Endosymbiont-Bearing Trypanosomatid		
10:50	Naoji Yubuki, Vladimir Hampl, Brian S. Leander & Anna Karnkowska: Reconstruction of Chloroplast Proteome of the Earliest Bran- ching Phototrophic Euglenid, <i>Rapaza viridis</i> Based on Transcrip- tomic Data		
11:10	Johana Rotterová, Ludmila Nováková & Ivan Čepička: Mapping the Diversity of Metopids and Revealing New Marine Anaerobic Ciliates Hosting Symbionts		
11:30	Ivan Čepička: Diversity of Metamonads		
11:50	Lunch		
13:00	Trip to Červená Řečice		
19:00	Banquet!		

Thursday May 14, 2015			
8:00	Breakfast		
	Molecular Biology of Protozoa		
9:00	Alexander Schlacht, <u>Anna Karnkowska</u> , Vladimir Hampl & Joel B. Dacks: Membrane Trafficking Proteins Evolution – Insight from Arf GEF Protein Family		
9:20	Dionigia Meloni, Jun-ichi Inoue, Satoko Noda & Moriya Ohkuma: Ho- rizontal Gene Transfer of Non-Phosphorylating Glyceraldehy- de-3-Phosphate Dehydrogenase from Bacteria in the Termite- -Gut Symbiotic Protists: a Probable Alternative Glycolytic Pathway?		
9:40	<u>Eva Martincová</u> , Luboš Voleman, Vojtěch Žárský, Jan Pyrih, Jan Tachezy & Pavel Doležal: <b>Molecular Analysis of the Mitosomal Protein</b> <b>Import in</b> <i>Giardia intestinalis</i>		
10:00	Darja Stojanovová, Jan Pyrih & Jan Tachezy: Reconstruction of Anaerobic CIA Pathway in Trichomonas vaginalis		
10:20	Coffee break		
	Molecular Biology of Protozoa		
10:40	<u>Jitka Kručinská</u> , Lilach Sheiner, Luděk Kořený, Boris Striepen & Miroslav Oborník: <b>Localization of</b> <i>Chromera velia</i> <b>heme pathway enzymes</b>		
11:00	<u>Heather J. Esson</u> , Aleš Horák, Petra Dufková, Roman Sobotka, Peter Ko- ník & Miroslav Oborník: <b>Exploring the Functional Evolution of</b> <b>Thylakoid Membrane Complexes: Nuclear Gene Transfer, Gene</b> loss, and Reconstructing Photosystem I in <i>Chromera velia</i>		
11:20	Pavel Flegontov, <u>Jan Michálek</u> , Jan Janouškovec, De-Hua Lai, Milan Jirků, Eva Hajdušková, Aleš Tomčala, Thomas D. Otto, Patrick J. Kee- ling, Arnab Pain & Miroslav Oborník, Julius Lukeš: <b>The Weird Respi-</b> <b>ratory Chain of Chromerid</b> <i>Chromera velia</i>		
11:40	Lenka Horváthová, Vojtěch Žárský, Markéta Petrů, Alžběta Krupičková, Gerard Huysmans, Mohamed Chami, Olivera Francetic & Pavel Doležal: Reduced Version of Bacterial Secretion System in the Mitochon- drion of Excavates		
12:00	Lunch		
13:00	<u>Hana Váchová</u> , Karolína Šubrtová, Ondřej Gahura, Brian Panicucci, Alena Zíková: Functional Analysis of Novel $F_1$ ATPase Subunit in <i>Trypanosoma brucei</i>		
13:20	Aygul Ishemgulova, Pavel Flegontov, Jana Hlaváčová, Alexei Kostygov, Jan Votýpka, Petr Volf, Julius Lukeš & Vyacheslav Yurchenko: Factors of Leishmania infectivity		

13:40	Jiří Týč, Lucie Ridlon, Dmitri Maslov & Julius Lukeš: Conserved Pu- tative Mitochondrial Ribosomal Proteins Important for Mito- chondrial Translation in <i>Trypanosoma brucei</i>
14:00	Anzhelika Butenko, Aygul Ishemgulova, Natalia Kraeva, Fred Opperdoes, Vyacheslav Yurchenko, Dmitry Filatov, Pavel Flegontov & Julius Lukeš: Genomes of Monoxenous Species Shed Light on the Evolution of Parasitism in Trypanosomatids
14:20	Vyacheslav Yurchenko, Natalia Kraeva, Alexei Kostygov, Jav Votýpka & Julius Lukeš: Origin of Dixenous Trypanosomatids: Lessons from Their Monoxenous Cousins
14:40	Sponzor – KRD
14:50	Coffee break
15:10	Tomáš Skalický, Eva Dobáková, Pavel Flegontov, Martina Tesařová, Dag- mar Jirsová, Jan Votýpka, Vyacheslav Yurchenko & Julius Lukeš: Life Cycle and Ultrastructure of <i>Paratrypanosoma</i>
15:30	Petr Rada & Jan Tachezy: Targeting of C-tail Anchored Proteins into the Outer Membrane of the Hydrogenosome and Endoplas- mic Reticulum
15:50	Ondrej Gahura, Hana Vachova, Karolina Subrtova, Brian Panicucci, John E. Walker & Alena Zikova: Deep Proteomic Analysis of Trypanosoma brucei $F_0F_1$ -ATP synthase reveals unique features
16:10	<u>Marek Eliáš</u> : A Fraction of the Ancestral Eukaryotic Gene Com- plement Has Survived Only in Poorly Studied Dispersed Protist Lineages
17:00	Closing Remarks
18:00	Dinner
Friday May 15, 2015	
8:00	Breakfast
9:00	Departure of Participants

Speakers' names are underlined.

### Abstracts

#### Expansion of Meiotic Genes in Euglenozoa

#### Erik Birčák<sup>1</sup> & Juraj Krajčovič<sup>1</sup>

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Meiotic genes are basically two groups of genes either with their sole purpose in cell meiosis or closely related to this process. Their presence in genome or transcriptome can support the readiness of that particular organism to undergo meiosis. The phylum Euglenozoa contains various interesting classes with parasitic or free living organisms but there is not documented any experimentally proven meiosis what is uncommon for eukaryotic organisms. Therefore, we propose that these taxa should undergo cryptic sex which can be indicated by presence of meiotic genes in their genomes. In our approach we used currently available genomic and transcriptomic data from various euglenoids and tested it against a group of 60 potential genes to determine their presence or absence. This approach should detect not only closely related homologs of higher eukaryotic genes but also specific variants of meiotic genes thanks to comparative approach between single organisms in phylum. We present these results as an inventory of potential meiotic genes in various groups of euglenoids and their phylogenic relations.

#### Regulation of Gene Expression in Monocercomonoides sp.

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Regulation of gene expression plays crucial role in development, differentiation and homeostasis of every single cell. However, rather sparse amount of information is available for protists and our understanding of gene regulation is nearly limited to several model organisms. This study is focused on oxymonad *Monocercomonoides*. Members of this genus are nonpathogenic anaerobes, which live in digestive tract of invertebrates and vertebrates as well. Gene expression is modulated at multiple levels by many mechanisms. In this work we concentrate mainly on (i) length and structure of 5' untranslated regions, an essential part of post-transcriptional regulation, and (ii) annotation of basal transcription and translation initiation factors. Our results are compared to the closest studied relatives *Trichomonas vaginalis* and *Giardia intestinalis*.

#### Genomes of Monoxenous Species Shed Light on the Evolution of Parasitism in Trypanosomatids

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Trypanosomatidae is a large group of parasitic protists within the class Kinetoplastea, including Trypanosoma and Leishmania species pathogenic for humans. Trypanosomatids can be restricted to one host (monoxenous) or have a life cycle involving two hosts (dixenous). We have sequenced genomes of 4 monoxenous species: Blechomonas ayalai, Leptomonas seymouri, Leptomonas pyrrhocoris and Paratrypanosoma confusum. The genome of L. *pyrrhocoris*, parasite of Pyrrhocoridae bugs, was assembled almost to chromosome level. In addition, we generated differential gene expression data for L. seymouri, Leishmania major LV561 and Leishmania mexicana. In order to gain an insight into evolution of gene content in trypanosomatids, we mapped gene family gains and losses on the established phylogenetic tree of kinetoplastids (27 genomes in total). Gene gains dominate at the basal nodes of: trypanosomatids, Leishmaniinae, Leptomonas-Crithidia, American trypanosomes, T. cruzi, and T. brucei; the other internal nodes and leaves are either dominated by losses or have almost equal counts of gains and losses. Genomes of the monoxenous species provide essential outgroups for studying genome evolution in human-pathogenic species, especially in Leishmania. We overlapped gene gains/losses patterns and differential gene expression data. As a result, we delineated Leishmania-specific gene families and compiled a list of 39 novel candidates for Leishmania virulence factors, which represent targets for future knock-out and knock-down experiments. Selection analysis using genomes of 13 L. pyrrhocoris isolates was performed and 1,318 genes showing signs of positive selection were identified.

#### **Diversity of Metamonads**

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Metamonada is one of major eukaryotic lineages. Although the metamonads are not particularly species-rich (less than 1,000 species have been described), they exhibit a wide diversity of morphology, ultrastructure, and ecology. The last common ancestor of metamonads was most probably an anaerobic, free-living excavate flagellate equipped with four flagella. However, most of extant metamonds have lost the excavate features, particularly the ventral feeding groove. Moreover, the number of flagella has dramatically increased in several lineages, while a few metamonads are aflagellated amoebae. Metamonads are predominantly endobiotic living in the intestines of various vertebrates and invertebrates, and only a minority is free-living. The diversity of both, free-living and endobiotic, metamonads is still poorly understood. Here we report a discovery of several new lineages, which considerably extend our knowledge about morphological, phylogenetic, and ecological diversity of the metamonads.

# Diversity, Phylogeny and Biology of *Cryptosporidium* spp. Infecting Rodents of Genus *Apodemus*

 $\underline{\check{S}\acute{a}rka}$   $\underline{\check{C}ondlov\acute{a}}^{1,2},$  Michalea Horčičková $^{1,2},$  Martin Kváč $^{1,2},$  Bohumil Sak $^1$  & Dana Květoňová $^1$ 

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A total of 257 faecal samples from wild Apodemus spp. trapped in 13 areas in the Czech Republic, namely 232 samples of A. flavicollis and 25 of A. sylvaticus, were screened for presence of Cryptosporidium spp. using both light microscopy and molecular tools. Microscopy examination revealed the presence of Cryptosporidium oocysts in 30 samples of A. flavicollis and one sample of A. sylvaticus. Using the PCR amplifying partial sequence of SSU rRNA gene, 39 samples were detected positive for the presence of Cryptosporidium-specific DNA. The same results were obtained using PCR amplifying sequence of gene encoding actin. All microscopy positive samples were also PCR positive. Phylogeny analyses showed presence of two novel genotypes, first phylogenetically related to C. ubiquitum (one sample A. flavicollis and one sample A. sylvaticus) and the second genotype (consisting of several subgroups) related to C. canis (32 samples A. flavicollis). Moreover, concurrent infection with these genotypes was reported in four A. flavicollis and one A. flavicollis needs to be verified using experimental infection in the future. This is the first report of these Cryptosporidium genotypes in Apodemus spp.

