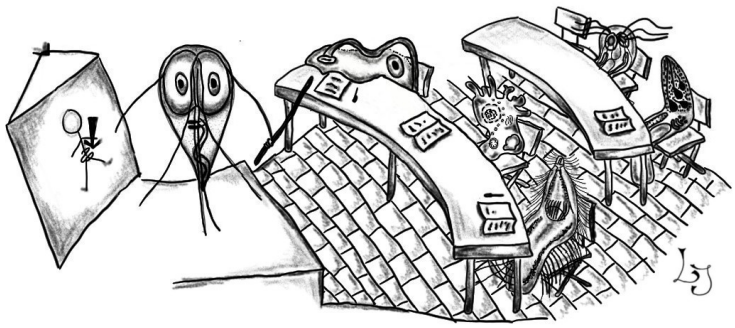
43rd Jírovec's Protozoological Days

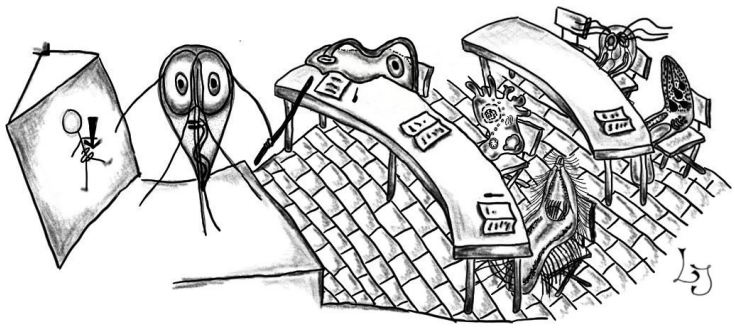
Conference Proceedings



Institute of Parasitology
Biology Centre of the Academy of Sciences of the Czech Republic, v. v. i.
České Budějovice 2013

43rd Jírovec's Protozoological Days

Conference Proceedings



Institute of Parasitology
Biology Centre of the Academy of Sciences of the Czech Republic, v. v. i.
České Budějovice 2013

43rd Jírovec's Protozoological Days
Conference Proceeding
This publication did not undergone language editing.

ISBN 978-80-260-4294-5

Obsah

Foreword

Dear Friends of Czech protozoology,

It is my honor and pleasure to welcome you to the 43rd Jírovec's Protozoological Days of Czech Society of Parasitology, specifically its Protozoological section. This year's meeting will be held at the scenic confluence of the Vltava and Lužnice rivers in South Bohemia, one of the important outposts of Czech protozoology.

Protodays is a traditional Czech conference and a meeting place for both beginning students, for which it is often the first experience with a conference environment, as well as their already seasoned older colleagues. For many years it is an important event, during which protistologists exchange their knowledge, share information and experience, and the unity and cohesion of the community is maintained.

Protodays have already undergone a long way and I am convinced that the time is ripe for another change and that is the transition to the language of science of today – the English. My ambition is not to transform the protodays into the international conference, contrariwise, I would like to keep it at the level as it is now with its family and relaxed atmosphere of friendship, where most people are already familiar with each other, but which still allows as to welcome new members among us as well. And that's the important point. Czech Republic is not anymore just a provincial small country on the border of two opposing and competing ideologies, far away from the rest of the world. On the contrary, we are back in the center of Europe, where we undoubtedly belong to. And we are not anymore close-knit community consisting only of Czechs and Slovaks. In our universities and institutions the number of students, postdocs and even PI's from abroad is increasing. And that is a really good thing for Czech science. These people want to be and they are a part of the Czech scientific and Protozoological community. However, their Czech is not at the level to attend a meeting in the Czech language. By clinging to the Czech language, we exclude these people from our midst, which cannot be beneficial for any of the parts.

This is related to the second, but not less important, reason. And that is the need of English language skills in the field of science for Czech students. Knowledge of English is a necessity today, but unfortunately, the Czech Republic still has not reached the level of Western Europe. English at protodays should be important for the students firstly to practice presenting in this language and secondly to realize the weak points and the need for improvement. Science always has been and will be based on communication. My intention

is the protodays to remain the Czech protozoologists conference, but not only for ethnic Czechs and Slovaks, but for all scientists working in the field of protozoology in the Czech and Slovak territory. And thus continues to fulfill its important function.

I hope that my efforts will meet with understanding and support from your side and I offer this topic for further discussion.

I wish you many unforgettable moments and a lot of new experiences at this year protodays.

Jiří Týč

List of Participants

Name	E-mail	Institut
Bartošová Pavla	bartosova@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Basu Somsuvro	somsuvro@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Birčák Erik	bircak@fns.uniba.sk	Comenius University in Bratislava, Faculty of Natural Sciences, Mlynská dolina, 842 15 Bratislava 4
Blanár Jozef	joziblanar@hotmail.com	University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Cihlář Jaromír	jerremy1@seznam.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Čepička Ivan	ivan.cepicka@centrum.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
David Vojtěch	mortis.gw@seznam.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Diakin Andrei	ondredyakin@gmail.com	Masaryk University, Faculty of Science, Department of Botany and Zoology, Kotlářská 267/2, 611 37 Brno
Dobáková Eva	dobakova@fns.uniba.sk	Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Mlynská dolina, 842 15 Bratislava 4

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Doležal Pavel	pavel.dolezal@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Drašnarová Zuzana	drasnarova.zuzana@seznam.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Dvořáková Nela	nelka@volny.cz	University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Hygiene and Ecology, Department of Biology and Wildlife Diseases, Palackého třída 1/3, 612 42 Brno
Eliáš Marek	marek.elias@osu.cz	University of Ostrava, Faculty of Science, Department of Biology and Ecology, Dvořákova 7, 701 03 Ostrava
Esson Heather	esson@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Ferencová Blanka	ferencovablanka@seznam.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Fiala Ivan	fiala@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Fialová Markéta	majki007@seznam.cz	Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Praha 2
Füssy Zoltán	zoltan@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Gondek Agata	a.gondek@student.uw.edu.pl	University of Warsaw, Faculty of Biology, Department of Plant Systematics and Geography, ul. Krakowskie Przedmieście 26/28, 00-927 Warszawa, Polska
Hadariová Lucia	luciahadariova@gmail.com	Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Mlynská dolina, 842 15 Bratislava 4
Hampl Vladimír	vlada@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Hofmannová Lada	hofmannoval@vfu.cz	University of Veterinary and Pharmaceutical Sciences, Department of Pathology and Parasitology, Palackého třída 1/3, 612 42 Brno
Horváth Anton	horvath@fns.uniba.sk	Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Mlynská dolina, 842 15 Bratislava 4
Horváthová Lenka	lenka@horvath.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Hostomská Jitka	jitka.hostomska@centrum.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Huang Zhenqiu	zhenhuang85@gmail.com	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Jalovecká Marie	maruska.jalovecka@seznam.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Jirků Milan	jirku@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Karnkowska Anna	karnkowa@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Kostka Martin	mkostka@centrum.cz	University of South Bohemia, Faculty of Science, Branišovská 31, 370 05 České Budějovice
Kotrbová Zuzana	z.kotrbova@centrum.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Kováčová Bianka	bian.kovacova@gmail.com	Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Mlynská dolina, 842 15 Bratislava 4
Krajčovič Juraj	krajcovic@fns.uniba.sk	Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Mlynská dolina, 842 15 Bratislava 4
Kručinská Jitka	jitka.krucinska@gmail.com	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Kulda Jaroslav	kulda@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Lorencová Markéta	marketalorencova@seznam.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
Lukeš Julius	jula@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Łukomska--Kowlaczyk Maja	majalukomska@biol.uw.edu.pl	University of Warsaw, Faculty of Biology, Department of Plant Systematics and Geography, ul. Krakowskie Przedmieście 26/28, 00-927 Warszawa, Polska
Martinek Jan	martinekjann@seznam.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Melicherová Janka	jankam@sci.muni.cz	Masaryk University, Faculty of Science, Department of Botany and Zoology, Kotlářská 267/2, 611 37 Brno
Michalek Jan	michalek@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Milanowski Rafał	milan@biol.uw.edu.pl	University of Warsaw, Faculty of Biology, Department of Plant Systematics and Geography, ul. Krakowskie Przedmieście 26/28, 00-927 Warszawa, Polska
Mutinová Petra	petra@muti.cz	Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Praha 2
Najdrová Vladimíra	najdrova.vladimira@seznam.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Novák Lukáš	niun42@gmail.com	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Nývltová Eva	evicka.ny@gmail.com	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Oborník Miroslav	obornik@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Pánek Tomáš	mistrpanek@seznam.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
Patra Sneha	snehampatra@gmail.com	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Pažoutová Marie	marie.pazoutova@gmail.com	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Polanská Nikola	polanskn@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Poliak Pavel	poliak@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Pomajbíková Kateřina	kpomajbik@gmail.com	University of Veterinary and Pharmaceutical Sciences, Department of Pathology and Parasitology, Palackého třída 1/3, 612 42 Brno
Procházková Kateřina	suri.kata@seznam.cz	Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Praha 2
Ptáčková Eliška	eliska.ptackova@centrum.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
Pyrih Jan	jan.pyrih@gmail.com	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Rada Petr	radapetr81@gmail.com	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Rašková Veronika	verona.rasca@centrum.cz	University of South Bohemia, Faculty of Agriculture, Studentská 13, 370 05 České Budějovice
Rmoutilová Eva	liska@rmail.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
Rotterová Johana	morganit@centrum.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
Roubalová Eva	roubalova@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Schneedorferová Ivana	ivana.schneedorferova@email.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Skalický Tomáš	tomas.skalicky@seznam.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Smejkalová Pavla	pvl.smejkalova@seznam.cz	Charles University in Prague, Faculty of Science, Department of Zoology, Viničná 7, 128 44 Praha 2
Smutná Tamara	vychodslnka@post.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Sojka Daniel	sojkadan@gmail.com	University of Geneva, Faculty of Medicine CMU/ Dpt MIMOL Rue Michel-Servet 1211 Genève 4 Suisse

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Soukal Petr	soukp4am@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Stojanovová Darja	darja.stojanovova@centrum.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Svobodová Milena	milena@natur.cuni.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Szabová Jana	janca.sz@centrum.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Ševčíková Tereza	TesaS@seznam.cz	Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Praha 2
Šíma Michal	michal-sima@seznam.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Šubrtová Karolína	caroli@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Ťápal Jiří	tapal.jiri@gmail.com	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Tomčala Aleš	a.tomcala@centrum.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Tůmová Pavla	ptumo@lf1.cuni.cz	Charles University in Prague, First Faculty of Medicine, Department of Tropical Medicine, Studničkova 7, 128 00 Praha 2

Continued on next page.

Continued from previous page.

Name	E-mail	Institut
Týč Jiří	imejl.xyz@centrum.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Vacek Vojtěch	messr@centrum.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Valigurová Andrea	andreav@sci.muni.cz	Masaryk University, Faculty of Science, Department of Botany and Zoology, Kotlářská 267/2, 611 37 Brno
Vanclová Anna	vanclova@gmail.com	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2
Verner Zdeněk	danken_mclaude@yahoo.com	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovicea Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Mlynská dolina, 842 15 Bratislava 4
Veselíková Michaela	veselmi@paru.cas.cz	Biology Centre ASCR, v. v. i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice
Vesteg Matej	vesteg@fns.uniba.sk	University of Ostrava, Faculty of Science, Department of Biology and Ecology, Dvořákova 7, 701 03 Ostrava/ Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Mlynská dolina, 842 15 Bratislava 4
Vobořilová Pavlína	pavlina.voborilova@seznam.cz	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2

Continued on next page.

Continued from previous page.