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#### Alternative U-indel Editing in Kinetoplastid Endosymbiont *Perkinsela*

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Perkinsela is an enigmatic early-branching kinetoplastid protist that lives as an obligatory endosymbiont inside Paramoeba (Amoebozoa). We have sequenced the highly reduced mitochondrial genome of Perkinsela, which possesses only six protein-coding genes (cox1, cox2, cox3, cob, atp6, and rps12), despite the fact that the organelle itself contains more DNA than is present in either the host or endosymbiont nuclear genomes. An *in silico* analysis of two Perkinsela strains showed that mitochondrial RNA editing and processing machineries typical of kinetoplastid flagellates are generally conserved, and all mitochondrial transcripts undergo U-insertion/deletion editing. We have developed software tools for accurate and exhaustive mapping of RNA-seq reads having extensive U-insertions/deletions, allowing a detailed investigation of RNA editing via deep sequencing. With these methods we show that up to 50% of reads for a given edited region seem to contain errors of the editing system or correspond to alternatively edited transcripts.

### A Fraction of the Ancestral Eukaryotic Gene Complement Has Survived Only in Poorly Studied Dispersed Protist Lineages

#### MAREK ELIÁŠ<sup>1</sup>

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Recent advances in phylogenomics have revealed that the last eukaryotic common ancestor was a surprisingly complex cell, but the exact constellation of molecular pathways and modules operating in it is yet to be worked out. An insufficiently appreciated aspect of the eukaryote evolution is a potentially substantial extent of recurrent gene loss, leading to preservation of some ancestral eukaryotic genes in only limited subsets of modern eukaryotes often composed of a few unrelated lineages. Genomic analyses by my group, our collaborators and others suggest that there might be a significant number of such ancestral sporadically distributed genes found only in obscure or understudied protists; these genes thus have so far remained neglected and functionally uncharacterized. Some such genes were inherited from prokaryotic ancestors, and in these cases function of these eukaryotic genes may be guessed at by considering the function of their prokaryotic homologs. I will present two examples from this group - the Min system, predicted to participate on mitochondrial division in a few protist lineages, and the putative mitochondrial type II secretion system found in malawimonads, jakobids and heterolobosean. Another category are sporadically preserved ancestral eukaryotic genes that are eukaryotic novelties. Here the cellular function often remains completely enigmatic, as is, for instance, the case of a number of proteins from the Ras-like GTPase superfamily. I argue that ignoring these genes imposes significant limitations to our understanding of how a (proto)typical eukaryotic cell looks like and works, and I plead for deploying less conventional or novel eukaryotic model organisms to fill this fundamental gap in eukaryotic cell biology.

#### Exploring the Functional Evolution of Thylakoid Membrane Complexes: Nuclear Gene Transfer, Gene Loss, and Reconstructing Photosystem I in *Chromera velia*

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In plants and algae, the light-dependent reactions of photosynthesis are carried out by four protein complexes in the thylakoid membrane: photosystem I (PSI), photosystem II (PSII), ATP synthase, and cytochrome b6/f. Despite the diversity and ecological importance of photosynthetic eukaryotes, little is understood about the evolutionary history and functional diversity of the thylakoid complexes. In order to determine how thylakoid membrane complexes have diversified throughout plastid evolution, we used BLAST to retrieve the sequences for 61 proteins associated with PSI, PSII, ATP synthase and cytochrome b6/f from NCBI, KEGG, and other sequence databases. Proteins were classified as plastid encoded, nuclear encoded, or absent, and results were compared across 29 taxa (24 eukaryotes and 5 cyanobacteria). Gene loss was most extensive in Chromera velia, a photosynthetic apicomplexan, with 21 missing proteins (34% of those sampled). To explore the structural effects of protein absences on PSI in C. velia, we purified this complex and identified individual components by two-dimensional electrophoresis and mass spectrometry. PSI in C. velia is atypical. The complex is tightly associated with two different superoxide dismutases and the conserved PsaD, PsaE and PsaF subunits contain extra C-terminal regions. Moreover, we detected an unusually large spectrum of light-harvesting antenna attached to PSI. These data indicate that C. velia rebuilt the PSI complex and uses a different strategy from cyanobacteria or plants to deal with environmental stresses.

# Deep Proteomic Analysis of Trypanosoma brucei $F_0F_1$ -ATP synthase reveals unique features

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Membrane-bound  $F_0F_1$ -ATP syntheses are central enzymes in the energetic metabolism of bacteria and eukaryotic organelles. We established procedures to isolate either the catalytic F<sub>1</sub>-ATPase moiety or the entire F<sub>0</sub>F<sub>1</sub>-ATP synthase from procyclic stage *T. brucei*. The first approach involves two purification steps – ion exchange followed by gel filtration chromatography. The second strategy relies on a GST-tagged inhibitory peptide (TbIF1) to tightly bind the F<sub>o</sub>F<sub>1</sub>-ATP synthase, which is subsequently purified using a GST-Trap column. Both methods have been optimized to yield very pure complexes that are currently undergoing structural studies using X-ray crystallography and high-resolution cryo-EM. The purification of functional  $F_1$ -ATPase reveals that it is comprised of all the usual eukaryotic components ( $\alpha\beta\gamma\delta\epsilon$ ), plus a multicopy subunit p18 that is essential. The silencing of p18 results in dramatic losses of  $F_1$  complexes. Furthermore, we mapped two cleavage sites in the sequence of subunit  $\alpha$ , which is split into two fragments in vivo. In addition to the  $F_1$  components, the isolated  $F_0F_1$ -ATP synthese contains only two homologous subunits of the eukaryotic  $F_0$ -moiety: the proton channel subunit c and OSCP. Additional identified subunits have no homology outside the Euglenozoa, suggesting that the peripheral stalk and membrane-bound subunits are either extremely divergent or replaced by other proteins. Quantitative mass spectrometry with isotope-labelled standards is being implemented to assess the stoichiometry of p18 and selected  $F_0$  subunits. Our data are in line with accumulating evidence from other non-model eukaryotes that the compositional diversity of functionally conserved F<sub>1</sub>F<sub>o</sub>-ATP synthases is significantly higher than previously thought.

#### Survey of Double-Stranded RNA Viruses in Trypanosomatidae

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A broad survey of double-stranded RNA (dsRNA) in trypanosomatid species was conducted. It was based on simultaneous DNase I and S1 nuclease treatment of total RNA with subsequent visualization of dsRNA in agarose gel. In total 77 individual isolates were screened, among them 12 were found positive. dsRNA of the positive isolates was of two types. Some, like those of *Crithidia otongatchiensis*, *Blechomonas ayalai* and *Crithidia* sp. isolate ZM were represented by a single band reminiscent of dsRNA of the well-characterized virus of *Leishmania guyanensis* (LRV1-4) that belongs to the family Totiviridae. Other isolates display not so distinctive profile with bands ranging from 1.2 to 4 kb or even multiple bands. This suggests that monoxenous trypanosomatids are prone to infection by different dsRNA viruses. The full-length genome of the *Phytomonas serpens* (isolate 9T) virus has been amplified and sequenced. Very little is known about role of dsRNA viruses in monoxenous trypanosomatids. The current study may shed light on novel strategies of virus-host interaction and coevolution in protists. To this end we have observed a gradual virus loss in *C. otongatchiensis* during prolonged continuous passaging in vitro which may indicate some connection to parasite proliferation inside insect host.

#### Experimental Chloroplast Genome Degradation of Photosynthetic flagellate *Euglena gracilis*

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Euglena gracilis is a unicellular photosynthetic flagellate belonging to the phylum Euglenozoa. Euglenozoa seems to be one of the evolutionary oldest eukaryotic group of organisms but the chloroplast compartment seems to be relatively very young. A treatment of E. gracilis by various physical and chemical agents leads the culture to form white colonies (bleaching), to switch from autotrophy to heterotrophy, and to damage the chloroplast and degrade its genome. In our study, we have studied the effect of two antibacterial drugs with a different mechanism of action (streptomycin – inhibitor of bacterial protein synthesis, and ofloxacin – inhibitor of bacterial replication) on the E. gracilis plastome during eight week lasting experiments. A presence of several plastid encoded genes for proteins involved in diverse processes (replication, transcription, translation and photosynthesis) as well as genes for ribosomal and transfer RNA, that cover whole chloroplast genome has been determined by semiquantitative PCR analysis. During the drug treatment we have observed successive degradation of certain plastid genes. In parallel, we have studied a capability of antihistaminic drugs (having very different mode of action in comparison to antibiotics or mutagenes) to induce bleached euglena mutants. We have obtained a few white mutants. PCR analyses of selected genes in these new mutants have confirmed lack of some plastid genes and presence of some others - their lists are very similar to the other white mutants induced by antibiotics or mutagenes analysed so far.

#### Captive Hedgehogs as Potential Source of Zoonotic Protists

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Two species of free ranging hedgehogs (Erinaceus europaeus and E. concolor) occur in the Czech Republic and at least two more species are kept as exotic pets. The numbers of native hedgehogs overwintering in animal shelters and growing popularity of "pet"Four-toed hedgehogs (Atelerix albiventris) lead to extensive contact of humans with captive hedgehogs. Present study investigated to what extend may the hedgehogs be a source of potentially zoonotic protists of the genus Cryptosporidium and Giardia. In cooperation with breeders and rescue centres, we acquired a set of 81 faecal samples of hedgehogs. The samples were examined both by standard coproscopic and molecular diagnostics. The presence of Cryptosporidium DNA was determined by double PCR detecting part of small subunit ribosomal rRNA gene and the presence of *Giardia* spp. was assayed by nested PCR for triosephosphate isomerase gene. All 30 examined samples of A. albiventris were negative. The results of parasitological examination of 31 E. europaeus and 20 E. concolor from animal shelteres showed the presence of common parasites as Isospora rastegaievae, Capillaria spp., Eucoleus aerophilus, Crenosoma striatum and undetermined acanthocephalans, but no Cryptosporidium oocysts nor Giardia cysts. PCR detection revealed a single E. europaeus positive for C. parvum. The obtained results show rather low risk of transmission of Cryptosporidium or *Giardia* as potentially zoonotic protists associated with keeping of hedgehogs in human care. Acknowledgment: Funding: IGA VFU 88/2014/FVL; CEITEC-Central European Institute of Technology from European Regional Development Fund (CZ.1.05/1.1.00/02.0068).

#### Highly Motile and Proliferating Quickly in Carp Blood: Is the Myxozoan *Sphaerospora molnari* an emerging pathogen?

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In recent years, we detected emerging numbers of myxozoan proliferative blood stages, at different carp culture sites in Central Europe. Using molecular diagnostics, we were able to demonstrate that most of these stages represent S. molnari, a parasite known to form spores in the epithelia of the gills and the skin. While spore-forming stages cause marked dystrophic changes and necrosis in the infected epithelia, the etiology of proliferative blood stages of S. molnari, which are able to reach any organ or tissue via the blood stream, is unknown to date. We determined S. molnari's dispersion in the blood system and its extravascular location in the fish host using in situ hybridisation, and we demonstrate that S. molnari serves as an important co-factor or precondition for the development of Swim Bladder Inflammation (SBI) in common carp, a disease which had previously been related to the kidney parasite S. dykovae, formerly known as S. renicola. Furthermore, PCR of monthly carp blood samples demonstrated that proliferative blood stages of S. molnari are present year-round while spore formation in the gills is restricted to the spring season. We detected extraordinary high densities of blood parasites during the summer months, and we determined that the proliferation of S. molnari in the blood is temperature dependent, using qPCR. This prompts us to predict emerging numbers of S. molnari as water temperatures are on the rise in Central European carp ponds. Due to the parasite's importance, present studies in our laboratory focus its extrapiscine life cycle and transmission pathways as well as on the discovery of parasite genes and proteins of particular importance for host exploitation, with the aim to develop antiparasitic strategies.