Name	**E-mail**	**Institut**
Wagnerová Pavla	pavlaacenta@seznam.cz	University of South Bohemia, Faculty of Agriculture, Studentská 13, 370 05 České Budějovice
Wencelová Monika	wencelova@saske.sk	Slovak Academy of Sciences, Institute of Animal Physiology, Šoltésovej 4–6, 040 01 Košice
Záhonová Kristína	zahonova.kristina@gmail.com	University of Ostrava, Faculty of Science, Department of Biology and Ecology, Dvořákova 7, 701 03 Ostrava
Žárský Vojtěch	zarsky1@gmail.com	Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Praha 2

Programm Schedule

Monday May 6, 2013	
16:00	Registration
18:00	*Dinner*

Thuesday May 7, 2013	
8:00	*Breakfast*
9:15	Opening of 43. Jírovec's Protozoological Days
	Molecular Biology of Protozoa
9:25	Somsuvro Basu, Daili J. Netz, Alexander Haindrich, Nils Herleth, Thibaut J. Lagny, Roland Lill, Antonio J. Pierik & Julius Lukeš: **Cytosolic Iron--Sulfur Cluster Assembly in *Trypanosoma brucei***
9:45	Jan Pyrih, Eva Martincová & Jan Tachezy: **How to Make Iron-Sulphur Clusters within the Mitosome of *Giardia intestinalis***
10:05	Vojtěch Vacek, Lukáš Novák, Zuzana Zubáčová, Miluše Hroudová, Čestmír Vlček & Vladimír Hampl: **Suf System for Iron Sulphur Cluster Assembly in *Monocercomonoides* (Oxymonads)**
10:25	*Coffee break*
	Veterinary and Human Protozoology
10:40	Kateřina Pomajbíková, Kateřina Schovancová, Petr Procházka, David Modrý, Petra Bolechová & Klára J. Petrželková: **Does Dietary Starch Have an Impact on *Neobalantidium coli* Infections in Captive Chimpanzees?**
11:00	Martin Kváč, John McEvoy, Martina Loudová, Brianna Stenger, Bohumil Sak, Dana Květoňová, Oleg Ditrich, Veronika Rašková, Elaine Moriarty, Michael Rost, Miloš Macholán & Jaroslav Piálek: **Coevolution of *Cryptosporidium tyzzeri* and the House Mouse**
11:20	Pavla Wagnerová, Bohumil Sak, Dana Květoňová, Martin Kváč & Iva Langrová: **Occurrence, Prevalence and Progression of Microsporidial Infection in Horses and Ponies from Czech Republic**
11:40	SPONZOR presentation – ROCHE
12:00	*Lunch*

Molecular Biology of Protozoa	
13:20	Jiří Ťápal, Lucie Kafková, Julius Lukeš & Hassan Hashimi: **Functional Analysis of TbFis1 Protein in *T. brucei***
13:40	Zuzana Kotrbová, Brian Panicucci, Dana Hocková & Alena Zíková: **Enzymes of Purine Salvage Pathway in *Trypanosoma brucei* and the Trypanocidal Action of Acyclic Nucleoside Phosphonates**
14:00	Vladimíra Najdrová & Pavel Doležal: **PUF proteins in *Giardia intestinalis***
14:20	SPONZOR presentation – Life Technologies
14:30	*Coffee break*
14:45	Poster Session A
Cell Biology of Protozoa	
16:00	Petr Rada, Ivan Hrdý & Jan Tachezy: **Glycolytic Enzyme Phosphofructokinase Is Targeted to Hydrogenosomes of *Trichomonas vaginalis***
16:20	Lenka Horváthová, Vojtěch Žárský & Pavel Doležal: **Bacterial Secretion System in the Eukaryote *Naegleria gruberi***
16:40	Jitka Hostomská, Jan Mach & Jan Tachezy: **Transport of Pyruvate into the Mitochondrion of *T. brucei***
17:00	*Coffee break*
Molecular Biology of Protozoa + Biodiversity, Phylogeny and Systematics of Protozoa	
17:15	Jan Martinek, Brian Panicucci, Harry P. de Koning & Alena Zíková: **Mechanism of *T. brucei* Cell Cytotoxicity by Benzophenone-Derived Bisphophonium Salts**
17:25	Karolína Šubrtová, Brian Panicucci & Alena Zíková: **Hypothetical Trypanosoma Protein Helps to Anchor the F_1-ATPase Moiety to the Mitochondrial Membrane**
17:45	Petr Soukal, Anna Karnkowska-Ishikawa, Štěpánka Hrdá, Jana Szabová, Miluše Hroudová, Čestmír Vlček & Vladimír Hampl: **Transcriptome of *Rhabdomonas costata* and the Testing of Plastid-Late Hypothesis for the Euglenid Plastid Origin**
18:05	*Dinner*
19:00	Meeting of Protistology Section
20:00 to 22:00	Anna Karnkowska: Workshop on Analysis of Next Generation Sequencing Data

Wednesday May 8, 2013	
8:00	*Breakfast*
Cell Biology of Protozoa	
9:35	Jitka Kručinská & Miroslav Oborník: **Formation and Exflagellation of Zoosporangia of a Coral Reef Alga *Chromera velia***
9:35	Janka Melicherová, Jana Ilgová & Andrea Valigurová: **Oocyst or Sporocyst? Another Enigma in the Development of Cryptosporidia**
9:55	Pavla Tůmová, Lenka Hudosová, Kristýna Marková, Gerhard Wanner & Eva Nohýnková: **Keywords in Karyotypes of *Giardia*: Aneuploidy, Heterogeneity, Minimalism**
10:15	Zuzana Zubáčová, Lukáš Novák, Jitka Bublíková, Vojtěch Vacek, Jan Fousek, Jakub Rídl, Jan Tachezy, Pavel Doležal, Čestmír Vlček & Vladimír Hampl: **Mitochondrion-Like Organelle of *Trimastix pyriformis***
10:35	*Coffee break*
Molecular Biology of Protozoa	
10:50	Vojtěch Žárský, Dušan Hurtoň & Jan Tachezy: **Evolution of Peroxisomes: Anything Can Happen**
11:10	Eva Nývltová, Zuzana Zubáčová, Ivan Hrdý, Jaroslav Kulda & Jan Tachezy: **Differences between Mitochondria-Like Organelles in Anaerobic Diplomonads *Spironucleus vortens* and *Spironucleus salmonicid***
11:30	Eva Martincová, Luboš Voleman, Vojta Žárský & Pavel Doležal: **Biogenesis of *Giardia intestinalis* Mitosomes**
11:50	SPONZOR presentation – Biotech
12:00	*Lunch*
13:20	Trip to Protivín
19:00	*Raut and free enjoyment of the evening*

Thursday May 9, 2013	
8:00	*Breakfast*
Biodiversity, Phylogeny and Systematics of Protozoa	
9:15	Andrei Diakin, Timur G. Simdyanov, Gita G. Paskerova & Andrea Valigurová: **Observations on Some Basal Apicomplexans from Marine Invertebrates**
9:35	Tomáš Pánek, Petr Táborský & Ivan Čepička: **'Anaeramoeba', a Novel Anaerobic Marine Amoeba with Uncertain Phylogenetic Position**
9:55	Pavla Smejkalová, Eva Nohýnková, Jaroslav Kulda & Ivan Čepička: **New Evidence for the Polyphyly of Retortamonads**
10:15	Jana Szabová, Richard E. Triemer, Naoji Yubuki & Vladimír Hampl: **Evolution and Distribution of MAT and MATX Genes in Euglenids**
10:35	*Coffee break*
Molecular Biology of Protozoa	
10:50	Tamara Smutná, Kateřina Pilařová, Ján Tarábek & Ivan Hrdý: **Novel Function of Bacterial-Type Iron-Sulfur Flavoprotein from *Trichomonas vaginalis* hydrogenosomes**
11:10	Daniel Sojka: **TgASP5 – and Analog of PEXEL Processing Plasmepsin V from *Toxoplasma gondii***
11:30	Marek Eliáš: **The Protist Perspective on the Ras GTPase Superfamily**
11:50	SPONZOR presentation – Baria
12:00	*Lunch*
Vectors and Protozoan Diseases	
13:20	Marie Jalovecká, Ondřej Hajdušek, Laurence Malandrin & Petr Kopáček: **Implementation of the *Babesia divergens* Transmission Model: an Essential Tool to Study *Babesia*-Tick Molecular Interactions**
13:40	Nikola Polanská & Iva Kolářová: **The Role of *Sergentomyia schwetzi* in Epidemiology of Visceral Leishmaniasis in Ethiopia**
14:00	Michal Šíma, Iva Kolářová & Petr Volf: **Characterization and Expression of *Phlebotomus orientalis* Salivary Antigens**
14:20	SPONZOR presentation – KRD
14:30	*Coffee break*
14:45	Poster Session B

Molecular Biology of Protozoa	
16:00	Lucia Hadariová, Eva Dobáková, Peter Kyseľ & Juraj Krajčovič: **Residual Plastid Genes in the Flagellate *Euglena gracilis* White Mutants**
16:20	Erik Birčák, Vladimir Klimeš, Kristína Záhonová, Matej Vesteg, Marek Eliáš & Juraj Krajčovič: **Transcriptome Analysis of the Colorless Flagellate *Euglena longa***
16:40	Marie Pažoutová, Fabio Rindi, Karolína Fučíková, Aleš Horák, Stephane Rombauts & Miroslav Oborník: **Everything You Always Wanted to Know About Sex of Greens but Were Afraid to Ask: Reviewing the Sexuality among Trebouxiophyte Green Algae**
17:00	*Coffee break*
17:15	Demonstration of Protists – Olympus Microscope
17:45	Announcement of Best Talks and Poster
18:00	*Dinner*
19:00 to 21:00	Anna Karnkowska: Workshop on RNA Seq in Non-Model Organisms

Friday May 10, 2013	
8:00	*Breakfast*
9:15	Minigolf Tournament

Speakers' names are underlined.

Poster Session

For posters highlighted with gray color is reserved in programm schedule Poster Session A. For the others is reserved Poster Session B.

Pavla Bartošová, Martina Loudová, Hana Pecková, Sneha Patra, Alena Kodádková & Astrid Holzer: **Hidden Biodiversity and Evolutionary Trends in the Malacosporean Parasites (Cnidaria: Myxozoa)**

Jozef Blanár & Pavol Mudroň: ***Cryptosporidium parvum* – an Unusual Cause of Chronic Diarrhoea in a Dairy Cow**

Jaromír Cihlář, Aleš Horák & Miroslav Oborník: **Porphobilinogen Deaminase in Phototrophic Eukaryotes and Its Mitochondrial Origin**

Vojtěch David, Pavel Flegontov, Hassan Hashimi, Evgeny S. Gerasimov, Ivan Fiala, Goro Tanifuji, Naoko T. Onodera, John Archibald & Julius Lukeš: **Assembly and Annotation of a Mitochondrial Genome of Kinetoplastid Protist *Perkinsela***

Nela Dvořáková, Jana Kvičerová & Pavel Široký: **Conspecificity of Blood Parasites of Genus *Haemogregarina* in Freshwater Turtles of Western Palearctic Region**

Blanka Ferencová, Jovana Sádlová, Aysheshm Kassahun, Jan Votýpka, Gad Baneth, Iva Kolářová & Petr Volf: **Rodents as Possible Reservoir Hosts of *Leishmania donovani***

Markéta Fialová & Jana Kulichová: **An Ecologigal View of the Diatom Morphology**

Ingrid Škodová, Zdeněk Verner, Fréderic Bringaud, Peter Fabian, Julius Lukeš & Anton Horváth: **Biochemical Characterization of FAD-Dependent Glycerol-3-Phosphate Dehydrogenases in *Trypanosoma brucei***

Zhenqiu Huang, Drahomíra Faktorová, Julius Lukeš & Hassan Hashimi: **MRB8620, the Unique Unessential Core Protein in RNA Editing Accessory Complex MRB1**

Martin Kostka, Tomáš Tyml, Hana Pecková & Iva Dyková: **Flabellulids, Their Weird Sequences and Phylogeny**

Zdeněk Verner, Petra Čermáková, Bianka Kováčová, Ingrid Škodová, Julius Lukeš & Anton Horváth: **Oxidative Phosphorylation in Trypanosomatids**

Markéta Lorencová, Pavla Smejkalová, Magdalena Uzlíková & Ivan Čepička: **Extensive Diversity of *Blastocystis* in Reptiles and Insects**

Maja Łukomska-Kowlaczyk, Anna Karnkowska, Małgorzata Korzeniecka & Bożena Zakryś: **DNA Barcoding of Autotrophic Euglenoids**

Jan Martinek, Brian Panicucci, Harry P. de Koning[3] & Alena Zíková: **Mechanism of *T. brucei* Cell Cytotoxicity by Benzophenone-Derived Bisphophonium Salts**

Jan Michálek, Marie Pažoutová & Miroslav Oborník: **Do Not Curse the Contamination – An Unexpected Discovery of the Novel Marine Fungus**

Rafał Milanowski, Anna Karnkowska, Takao Ishikawa & Bożena Zakryś: **Toward a Model of Euglenoid Non-Conventional Introns Structure**

Petra Mutinová & Jiří Neustupa: **Substrate Specificity of Epiphytic Communities of Diatoms (Bacillariophyceae)**

Sneha Patra, Astrid S. Holzer, Hana Pecková, Nathan P. Brennan, Carlos Yanes-Roca & Kevan L. Main: ***Sphaerospora motemarini* n. sp. Causes Glomerular Disease in Juvenile Grey Snapper *Lutjanus griseus* L.: A Reason for Host Population Declines in the Gulf of Mexico?**

Pavel Poliak, Jan Mach, Jan Tachezy & Julius Lukeš: **Mitochondrial Processing Peptidases in *Trypanosoma brucei***

Kateřina Procházková, Lira A. Gaysina, Martina Pichrtová, Alena Lukešová & Marek Eliáš: **The Diversity in the *Vischeria/Eustigmatos* Complex (Eustigmatophyceae): Morphological and Molecular Perspectives**

Eliška Ptáčková & Ivan Čepička: **The First Known Endobiotic *Carpediemonas*-Like Organism**

Eva Rmoutilová, Eliška Ptáčková & Ivan Čepička: **Evolutionary Significance of Free-Living Diplomonads**

Eva Roubalová, Zoltán Füssy & Miroslav Oborník: **Localisation and Functional Analysis of Heme Pathway in *Phaeodactylum tricornutum***

Ivana Schneedorferová, Aleš Tomčala & Miroslav Oborník: **Comparison of Glycerolipid Composition of Two Chromerida Species: *Chromera velia* and *Vitrella brassicaformis***

Pavel Flegontov, Jan Votýpka, Tomáš Skalický, Maria D. Logacheva, Alexey A. Penin, Goro Tanifuji, Naoko T. Onodera, Alexey S. Kondrashov, John M. Archibald & Julius Lukeš: ***Paratrypanosoma* – a Novel Ancestral Trypanosomatid**

Darja Stojanovová, Jan Pyrih & Jan Tachezy: **Knocking Out Genes in *Trichomonas vaginalis***

Tereza Ševčíková & Jana Kulichová: **The Relative Biovolume of Benthic Diatom Assemblages in Relation to Environmental Conditions**

Karolína Šubrtová, Brian Panicucci & Alena Zíková: **Hypothetical Trypanosoma Protein Helps to Anchor the F_1-ATPase Moiety to the Mitochondrial Membrane**

Jiří Týč, Tomáš Skalický, Somsuvro Basu & Julius Lukeš: **Mitochondrial Chaperone and kDNA**

Anna Vanclová, Róbert Šuťák & Vladimír Hampl: **Isolation of Chloroplasts and Chloroplast Membranes from *Euglena gracilis***

Zdeněk Verner, Ingrid Škodová, Simona Poláková, Vladislava Ďurišová-Benkovičová, Anton Horváth & Julius Lukeš: **Alternative NADH:Ubiquinone Oxidoreductase in Procyclic *Trypanosoma brucei*: an Intermembrane-Space-Oriented Counterpart of Mitochondrial Complex I**

Michaela Veselíková, Brian Panicucci & Alena Zíková: **Protein MIX as a Drug Target in *Leishmania major***

Matej Vesteg, Katarína Krnáčová, Vladimír Hampl, Čestmír Vlček & Anton Horváth: **Parasitic Trypanosomatids and the Phototroph *Euglena gracilis* Possess Common Motifs in Mitochondrial Targeting Presequences**

Pavlína Vobořilová, Jaroslav Kulda, Ivan Čepička & Jan Tachezy: **Trichomonads in Cats and Dogs**

Monika Wencelová, Zora Váradyová, Katarína Mihaliková, Svetlana Kišidayová & Dušan Jalč: **Exploring the Possibilities of Using Chitosan as Antiprotozoal Agent and Modulator of Rumen Fermentation**

Kristína Záhonová, Matej Vesteg & Juraj Krajčovič: **A Small Portion of Plastid Transcripts is Polyadenylated in the Flagellate *Euglena gracilis***

The names of the presenters are underlined.

Abstracts

Hidden Biodiversity and Evolutionary Trends in the Malacosporean Parasites (Cnidaria: Myxozoa)

Pavla Bartošová[1], Martina Loudová[1], Hana Pecková[1], Sneha Patra[1], Alena Kodádková[1] & Astrid Holzer[1]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

Malacosporeans represent a small fraction of myxozoan biodiversity with only three described species belonging to two genera. They cycle between the bryozoans and freshwater fish. In this study, we (i) PCR screen different freshwater/marine fish species from various geographic locations; (ii) perform the rDNA and EF-2 based phylogenetic analyses of all available malacosporean data, and (iii) trace the host species and geographic data on the phylogenetic tree to improve the understanding of the biodiversity, distribution and evolutionary trends within malacosporeans. In all analyses, malacosporeans created a sister lineage to the myxosporeans and showed a partial trend in their clustering according to the host species and biogeography. We discovered the existence of six new *Tetracapsuloides* species, three new *Buddenbrockia* species and one new malacosporean genus. Co-infections of up to three malacosporean spp. were found in one fish specimen of several fish species. Significantly increased species richness in the Malacosporea (5 times) shown in present study points out on the hidden biodiversity within this parasitic group. The finding of a new *Buddenbrockia* species in the marine fish indicates that malacosporean life cycles might exist in the marine environment which would be reasonable due to the fact that the major part of bryozoans are marine species.

Cytosolic Iron-Sulfur Cluster Assembly in *Trypanosoma brucei*

Somsuvro Basu[1,2], Daili J. Netz[3], Alexander Haindrich[2], Nils Herleth[3], Thibaut J. Lagny[3], Roland Lill[3], Antonio J. Pierik[3] & Julius Lukeš[1,2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]Philipps-Universität, Institut für Zytobiologie und Zytopathologie, Marburg, Germany

In *Trypanosoma brucei* we are studying the cytosolic iron-sulfur (Fe-S) cluster assembly (CIA) pathway, which is conserved there as in other aerobic eukaryotes. A homology search using the yeast and human CIA proteins identified all 9 orthologues in the *T. brucei* genome – Cfd1, Nbp35, Nar1, Cia1, Cia2A, Cia2B, Met18, Tah18, and Dre2. Cell lines were generated for the bloodstream and procyclic stage, in which each of these genes is targeted for RNAi-mediated depletion. Unexpectedly, for both stages of *T. brucei* most of the CIA proteins appeared to be non-essential. Next, we have prepared trypanosomes, in which two CIA components were RNAi-targeted in parallel. In most cases, interacting partners were selected for ablation, which was detrimental for the viability of both life stages, proving the essentiality and functional redundancy of these candidates. The presence of the CIA pathway in this protist is supported by complementation of CIA-factor depleted *S. cerevisiae*, which showed that *T. brucei* Cia2A, Cia1 or Tah18+Dre2 (co)overexpression efficiently rescued growth of yeast depleted for the respective orthologues. For mass spectrometric analysis of proteins binding to the *T. brucei* CIA targeting complex Cia1, Cia2A, Cia2B, Met18 have been PTP-tagged. We aim to identify CIA components unique for *T. brucei* and to fish for hitherto unidentified trypanosomal Fe-S proteins.

Transcriptome Analysis of the Colorless Flagellate *Euglena longa*

Erik Birčák[1], Vladimir Klimeš[2], Kristína Záhonová[2], Matej Vesteg[1,2], Marek Eliáš[2] & Juraj Krajčovič[1]

[1]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Bratislava

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

Euglena longa is a naturally occurring freshwater colorless non-photosynthetic flagellate closely related to the green photosynthetic *Euglena gracilis*. Nuclear genomes of both those euglenoids remain largely a mystery (as well as mitochondrial ones). *E. longa* transcriptome analysis is an initial step towards a characterization of many nuclear genes and examining their expression under different conditions. We have obtained two different versions of transcriptome using mRNA from cells of *E. longa* grown under light and dark conditions. Next-generation sequencing method and transcriptome assembly with ABySS/Trans-AbySS and Trinity have been used. Evaluating the quality of both transcriptormes we have been searching for the presence of different groups of genes. The obtained data showed the presence of many interesting groups of genes, such as calpains or meiotic-specific genes, which were transcribed in both versions of transcriptome. Both transcriptomes has roughly the same size and did not differ significantly by the presence or absence of genes studied, suggesting that cultivation in the dark may not have a significant effect on the expression of nuclear genes in this flagellate. Nevertheless, results of this analysis in *E. longa* may have implications for understanding the gene expression and regulation with its close relatives, including *E. gracilis* as well as evolution of those ancient eukaryotes.

Cryptosporidium parvum – an Unusual Cause of Chronic Diarrhoea in a Dairy Cow

Jozef Blanár[1] & Pavol Mudroň[1]
[1]University of Veterinary Medicine and Pharmacy in Košice, Košice

Cryptosporidia are gastrointestinal, coccidian, protozoan parasites of warm-blooded and cold-blooded animals. These parasites do not require an external development stage, but are immediately infective when passed in the faeces as thick-walled oocysts. Intestinal cryptosporidiosis of livestock causes a brief diarrheal disease and probably does not hinder lifetime production in most cases. Cryptosporidiosis has become a concern for dairy producers because of the direct losses due to calves not performing well and the potential for environmental contamination with *C. parvum*. *Cryptosporidium parvum* is a zoonotic protozoan recognized as one of the primary pathogens causing diarrhoea in neonatal calves. Cryptosporidia almost always can be found among diarrheic calves, but the rule is that other known serious enteric pathogens can be found, too, if sought. Chronic diarrhoea in dairy cows is not a healthy disorder characterized by extremely high incidence, however, its occurrence can be both a serious diagnostic challenge for surgeons and a signal of possible future outbreaks of the disease in the herd. The most frequent causes of chronic diarrhoea in dairy cows are discussed: GIT helminths, paratuberculosis, salmonellosis, BVD, chronic kidney (amyloidosis) and liver diseases, chronic rumen acidosis, and abomasal displacement. A rare case of chronic diarrhoea in 5-year-old dairy cow, with *Cryptosporidium parvum* as the only causative agent found, is described in this paper.

Acknowledgment: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0701-11.

Porphobilinogen Deaminase in Phototrophic Eukaryotes and Its Mitochondrial Origin

Jaromír Cihlář[1], Aleš Horák[1] & Miroslav Oborník[1]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

Porphobilinogen deaminase (PBGD) is an enzyme involved in tetrapyrrole biosynthesis. This enzyme is present in both heterotrophic and autotrophic routes. However, its origin is different in both groups. While in heterotrophs the gene coding for PBGD originates in the ancient eukaryote nucleus, in phototrophs it shows a mitochondrial (alphaproteobacterial) origin. We performed phylogenetic analyses in order to confirm this somewhat surprising origin of the PBGD in phototrophic organisms and to get insight into the evolution of the pathway in eukaryotic phototrophs. Based on these analyses we suggest that mitochondrial origin of this gene is a result of endosymbiotic processes during which eukaryotes acquired plastids and mitochondria. We propose that three independent enzymatic pathways could have coexisted in early ancestors of eukaryotic phototrophs. Endosymbiotic gene transfer during consecutive endosymbiotic events probably resulted in mixing of genes with different origin which led to the current state in eukaryotic phototrophs.

Assembly and Annotation of a Mitochondrial Genome of Kinetoplastid Protist *Perkinsela*

Vojtěch David[1], Pavel Flegontov[1], Hassan Hashimi[1], Evgeny S. Gerasimov[2], Ivan Fiala[1], Goro Tanifuji[3], Naoko T. Onodera[3], John Archibald[3] & Julius Lukeš[1]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]Lomonosov Moscow State University, Biological Faculty, Department of Molecular Biology, Moscow, Russia
[3]Canadian Institute for Advanced Research, Integrated Microbial Biodiversity Program, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada

The evolutionary picture of uridine insertion-deletion RNA editing is still incomplete as 'basal' Kinetoplastida remain poorly studied. Therefore we are aiming for comprehensive analysis of mitochondrial genome and transcriptome of *Perkinsela*-like or *Ichthyobodo*--related organism, which is an obligate endosymbiont of *Neoparamoeba pemaquidensis* (CCAP1560/4). Illumina reads of the transcriptome were mapped on genomic contigs assembled from paired-end and mate-pair Illumina data. *Perkinsela* apparently lacks any trace of genes for complex I of the electron transport chain. Three protein-coding genes were identified in the mitochondrial genome: cox1, cox3, cob, and another three unidentified transcripts were detected using RNAseq data. Two of the transcripts having extremely high RNAseq coverage might represent mitochondrial rRNAs diverged beyond recognition. Cox1,cox3, cob, and the two high-coverage transcripts are edited with U-insertions/deletions in relatively short regions at both transcript ends. U-insertion/deletion editing pattern in *Perkinsela*. apparently differs greatly from that of Trypanosomatida, in which RNA editing was discovered. Alternatively edited transcripts are apparently common in *Perkinsela*.

Observations on Some Basal Apicomplexans from Marine Invertebrates

Andrei Diakin[1], Timur G. Simdyanov[2], Gita G. Paskerova[3] & Andrea Valigurová[1]
[1]Masaryk University, Faculty of Science, Department of Botany and Zoology, Brno
[2]Lomonosov Moscow State University, Biological Faculty, Department of Invertebrate Zoology, Moscow, Russia
[3]Saint-Petersburg State University, Faculty of Biology and Soil Science, Department of Invertebrate Zoology, St. Petersburg, Russia

Apicomplexans represent a very successful group of unicellular parasites consisting entirely of parasitic species infecting different vertebrates and invertebrates. Most of them are well known as agents causing human and animal diseases (e. g. malaria, toxoplasmosis, cryptosporidiosis), which are intensively studied in different aspects of biology and medicine. In contrast, deep-branching apicomplexans are generally considered of no practical importance and thus remain poorly investigated. These groups, however, are crucial in our understanding of evolutionary pathways of the phylum Apicomplexa. Here we present observations on several apicomplexan parasites from marine invertebrates of the White Sea: protococcidian *Eleutheroschizon dubosqui* and blastogregarine *Siedleckia nematoides* from intestine of polychaete *Scoloplos armiger*, agamococcidian *Rhytidocystis* sp. (presumably new species) from intestine of polychaete *Travisia forbesii*, eugregarines *Urospora travisiae* and *U. ovalis* parasitizing the body cavity of the same host. *U. chiridotae* inhabiting blood vessel of holothurian *Chiridota laevis* and intestinal archigregarine *Selenidium* sp. from polychaete *Pygospio elegans*. Studied parasites differ in their morphological aspects, localization and in the mode of movement: i. e. gliding (*U. travisiae*), metaboly (*E. dubosqui* and *U. ovalis*), nematode-like movement (*Selenidium* and *Siedleckia*). They seem to show parallel pathways of evolution realized by various morphological and probably functional adaptations. Combined morphological and molecular-phylogenetical analysis supports our hypothesis that there are several early emerging branches of Apicomplexa.
Acknowledgment: Financial support provided by Czech Science Foundation, project No. P505/12/G112 (ECIP).