#### Reduced Version of Bacterial Secretion System in the Mitochondrion of Excavates

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Mitochondria of all eukaryotes have originated from a single alphaproteobacterial ancestor. Evolution of the organelle was accompanied by extensive transfer of genes from the ancestral endosymbiont to the host cell nucleus, as well as dramatic renewal at the level of its proteome. The protein flow across the two membranes of the evolving mitochondrion reversed. New pathways importing proteins into the organelle had to be installed, while bacterial machineries originally used for protein secretion to the cell exterior were abandoned, but not entirely. Searching the eukaryotic genome data we have found that one group of eukaryotes (Excavata) still possesses components of one of the bacterial secretion systems: type II secretion system (T2SS). The eukaryotic version of the system is minimalist, consisting of only 4 of 12–15 different protein components normally present in bacteria. Nevertheless it can be still functional, as each of these proteins represents a core subunit of 4 T2SS subassemblies that span both the outer and inner membranes. We focus on characterization of two proteins (i) GspD, a homologue of bacterial secretin, that forms multimeric outer-membrane channel and (ii) GspG, a homologue of bacterial major pseudopilin, a basic building block of periplasmic pseudopilus. We show their localization within mitochondrion of Naegleria gruberi, a free living amoeba that is our model organism. According to our data from blue native PAGE, electron microscopy and bacterial two hybrid system, both proteins are able to form multimers, suggesting their functionality within the reduced secretion system that is still able to secrete proteins to the cytosol of the cell.

#### Assembly and Annotation of Full Mitochondrial Genome of Green Alga *Pyramimonas parkeae*

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The complete mitochondrial DNA of *Pyramimonas parkeae* was sequenced (43,295 bp). The genome is circular and very compact, 71 % is covered by protein-coding genes. We have identified 34 protein-coding genes, 2 rRNA genes, 25 tRNA genes and 2 ORFs of unknown function. The circular DNA contains two inverted repeats (6,672 bp) without genes coding rRNAs. The gene content is very similar to those of *Nephroselmis olivaceus* but the gene order resembles more closely another related prasinophyte *Prasinoderma coloniale*. The single identified intron is located in Cox1 gene. Interestingly, the two exons of Cox1 are encoded on the genomic DNA in the reverse order, which indicates that the mature mRNA is formed by trans-splicing. *P. parkeae* is considered to be the closest known relative of the euglenid chloroplasts. Therefore, we have searched the transcriptomes of *Eutreptiella gymnastica* and *Euglena gracilis* for close homologues of *P. parkeae* mitochondrial genes but did not identify any. This result indicates that that the mitochondrial genes but did not identify any. This result indicates that that the mitochondrial genome of the endosymbiont has been lost and did not contribute by endosymbiotic gene transfer to the nuclear genomes of photosyntetic euglenids.

#### Host Specificity, Pathogenicity and Mixed Infections of Trypanosomes and Trypanoplasms from Freshwater Fishes

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This work summarizes results of the eight years study focused on *Trypanosoma* sp. and Trypanoplasma sp. parasitizing freshwater fishes in the vicinity of Kyiv, Ukraine. About 1,370 fish specimens of eight different species and 570 fish specimens of 2 different species were examined for the presence of trypanosomes and trypanoplasms, respectively. Out of investigated samples 921 individuals specimens were found to be infected by trypanosomes and 440 individuals – by trypanoplasms. The prevalence of infection ranged from 24% in freshwater bream, Abramis brama (Linnaeus), to 100% in spined loach, Cobitis 'taenia' Linnaeus. The level of parasitaemia also varied significantly between generally mild infections in pikeperch, Sander lucioperca (Linnaeus), and heavy ones in C. 'taenia'. In most cases the infections with trypanosomes and trypanoplasms were asymptomatic. Cases of co-infection with species of Trypanoplasma Laveran et Mesnil, 1901 were documented for five out of eight examined host species. Molecular analysis of the 18S rDNA sequences revealed that four hosts, namely northern pike, Esox lucius Linnaeus, freshwater bream, spined loach and European perch, Perca fluviatilis Linnaeus, were simultaneously infected with two different trypanosome species. Our findings advocate the view that to avoid the risk posed by mixed infections, subsequent molecular taxonomic studies should be performed on clonal lines derived from laboratory cultures.

#### Factors of Leishmania infectivity

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Leishmania is a trypanosomatid parasite responsible for the disease leishmaniasis. This study is devoted to genes involved in infectivity of Leishmania major and Leishmania mexicana. We applied next-generation sequencing approach to investigate genes overexpressed in virulent strain when compared with attenuated strain of L. major and genes overexpressed in infective developmental stages when compared with non-infective stages of L. mexicana. Avirulent line LV561/AV with attenuated infectivity for mice and sand fly vectors was obtained by long-term cultivation of the L. major virulent strain LV561 (LRC-L137; MHOM/IL/1967/Jericho-II). Whole transcriptome analysis revealed that 53 transcripts are up-regulated in LV561/V (virulent) strain. Procyclic promastigote, metacyclic promastigote and amastigote stages were obtained by differentiation of L. mexicana in axenic culture. RNA-seq analysis revealed that 8 genes are up-regulated in infective for human metacyclic promastigotes; 276 genes are up-regulated in a blood-dwelling amastigotes. Genes encoding for major surface protease gp63, casein kinase I, pteridine transporters and several hypothetical proteins are found to be overexpressed both in virulent strain of L. major and metacyclic promastigotes or amastigotes of L. mexicana.

### Implementation of the *Babesia microti* Transmission Model: an Essential Tool to Study *Babesia*-tick molecular interactions

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Babesiosis, a zoonosis caused by the apicomplexan protozoa of genus Babesia, is a tick--borne malaria-like disease of various vertebrate hosts. Despite a growing attention paid to the babesiosis as an emerging disease from the aspects of veterinary and human medicine, our knowledge about interactions between *Babesia* and tick is still insufficient. In order to better understand multiple molecular interactions between *Babesia* and the tick vector, we implemented laboratory babesiosis transmission model using Babesia microti, the primary cause of human babesiosis in the United States. Our in vivo transmission model of B. microti employs infected laboratory mice and immature tick stages of *Ixodes ricinus*. To visualize journey of parasite inside the vector, we developed FISH (fluorescence in-situ hybridization) technique, which targets B. microti mRNA and highlight parasite developmental stages inside the vector tissues. Special emphasis is paid to sporozoites inside the salivary glands, the primary site responsible for *Babesia* transmission from the vector into the host. Introduction of *B. microti* laboratory model together with detailed knowledge of parasite stages inside the vector tissues open a new way for research of mutual molecular interactions between Babesia and the tick. Identification and characterization of molecules playing a role in Babesia persistence within the tick vector and parasite transmission could significantly contribute to the control of babesiosis.

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#### Membrane Trafficking Proteins Evolution – Insight from Arf GEF Protein Family

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Vesicular transport has central importance to the organization of eukaryotic cells. Understanding the molecular mechanisms that control vesicle packing, budding, and fusion is a major interest in eukaryotic cell biology. Arf GEF (guanine-nucleotide exchange factor for ADP-ribosylation factor GTPases) protein family is involved in regulation of these processes. The protein family shares a conserved Sec7 domain and is essential for vesicular trafficking in all eukaryotic kingdoms. In humans, six subfamilies of Arf GEFs have been identified and two of them (BIG and GBF) were suggested to be present in the Last Eukaryotic Common Ancestor (LECA).

Here we performed evolutionary analyses of Arf GEFs based on broad taxonomical sampling. We searched for the Sec7 domain containing proteins and identified Arf GEF proteins in all eukaryotic taxa sampled including recently sequenced *Monocercomonoides* sp., which possessed 11 Arf GEFs robustly classified as either subfamilies BIGs or Cytohesins. Phylogenetic analyses and clustering analyses suggested that three subfamilies (BIG, GBF, and Cytohesin) were most likely present in the LECA. By contrast, three remaining subfamilies have a limited distribution and more recent origin. FBXO8 was identified only in vertebrates, but the origins of two other subfamilies (BRAG and EFA) remain unclear.

# The Closest Monoxenous Relative of *Leishmania* Is an Endosymbiont-Bearing Trypanosomatid

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According to the generally accepted hypothesis, leishmaniae arose in Neotropics. Therefore, their closest relatives whose study could shed light on the origin of the genus should live there. We performed molecular analysis of cultures of Leishmaniinae isolated in the center of Neotropics (Ecuador) using 18S rRNA gene. One of them (Trypanosomatidae sp. 262AT) showed close phylogenetic relationship to Leishmania that was supported by other molecular markers (28S rRNA, HSP83 and SL-RNA genes). Morphologically this species is similar to its dixenous counterpart since it has not only promastigotes that are common for Leishmaniinae but also typical amastigotes. Moreover it is able to grow at lowered pH (5.5)though cannot endure elevated temperature as do leishmaniae. All these facts should make the novel parasite an ideal candidate for studying the origin of dixeny in Leishmania with the use of comparative approach. However we found that it has bacterial endosymbionts. To date it is the only member of the species-rich Leishmaniinae subfamily bearing bacteria in the cytoplasm. Its endosymbiont is different from *Kinetoplastibacterium* spp. (fam. Alcaligenaceae) living within trypanosomatids of the subfamily Strigomonadinae. It belongs to the genus Pandoraea (fam. Burkholderiacea) whose other known members are either free-living or associated with opportunistic infections in humans. The number of bacterial cells in the cytoplasm of Trypanosomatidae sp. 262AT is inconstant that shows the absence of the coordinated division. Summing up, we suggest that Trypanosomatidae sp. 262AT acquired its endosymbiont quite recently, so it is a perfect model to study the formation of trypanosomatid-bacterium symbiotic relationships.

## The CCAP 1532/1 Culture of *Gruberella flavescens* Is a Complex Ecosystem of Unusual Eukaryotes

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Gruberella flavescens is a relatively large multinucleated marine amoeba. It is believed to be a heterolobosean based on its gross morphology and ultrastructure; details of its mitotic division resemble *Stachyamoeba*, which is treated as the only other genus of Gruberellidae. Unlike Stachyamoeba, Gruberella was never observed to form flagellated stages or cysts. Gruberella flavescens was studied in detail by Page in 1980s; the culture was deposited in CCAP under the code number 1532/1. We obtained and studied the culture and attempted to obtain first sequence data belonging to Gruberella. Our effort to sequence its SSU rDNA (or any other gene) has been hindered by the presence of other eukaryotic organisms in the culture: there are (at least) 5 other eukaryotes contained. Some of them are readily observed (scuticocilates and dinoflagellates), but others are hardly noticeable tiny amoebae (a new species of Parvamoeba and another "pikoamoeba" of uncertain affinity) and Ministeria vibrans (a close "heliozoan" relative of metazoans). We managed to obtain sequence data for all of them and we believe we also have a partial SSU rDNA sequence of Gruberella available. The sequence belongs among Heterolobosea, but is not sister to Stachyamoeba. Before any conclusions are drawn, it must be shown properly that the sequence really belongs to Gruberella – a FISH experiment is under way.

# Latent *Encephalitozoon cuniculi* Infection is Reactivated after Factitious Immunodeficiency in Mice

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The different course of microsporidiosis depending on the immunological status of the host and the strain of *Encephalitozoon cuniculi*, which was reported in clinical cases has been successfully imitated in murine models using molecular detection of the parasite in numerous tissues of the host. Whereas E. cuniculi strain II caused lethal microsporidiosis in SCID and CD4 knock-out mice, the infection in BABL/c and C57Bl/6J mice remained asymptomatic despite parasite dissemination into many organs during the acute infection phase. CD8 knock-out mice survived the infection despite multiple organs affected. This fact opened the question about the reliability of generally accepted protective effect of cellular part of immune response against microsporidiosis caused by E. cuniculi. The differences between E. cuniculi strains were very striking. While course of microsporidiosis caused by E. cuniculi II in immunocompetent mice had the peak of infection 35. DPI and remained only in spleen in chronic stage of infection, EC III spread into all host organs already 7 DPI and infection persisted in many organs also after acute phase. While treatment with albendazole led to disappearance of EC II from all examined organs in BALB/c mice, only temporary reduction of number of affected organs was observed in mice infected by EC III. Moreover, immunosuppression by dexamethasone in infected BALB/c mice pre-treated with albendazole caused expansion of parasite. Although the number of affected organs was not so high, the fact that the infection is able to be reactivated virtually from zero, is groundbreaking, implying potential risk especially for transplant recipients of organs originating from infected donors. Acknowledgment: unding: GAČR P302-14-20684S and GAJU 011/2013/Z.

#### Protein Damage in Euglena gracilis after UVC Irradiation

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Euglena gracilis is a robust organism in general. It is able to survive a large spectrum of environmental conditions (pH, pollution, aerobic/anaerobic, light/dark). Euglena can grow and absorb heavy metals such as  $Hg^{2+}$ ,  $Cd^{2+}$ , Cr(VI), Cr(III),  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Cd^{2+}$ ,  $Pb^{2+}$ ,  $Tc^{2+}$ . Euglena has also relatively strong resistance to UV and ionizing radiation. Nutritional value of euglena is sufficient to support some animal life (mice, rats). Given the high photosynthetic efficiency, nutritional quality and radiation resistance, it has been proposed that this organism might be used to supply oxygen and food in close ecological life support system. In our study we monitored the influence of UV radiation on *Euglena gracilis*. We used UVC radiation as the part of UV light characterized by highest energy. We measured protein carbonylation as a major irreparable type of protein damage under stress conditions. While we could see high increase of carbonyls in total cellular proteins extract, we did not find any significant differences in carbonylation of oxidative phosphorylation enzymes in cells irradiated by UVC (range 10–900 J m<sup>-2</sup>). Our results indicate that proteins participating in oxidative phosphorylation (membrane bound ones) are more resistant to damage after UVC irradiation in range 10–900 J m<sup>-2</sup> than other one.

#### Localization of Chromera velia Heme Pathway Enzymes

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Chromera velia is a close photosynthetic relative of apicomplexan parasites. One of the features common for C. velia and apicomplexans is a secondary plastid of red-algal origin. Although apicomplexans are obligatory parasites their existence is still strictly dependent on this nowadays non-photosynthetic plastid, because essential biochemical pathways such as isoprenoid, fatty acid and heme syntheses are localized here. Heme biosynthesis is well conserved throughout the tree of life. However differences in synthesis of the first common precursor of the pathway,  $\delta$ -aminolevulinic acid (ALA) have been described. Contrary to other phototrophs C. velia synthesizes ALA via C4 pathway homologously to related apicomplexans. Origins and localizations of the particular enzymes of the heme pathway also vary among organisms. Enzymes of the C. velia heme pathway are of different origin including  $\alpha$ -proteobacterial, cyanobacterial origins, and the origin in primary or secondary host nucleus. Besides mitochondrially localized ALA synthase all remaining enzymes have well predicted bipartite targeting sequence suggesting their localization in the secondary plastid of C. velia. Here we show our data from experimental localization of selected heme pathway enzymes (ALA synthase, two ALA dehydratases, uroporphyrinogen synthase, and two ferrochelatases) by xenotransfection in phototrophic diatom *Phaeodactylum tricornutum* and apicomplexan parasite Toxoplasma gondii. Due to striking differences between results of two xenotransfection systems we used direct localization in C. velia by specific antibodies that shows unusual mitochondrial localization of all selected enzymes.

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# Type II Secretion System in the Mitochondria of Naegleria gruberi

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The type II secretion system is a complex of proteins, which is used by gram-negative bacteria for the transport of the folded proteins across the outer membrane into the extracellular milieu. Several core type II secretion proteins were found in the mitochondria of free-living protists *Naegleria gruberi* and *Malawimonas jakobiformis*. Our aim is to characterize these proteins using the heterologous system of *Saccharomyces cerevisiae*. We employ the yeast two hybrid system to study the protein interactions of the identified components and the *in vivo* and *in vitro* import techniques to study the mitochondrial transport and the assembly of the the putative mitochondrial type II secretion system.

### Lipid Content Modulation of Apicomplexan Cousin Chromera velia by Nitrogen Addition

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Apicomplexans parasites represented by genus *Plasmodium* causes severe health and economic losses. Recently found alveolate *Chromera velia* was proved as the closest known photosynthetic relative of apicomplexan parasites thus becoming an excellent model for study of the evolution of the parasitism. Intensive studies on genomics, physiology, and metabolomics of the alga revealed surprisingly high ability of *C. velia* to produce fatty acids and to accumulate storage lipids. Furthermore changing the concentration of anorganic nitrogen in medium modulate amount of biomass, amount of accumulated lipids and even proportion of n-3 polyunsaturated fatty acids.

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### New Markers for Resolving Myxozoan Phylogenetic Relationships

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Current myxozoan phylogeny relies on single SSU rDNA or concatenated analysis of limited number of SSU + LSU rDNA and EF2 gene sequences. These markers, however, are not sufficient to reconstruct the pattern of some early branching nodes that are important for the understanding of the main phylogenetic trends of Myxozoa. These weakly supported nodes show unstable branching patterns or large polytomies. We expect that multigene analysis with large taxon sampling will resolve most of the polytomic phylogenies as observed in other biological groups and provide greater stability in myxozoan interspecies relationships. We thus used publically available genomic/transriptomic data as well as our own Sphaerospora *molnari* transcriptome to select new candidate genes that are phylogenetically informative and suitable for myxozoan phylogenetic reconstruction. Our genome/transcriptome mining resulted in the selection of the following myxozoan genes for further analysis: V-ATPase, PAX-B (paired box), Elongation Factor  $1\alpha$ , PGD (6-phosphogluconate dehydrogenase), SNF (sans fille), TPI (triosephosphate isomerase), as well as three nematogalectins previously identified in Myxozoa. The phylogenetic tree based on V-ATPase and two nematogalectin genes corresponds to known rDNA-based myxosporean phylogeny. The following research will include design of primers that will be able to amplify selected genes of myxosporean representatives from our DNA collection to provide large scale multigene analysis of SSU rDNAs, EF2, V-ATPase, nematogalectins and other suitable genes.

#### Search for the Donor of Euglenid Chloroplast

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Photoautotrophic clade of euglenids acquired chloroplast via secondary endosymbiosis with a green alga as the plastid donor. Chloroplast genome of *Pyramimonas parkeae*, the closest known relative to euglenid chloroplast, shares with euglenids several gene clusters with conserved order of genes. Some of these clusters are unique for euglenids and *Pyramimonas*. It points to a member of Pyramimonadales as the source of the photosynthetic organelle. In this work, we investigate the identity of plastid donor by two approaches. In the firts approach, we use specific PCR for unique gene clusters to amplify parts of plastid genomes from environmental samples and to investigate phylogenetic position of these chloroplast parts. So far, we have not obtained any environmental chloroplast sequence, which could be regarded as either a deep euglenid lineage or a green alga closely related to euglenid plastid. Our second approach is based on phylogenetic analysis of plastid 16S rRNA gene using extensive sampling of anotated as well as environmental sequences. The statistical support for the phylogenetic tree of euglenids and green algae is generally low, however, it showed genus *Pterosperma* and its relatives as another candidate for plastid donor.

# 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 identified homologs of both MPC subunits in the genome of *Trypanosoma brucei* and confirmed localization exclusively in the mitochondrial membrane. We created MPC knock-out cell lines in both procyclic and bloodstream stages and discuss the changes in the end-products metabolism, pyruvate import to the mitochondrion and the effect on viability of infected mice.

## Molecular Analysis of the Mitosomal Protein Import in Giardia intestinalis

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The acquisition of mitochondria was a key event in early eukaryotic evolution. During the endosymbiont transformation, highly effective system for targeting and translocation of mitochondrial proteins from cytosol has evolved. In yeast and mammalian cells the protein import machinery is very complex, comprising more than 30 protein components. As model organism for studying mitochondrial evolution we chose an unicellular human pathogen Giardia intestinalis. Due to its anaerobic lifestyle it has highly reduced mitochondria. Those tiny organelles called mitosomes have completely lost their energy metabolism and genome. Mitosomal proteins thus have to be transported from cytosol. However, mitosomal protein import machinery comprises only four components known so far. More surprisingly, no inner membrane translocase was ever identified. Due to highly divergent sequences we are not able to identify more components of protein import pathway bioinformatically. Therefore we developed efficient method to identify the proteins directly. We established new method for isolation of interacting proteins in giardia. The system is based on highly specific in vivo protein biotinylation using enzyme biotin-ligase. Biotinylated proteins were crosslinked and purified by streptavidin affinity capture. We used two already known membrane components of the protein import pathway as biotinylation targets – Pam18 and Tom40. Using this method we managed to identify highly divergent Tim44 homologue, possible outer membrane receptor and other new mitosomal proteins. Moreover, we used this method for studying mitosomal import dynamics. Mitosomal proteins were successfully biotinylated by cytosolic biotin-ligase, which reveals posttranslational mode of transport.

### Horizontal Gene Transfer of Non-Phosphorylating Glyceraldehyde-3-Phosphate Dehydrogenase from Bacteria in the Termite-Gut Symbiotic Protists: a Probable Alternative Glycolytic Pathway?

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Termites play a pivotal role within the forest ecosystem because of their ability in the bio-recycling of lignocellulose. This capacity depends on the microbial community composed of two groups of anaerobic and amitocondriate flagellated protists that belong to the phyla Parabasalia and Preaxostila, and hundreds of bacterial species. The close relationship probably facilitates Horizontal Gene Transfers from bacteria to protists. In this study we analyzed a particular gene, the non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN) (EC 1.2.1.9) acquired by protists by Horizontal Gene Transfer. This enzyme catalyzes the irreversible oxidation of glyceraldehyde-3-phosphate to 3-phosphoglycerate by the reduction of NADP<sup>+</sup> to NADPH and could replace some enzymes of the standard glycolytic pathway and create a new probable alternative pathway. The GAPN activity has been detected in some bacteria that lack enzymes in the oxidative pentose phosphate pathway usually responsible for the production of NADPH, and now for the first time in protists. Probably, the gut protists, like many anaerobic microorganisms, do not have the oxidative pentose phosphate pathway because genes for this pathway were seldom found in their Expressed Sequences Tag data. In conclusion, the acquisition of non-phosphorylating glyceraldehyde-3--phosphate dehydrogenase gene by Horizontal Gene Transfer is likely important for the gut protists to produce NADPH necessary for the biosynthesis of cellular components.