Biogenesis of *Giardia intestinalis* Mitosomes

Eva Martincová[1], Luboš Voleman[1], Vojta Žárský[1] & Pavel Doležal[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Mitosomes represent extreme mitochondrial adaptations that have evolved in anaerobic eukaryotes such as the human unicellular parasite *Giardia intestinalis*. Our laboratory studies mainly two aspects of these mitochondria in miniature (i) the organelles dynamics and inheritance and (ii) the import of the proteins and the metabolites from the cytosol. To this aim we have designed several molecular and cell biology tools, which greatly facilitate our efforts towards the characterization of the mitosomal proteome and the live organelle imaging. We believe that mitosomes carry bare bones of otherwise intricate processes occurring in the mitochondria of aerobic eukaryotes.

Conspecificity of Blood Parasites of Genus *Haemogregarina* in Freshwater Turtles of Western Palearctic Region

Nela Dvořáková[1], Jana Kvičerová[2] & Pavel Široký[1]
[1]University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Hygiene and Ecology, Department of Biology and Wildlife Diseases, Brno
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

Hemogregarines s. l. are a group of more than 400 species of heteroxenous blood parasites infecting mostly aquatic vertebrates. Turtle, as a host of these parasites, is infected by blood sucking vector (and definitive host), usually a leech. In our project, group of aquatic turtles of the species *Emys orbicularis*, *Mauremys caspica* and *M. rivulata* originally from areas of Bulgaria, Iran, Syria and Turkey were examined. Presence of gamonts morfologically similar to genus *Haemogregarina* was detected in turtle's blood, using light microscopy. In principle they were coincident with the species *H. stepanowi*. In total 47 (64.4 %) of 73 studied turles were infected with blood parasites. Prevalence varied between 93.3 % of *E. orbicularis*, 47.1 % *M. caspica* and 59.5 % *M. rivulata*. Samples were investigated by PCR--based methods, obtained 1500 bp long sequences of 18S rDNA of all our isolates were used for phylogenetic analyses, which confirmed their genetic identity and concurrently low host specificity of this blood parasite. According to our results we conclude that that the presence of *H. stepanowi* is probably strictly bound to the vector and definitive host – leech of the genus *Placobdella*. Turtle in the development cycle represents less species-specific role of intermediate hosts. Diagnosis by PCR method showed the same sensitivity, and therefore was as reliable as microscopic examination.

Acknowledgment: Práce byla podpořena granty IGA VFU číslo 11/2012/FVHE a GAČR P506/11/1738.

The Protist Perspective on the Ras GTPase Superfamily

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

The Ras GTPase superfamily is a vast grouping of proteins sharing a particular variant of a more general GTPase domain typified by the human protooncoprotein Ras. While rather inconspicuous in prokaryotes, the superfamily has massively radiated in eukaryotes to comprise up to hundreds of paralogs in some species. Studies on a limited array of model species, primarily representing just three eukaryotic lineages – metazoans, fungi, and land plants, have led to a scheme classifying the superfamily into several subgroups, each with a characteristic cellular role. In my talk I would like to show that focusing onto a few model species leads to a biased and incomplete perception of the actual scope of phylogenetic and functional diversity of the Ras superfamily. I will discuss two aspects that provide more general lessons about the evolution of eukaryotes and their genomes and cells. First, analyses of phylogenetically diverse protist genomes have uncovered a number of apparently ancient Ras superfamily paralogs that have been lost from most or all established model species, pointing towards a wealth of hitherto unnoticed cellular processes retained in some eukaryotic lineages since the last eukaryotic common ancestor. Interestingly, sequence characteristics or phyletic patters on some of these uncharacterised ancestral GTPase paralogs suggest their possible function e. g. in pathogen defence or in biogenesis of the flagellum. Second, lineage-specific evolutionary events in protists have modelled the Ras superfamily and its individual members to generate unprecedented paralog expansions or sequence and structural characteristics, contributing to the sheer diversity of incarnations of the eukaryotic cell.

An Ecolocigal View of the Diatom Morphology

Markéta Fialová[1] & Jana Kulichová[1]
[1]Charles University in Prague, Faculty of Science, Department of Botany, Praha

Many recent studies are interested in the problematic of protist cryptic diversity. Species defined by traditional morphological concept do not often correspond with real count of species, which is so usually underestimated. Combination of different approaches have already discovered a few (semi)cryptic species complexes. Multidisciplinary approach to the protist taxonomy also showed that the molecular diversity does not always correlate with ecological data, while the individual morphotypes show different ecological preferences. This study focuses on the morphology of natural populations of diatom species complex *Frustulia rhomboides* and it engages how the morphology reflects the effects of environmental conditions. Using methods of geometric morphometrics the morphological variability of diatom frustules of this species complex from various peat bog habitats in the Czech Republic were analyzed. Environmental parameters were measured and the species composition of diatom communities present in the sample was investigated. These data were used to analyze the relationship between environmental conditions and cell shape variability within the complex. We demonstrated some trends that could help with using of the shape of natural populations for ecological studies without applying a multidisciplinary approach.

Residual Plastid Genes in the Flagellate *Euglena gracilis* White Mutants

Lucia Hadariová[1], Eva Dobáková[1], Peter Kyseľ[1] & Juraj Krajčovič[1]
[1]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Bratislava

Growth of the flagellate *Euglena gracilis* in the presence of specific inhibitors of bacterial DNA, RNA, and protein synthesis has no effect on cell viability but leads to the permanent loss of the ability to form green colonies, a process termed bleaching. The loss during bleaching of most if not all of the plastid genome in the absence of nuclear gene loss makes *E. gracilis* an attractive model to study the reductive evolution of plastids. *E. gracilis* is possibly the only plastid-containing organism whose growth and viability are independent of a functional plastid genome. There are several experimentally induced/bleached *E. gracilis* white mutants. To gain further insight into the overall functional organisation of the *Euglena* plastid chromosome we have analyzed a presence of the plastid genes in three stable white mutants – W3BUL, W10BSmL and WgmZOflL. All 96 genes of the circular plastid chromosome of *E. gracilis* strain Z (encoding rRNA, tRNA, known proteins, ORFs, ycfs) have been studied by a semi-quantitative PCR-based approach. PCR analysis using total cellular DNA showed presence of some plastid genes in all three white mutants. W3BUL mutant contains almost a half of the wild type plastome gene set (48, i.e. 49 %), WgmZOflL (6, i.e. 6.1 %), and W10BSmL only 5 genes (5.1 %). We did not detect any photosynthetic genes in W10BSmL and WgmZOflL mutants. Some tRNA genes have been retained in all mutants and genes were lost independently of their position on the *E. gracilis* circular plastid chromosome.

Biochemical Characterization of FAD-Dependent Glycerol-3--Phosphate Dehydrogenases in *Trypanosoma brucei*

Ingrid Škodová[1,2], Zdeněk Verner[1,2,4], Fréderic Bringaud[3], Peter Fabian[1], Julius Lukeš[2,4] & Anton Horváth[1]

[1]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Bratislava

[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

[3]Centre de Resonance Mgnetique des Systemes Biologiques, UMR 5536, Universite Bordeaux Segalen, CNRS, Bordeaux, France

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

Glycerol-3-phosphate dehydrogenases (G3PDHs) constitute a shuttle that serves for regeneration of NAD^+ reduced during glycolysis. NAD-dependent enzyme is employed in glycolysis and produces glycerol-3-phosphate from dihydroxyacetone phosphate while its FAD-dependent homologue catalyzes a reverse reaction coupled to respiratory chain. *Trypanosoma brucei* possess two FAD-dependent G3PDHs. While one of them has been attributed to mitochondrion and seems to be directly involved in G3PDH shuttle reactions (mtG3PDH), function of the other one remains unknown (putG3PDH). In the presented work, we employed RNA interference and protein over-expression to shed a light on relative contribution of both FAD-G3PDHs to overall cellular metabolism. Our results indicate that mtG3PDH is essential for bloodstream stage of *T. brucei*. In procyclic stage the enzyme is dispensable in presence of an alternative NADH:ubiquinone oxidoreductase whose contribution to respiration was elevated upon depletion of mtG3PDH. Surprisingly, expressed putG3PDH-V5 construct showed mitochondrion localization too. Based on our data obtained from digitonin permeabilization followed by Western analysis, we propose putG3PDH being located within an outer mitochondrial membrane thus not contributing to mitochondrial respiratory chain.

Bacterial Secretion System in the Eukaryote *Naegleria gruberi*

Lenka Horváthová[1], Vojtěch Žárský[1] & Pavel Doležal[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Mitochondrion has evolved from an endosymbiotic Gram-negative bacterium and has become central compartment of the eukaryotic cell. The type II secretion system (T2SS) constitutes the main secretory channel across the outer membrane of many Gram-negative bacteria. During the conversion of the endosymbiont into the genetically dependent organelle bacterial secretory pathways were forsaken, but, as we show here, not entirely. Our identification of four proteins homologous to the components of bacterial T2SS in the genome of the eukaryote *Naegleria gruberi* may shed light on early steps in the origin and evolution of mitochondria. These four Gsp (general secretory pathway) proteins could provide for very minimalist but still functional secretory apparatus as they constitute all the essential part of the pathway. We show that NgGsp proteins are specifically targeted to *S. cerevisiae* mitochondria. Our work now aims to confirm mitochondrial localization of endogenous proteins in *N. gruberi* and to follow the route and the assembly of secretion complex.

Transport of Pyruvate into the Mitochondrion of *T. brucei*

Jitka Hostomská[1], Jan Mach[1] & Jan Tachezy[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

In the mitochondrion, pyruvate is the principal substrate for acetyl coenzyme A formation. Mitochondrial acetyl coenzyme A in turn is a key entry substrate of the citrate cycle as well as several important biosynthetic reactions. Therefore the transport of pyruvate across mitochondrial membrane represents a branching point in cellular metabolism important for balancing glycolysis and oxidative phosphorylation. Transport of pyruvate across the inner mitochondrial membrane is independent of members of both mitochondrial carrier family and monocarboxylate transporter family. The specific carrier of pyruvate was only identified last year in mitochondria of yeast, fruit fly and human. It acts as a heterodimer consisting of two small hydrophobic proteins, MPC1 and MPC2/3. We found homologs of both MPC1 and MPC2 in *Trypanosoma brucei*, a kinetoplastid whose mitochondrion undergoes remarkable remodelling between bloodstream and procyclic forms. We confirmed the mitochondrial localization of V5-tagged MPC1 in procyclic and bloodstream forms. No MPC1-V5 was observed in the cellular membrane of bloodstream forms, suggesting that the MPC complex does not participate in the excretion of pyruvate, the major metabolic end product in bloodstream forms. Cell lines allowing inducible RNAi of MPC1 were generated in procyclic *T. brucei*. We detected no growth phenotype upon RNAi induction in standard culture medium containing 11 or 5 mM glucose. We assume that upon RNAi induction, the availibility of pyruvate in the mitochondrion is compromised and we would like to study the adaptations of mitochondrial metabolism in this model by end product analysis.

MRB8620, the Unique Unessential Core Protein in RNA Editing Accessory Complex MRB1

Zhenqiu Huang[1], Drahomíra Faktorová[1], Julius Lukeš[1] & Hassan Hashimi[1]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

The uridine insertion/deletion RNA editing in the single mitochondrion of *Trypanosoma brucei* is performed by a 20S editosome and multiple protein complexes. The multiprotein mitochondrial RNA-binding complex 1 (MRB1) is essential for the RNA editing process and contains a core complex comprised of six proteins via direct interactions. MRB8620, as one among the six core proteins, when silenced by RNAi did not affect either the growth of *T. brucei* in the procyclic stage or the stabilization of certain RNA transcripts. Here is the first time to report an unessential core protein in MRB1. With the knockout of MRB8620, we can further confirm the function of this protein in the life cycle of *T. brucei*.

Implementation of the *Babesia divergens* Transmission Model: an Essential Tool to Study *Babesia*-Tick Molecular Interactions

Marie Jalovecká[1,2,3], Ondřej Hajdušek[2], Laurence Malandrin[3] & Petr Kopáček[1,2]
[1]University of South Bohemia, Faculty of Science, České Budějovice
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[3]UMR INRA/Oniris 1300 BioEpAR, Nantes, France

Babesiosis, zoonosis caused by *Babesia*, is a tick-borne malaria-like disease of various vertebrate hosts and is considered among the emergent diseases from the aspects of human and veterinary medicine. Currently the great attention is paid to the increasing incidence of *Babesia* parasites. Interplay between the *Babesia* and the tick vector represents a complex system of multiple molecular interactions. To date only a limited number of genes and proteins have been demonstrated to play roles in these interactions. Nevertheless, so far no research in this area has ever been focused on the model of the in Europe common species *Babesia divergens* and its tick vector *Ixodes ricinus*. An implementation of *B. divergens* transmission model will enable to investigate molecular mechanisms of transmission and persistence of the parasite in the vector organism. The research aimed to identify and characterize molecular interactions between *B. divergens* and *I. ricinus* represents promising direction that can lead to the discovery of effective therapies or vaccines, and thus to reduce diseases caused by *Babesia*.