#### The Weird Respiratory Chain of Chromerid Chromera velia

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The respiratory chain is the main metabolic feature of most mitochondria. In several cases of mitochondria and derived organelles, the sets of respiratory complexes were reduced or lost. Using experimental and bioinformatic approaches we have described a uniquely reduced mitochondrion of *Chromera velia*, a closest known phototrophic relative of apicomplexan parasites. Mitochondrial genome of *Chromera* encodes only cox1 nad cox3 on linear molecules and respiratory complexes I and III are completely absent from the respiratory chain which is hereby split in two parts. The function of missing complex III appears to be supplemented by an alternative mechanisms engaging putative lactate:cytochrome c oxidoreductases. In contrast, the second known chromerid alga *Vitrella brassicaformis* possesses respiratory chain similar to apicomplexans as well as mitochondrial-encoded cox1, cox3 and cob.

# *Hepatozoon* spp. in Carnivores from Czech Republic with Focus on Canidae

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Apicomplexan protozoa of the genus *Hepatozoon* are common intracellular parasites of carnivores and are transmitted by ingestion of hematophagous arthropod definitive hosts. Recently the most discussed species in Europe is *H. canis* because of its importance in veterinary medicine. This parasite is frequently documented in dogs in the Mediterranean area where its only known vector tick Rhipicephalus sanguineus is present. In wild Canidae, Hepatozoon sp./H. can is seems to be common all over Europe. However, information about its taxonomy and transmission is still lacking. The aim of this study was to primary investigate the presence of *Hepatozoon* spp. in wild carnivores from the Czech Republic and to define those ectoparasites acting as potential vectors. We sampled and examined blood or tissue specimen of animals from the families Canidae (40, positive 24), Mustelidae (7, positive 2) and Procyonidae (16, positive 0). Furthermore phylogenetic analysis based on entire 18S rRNA genes including sequences from wild Canidae and dogs (Austria, Romania, Guinea Bissau) from our previous studies was performed, to reveal the diversity of *Hepatozoon* spp. in carnivores. In H. canis/Hepatozoon sp. from Canidae, 18S rRNA appeared to be with very limited variability to explain the biological and epidemiological discrepancies between wild canides and domestic dogs. Our next aim is to develop PCR techniques amplifying mitochondrial or apicoplast genes of *Hepatozoon* and to collect more ectoparasites from wild carnivores to search for potential competent vectors in the *Rhipicephalus*-free areas.

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### Do Cryptosporidium spp., Encephalitozoon spp., Enterocytozoon bieneusi and Giardia spp. Pose Any Risk to Orangutans?

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The orangutans (*Pongo abelii* and *Pongo pygmaeus*) are critically endangered mainly as the result of loss and fragmentation of their natural habitat which leads to their closer contact with human population and consequently to the higher risk of pathogen transmission. Consequently, this proximity can pose another serious threat as orangutans are susceptible to many human pathogens and vice versa. Therefore, two hundred and twenty seven orangutans at six different sites on Sumatra and Borneo were sampled to the occurrence of Cryptosporidium spp., Encephalitozoon spp., Enterocytozoon bieneusi and Giardia spp. Out of the total of 228 examined animals 37 were positive for tested parasites (16.2%). The most prevalent was Encephalitozoon cuniculi genotype II found in 25 animals (11%); Enterocytozoon bieneusi genotype D was detected in seven individuals (3.1%) and Cryptosporidium spp., including C. muris and C. parvum type A and B, in eight animals (3.5%). To the best of our knowledge, these parasites were documented in orangutans for the first time. Giardia intestinalis was identified in single individual (assemblage B, subtype MB6). The most of Cryptosporidium spp. infections were detected in individuals with close human contact. In addition, the findings revealed higher prevalence of E. cuniculi at localities on Sumatra. Acknowledgment: Funded by Grant Agency of the Czech Republic (P505-11-1163) and Foundation "UMI-Saving of Pongidae".

#### Get Pathway in Giardia intestinalis

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Special class of membrane proteins carries a single C-terminal transmembrane domain that anchors them to membrane of organelles in secretory and endocytic pathway. These so called tail-anchored (TA) proteins 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 post-translational insertion of TA-proteins into the endoplasmic reticulum membrane. Our bioinformatics analyses revealed the absence of key GET proteins in most eukaryotic lineages except opisthokonts. We are using *Giardia intestinalis* in order to characterize putative distinct GET machinery common to all other lineages.

# Evolutionary History of the Arginine Deiminase Pathway among Eukaryotes

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Multiple prokaryotic lineages use the arginine deiminase (ADI) pathway for anaerobic energy production by arginine degradation. Eukaryotes have been thought to be generally devoid of this pathway, with two notable exceptions: trichomonads and diplomonads, closely related groups of protists living in low-oxygen niches. Our survey of newly available genomic and transcriptomic data shows that the complete ADI pathway is present also in representatives of Preaxostyla, Heterolobosea, Breviatea, Amoebozoa, and Chlorophyta, while individual enzymes constituting the pathway are distributed among almost all other major eukaryotic groups in a patchy pattern. The subsequent phylogenetic analyses suggest a complicated evolutionary history of the ADI pathway enzymes within the eukaryotic domain driven by multiple losses of the individual enzymes, duplications, and lateral gene transfers. Two points arise as the most important from these analyses: 1) the ADI pathway is likely an ancestral feature of Metamonada and 2) the results are consistent with presence of the ADI pathway in LECA as a result of vertical inheritance from the archaeal ancestors of eukaryotes.

#### Peculiarities of Queuosine Biosynthesis in Trypanosomes

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A general feature of tRNAs is a high number of nucleotide chemical modifications that are introduced post-transcriptionally. Queuosine (Q) is one of the most complex tRNA modifications found at the first position of the anticodon (wobble base) of several tRNAs. Despite its omnipresence in bacteria and eukaryotes, the function of Q in tRNA is not completely clear. In this study, we have used the protozoan parasite Trypanosoma brucei as a model for a comprehensive analysis of the tRNA guanine transglycosylase (TGT), the enzyme responsible for Q-tRNA formation in eukaryotes. Unlike its bacterial counterpart, in most eukaryotes TGT predominantly functions as a heterodimeric enzyme. In order to investigate the composition and function of the trypanosomal TGT, we used the sequence of the human TGT to search for potential homologues in the T. brucei genome. Analogous to humans, is the presence of two homologues of the TGT enzyme (TbTGT1 and TbTGT2) in T. brucei. However, we showed using RNAi knock-down strategy that only the most divergent TbTGT2 is responsible for Q-tRNA formation, while Q-tRNA levels in the RNAi cells of the conserved subunit TbTGT1 remained minimally affected. This striking observation suggests that in contrast to mammals, the trypanosomal TGT subunits don't form a complex. These observations are discussed in the context of the possible roles of the differential intracellular localization of Q-tRNA and modification enzymes in respect to their role in cytosolic and mitochondrial translation.

#### Contrasting Patterns in the Evolution of the Rab GTPase Family in Archaeplastida

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Rab GTPases are a vast group of proteins serving a role of master regulators in membrane trafficking in eukaryotes. Previous studies delineated some 23 Rab and Rab-like paralogs ancestral for eukaryotes and mapped their current phylogenetic distribution, but the analyses relied on a limited sampling of the eukaryotic diversity. Taking advantage of the recent growth of genome and transcriptome resources for phylogenetically diverse plants and algae, we reanalyzed the evolution of the Rab family in eukaryotes with the primary plastid, collectively constituting the presumably monophyletic supergroup Archaeplastida. Our most important novel findings are as follows: (i) the ancestral set of Rabs in Archaeplastida included not only the paralogs Rab1, Rab2, Rab5, Rab6, Rab7, Rab8, Rab11, Rab18, Rab23, Rab24, Rab28, IFT27, and RTW (=Rabl2), as suggested previously, but also Rab14 and Rab34, because Rab14 exists in glaucophytes and Rab34 is present in glaucophytes and some green algae; (ii) except in embryophytes, Rab gene duplications have been rare in Archaeplastida. Most notable is the independent emergence of divergent, possibly functionally novel, in-paralogs of Rab1 and Rab11 in several archaeplastidial lineages; (iii) recurrent gene losses have been a significant factor shaping Rab gene complements in archaeplastidial species; for example, the Rab21 paralog was lost at least six times independently within Archaeplastida, once in the lineage leading to the "core" eudicots; (iv) while the glaucophyte Cyanophora paradoxa has retained the highest number of ancestral Rab paralogs among all archaeplastidial species studied so far, rhodophytes underwent an extreme reduction of the Rab gene set along their stem lineage, resulting in only six paralogs.

# Multilocus Genotyping of *Cryptosporidium* in Two European House Mice (*Mus musculus musculus* and *Mus m. domesticus*): Preliminary Results

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A total of 578 faecal samples collected from East-European House Mice (Mus musculus musculus, MMM) and West-European House Mice (Mus m. domesticus, MMD) on the border of the Czech Republic and Germany were screened for the presence of *Cryptosporidium* spp. using microscopic examination, PCR amplifying small-subunit rRNA, 60 kDa glycoprotein (gp60) genes and microsatellites MS1, MS2 and MS16, followed by DNA sequencing. Of these, 153 specimens were Cryptosporidium PCR positive. Altogether, 5 Cryptosporidium species, namely Cryptosporidium muris, C. parvum, C. tyzzeri, C. hominis and C. ubiquitum were found in both house mice subspecies. Within C. muris two various strains C. muris RN66 and C. muris TS03 were detected by SSU. In addition, 3 subtypes of C. muris RN66 were revealed in MS1, 3 subtypes in MS2 and 2 subtypes in MS16 locus, while C. muris TS03 represented only 2 subtypes in MS1 locus and 1 subtype in MS2. In MS16 locus C. muris TS03 was not successfully amplified. Two subtypes of gp60 within C. parvum and 13 subtypes within C. tyzzeri were detected. Novel family (XIIg) within C. ubiquitum was established. No difference between males and females either in MMD or MMM, were observed. MMD was significantly more often infected with Cryptosporidium spp. Also C. muris was more frequently detected in this mice subspecies than in MMM. The results of this study well document the Cryptosporidium diversity in both studied mice subspecies and imply the importance of synanthropic rodents as a potential source of *Cryptosporidium* for human. Acknowledgment: The study was funded by GACR 15-01090S, 206/08/0640 and GAJU 011/2013/Z.

#### Diversity of *Cryptosporidium* of Wild Rodents Genus *Rat*tus in the Czech Republic, Slovakia and Kenya

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Cryptosporidium rat genotypes I-IV, C. parvum, C. suis-like, C. scrofarum, C. tyzzeri and C. muris were described in wild rats before using molecular techniques. In our research we collected samples of rat faeces from 36 locations in Czech Republic, three locations in Slovakia and three locations in Kenya. We tested these samples by molecular and microscopical methods. There were not any Cryptosporidium positives from 46 samples from Slovakia and 31 samples from Kenya. We tested 233 samples from all over the Czech Republic and there were 24 (10.30%) positives for Cryptosporidium. None of the positive rodents had diarrhoea. Intensity of infection was microscopically determined in 6 cases ranging from  $2 \times 10^3 - 4 \times 10^5$ OPG. Phylogenetic analyses based on sequences of genes encoding small subunit rRNA, actin and Cryptosporidium occyst wall protein revealed that 18 (7.72%) wild living rats in the Czech Republic had Cryptosporidium genotype I, two (0.86%) had genotype IV, one (0.43%) had Cryptosporidium genotype II and one (0.43%) had C. andersoni. Cryptosporidium andersoni could not be simply passed through rats GIT accidentally by cross-contamination because those animals were from inside husbandry held and strictly closed for three years. It is first finding of C. and ersoni in genus Rattus. In oposite of our expectations we didn't find any other species or genotypes of Cryptosporidium including C. muris.

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### Targeting of C-tail Anchored Proteins into the Outer Membrane of the Hydrogenosome and Endoplasmic Reticulum

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The  $\alpha$ -helical C-tail anchored proteins represent a heterogenous group of membrane proteins with a large functional N-terminal domain exposed to the cytosol and a short membrane insertion at their C-terminus. These include components of the outer membrane of the organelle such as mitochondria and its relatives, namely hydrogenosomes and mitosomes, as well as some membrane proteins of the endoplasmic reticulum. Targeting signals that direct proteins into the endoplasmic reticulum and the organelle are located at the C-terminus of examined proteins. These signals consist of transmembrane domain and its flanking region with a high presence of charged residues. Targeting signals that discriminate the insertion of C-tail anchored proteins into organelles and ER are poorly understood. We partially determined signals which are in trichomonads required for (i) delivery into the hydrogenosome and endoplasmic reticulum and (ii) we targeted proteins from endoplasmic reticulum into the hydrogenosome and vice versa, from the organelle into endoplasmic reticulum.

### Mapping the Diversity of Metopids and Revealing New Marine Anaerobic Ciliates Hosting Symbionts

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Remarkably many ciliates live in marine or freshwater anoxic sediments. The ecological importance of these ciliates is indisputable, yet understanding of the diversity and their role in anoxic sediments is still very limited. Anaerobiosis has independently arisen in several lineages of ciliates; so far, anaerobes have been found in at least six ciliate classes. To deepen our knowledge about their diversity, we have cultivated over 100 strains from fresh water, brackish, and marine anoxic sediments worldwide. We determined their SSU rDNA sequences, performed protargol staining techniques, and studied light-microscopic morphology. In addition, we used transmission electron microscopy to assess the ultrastructure of some of the strains and confirmed the presence of endosymbiotic methanogens by fluorescence microcopy. We have identified over 30 species of metopids, the free-living anaerobic ciliates of the class Armophorea, and three novel species were determined. Importantly, we present new outcomes about the new deep lineage of marine anaerobic ciliates (MURANO) that we have discovered. They go through a complex life cycle, host prokaryotic endo- and ectosymbionts and their hydrogenosomes do not possess cristae. According to the SSU rDNA analysis, they are related to SAL group (Spirotrichea, Armophorea, Litostomatea) and Cariacotrichea, but form a separate lineage, possibly a novel class.

# Interdependence of tRNA Editing and Methylation at a Single Site: Keeping a Mutagenic Enzyme in Check

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In all cells tRNAs undergo extensive post-transcriptional modifications, which collectively play important roles in tRNA structure and function. RNA editing, a specialized form of modification, has a wide phylogenetic distribution and in tRNAs may either expand their decoding capacity or generate conserved structural features. We showed that tRNAThrAGU undergoes both adenosine to inosine (A to I) and cytosine to uridine (C to U) editing in the anticodon loop, while robust A to I editing was reconstituted *in vitro*, C to U editing activity was not. Yet *in vivo* the TbADAT2/3 deaminase was essential for both editing events. Adding to the puzzle was the finding that the C to U edited position is also methylated leading to formation of 3-methylcytosine (m3C) and 3-methyluridine (m3U), respectively. This finding raised the possibility that editing and methylation are interconnected events. Here we present the identification of the tRNA methylase for position 32 in tRNAThr. We show that Treconstitution of methylase activity requires the presence of the TbADAT2/3 deaminase. These findings are discussed in the context of the intracellular localization of both enzymes and how the potentially mutagenic deaminase is kept in check while it traffics to the nucleus of *T. brucei*.

#### You Are What You Eat: Metabolic Specialisation Drives Genome Evolution in Parasitic Microorganisms

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Kinetoplastids are a group of single celled eukaryotic parasites that infect an broad range of hosts, from crocodiles to coconuts. Within the kinetoplastida, different species have evolved to inhabit a diverse range of host micro-environments and each obtain their energy from a limited pool of host biomolecules. Using comparative bioinformatic approaches we show this metabolic specialisation has a significant impact on genome evolution. Specifically, we show that the ability to obtain bioligcally available nitrogen as a biproduct of energy metabolism imparts a major selective pressure on genes, non-coding sequences, protein sequences and transcript expression. By extending our analysis to bacterial parasites we demonstrate that this novel discovery is not unique to kinetoplastids, but is a general phenomenon applicable to other parasitic groups. Furthermore, we show that analysis of raw nucleotide sequences can be used to accurately predict major metabolic inputs of these parasitic microorganisms. Taken together this work provides significant new insight into the evolution of genes and genome sequences that occurs in response to metabolic specialisation.

#### Investigation of Lipid Biosynthesis in Chromera velia

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Photosynthetic alveolates *Chromera velia* and *Vitrella brassicaformis* constitute together with colpodellids the new group chrompodelids and are the closest relatives of apicomplexans. *C. velia* and *V. brassicaformis* are the photoautotrophic algae with signs of heterotrophy and facultative symbiotic relationship with stony corals. *C. velia* is easily cultivable which makes it a model organism for studying biochemistry, genetics and evolution of alveolate protist with the respect to apicomplexan parasites. Genomic research, mass spectrometry, and treatment by selective lipid pathway inhibitors were employed to obtain sufficient data for lipid pathway determination. The administration of triclosan (the selective inhibitor of bacterial FAS II) showed that thylakoid membrane collapsed due long-chain unsaturated fatty acids insufficiency. TLC caused the loss of ability to synthesize *de novo* saturated and mainly unsaturated FA longer than 16 carbons. This effect of TCL arose in 0.1 mM concentration. The impact of cerulenin (the inhibitor of different types of fatty acid synthetases) was observed only in the highest used concentration – 0.6 mM. Draw up the detailed description of FA/Lipid synthesis pathways in *C. velia* in order to identify key metabolic changes playing substantial roles in evolution from free living autotrophy to obligate parasite.

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#### Life Cycle and Ultrastructure of Paratrypanosoma

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Paratrypanosoma confusum is a novel uniflagellar monoxenous kinetoplastid discovered in the gut of mosquito *Culex pipiens*. As the most basal trypanosomatid lineage, it represents a possible evolutionary link between the free-living bodonids and obligatory parasitic trypanosomatids. In axenic culture, P. confusum occurs in at least three morphologically distinct stages. The promastigote-like motile stage with a long external flagellum transforms under certain conditions into a sessile stage equipped only with a short internal flagellum. Moreover, P. confusum forms an oval amastigote-like stage with a very short external flagellum on agar plates. Time-lapse videos proved transformation of motile stage into sessile cells, their division and shortening and disappearance of external flagellum. Presence of homologs of Ago1, Piwi1 and DCL1 genes both in genome and transcriptome together with confirmed presence of dsRNA viruses within P. confusum cells suggests that both dsRNA viruses and RNA interference coexists in ancient species which later evolved to retain only one. Paratrypanosoma exhibits social motility behavior with polarized movement of thin rays radiating from center and halting or diverting their movement to avoid contact with another cell projections. This suggests some sort of cell signaling between cells to control this group behavior. Preliminary analyzes suggest that one of the signaling molecules could be Biopterin. P. confusum is not forming sessile stage in medium without Biopterin and its absence is also affecting social motility behavior. Moreover, differential expression experiments performed on transcriptomes of promastigote-like stage and sessile stage revealed overexpression of more than 1200 genes in sessile stage.

#### Gene Transfer Accompanying the Secondary Endosymbiosis of Euglenid Plastid

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Euglenozoa consist of four groups (Kinetoplastea, Diplonemea, Symbiontida and Euglenoidea) and use various trophic strategies including autotrophy. The autotrophic euglenids contain secondary green plastids derived from a prasinophyte green alga. The fact that the plastid is specific for one clade supports the plastid-late hypothesis postulating that the plastid was gained by the ancestor of the autotrophic clade, but hypothetical scenario that the plastid acquisition happened much earlier cannot be ruled out.

Since the process of organelle acquisition should be accompanied by transfer of genes from endosymbiont to host (EGT), the presence of such genes provides an indication of past endosymbiosis. We are analyzing transcriptomes of primarily osmotrophic euglenid *Rhabdomonas costata* and autotrophic *Eutreptiella gymnastica*, *Euglena gracilis* for the amount of EGT derived genes. Using semiautomatic pipeline, we have selected transcripts of genes putatively related to algae. The selection was based on BLASTing against local database followed by maximum likelihood phylogenetic analysis of euglenid gene together with its homologues from the local database. The phylogenetic position of selected candidates was verified by re-analysis to determine bootstrap support. In case of *Rhabdomonas* and *Eutreptiella*, 63 and 7,508 candidates, respectively, were produced by the first round of selection which represents 0.9% and 10% of transcripts. In case of *Rhabdomonas*, only 11 genes were found robustly (BS  $\geq 75\%$ ) related to algae after the re-analysis of *Eutreptiella* candidates and *Euglena* is in progress. The preliminary results support the plastid-late hypothesis for euglenid plastid origin.

#### Reconstruction of Anaerobic CIA Pathway in *Trichomo*nas vaginalis

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The key pathways of iron-sulfur (FeS) cluster assembly are extensively studying in model organisms such as Saccharomyces cerevisiae. However, their character is not fully elucidated in anaerobic protists such as a human pathogen Trichomonas vaginalis. Although both the hydrogenosomal and the Cytosolic Iron-Sulfur assembly (CIA) pathways in trichomonads are similar to the corresponding yeast machineries, they are different in two characters: (i) most of key protein components are present in multiple copies, while (ii) other componets are absent. Here we focused on investigation of the CIA pathways. We have identified 8 CIA components including two paralogs for Cia1 (A,B), Nbp35 (A,B) and Cfd1 (A,B) proteins, while Nar1 and Cia2 (Mip18) are present in a single copy. Cell localization studies of Ha-tag CIA components confirmed cytosolic localization for Cfd1-A, Cia1-A, Nar1 and Nbp35-A, B proteins. Interestingly, Nar1 and Cfd1-A are partially associated with outer hydrogenosomal membrane. Moreover, Cia1-A protein seems to be dually localized in the cytosol and the hydrogenosomal matrix. The CIA components that are apparently absent in T. vaginalis include Dre2, MMS19, Grx3/4. Tah18 was previously suggested to be fused with Nar1. However, our analysis revealed that Tah18 is absent and the previously reported fused protein represents most likely the hydrogenase with cytochrome p450 reductase domain. Absence of Dre2 and Tah18 seems to be a general trend of anaerobic protists.