Flabellulids, Their Weird Sequences and Phylogeny

Martin Kostka[1,2], Tomáš Tyml[1], Hana Pecková[1,2] & Iva Dyková[3]
[1]University of South Bohemia, Faculty of Science, České Budějovice
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[3]Masaryk University, Faculty of Science

Flabellulidae (Bovee, 1970) Page, 1987 are a group of amoebae belonging among Amoebozoa, Tubulinea, Leptomyxida. They are usually flattened marine amoebae with a tendency to have more nuclei per cell. Although having a relatively distinct morphology (enabling their light-microscopy-based identification), SSU rDNA sequences obtained from some of them form very long branches in phylogenetic trees. These strains do not cluster with other flabellulids in the SSU rDNA trees. One could thus question the monophyly of flabellulids. Here, we show results of phylogenetic analyses of SSU rDNA sequences as well as sequences of other genes – which are in conflict. We discuss possible monophyly/polyphyly and general phylogeny of flabellulids and their closest relatives.

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

Zuzana Kotrbová[1,2], Brian Panicucci[2], Dana Hocková[3] & Alena Zíková[1,2]
[1]University of South Bohemia, Faculty of Science, České Budějovice
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[3]Institute of Organic Chemistry and Biochemistry ASCR, v.v.i., Praha

Trypanosoma brucei is a tropical protozoan parasite belonging to the group Kinetoplastida and it causes serious disease in human (sleeping sickness) and livestock (Nagana). Since commonly used drugs are toxic and inefficient against all stages of the disease, it is necessary to search for new therapeutic alternatives. Unlike mammals, *T. brucei* cannot synthesize purines *de novo* and it depends strictly on the uptake, transformation and incorporation of purines from extracellular sources using the purine salvage pathway (PSP). Hypoxanthine-guanine phosphorybosyltransferase (HGPRT) and xanthine phosphorybosyltransferase (XPRT) are two key enzymes of the redundant PSP in *T. brucei*. The simultaneous RNAi silencing of both enzymes (HGPRT and XPRT) in the bloodstream form of *T. brucei* was lethal when hypoxanthine or guanosine were the only source of purines in the media. On the other hand, when adenosine was added back to the media, the growth phenotype was slightly rescued, indicating that the adenosine-dependent enzymes of the PSP are less important for cell survival. Additional immunofluorescence assays and digitonin fractionations suggest that both enzymes are localized in the glycosome. Finally, we screened 100 acyclic nucleoside phosphonates (ANPs), which are potential inhibitors of HGPRT and XPRT, and found ten compounds with an effective 50 % inihibitory concentration (EC50) in the single micromolar range. Importantly, when HGPRT was over-expressed in *T. brucei*, the ANPs with a guanine or hypoxanthine base had a significantly increased EC50 value, indicating that this enzyme is the target of the tested ANPs.

Oxidative Phosphorylation in Trypanosomatids

Zdeněk Verner[1,2,3], Petra Čermáková[1], Bianka Kováčová[1], Ingrid Škodová[1,2], Julius Lukeš[2,3] & Anton Horváth[1]
[1]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Bratislava
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[3]University of South Bohemia, Faculty of Science, České Budějovice

Trypanosomatids (Euglenozoa, Kinetoplastida) are obligate parasites of various organisms. Here, we studied oxidative phosphorylation in procyclic stage *Trypanosoma brucei*, *Leishmania tarentolae*, *Crithidia fasciculata* and *Phytomonas serpens*. Activities of NADH:ubiquinone oxidoreductase, succinate dehydrogenase, cytochrome c reductase and cytochrome c oxidase as well as activity of ATP synthase were detected by histochemical staining and/or measured spectrophotometrically and correlated with a rate of oxygen consumption. We used TMRE-stained cells and cells treated with an uncoupler FCCP to measure mitochondrial membrane potential in each organism. Interestingly, this method is not suitable for staining of *C. fasciculata*, we speculate that this is due to a negatively charged surface of this parasite. Composition of respiratory chain enzyme complexes and ATP synthase was elucidated using 2D BN/SDS-PAGE.

Formation and Exflagellation of Zoosporangia of a Coral Reef Alga *Chromera velia*

Jitka Kručinská[1,2] & Miroslav Oborník[1,2,3]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]ASCR, v. v. i., Institute of Microbiology, Praha

Chromera velia, the closest known photosynthetic relative of apicomplexan parasites, was found in Sydney harbor in 2008. Since then, its morphology and ultrastructure was described in detail. *C. velia* life cycle, where one zoospore is formed directly from one vegetative cell, was published as well. Here we unveil formation of large zoosporangia followed by exflagellation of multiple zoospores. We also outline new life cycle of *Chromera velia* in connections to recently published data about its endosymbiotic life strategy in reef corals.
Acknowledgment: This work was supported by Grant Agency of the Czech Republic No. 540/3158 and 540/3170.

Extensive Diversity of *Blastocystis* in Reptiles and Insects

Markéta Lorencová[1], Pavla Smejkalová[1,2], Magdalena Uzlíková[3] & Ivan Čepička[1]
[1]Charles University in Prague, Faculty of Science, Department of Zoology, Praha
[2]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha
[3]Charles University in Prague, First Faculty of Medicine, Department of Tropical Medicine, Praha

Blastocystis is an anaerobic protist that lives in the intestine of many animals including humans. Since members of the genus have completely lost flagella, the phylogenetic position of *Blastocystis* was puzzling until relatively recently. It is currently universally accepted that *Blastocystis* is a member of the Stramenopiles and is closely related to opalinids. Although morphologically uniform, *Blastocystis* displays enormous genetic diversity. In contrast, trophozoites of all *Blastocystis* lineages are considered morphologically identical. Isolates from birds and mammals are relatively well studied. On the other hand, almost nothing is known about *Blastocystis* in invertebrates and poikilotherm vertebrates. We have isolated approximately 30 *Blastocystis* strains from feces and intestines of tortoises, lizards, cockroaches, beetle larvae, and millipedes, and analyzed their SSU rDNA sequences and light-microscopic morphology. Our strains form six independent lineages across *Blastocystis* phylogenetic tree including the most basal branches. Our results show a considerable genetic and probably morphological diversity of *Blastocystis* in reptiles.

DNA Barcoding of Autotrophic Euglenoids

Maja Łukomska-Kowlaczyk[1], Anna Karnkowska[1], Małgorzata Korzeniecka[1] & Bożena Zakryś[1]
[1]University of Warsaw, Faculty of Biology, Department of Plant Systematics and Geography, Warszawa

DNA barcoding is a molecular identification method, which gains popularity recently. Barcoding uses a short genetic marker, which enable identification of species. The good barcode provide a large variation between species yet a relatively small amount of variation within a species. There is no universal barcode which could be effective in all groups of living organisms, therefore different fragments of DNA were chosen for different groups. The most popular barcodes for protists are 18S rDNA, COI (cytochrome c oxidase) gene and ITS sequences. For autotrophic euglenoids it is known, that ITS sequences are extremely variable, thus this marker is not suitable. We obtained sequences from 18S rDNA and COI and analysed intra and interspecific variability within 330 sequences of 18S rDNA and 65 sequences of COI. However the interspecific variability of COI gene was sufficient, we rejected this sequence as a barcode, because we were unable to develop universal primers for it. 18S rDNA also exhibited suitable variability and was much easier to amplify with set of universal primers. The whole sequence was too long (aprox. 1800 bp) therefore based on analysis of sequences we have chosen variable region V4 as the most appropriate as a barcode for autotrophic euglenoids.

Mechanism of *T. brucei* Cell Cytotoxicity by Benzophenone-Derived Bisphophonium Salts

Jan Martinek[1,2], Brian Panicucci[1,2], Harry P. de Koning[3] & Alena Zíková[1,2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom

A recent report claims that succinate dehydrogenase, respiratory complex II (cII), is the target of a subset of benzophenone-derived bisphophonium salts that inhibit *Leishmania donovani* proliferation in a low micromolar concentration range. However, this was suggested from the interpretation of broad phenotypes in treated cells and indirect evidence. We show that these compounds are also very potent inhibitors of both the insect (PS) and mammalian (BS) life stage of *Trypanosoma brucei*. Since cII is not essential in either stage of *T. brucei*, we explored the mechanism of cell death in this very closely related parasite. RNAi knockdown cell lines of a critical subunit of cII was generated in both life stages and analyzed for growth phenotype, mitochondrial membrane potential ($\Delta\Psi m$), cII assembly and cII activity. Furthermore, wild type and cII RNAi induced *T. brucei* cells were treated with two of these compounds and then monitored for their effects on ($\Delta\Psi m$) and cII activity. We propose that while these new trypanocidal drugs can directly inhibit cII, this is most likely not the major cause of cell death.

Oocyst or Sporocyst? Another Enigma in the Development of Cryptosporidia

Janka Melicherová[1], Jana Ilgová[1] & Andrea Valigurová[1]
[1]Masaryk University, Faculty of Science, Department of Botany and Zoology, Brno

Developmental stages of the gastric parasite, *Cryptosporidium muris* (strain TS03), were obtained from stomachs and faeces of experimentally inoculated *Mastomys coucha*. The development of *C. muris* oocysts was mapped in detail using a combined microscopic approach, supported by a freeze etching. Two types of wall forming bodies (WFB) of different electron density occurred in mature macrogamonts and zygotes. The WFB, located beneath the zygote pellicle, disintegrated into small particles and migrated into the space between pellicular membranes. Additional membranes seemed to develop beneath the pellicle so that four or more membranes could be seen enveloping more advanced zygote stages. Developing oocysts were enveloped by a parasitophorous sac and their wall comprised three layers. The outermost one, considered to be a 'true oocyst', was very fragile and this could be the reason that it usually remains unnoticed. In endogenous stages, this layer was usually separated from inner two layers and often almost unnoticeable as it was adjacent to the inner membrane of parasitophorous sac. The middle thin and the innermost thick layers, on which the characteristic suture could be seen, form the wall of a 'sporocyst'. Fully sporulated 'sporocysts', found either in stomach or faeces, were released from parasitophorous sac and frequently lacked the outermost layer ('oocyst'). Under scanning electron microscope, they exhibited either smooth or wrinkly surface, presumably depending on the wall thickness. Furthermore, due to a high variability in wall thickness, authors call into question the existence of two independent types of oocysts (thin- and thick-walled) in cryptosporidia.

Acknowledgment: Financial support provided by projects No. P506/10/P372 and MUNI/A/0937/2012.

Do Not Curse the Contamination – An Unexpected Discovery of the Novel Marine Fungus

Jan Michálek[1,2], Marie Pažoutová[1,2] & Miroslav Oborník[1,2]
[1]University of South Bohemia, Faculty of Science, České Budějovice
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

Chromerid algae *Chromera velia* and *Vitrella brassicaformis* are closest known autothrophic relatives of apicomplexans. After few years of cultivation of chromerid alga *V. brassicaformis* we have encountered a widespread mold contamination in the cultures. Presence of this fungus in the original sample of *V. brassicaformis* was confirmed. Morphological structures which arise on minimal medium with presence of *V. brassicaformis* remind assexual stages of enthomopathogenic ascomycetes of the group *Cordycipitaceae*. Analysis of nuclear ribosomal ITS region showed the highest similarity to several species of incorrectly determined marine fungi collected from various marine environments and to the *Beauveria* species (*Cordycipitaceae*). Further sequencing of large nuclear ribosomal subunit confirmed affiliation to the family *Cordycipitaceae*. Besides the unexplained ecological relationship to the chromerid algae or rather to the coral reef environment we are interested in this organism because marine fungi are currently being explored as a new source of bioactive compounds such as antibiotics and cytostatics.

Toward a Model of Euglenoid Non-Conventional Introns Structure

Rafał Milanowski[1], Anna Karnkowska[1], Takao Ishikawa[2] & Bożena Zakryś[1]
[1]University of Warsaw, Faculty of Biology, Department of Plant Systematics and Geography, Warszawa
[2]University of Warsaw, Faculty of Biology, Department of Molecular Biology, Warszawa, Polska

In nuclear genes of euglenoids three types of introns occur: (1) conventional spliceosomal introns, (2) non-conventional introns for which a splicing mechanism is unknown and (3) so-called intermadiate introns, which have some features of conventional and non-conventional introns. Very little is known about non-conventional introns – we do not know the mechanism of removal nor any factor involved in this process. It seems that they are excised by spliceosome-free mechanism, because the 5' ends of intron sequences are not complementary to U1 snRNA. It was just noticed that all introns of this type have non--canonical, variable borders and form a stable secondary structure bringing together both splice sites, what is probably needed for their proper removal. However this structure seems not to be conserved and shows no common features with self-splicing group I, II or III introns. To find the most conserved features of non-conventional introns the comparison of all known sequences was done. As a result, the most common elements in their sequence and secondary structure were indicated.

Substrate Specificity of Epiphytic Communities of Diatoms (*Bacillariophyceae*) and Desmids (*Desmidiales*)

Petra Mutinová[1] & Jiří Neustupa[1]
[1]Charles University in Prague, Faculty of Science, Department of Botany, Praha

Epiphytic community is an important component of aquatic ecosystems. The structure of epiphyton, as well as of other benthic communities, is influenced by abiotic factors and biotic interactions. However, it is not clear whether epiphytic community is influenced by its substrate, i. e. host plant, and how. Previous scanty publications showed that host plant could affect epiphyton positively or negatively, or alternatively, host pant is just a neutral substrate, as there are no biological and chemical influences of host plant on epiphyton.