#### Bioenergetics of the Bloodstream *T. brucei* Mitochondrion: a New Look

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The infective bloodstream stage (BS) of Trypanosoma brucei possesses a single mitochondrion that lacks a cytochrome-mediated respiratory chain and thus employs the hydrolytic activity of  $F_0F_1$ -ATPase to maintain the essential mitochondrial (mt) membrane potential ( $\Delta\psi$ m). Meanwhile, dyskinetoplastic (Dk) trypanosomes lacking the mt encoded A6 that is essential for the functional  $F_0$  proton pore alternatively maintain their  $\Delta\psi$ m by combining the hydrolytic activity of the matrix-facing  $F_1$ -ATPase and the electrogenic exchange of ATP4- for ADP3- by the ADP/ATP carrier (AAC). Interestingly, the EC50 values of AAC inhibitor atractyloside are approximately 100× higher in BS cells compared to EC50 values measured for Dk trypanosomes. This result would suggest that AAC activity is not as important for BS as for Dk cells and thus the ATP for maintaining the  $\Delta\psi$ m in BS cells is provided by mt substrate phosphorylation pathway(s). Indeed, RNAi silencing of AAC in BS trypanosomes has no effect on growth *in vitro*. The alternative sources of mt ATP will be discussed and revisited mt energy metabolism map of the infectious stage of the parasite will be presented.

### The Morphological and Molecular Characterisation of *Ce*ratomyxa Species from Clinid Fish from the Tip of Africa

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Intertidal rock pools harbour large numbers of fishes and invertebrates, which are well adapted to life in harsh conditions with a strong fluctuation of water temperature and salinity. These rock pools with close contact of fish and invertebrates may represent ideal conditions for myxosporean life cycles. We focused on different host species – clinid fish (Clinus acuminatus, Clinus brevicristatus, Clinus cottoides, Clinus superciliosus, Muraenoclinus dorsalis) collected from the rock pools from different localities along the temperature gradient of the South African coast in the Atlantic and Indian Oceans. Species of the genus Ceratomyxa were found in host gall bladders. *Ceratomyxa*, the second most numerous myxosporean genus, has crescent shape of spores with two polar capsules. Its species are generally strongly host specific. Ceratomyxids are morphologically uniform and individual species commonly differ by host preference and sequences. The intraspecific variability in ceratomyxids based on their SSU rDNA is lower (up to 0.3%) than in the remaining myxozoan genera (1%). We morphologically and molecularly characterise *Ceratomyxa* species from several clinid fish species and localities. Based on molecular data (rDNA), seven species were found in five fish hosts in single as well as in co-infections. Four of *Ceratomyxa* species were strictly host specific, on the contrary, one ceratomyxid was found in four fish hosts. Interestingly, Clinus superciliosus was infected by six Ceratomyxa species in three different localities. Phylogenetically, all ceratomyxids from clinid fish are closely related and sister to ceratomyxids from labrid fishes.

#### Diversity of the Genus Monocercomonoides

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Monocercomonoides sp. is a genus of small flagellates belonging to the order Oxymonadida. They live as intestinal endosymbionts of insects, but some of them can be found also in the intestine of vertebrates. In this work we sequenced the SSU rRNA of 25 different strains of *Monocercomonoies* isolated from various insect and vertebrate hosts and from unused cesspit. We have performed phylogenetic analysis in order to understand the diversity of this genus. Our preliminary results indicate large variation among strains at the genetic level (up to 53.7% of nucleotide differences). All strains formed one clade, however, strains NAU1, NAU3 and Z17NC from cockroach *Nauphoeta cinerea* and Z31SE from cockroach *Enterobius* sp. are relatively distant to the rest and they may represent a new genus. Morphological analysis of selected strains using light and electron microscopy is under way.

### Conserved Putative Mitochondrial Ribosomal Proteins Important for Mitochondrial Translation in *Trypanosoma brucei*

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Mitochondrion is an indispensable organelle for most eukaryotic cells. The most important role of a typical mitochondrion is production of ATP. Since a handful of proteins is coded by the mitochondrion the organelle had to keep vast replication, transcription and translation machineries. In our survey in which we were looking for highly conserved mitochondrial proteins with unknown function between human and T. *brucei*, we have found three proteins that according to TAP-tag analysis have putative connection to the mitochondrial ribosomes. T. *brucei* is a suitable model organism for studying mitochondrial ribosomes and their function because RNA interference and assays addressing mitochondrial translation and ribosome stability are already available. Down-regulation of targeted genes was shown by qPCR. Preliminary data shows effect on mitochondrial translation *in vivo* with intact transcription and editing machineries. Levels of 9S or 12S rRNAs were down-regulated suggesting that ribosome integrity has be affected.

#### Iron Sulphur Cluster Assembly in Monocercomonoides

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Oxymonads are a group of anaerobic or microaerophilic protists living in guts of insects and vertebrates. They are considered to be the largest group of eukaryotes without confirmed presence of mitochondrion or related organelle, although their closest free-living relative Trimastix has organelles which are similar to hydrogenosomes. In the search for mitochondrion in oxymonads we have sequenced the genomes and transcriptomes of the oxymonad Monocercomonoides strain Pa203. Surprisingly we did not identify any transcripts/genes involved in the mitochondrial pathway of iron-sulphur (FeS) cluster assembly (ISC) which is ubiquitous in eukaryotes. Instead, we found genes for subunits of Sulphur mobilisation machinery (SUF) and a set of genes for proteins functioning in cytosolic FeS cluster assembly (CIA) pathway. SUF pathway is in eukaryotes known only from plastids and two protists unrelated to oxymonads – Blastocystis hominis and Pygsuia biforma. In the genome of Monocercomonoides we identified four subunits of SUF system. Two subunits SufS and SufU are fused into one gene. Genes for SufC and SufSU contain classical spliceosomal introns. All subunits of SUF system in *Monocercomonoides* seem to contain all important catalytic sites needed for their function in FeS cluster assembly. Phylogenetic analysis of concatenated subunits of SufC, SufB and SufS proved that Monocercomonoides acquired SUF system from a different source than Pygsuia, Blastocystis and plastid containing eukaryotes. Heterologous localisation of SufB and SufC in Trichomonas vaginalis expression system showed cytosolic localisation and this localisation was confirmed by specific antibodies against SufSU in the cell of Monocercomonoides. Our results indicate that Monocercomonoides is the first known organism, which assemble FeS clusters in the cytosol by concerted action of SUF and CIA pathways.

### Functional Analysis of Novel $F_1$ ATPase Subunit in Trypanosoma brucei

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 $F_0F_1$ -ATP synthase is a splendid molecular machine that produces ATP in bacteria and eukaryotic organelles. This complex is composed of the catalytic  $F_1$  moiety and membrane-bound  $F_{o}$  moiety. The composition of the catalytic  $F_{1}$  ATPase is extremely conserved between eukaryotes and includes subunits  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ . No additional subunits were ever assigned to this moiety in any of the studied model organisms. In a parasitic protist, Trypanosoma brucei, the  $F_0F_1$  ATP synthase/ATPase, differs in its function and activity between the insect (procyclic form, PF) and the mammalian (bloodstream form, BF) life stages. In PF cells this complex contributes to the total ATP production by oxidative phosphorylation while in BF cells it maintains the mitochondrial membrane potential ( $\Delta \psi m$ ). In contrast to other eukaryotes, a novel euglenozoa-specific subunit (p18) was purified together with the T. brucei  $F_1$  ATPase. To investigate if this protein is a bona fide subunit of  $F_1$  ATPase, the p18 expression was knocked-down in PF and BF trypanosomes. Our data suggest that p18 is important for growth of PF cells and crucial for BF cells viability. This observation is in agreement with the proposed essential function of  $F_0F_1$  ATPase in the BF cells. Importantly, the stability of the  $F_1$  moiety was strongly affected in both PF and BF RNAi induced cells suggesting that p18 subunit is criticial for F1 ATPase structural integrity. As expected, the  $\Delta \psi$ m was increased in PF cells while significantly decreased in BF cells in the absence of p18. In conclusion, our data suggest that p18 is novel subunit of  $F_1$  ATPase and this proposition breaks the long-standing conception of the strict conservancy of the  $F_1$ -ATPase complex in eukaryotes.

## *In silico* Evidence for Tic21 and Tic32 Subunits of TIC Translocase Complex in Euglenids

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Euglenophytes are inner group of euglenids characterized by photoautotrophy and presence of green three-membrane-bound secondary plastid derived from prasinophyte ancestor. Secondary and higher plastids are present in many unrelated protist groups yet their protein targeting seems to have developed in a remarkably convergent way, reflecting the import system already present in the primary plastid and the general endosymbiont-host system features. Components of the TOC/TIC system of the innermost membranes, which are homologous to primary plastid envelope, have been identified in most secondary and higher algae at least on the genomic level. Import across the additional membrane(s) often proceeds through ER or ER-derived pathway. The presence of targeting sequence in majority of transcripts destined to plastid suggests that TOC/TIC-based system mediates protein transport across the inner membranes of euglenid plastid. Both complexes are probably reduced and/or divergent in primary structure because no component has been identified yet. Import across the third membrane is known to involve vesicles whose recognition and fusion mechanism remains unclear. Using HMM-based algorithm we found in silico evidence for Tic21 and Tic32 subunits in *Euglena gracilis* transcriptome. Both subunits were also found in Eutreptiella gymnastica (marine species, phylogenetically distant from E. gracilis), Tic32 was found in Euglena longa (secondarily heterotrophic species closely related to E. gracilis) and Tic21 in Rapaza viridis (mixotrophic euglenid, sister taxon to all other euglenophytes). Localization experiments in *E. gracilis* are underway.

### Hydrogenosomes of Trichomonas vaginalis and tRNA

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Trichomonas vaginalis is an anaerobic parasite of human urogenital tract. Instead of a regular mitochondria, the parasite bears hydrogenosomes. Living under anaerobic conditions led to loss of an organellar DNA and subsequently of the classical respiratory chain enzymes and associated proteins and metabolic pathways. Also, the loss of the DNA was inevitably followed by a loss of transcription and translation machineries including ribosomes, r- and tRNAs. Surprisingly, a tRNA was identified by sequencing of RNAs protected from RNAse treatment in isolated hydrogenosomes. This led us to further study the observed RNAse protection and the putative import of an in vitro-synthetized tRNA into isolated hydrogenosomes of T. vaginalis.

# Bloodstream *T. brucei* Adapts to Decreased Levels of Functional $F_0F_1$ -ATPase subunit A6

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The infective bloodstream stage (BS) of Trypanosoma brucei possesses a single mitochondrion that lacks cytochrome-containing respiratory complexes III and IV and thus employs the F<sub>o</sub>F<sub>1</sub>-ATPase to hydrolyze matrix ATP to maintain the essential mitochondrial (mt) membrane potential ( $\Delta \psi m$ ). Due to this reduced organellar function, it is currently presumed that out of the 18 (mt) encoded proteins, only A6 (a subunit of  $F_0F_1$ -ATPase) and possibly RPS12 (a ribosomal subunit) are essential in BS trypanosomes. However, dyskinetoplastic trypanosomes, parasites locked in the bloodstream stage because they have lost their mtDNA, have found a way to maintain their  $\Delta \psi$ m with F<sub>o</sub>F<sub>1</sub>-ATPase lacking the A6 proton pore. To explore how BS T. brucei cope with a decrease in functional A6, we generated a double knock-out cell line for the mt peptide release factor ( $\Delta TbMrf1$ ). This interference with the mt translation machinery resulted in a  $\Delta$ TbMrf1 cell line with a decreased  $\Delta \psi$ m, a decreased abundance of the  $F_0F_1$ -ATPase, and an increased sensitivity to  $F_0F_1$ -ATPase and ATP/ADP carrier inhibitors. Furthermore, while the  $\Delta$ TbMrf1 cells are viable when cultivated in vitro, it appears that they are less virulent in the mouse model, suggesting a greater reliance on mt metabolism in vivo. Importantly, in vitro cultivation over several weeks dissipates the decreased  $\Delta \psi m$  phenotype, perhaps as the cells adapt to the depletion of functional A6 by unknown mechanism. Recently, the release factor family protein ICT1 was described to act as a mito ribosome recycling factor in case of stalled translation in human mitochondria. The function of TbICT1 homologue in  $\Delta$ TbMrf1 background is currently investigated.