This work is focused on the comparison of influence of substrate, site and environmental conditions on freshwater algal epiphyton. The research concerns two monophyletic, unrelated and ecologically very important groups of microscopic algae - diatoms (*Bacillariophyceae*) and desmids (*Desmidiales*). Besides the analysis of species composition, the influence of substrate on the phylogenetic structure of communities and their size structure will be studied. The results of this research should answer the drafted questions and determine whether there are parallel or contrast strategies of studied algal groups, therefore to which extent the discovered trends could be generalized for the entire microphytobentic community.

PUF proteins in *Giardia intestinalis*

Vladimíra Najdrová[1] & Pavel Doležal[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Giardia intestinalis is an anaerobic protozoan pathogen causing giardiasis, an intestinal disease of humans and animals. To date only limited data exist on the regulation of gene expression in *G. intestinalis* with the exception of the variable surface proteins, which constitute the immunoprotective coat of the cell. Thus, we have decided to characterize the family of RNA-binding proteins called PUF. By the sequence-specific binding to 3´untranslated regions (UTRs) of mRNAs PUF proteins control the repression, activation or sequestration of the target transcripts. These eukaryotic proteins are evolutionarily conserved from protists to aminals and plants. Five putative PUF proteins can be found in *G.intestinalis*. In order to reveal their function in *G. intestinalis* biology, we study their cellular localization as well as search for their cognate mRNAs.

Mitochondrion-Like Organelle of *Trimastix pyriformis*

Zuzana Zubáčová[1], Lukáš Novák[1], Jitka Bublíková[1], Vojtěch Vacek[1], Jan Fousek[2], Jakub Rídl[2], Jan Tachezy[1], Pavel Doležal[1], Čestmír Vlček[2] & Vladimír Hampl[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha
[2]Academy of Sciences of the Czech Republic, Institute of Molecular Genetics, Praha

Free-living, microaerophilic flagellate *Trimastix pyriformis* is closely related to oxymonads with which it constitutes group Preaxostyla within Metamonada, Excavata. Unlike in oxymonads, which are candidates for secondarily-amitochondriate eukaryotes, there has been found an enigmatic mitochondrion-like organelle in the cell of *Trimastix*. We have identified number of transcripts in the transcriptome of *Trimastix* whose products are putatively transported into the organelle. Among them are transcripts coding for proteins of the glycine cleavage system (GCS). We have conducted experiments which showed that proteins of this amino acid metabolism pathway are localized in the organelle. This makes GCS the only function of *Trimastix*s reduced mitochondrion known yet. Results of new experiments regarding the energy metabolism of *Trimastix* mitochondrion-like organelle will also be presented and discussed.

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

Tomáš Pánek[1], Petr Táborský[1] & Ivan Čepička[1]
[1]Charles University in Prague, Faculty of Science, Department of Zoology, Praha

We have isolated and cultured five strains of amoebae ('Anaeramoeba') from anoxic/microoxic marine coastal sediments worldwide. The strains morphologically represent two distinct, but similar species that are highly reminiscent of the genera *Flamella* (Gracilipodida), *Flabellula* or *Paraflabellula* (Tubulinea) by having extremely flattened, fan-shaped cells with trailing uroidal filaments. On the other hand, 'Anaeramoeba' displays several unique ultrastructure features. It possesses acristate mitochondrial derivates and a peculiar paranuclear body whose structure is yet not well understood. Phylogenetic analyses of SSU rDNA of three strains showed that 'Anaeramoeba' is related to neither of the aforemetioned genera. Instead, it forms an independent lineage with unclear phylogenetic affinities. Nevertheless, in most analyses it weakly clustered with the archamoebae, the major anaerobic clade of Amoebozoa. 'Anaeramoeba' thus represents either the closest known relative of the archamoebae, or independently arisen anaerobic lineage of the Amoebozoa.

Sphaerospora motemarini n. sp. Causes Glomerular Disease in Juvenile Grey Snapper *Lutjanus griseus* L.: A Reason for Host Population Declines in the Gulf of Mexico?

Sneha Patra[1], Astrid S. Holzer[1], Hana Pecková[1], Nathan P. Brennan[2], Carlos Yanes-Roca[2] & Kevan L. Main[2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]Mote Marine Laboratory, Sarasota, Florida, USA

In the eastern Gulf of Mexico, off the coast of Florida, grey snapper *Lutjanus griseus* was found to be infected with the myxozoan parasite *Sphaerospora motemarini* sp.n., with high prevalence (83 %) and intensity of infection occuring in 0^+ fish. The morphological, molecular and phylogenetic characterisation of the myxozoan showed that it is a member of the typically marine, polysporoplasmid *Sphaerospora* spp. which form a subclade within the *Sphaerospora sensu stricto* clade of myxozoans, characterised by large expansion segments in their SSUrDNA sequences. With specific PCR assay, invasive presporogonic stages of the parasite were detected in blood. Pseudoplasmodia and spores were found to develop in the renal corpuscles of the host, causing their massive expansion. Macroscopic and histopathological changes showed that *S. motemarini* n. sp. causes severe glomerulonephritis in *L. griseus* which makes it more susceptible to stress and leads to mortalities. As populations of *L. griseus* are declining in the south of Florida, in the future, we aim to determine prevalence and intensity of infection in juvenile *L. griseus* in different areas of the Gulf of Mexico in order to be able to estimate the temperature dependence of *S. motemarini* n. sp. proliferation and to predict its distribution and severity during climatic changes in the Gulf.

Everything You Always Wanted to Know About Sex of Greens but Were Afraid to Ask: Reviewing the Sexuality among Trebouxiophyte Green Algae

Marie Pažoutová[1,2], Fabio Rindi[3], Karolína Fučíková[4], Aleš Horák[1,2], Stephane Rombauts[5] & Miroslav Oborník[1,2]

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

[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

[3]Università Politecnica delle Marche, Dipartimento di Scienze della Vita e dell'Ambiente, Ancona, Italy

[4]University of Connecticut, Department of Ecology and Evolutionary Biology, USA

[5]VIB/Ghent University, Bioinformatics & Systems Biology, Gent, Belgium

Sex, a process of reproduction that comprises meiosis and syngamy, is one of the key features of eukaryotes. Sex is ancient and widespread, yet very costly. The costs vs. benefits intellectual conundrum has been called "the queen of problems in evolutionary biology" and has been under endless discussion over many decades. The use of molecular methods in population genetics and the rise of genomics brought evidence for sexuality (or cryptic sexuality) among some of the putatively asexual groups of organisms. The question that emerges is, how many of the lineages in the eukaryotic tree of life do have the capacity for sex and if they have it, whether and how they use it. Are there true "ancient asexuals" at all or are the little beasts just extremely shy? Our study focuses on the trebouxiophyte green algae as a model group of understudied and putatively asexual microorganisms, where different evidence of sexual process (direct observation, genetics, genomics) is already available. In accordance with the *Chlorella* and *Coccomyxa* genome revelations, the partial draft genome of *Prasiola crispa* shows presence of several meiosis-specific proteins. We suspect that among trebouxiophytes, the ability for sexual reproduction is rather common, albeit overlooked.

The Role of *Sergentomyia schwetzi* in Epidemiology of Visceral Leishmaniasis in Ethiopia

Nikola Polanská[1] & Iva Kolářová[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Phlebotominae sand flies (Insecta, Diptera) are widespread in subtropical and tropical regions. They are vectors of *Leishmania* parasites (Kinetoplastida), the causative agent of leishmaniasis that can affect vertebrates, including humans and veterinary important animals. The clinical signs range from small self-healing cutaneous lesions to life-threatening visceral manifestations. The life cycle of the parasites can include reservoir hosts, which can be both the wild and domestic animals. The reservoir hosts typically serve as a source of infection for the sand fly females. This phenomenon helps to establish the endemic focus and higher the risk of transmission to humans.

Ethiopia is one of the several countries endemic for visceral leishmaniasis caused by *Leishmania donovani*. *Phlebotomus orientalis* is one of the main vectors in this area, but the most abundant are sand flies of the genus *Sergentomyia*. *Sergentomyia* females prefer to feed blood from poikilothermic vertebrates, but several studies showed also mammals as an important source of blood. The main aim of this study was to test, whether *Sergentomyia schwetzi* is able to feed blood on mammals, domestic animals in particular, and could be thus possibly involved in *L. donovani* life cycle and in transmission to humans.

Mitochondrial Processing Peptidases in *Trypanosoma brucei*

Pavel Poliak[1,2], Jan Mach[3], Jan Tachezy[3] & Julius Lukeš[1,2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Most mitochondrial proteins encoded in the nucleus are synthesized on cytoplasmic ribosomes as larger precursors with amino-terminal extension peptides for targeting into mitochondria. During or shortly after import of the precursors into mitochondria, the extension peptides are proteolytically removed by different types of processing peptidases (Gakh et al. 2002). Mitochondrial presequences on trypanosomatid precursors have been found to be either 8–9 or 15–31 amino acids long (Hausler et al. 1997, Maslov et al. 2002) and contain positively charged and hydroxylated amino acid residues with almost no acidic amino acids (Hausler et al. 1997). The major presequence protease is the mitochondrial processing peptidase (MPP) located in the matrix (Taylor et al. 2001, Neupert and Herrmann 2007). The mitochondrial intermediate peptidase (MIP) is a soluble mitochondrial matrix protein which functions after MPP and typically removes an octapeptide from several preproteins (Gakh et al. 2002). In contrast to MIP, which can cleave its substrate only after initial processing by MPP, the inner membrane peptidase (IMP) can remove a hydrophobic sorting signal from mitochondrial precursor proteins independent of MPP (Gakh et al. 2002, Neupert and Herrmann 2007, Mossmann et al. 2012). Recently, a mitochondrial aminopeptidase was identified – the intermediate cleaving peptidase ICP55 – that typically removes a single amino acid residue after processing by MPP (Vögtle et al. 2009). ICP55 has not yet been purified and therefore the mechanism of substrate recognition and catalysis is presently unknown. Interestingly, ICP55 was reported to be dual localized to both mitochondria and the nucleus.

Does Dietary Starch Have an Impact on *Neobalantidium coli* Infections in Captive Chimpanzees?

Kateřina Pomajbíková[2], Kateřina Schovancová[1], Petr Procházka[3], David Modrý[2,4,5], Petra Bolechová[6] & Klára J. Petrželková[2,3,4,6]

[1]Masaryk University, Faculty of Science, Department of Botany and Zoology, Brno

[2]University of Veterinary and Pharmaceutical Sciences, Department of Pathology and Parasitology, Brno

[3]ASCR, v. v. i., Institute of Vertebrate Biology, Brno

[4]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

[5]Central European Institute for Technology, Brno

[6]Liberec Zoo, Liberec

Although the infections by *Neobalantidium coli* in humans were recognized more than one and half century ago, many aspects of the epidemiology or mechanisms of pathogenicity remain unknown. Infections are asymptomatic in most hosts, but in humans and captive African great apes clinical infections manifested mainly by dysentery occasionally occur. Thus, *Balantidium* infections in captive African great apes offer an interesting model, which helps us to unravel the factors contributing to development of clinical balantidiasis in humans. Several almost forgotten experimental studies pointed to a possible effect of diet on *N. coli* infections in pigs and rats with the emphasis on the starch content. We studied the effect of dietary starch on the intensities of infection by *N. coli* in two groups of captive chimpanzees from the Hodonín Zoo and from the Brno Zoo, Czech Republic. We fed adult chimpanzees infected by *N. coli* with a high starch diet (HSD) (average 13.5 % of starch), followed by a five-day transition period and subsequently with a period of low starch diet (LoSD) (average 0.1 % of starch). We collected fecal samples during the last seven days of HSD and LoSD and stored them in 10 % formalin. We quantified trophozoites of *N. coli* using the FLOTAC method. Generalized linear mixed-effects model showed significantly lower numbers of the *N. coli* trophozoites in the feces during the LoSD in comparison to the HSD. We conclude that a starch-rich diet can be responsible for high intensities of infection of *N. coli* in captive chimpanzees and might predispose them to clinically manifested balantidiasis. We discuss the potential nutritional modifications to diets that can be implemented in part to control *N. coli* infections in captive apes. Our finding opens also the question of the similar dietary effect on *N. coli* infection in humans.

Acknowledgment: We would like to express our sincere thanks to the Hodonín and Brno Zoos for enabling us to conduct this study. We are particularly grateful to Jaroslav Hyjánek, Jiří Ingr, Petra Bílková, Lukáš Baránek (Zoo Hodonín) and Martin Hovorka, Jiří Vítek, Iva Slaninová, Marcela Němečková, Mariana Hubíková (Zoo Brno). The manuscript benefited from valuable comments and language revision by Dr. J. M. Rothman from the Department of Anthropology, Hunter College at the City University of New York (USA).

The Diversity in the *Vischeria/Eustigmatos* Complex (Eustigmatophyceae): Morphological and Molecular Perspectives

Kateřina Procházková[1], Lira A. Gaysina[2], Martina Pichrtová[1], Alena Lukešová[3] & Marek Eliáš[1,4]

[1]Charles University in Prague, Faculty of Science, Department of Botany, Praha
[2]M. Akmullah Bashkir State Pedagogical University, Department of Botany, Russia
[3]Biology Centre ASCR, v. v. i., Institute of Soil Biology, České Budějovice
[4]University of Ostrava, Faculty of Science, Department of Biology and Ecology, Ostrava

Vischeria spp. and *Eustigmatos* spp. are closely related coccoid algae common in terrestrial habitats. The two genera were distinguished by relatively subtle morphological features (the cell surface raised into projections or ridges, or smooth, respectively). Three species in *Eustigmatos* were recognised, but their discrimination proved difficult in practise. Twelve species were described in *Vischeria*, but nine of them have been rarely, if ever, observed since the original description. To reassess the diversity and taxonomy of the *Vischeria/Eustigmatos* complex, we studied a wide set of strain from public algal collections, including type strains of two *Eustigmatos* and three *Vischeria* species, and of strains newly isolated from places distributed all over the globe. Sequencing of the nuclear ITS rDNA region and the plastid rbcL gene showed that: 1) maintaining *Vischeria* and *Eustigmatos* as separate genera is not tenable 2) the five species represented by type strains are indeed genetically distinct from each other 3) there is a large number of additional lineages of a similar degree of phylogenetic separation, few of which can, however, be identified as some of the remaining *Vischeria/Eustigmatos* species described previously. Our results thus indicate that the morphological species concept cannot be easily applied in this algal group.

The First Known Endobiotic *Carpediemonas*-Like Organism

Eliška Ptáčková[1] & Ivan Čepička[1]
[1]Charles University in Prague, Faculty of Science, Department of Zoology, Praha

The taxon Fornicata represents one of the major eukaryotic lineages. It comprises predominantly parasitic/commensal diplomonads and retortamonads, and six lineages of exclusively free-living, marine *Carpediemonas*-like organisms (CLOs). We have isolated strain PHEM1 from feces of a gecko. Cells of PHEM1 are biflagellate and often display uncommon spiral morphology. Phylogenetic analyses of SSU rDNA unexpectedly showed that PHEM1 is a fornicate and belongs to the lineage CL3. The closest relative of PHEM1 is the organism PCS that represents an undescribed fornicate genus and shows some morphological similarities with PHEM1. The organism PHEM1 is the first known endobiotic member of CLOs. In addition, it was isolated from a terrestrial host, while all other to date discovered CLOs are marine. Our data show that the endobiotic style of life has arisen at least three times independently within the Fornicata.

Glycolytic Enzyme Phosphofructokinase Is Targeted to Hydrogenosomes of *Trichomonas vaginalis*

Petr Rada[1], Ivan Hrdý[1] & Jan Tachezy[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Phosphofructokinase (PFK) is a key regulatory enzyme of glycolysis that generally occurs within the cytosol. There are two types of PFK: ATP-dependent (ATP-PFK) and PPi-dependent PFK (PPi-PFP). In terms of glycolysis, *Trichomonas vaginalis* represents a unique organism, because its genome is coding for 4 homologues of ATP-PFK and 7 homologues of PPi-PFP. Interestingly, three paralogues of ATP-PFK were detected in the proteome of *T. vaginalis* hydrogenosome (Rada et al., 2011). To validate the proteomic analysis, epitope-tagged ATP-PFK was expressed in trichomonads, which confirmed hydrogenosomal localization of this protein. Then we searched for hypothetical partners of ATP-PFK within hydrogenosome and identified its two paralogues, which one of them was previously detected in the proteome. Sequence alignment of *T. vaginalis* ATP-PFK with its bacterial homologues revealed striking similarity with N-terminus of ATP-PFK from *Escherichia coli*. To investigate, whether *E. coli* ATP-PFK is capable for import into hydrogenosome, we expressed this protein in trichomonads and demonstrated its localization within the organelle.

Coevolution of *Cryptosporidium tyzzeri* and the House Mouse

Martin Kváč[1], John McEvoy[2], Martina Loudová[3], Brianna Stenger[2], Bohumil Sak[4], Dana Květoňová[4], Oleg Ditrich[4], Veronika Rašková[1], Elaine Moriarty[5], Michael Rost[6], Miloš Macholán[7] & Jaroslav Piálek[8]
[1]University of South Bohemia, Faculty of Agriculture, České Budějovice
[2]North Dakota State University, Department of Veterinary and Microbiological Sciences, USA
[3]University of South Bohemia, Faculty of Science, České Budějovice
[4]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[5]Christchurch Science Centre, Institute of Environmental Science and Research (ESR) Ltd, Christchurch, New Zealand
[6]University of South Bohemia, Faculty of Economics, České Budějovice
[7]ASCR, Institute of Animal Physiology and Genetics, Laboratory of Mammalian Evolutionary Genetics, Brno
[8]ASCR, v. v. i., Institute of Vertebrate Biology, Department of Population Biology, Brno

Two house mouse subspecies occur in Europe, eastern and northern *Mus musculus musculus* (Mmm) and western and southern *M. m. domesticus* (Mmd). Where their ranges meet a secondary hybrid zone occurs, running from Scandinavia to the Black Sea. In this paper, we tested a hypothesis that the apicomplexan protozoan species *Cryptosporidium tyzzeri* has coevolved with the house mouse. More specifically, we assessed to what extent the evolution of this parasite mirrors divergence of the two subspecies. In order to test this hypothesis, we analyzed sequence variation at five genes (small subunit rRNA, COWP, TRAP-C1, actin, and gp60) in *C. tyzzeri* isolates from Mmd and Mmm sampled along a transect across the hybrid zone from the Czech Republic to Germany. Mmd samples were supplemented with mice from New Zealand. We found two distinct isolates of *C. tyzzeri*, each occurring exclusively in one of the mouse subspecies (*C. tyzzeri*-Mmm and *C. tyzzeri*-Mmd). In addition to genetic differentiation, oocysts of the *C. tyzzeri*-Mmd subtype (mean: $4.24 \times 3.69\,\mu m$) were significantly smaller than oocysts of *C. tyzzeri*-Mmm (mean: $4.49 \times 3.90\,\mu m$). Mmm and Mmd were susceptible to experimental infection with both *C. tyzzeri* subtypes however, the subtypes were not infectious for the rodent species *Meriones unguiculatus*, *Mastomys coucha*, *Apodemus flavicollis*, or *Cavia porcellus*. Overall, our results support the hypothesis that *C. tyzzeri* is coevolving with Mmm and Mmd.

Evolutionary Significance of Free-Living Diplomonads

Eva Rmoutilová[1], Eliška Ptáčková[1] & Ivan Čepička[1]
[1]Charles University in Prague, Faculty of Science, Department of Zoology, Praha

Diplomonads (Diplomonadida) is a group of excavate flagellates belonging to the recently established taxon Fornicata. Most diplomonads are diplozoic, i. e. have a double set of cell structures, though some unizoic diplomonads have been discovered as well. Evolutionary relationships between unizoic and diplozoic diplomonads have not yet been elucidated. Large proportion of diplomonads lives endobiotically, but some species are free-living. Remarkably, it is believed that they are secondarily free-living. Diversity of free-living diplomonads has not been studied in detail until recently. We obtained approximately 30 isolates of free-living diplomonads mostly from freshwater anoxic sediments worldwide and analyzed their SSU rDNA and light-microscopic morphology. Our preliminary results show that the diversity of free-living diplomonads is bigger than expected and support the hypothesis that they are secondarily free-living. Among others, we identified a novel lineage *Trepomonas* which is closely related to the unizoic genus *Trimitus*.

Localisation and Functional Analysis of Heme Pathway in *Phaeodactylum tricornutum*

Eva Roubalová[1], Zoltán Füssy[1] & Miroslav Oborník[1,2,3]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, Laboratory of Evolutionary Protistology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]ASCR, v. v. i., Institute of Microbiology, Praha

Heme biosynthesis belongs to the most essential metabolic pathways, however the subcellular localisations of heme pathway enzymes in the model diatom *Phaeodactylum tricornutum* have not been studied experimentally yet. Since not all gene annotations are complete in *P. tricornutum*, we have revised all the pathway genes using available genomic and RNA-seq data. RACE technique is being implemented to determine the sequences of 5' ends of the transcripts in question. Within respective predicted proteins, we have particularized all the candidate N-terminal targeting signals by *in silico* predictions of ER signal peptides and transit peptides. To verify the *in silico*-predicted localisations, we utilize transfection of *P. tricornutum* by vectors expressing N-terminal domains of studied proteins fused to GFP. Notably, there are more gene variants coding for uroporphyrinogen decarboxylase (UROD) and coproporphyrinogen oxidase (CPOX) present in the genome, with different origins and not fully understood functions. We plan to employ functional genomics approaches (RNAi/antisense RNA) to inquire into functions of individual heme pathway enzymes, including different variants of UROD and CPOX. Furthermore, we intend to complement particular knockdowns using corresponding genes of *Chromera velia*, a phototrophic relative of apicomplexans, thus contributing to assess the degree of functional universality among various protist groups.

Acknowledgment: This publication was supported by the project Postdok_BIOGLOBE (CZ.1.07/2.3.00/30.0032) co-financed by the European Social Fund and the state budget of the Czech Republic (ER) and by GAP501/13-33039S (ZF).

Comparison of Glycerolipid Composition of Two Chromerida Species: *Chromera velia* and *Vitrella brassicaformis*

Ivana Schneedorferová[1,2], Aleš Tomčala[1] & Miroslav Oborník[1,2,3]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, Laboratory of Evolutionary Protistology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]ASCR, v. v. i., Institute of Microbiology, Praha

Malaria is deadly disease of humans and animals caused by protists of the genus *Plasmodium* belonging to apicomplexans. *Chromera velia* and *Vitrella brassicaformis* are photoautotrophic alveolates recently found in Australian corals. The ultrastructure, photosynthetic pigment profiles and phylogenetic analyses revealed that Chromerida are the closest known photosynthetic relatives of apicomplexan parasites. Lipidomic profiles of both these algae help to understand the lipid biosynthesis and degradation, and particularize annotation of genes involved in lipid biosynthesis in these organisms. Extraction, separation, and mass spectrometry methods were adapted for this purpose. Total lipid extraction followed by solid phase extraction (SPE) separation was performed to acquire two fractions of nonpolar and polar lipids. Liquid chromatography (LC) coupled with mass spectrometry (MS) are able to reveal intact lipid molecules. On the other hand the complementary technique of gas chromatography mass spectrometry (GC/MS) is suitable for determination of double bound position in fatty acid incorporated in lipids molecules. Preliminary studies supported by multivariate statistic analysis revealed extremely higher content of storage triacyglyceroles in *C. velia* in comparison to *V. brassicaformis*.
Acknowledgment: Financial support by the Czech Science Foundation (P506/12/1522) is gratefully acknowledged.

Paratrypanosoma – a Novel Ancestral Trypanosomatid

Pavel Flegontov[1,2], Jan Votýpka[1,2,3], Tomáš Skalický[1,2], Maria D. Logacheva[4], Alexey A. Penin[4], Goro Tanifuji[5], Naoko T. Onodera[5], Alexey S. Kondrashov[4,6], John M. Archibald[5] & Julius Lukeš[1,2]

[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

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

[3]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

[4]Lomonosov Moscow State University, Moscow, Russia

[5]Dalhousie University, Halifax, Nova Scotia, Canada

[6]University of Michigan, Life Sciences Institute, Ann Arbor, USA

The kinetoplastids are widespread and important unicellular eukaryotes, many of which are devastating parasites. In the gut of *Culex pipiens*, we have discovered a new insect trypanosomatid which GAPDH and SSU rRNA-based phylogenetic analyses place into a separate branch between free-living *Bodo saltans* and parasitic *Trypanosoma* species. From draft genome sequence data we identified 114 protein genes shared between the new isolate, 15 trypanosomatid species, *B. saltans*, and *Naegleria gruberi*, as well as 129 protein genes shared with the early-branching kinetoplastid *Perkinsela*. Protein-by-protein phylogenies together with analysis of concatenated alignments show that the new isolate branches at the base of the family Trypanosomatidae. Thus, this newly identified insect flagellate, here named *Paratrypanosoma culicis* n. gen., n. sp., represents a missing link between free-living bodonids and obligatory parasitic trypanosomatids, further analysis of which should provide insight into the emergence of parasitism in this medically important group.

New Evidence for the Polyphyly of Retortamonads

Pavla Smejkalová[1,2], Eva Nohýnková[3], Jaroslav Kulda[2] & Ivan Čepička[1]
[1]Charles University in Prague, Faculty of Science, Department of Zoology, Praha
[2]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha
[3]Charles University in Prague, First Faculty of Medicine, Department of Tropical Medicine, Praha

Retortamonads (genera *Retortamonas* and *Chilomastix*) are a small group of heterotrophic flagellates living mostly as intestinal commensal of vertebrates and invertebrates. Although retortamonads were originally proposed to be monophyletic on the basis of light-microscopic morphology, recent molecular analyses have brought new data indicating polyphyly of retortamonads. The discrepancy between results of morphological and molecular analyses could be explained by the fact that the previous TEM studies were performed on *Retortamonas* spp. from insects while molecular phylogenetic studies were based on *Retortamonas* spp. from vertebrates. Our new morphological, ultrastructural and molecular data confirmed polyphyly of retortamonads and suggested the existence of two unrelated lineages of the genus *Retortamonas*. While strains isolated from vertebrates formed a sister branch to diplomonads, isolates from insects branched within *Chilomastix*. In addition, we revealed an extensive variability in the length of SSU rDNA of *Chilomastix* ranging from ca. 1500 to more than 4000 bp. Analysis of the secondary structure of SSU rRNA detected the presence of five hypervariable regions. On the basis of our results we propose a taxonomic revision of retortamonads.