# Equine Cryptosporidiosis and Microsporidiosis: Minire-view

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A total of 1176 individual fecal samples from horses (n=648 from Czech Republic, n=81 from Poland, n=90 from USA, n=357 from Algeria) were screened for the presence of *Cryptosporidium* spp., *Encephalitozoon* spp. and *Enterocytozoon bieneusi* DNA by genus-specific nested PCR during the years 2011–2015. The highest prevalence of screened parasites was observed in horses kept in Czech Republic (microsporidia) and USA (*Cryptosporidium*). Three genotypes of *E. cuniculi* (I, II and III) and 16 genotypes of *E. bieneusi* including seven previously described and nine novel genotypes were detected. The most common genotype detected was *E. bieneusi* genotype D identified in 43.2% (35/81) of positive horses. Our results revealed the susceptibility of horses to six *Cryptosporidium* species: *Cryptosporidium* horse genotype, *C. parvum*, *C. muris*, *C. tyzzeri*, *C. erinacei* and *C. hominis*. The infections were not associated with diarrhoea. In addition, to determine the course of infection, 9 one year old ponies were used for the experimental infection with *E. cuniculi* genotype II. Acute microsporidiosis in ponies was characterized by the dissemination of microsporidia into almost all organs and significant increase of concentration of specific antibodies in blood was observed from 28 to 42 DPI.

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# Reconstruction of Chloroplast Proteome of the Earliest Branching Phototrophic Euglenid, *Rapaza viridis* Based on Transcriptomic Data

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The chloroplasts of phototrophic euglenids originated through a secondary endosymbiotic relationship between a phagotrophic euglenid and a prasinophyte-like green alga. Molecular phylogenetic analyses revealed that the chloroplast of *Pyramimonas* is a close approximate of the most recent ancestor of all euglenid chloroplasts. Nonetheless, there is still substantial missing data that limit our ability to fully portray the origin and early evolution of euglenid plastids. A mixotrophic (phototrophy plus phagotrophy) euglenid, Rapaza viridis, was described as a new species in 2012. This microalga possesses functional chloroplasts and consume a specific strain of a prasinophyte alga, *Tetraselmis* sp. Behavioral and ultrastructural data, and molecular phylogeny analyses of this euglenid flagellate demonstrated the intermediate features between phototrophic euglenids and phagotrophic lineages. In order to study the evolutionary history of secondary plastid endosymbiosis in euglenids, we sequenced transcriptome from Rapaza viridis and assembled it into 107,092 transcripts. 8,875 transcripts contained euglenid specific splice leader at the 5' end indicating their completeness and these were selected for further analyses. Based on automatic annotations and further manual analyses, we hitherto identified 66 sequences encoding putative plastid-targeted proteins. Phylogenetic analyses of this dataset will help to indentify algae that provided the plastid to Rapaza as well as infer the evolutionary history of plastid targeted proteins among phototrophic euglenids.

## Origin of Dixenous Trypanosomatids: Lessons from Their Monoxenous Cousins

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The origin of the dixenous (= two hosts) life cycle in trypanosomatids still remains a mystery. It is generally accepted that dixenous species have evolved from their monoxenous kins and this transition has happened several times during evolution of Trypanosomatidae -- independent origin of genera Trypanosoma, Leishmania and Phytomonas. This suggests that such an evolutionary event might be "caught in action", as some (presumably) monoxenous species may occasionally try switching to dixeny. Indeed, the presence of monoxenous trypanosomatids in vertebrates has been noted about 100 years ago. Clinical cases documented late are more solid. They identified several monoxenous species of Trypanosomatidae belonging to genera Herpetomonas, Crithidia, Leptomonas and Blechomonas in humans. Importantly, most such cases involved immune-compromised individuals leading to a hypothesis that these usually non-infectious species may explore new ecological niches when the host's immune system is suppressed. Within this paradigm, numerous cases of monoxenous trypanosomatids co-infecting along with *Leishmania* spp. have been reported. Most of them implicated causative agents of visceral leishmaniasis of the L. donovani complex. Both dixenous and monoxenous parasites may potentially be transmitted by the same Phlebotomus spp. vector. The species most often recovered in co-infections with *Leishmania* is *Leptomo*nas seymouri. In this work have demonstrated that Leptomonas seymouri can withstand elevated temperatures in vitro, sequenced its genome, and assessed transcriptional profiles of the parasites cultivated in different conditions, as well as showed its capacity to survive in the *Phlebotomus* vectors implicated in *Leishmania* transmission.

# Morphological and Molecular Diversity of the Neglected Genus *Rhizomastix* Alexeieff, 1911 (Amoebozoa: Archamoebae)

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The poorly-known genus Rhizomastix comprises a moeboid heterotrophic flagellates living mostly as intestinal commensals of insects, amphibians or reptiles. One species was also found in human feces, and two Rhizomastix species were isolated from organic freshwater sediments. Recent phylogenetic analyses confirmed a previous hypothesis that the genus belongs to the Archamoebae and might be closely related to the parasitic genus Entamoeba. We determined six new SSU rRNA and 15 actin gene sequences of strains of Rhizomastix. We examined morphology of five of these strains by light-microscopy and ultrastructure of one of them. Four new species of this genus were described. Our data show that Rhizomastix is monophyletic and splits into free-living and endobiotic lineage. In addition, Rhizomastixidae form a sister branch of Entamoebidae. Based on our results we reveal that the endobiotic lifestyle has arisen at least twice, but likely more times, independently within the Archamoebae.

### Unraveling the Enigmatic Non-Photosynthetic Plastid of Euglena longa (Euglenophyceae, Euglenozoa)

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Euglenophytes bear a secondary plastid of green algal origin. Euglena longa, a relative of the model photosynthetic species Euglena gracilis, possesses a cryptic non-photosynthetic plastid, whose genome surprisingly encodes the large subunit of the CO<sub>2</sub>-fixing enzyme Ru-BisCO (RBCL). Although our phylogenetic analysis demonstrates that the E. longa RBCL sequence is highly divergent, transcriptome sequencing revealed the existence of a nuclear gene for RbcS (RuBisCO small subunit) that, similarly to E. gracilis, encodes a precursor polyprotein comprising an N-terminal plastid-targeting sequence and an array of RBCS units separated by decapeptide linkers. A plastid-targeted RuBisCO activase is also encoded by the E. longa nuclear genome. Heterologous anti-RBCL and anti-RBCS antibodies confirmed the synthesis of RBCL and the RBCS precursor in E. longa, but at a much lower abundance than in E. gracilis, and no RBCS monomer could be detected. While the rbcL transcript level correlated with the protein abundance in the two Euglena species, RbcS transcript levels were comparable and the low RBCS abundance in E. longa was due to its rapid turnover. Surprisingly, the E. longa transcriptome revealed the presence of enzymes for the synthesis of galactolipids (MGDG and DGDG) that are critical for proper thylakoid function. Interestingly, at least one of these enzymes is phylogenetically affiliated with homologs from haptophytes rather than green algae. Both galactolipids were detected in lipid extracts from E. longa and an anti-DGDG antibody labelled a number of spots in E. longa cells that might be the first visualization of its plastids. The presence of attributes of photosynthetic plastids like RuBisCO and galactolipids in E. longa remains puzzling.

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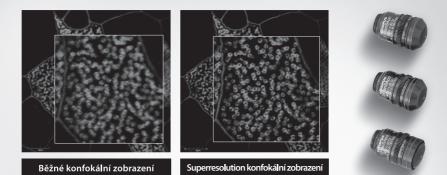




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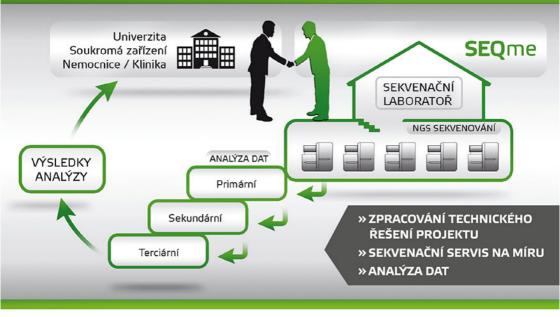
- > Nová ergonomie madla umožní snížení sil potřebných na otevření a zavření dveří
- > Vnitřní izolované dveře jsou zajištěny magnetickými bezpečnostními uzávěry

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# NEXT-GENERATION Sekvenování



# NABÍZÍME SPOLUPRÁCI PŘI NÁVRHU VAŠICH SEKVE-NAČNÍCH PROJEKTŮ, DOPORUČÍME VÁM OPTIMÁLNÍ TECHNICKÉ ŘEŠENÍ A PROVEDEME SEKVENOVÁNÍ TECHNOLOGIEMI NGS VČETNĚ ANALÝZY DAT.

- » All-inclusive Dodáte vstupní materiál (DNA, RNA). My zajistíme zpracování vašich vzorků, přípravu knihoven, sekvenování na vybrané platformě a v případě zájmu i analýzu dat.
- » Ready-to-Run Pokud máte zkušenosti s NEXT-GENERATION sekvenováním, můžete si zajistit přípravu knihoven sami. Na nás je vlastní sekvenování a v případě zájmu i analýza dat.
- » Analýza dat Máte již data od jiného poskytovatele a nevíte si rady s jejich zpracováním? Provedeme pro vás a v těsné spolupráci s vámi jejich analýzu.

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#### PŘÍSTROJOVÉ PLATFORMY:

- » HiSeq2500 (Illumina) Nejvyšší kapacita. Nejnižší cena za bázi.
- » Ion Proton (Life Technologies) Projekty nižší náročnosti. Zlatá střední cesta.
- » Ion PGM (Life Technologies) Ekonomické řešení pro malé projekty.

NGS sekvenování lze kdykoliv předplatit!!!



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## Laboratory equipment:

pipettes, balances, pH meters centrifuges, cleaners, pumps bathes, shakers, thermoblocks sterilization, decontamination (ClO<sub>2</sub>) refrigerators, freezers, deep freezers laminary flow and biosafety cabinets plantgrowth and climate chambers bioreactors, fermentors water purification

# **Oligonucleotide synthesis:**

primers and assays probes and quenchers DNA/RNA synthesis modifications and freedom dyes custom gene synthesis gBlocks gene fragments

# Special devices:

freeze dryers, concentrators PCR devices, cell counters electrophoresis and blotting power supplies, microscopes nucleid acid isolators detection of mutation microarray spectrophotometers in-vivo imaging, software

## **Reagents:**

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# Laboratory plastic:

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