TgASP5 – and Analog of PEXEL Processing Plasmepsin V from *Toxoplasma gondii*

Daniel Sojka[1]
[1]University of Geneva, Faculty of Medicine, Geneva, Suisse

TgASP5 is a member of an evolutionary distinguished 'group C' of apicomplexan aspartic peptidases comprising homologous Plasmepsin V facilitating effector plasmodial proteins for transportation to host erythrocytes by cleaving a short N-terminal RxLxE/Q/D motive (PEXEL). PEXEL containing proteins of *T. gondii* are probably not targeted to the host cell, but PEXEL could be important for targeting to the parasitophorous vacuole membrane (PVM). Our scientific goal is to verify the role of TgASP5 in this process. Using gene targeting by a single homologous recombination (knock-in), we have constructed a *T. gondii* line expressing C-terminal -Ty epitope tag from the endogenous TgASP5 locus. Unlike plasmepsin V located in the endoplasmatic reticulum TgASP5 localizes specifically to Golgi. We have also used the TgASP5 gene knock-in/knock-out strategy to interrupt the reading frame of the endogenous TgASP5 gene and demonstrated its essential role for Toxoplasma. Conditional gene knock-down including expression of the TgASP5 gene from a tetracycline operon controlled promoter, introduction of a destabilization domain (DD) or utilizing the Cre-lox site-specific recombinase are used to study the enzyme specific role. In parallel, immunopurified and recombinantly expressed TgASP5 are characterized in-vitro and used for cleavage preference determination in assays with unique peptidyl substrate libraries.

Transcriptome of *Rhabdomonas costata* and the Testing of Plastid-Late Hypothesis for the Euglenid Plastid Origin

Petr Soukal[1], Anna Karnkowska-Ishikawa[1], Štěpánka Hrdá[1], Jana Szabová[2], Miluše Hroudová[2], Čestmír Vlček[1] & Vladimír Hampl[1]
[1]Faculty of Science, Charles University in Prague, Prague, Czech Republic
[2]Institute of Molecular Genetics, Academy of Science of the Czech Republic, Prague, Czech Republic

Euglenida comprise species with various forms of nutrition – bacteriovores, eukaryovores, osmotrophs, myxotrophs and photoautotrops. The photoautotrophic and mixotrophic euglenids contain a secondary plastid of green-algal origin. Because the plastid-containing species form one well-supported clade it is hypothesized that the plastid was acquired by the common ancestor of this clade – plastid-late hypothesis. Contrary to this, plastid-early hypothesis postulates that plastid was acquired earlier even in the common ancestor of Euglenozoa. Plastid late hypothesis would be falsified if it was proven that early branching lineages contain substantially high number of genes acquired from the plastid endosymbiont. We have performed transcriptome survey of an osmotrophic species *Rhabdomonas costata* branching outside the clade of plastid-containing species. Using BLAST against our local database, we have selected 351 potential candidates for genes originating from the algal endosymbiont. Phylogenetic trees for these candidates will be reconstructed to reveal their evolutionary origin.

Knocking Out Genes in *Trichomonas vaginalis*

Darja Stojanovová[1], Jan Pyrih[1] & Jan Tachezy[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Methods of reverse genetics are crucial for functional studies of genes identified in genomes of parasitic protists such as *Trichomonas vaginalis*. However, neither gene silencing nor gene knockout is routinely used in this parasite. All attempts to establish RNA interference in *T. vaginalis* and utilization of antisense oligonucleotides seems to be inefficient. Therefore we decided to establish a technique of gene knockout in *T. vaginalis* that was successfully employed by others, although only two genes (ferredoxin 1 by Land et al. 2004, and a hydrogenosomal membrane protein Hmp23 by Bras et al. 2013) were inactivated by this method thus far. Currently we attempt to reproduce the knockout of ferredoxin gene published by Land et al., 2004. We have used the plasmid pKO-Fdx-Neo, in which the neomycin resistance cassette is surrounded by ferredoxin flanking regions (2 and 2.6 kbp). The plasmid was digested by XhoI, SacI and ScaI restriction enzymes and used for *T. vaginalis* transfection by electroporation. A circular plasmid was used as an electroporation control. Positive transformants were selected for two weeks with $90\,\mu g \cdot ml^{-1}$ G418 in TYM medium. However, no viable ferredoxin knockout cells were selected using the digested plasmid so far. Currently, we are testing an optimal concentration of antibiotic that appeared to be critical for initial selection of resistant clones.

Evolution and Distribution of MAT and MATX Genes in Euglenids

Jana Szabová[1], Richard E. Triemer[2], Naoji Yubuki[3] & Vladimír Hampl[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha
[2]Michigan State University, Department of Plant Biology, USA
[3]The University of British Columbia, Department of Botany, Vancouver, British Columbia, Canada

Methionine adenosyltransferase (MAT) is a ubiquitous enzyme that synthesizes S--adenosylmethionine, which is one of the most important metabolites in all living cells. This essential enzyme occurs in eukaryotes in two relatively divergent paralogs, MAT and MATX. MATX was found in a variety of unrelated organisms, but its distribution is punctuated and except of few organisms is mutually exclusive. This could have arisen by differential losses of old paralogs or by horizontal gene transfers of one of them between eukaryotes. Our aim was to map the distribution of MAT/MATX genes in the group of euglenids and thus help to clarify the evolutionary history of MATX in this clade. We gained 26 new sequences from 23 various euglenids and one prasinophyte alga *Pyramimonas parkae*. MATX was found only in the photoautotrophic euglenids. The mixotroph *Rapaza viridis* and the prasinophyte alga *Pyramimonas parkae*, the closest known relative of plastid ancestor in euglenids, both possess only MAT form of the gene. In two euglenid species (*Monomorphina pyrum* and *Phacus orbicularis*) we found both types MAT and MATX. However, these MAT genes were unrelated to ancestral euglenid MATs. Our results suggest that the MATX distribution in euglenids is restricted to photoautotrophs. The distribution of MAT/MATX in euglenids can be explained by only one HGT of MATX that happened after the origin of euglenid secondary plastid and by two HGTs of MAT into two photoautotrophic species.

The Relative Biovolume of Benthic Diatom Assemblages in Relation to Environmental Conditions

Tereza Ševčíková[1] & Jana Kulichová[1]
[1]Charles University in Prague, Faculty of Science, Department of Botany, Praha

Benthic diatom communities are widely used as indicators of water quality. Long-term biomonitoring of ecological status of fresh waters is anchored in the legal system of national and transnational level (NAWQA, WFD). Many research groups deal with the issue in assessing the relationship between biovolume of diatom communities and various parameters (content of dissolved nutrients, water temperature, water colour, pH, conductivity). There are several main theories explaining the response of relative biovolume to environmental conditions often based on an advantage of S/V ratio, sedimentation, or shading; but still an important part of the observed responses of diatom communities remains unclear. The main questions of my study are: i) whether the correlation between environmental variables (pH, conductivity) and biovolumes of benthic diatoms is significant; ii) whether the response of relative biovolume between two geographical regions (Czech Republic, Norway) is different; iii) whether the relative biovolume of diatom communities is a good predictor of environmental variability than as the species composition and diversity of diatoms.

Characterization and Expression of *Phlebotomus orientalis* Salivary Antigens

Michal Šíma[1], Iva Kolářová[1] & Petr Volf[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Sand flies (Phlebotominae) are bloodfeeding insects and vectors of *Leishmania* (Trypanosomatidae), the causative agents of leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Repeated exposures to sand fly saliva elicit anti-saliva antibodies that could be used in epidemiological studies as a marker of exposure to assess the effectiveness of anti-vector campaigns and to assess the risk of *Leishmania* transmission. The anti-saliva host immunity has been also shown to protect the host from *Leishmania* infection, thus salivary proteins are under consideration as a part of anti-leishmania vaccine.

The main aim of this study is to characterize and express the salivary gland antigens of *Phlebotomus orientalis*, the important vector of life-threatening visceral leishmaniasis in East Africa. The main antigens were determined by SDS-PAGE and immunoblot using antibodies from hosts repeatedly bitten by this sand fly species in Ethiopia. Based on the cDNA library and proteome analysis, we identified eight antigens with molecular weight from 26 kDa to 42 kDa from five different protein families. All of these proteins are antigenic for dogs but only four of them for humans (apyrase, yellow-related protein, antigen 5-related protein, D7-related protein). These antigens were expressed in the bacterial expression system. Final products will be compared in their ability to elicit antibody response and to bind specific antibodies in sera from hosts repeatedly bitten by *P. orientalis*. The selected recombinant antigen(s) could be utilized in larger epidemiological studies.

Hypothetical Trypanosoma Protein Helps to Anchor the F_1-ATPase Moiety to the Mitochondrial Membrane

Karolína Šubrtová[1,2], Brian Panicucci[2] & Alena Zíková[1,2]
[1]University of South Bohemia, Faculty of Science, České Budějovice
[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

Trypanosoma brucei is a medically important parasite that infects humans and livestock. Interestingly, the mitochondrial (mt) F_oF_1-ATPase activity is essential in the infectious form of this pathogen as it hydrolyzes ATP to pump protons into the mt inner membrane space to maintain the mt membrane potential (mt $\Delta\Psi$) in the absence of a traditional cytochrome mediated respiratory chain. Unlike the well conserved higher eukaryotic F_oF_1-ATP synthases, the *T. brucei* F_oF_1-ATP synthase contains several trypanosoma specific subunits with unknown function. One of the largest novel subunits, Tb2930 (43 kDa), is membrane-bound and localizes into monomeric and multimeric assemblies of the F_oF_1-ATPase. RNAi silencing of Tb2930 led to a significant decrease of the mt $\Delta\Psi$ and consequently to a major growth phenotype, indicating that the F_oF_1-ATPase is not functioning properly even though its structural intergrity seems unchanged. To further explore the function of this protein, we silenced the expression of Tb2930 in a strain of trypanosoma lacking mitochondrial DNA (dyskinetoplastic, Dk) and thus subunit a, an essential component of the F_o moiety and proton pore. Dk cells maintain mt $\Delta\Psi$ by the electrogenic exchange of ATP4-/ADP3- by the ATP/ADP carrier (AAC) and the hydrolytic activity of the F_1-ATPase. The depletion of Tb2930 in Dk cells resulted in a significant growth phenotype caused by a decreased mt $\Delta\Psi$. Importantly, subfractionation of the Dk mitochondria showed that in Tb2930 knockdown cells, the F_1 moiety is more loosely attached to the membrane. In conclusion, Tb2930 is responsible for connecting the F_1-ATPase to the mt membrane in the absence of the F_o moiety, thus increasing the efficiency of the functional association between F_1-ATPase and AAC.

Keywords in Karyotypes of *Giardia*: Aneuploidy, Heterogeneity, Minimalism

Pavla Tůmová[1], Lenka Hudosová[1], Kristýna Marková[1], Gerhard Wanner[2] & Eva Nohýnková[1]

[1]Charles University in Prague, First Faculty of Medicine, Department of Tropical Medicine, Praha

[2]Ultrastructural Research, Biozentrum der LMU München, Germany

The presence of two transcriptionally active nuclei within a cell is a characteristic feature of trophozoites of the parasitic diplomonad *Giardia intestinalis*. Cytogenetic analysis revealed different chromosome numbers (8–14 per nucleus), varying among individual clinical isolates and laboratory lines from diploidy ($2n = 10$) to a stable transmitted aneuploidy as shown by karyotyping and FISH analysis. Moreover, different patterns of aneuploidy coexist in both nuclei within a single cell, without preventing proliferation, long term cultivation and ability to encyst. The non-canonical course of mitosis and the absence of spindle assembly checkpoint leading to putative chromosome non-disjunctions are likely to underlie the described aneuploidy in *Giardia*. The apparent heterogeneity seen among different laboratory lines and clones derived from WB isolate taken from a patient with clinically resistant giardiasis indicates a rapid karyotype differentiation, suggesting a clonal propagation of newly emerging karyotypes. The genomic minimalism unwound by the sequencing project is obvious also from the simple ultrastructure of *Giardia* tiny chromosomes, belonging to the smallest eukaryotic chromosomes visualized by high-resolution scanning electron microscopy.

Mitochondrial Chaperone and kDNA

Jiří Týč[1,2], Tomáš Skalický[1,2], Somsuvro Basu[1,2] & Julius Lukeš[1,2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice

Mitochondrial chaperone Hsp70 (mtHsp70) takes part in many essential processes in the mitochondrion – folding of newly synthesized proteins and folding and degradation of damaged and denatured proteins. Moreover, this mitochondrial version of Hsp70 gained new functions such as in Fe-S cluster biogenesis and protein import into the organelle. Another poorly explored aspect of mtHsp70 is its association with mitochondrial DNA (mtDNA). This finding was never properly addressed in eukaryotes, while the bacterial homolog (DnaK) of mtHsp70 was proven to act in replication of both chromosomal and plasmid DNA, as well as the bacteriophage DNA. *Trypanosoma brucei* is a suitable model for studies of mtDNA, since there is only one large mitochondrion per cell and its mtDNA, represented by a dense huge network of circular DNA molecules – the kinetoplast (kDNA) is located close to the basal body of the flagellum and can be observed using light microscopy. Our preliminary data show that in cells depleted for mtHsp70, kDNA is getting smaller and eventually disappears completely. More detailed examination by electron microscopy revealed that the ultrastructure of kDNA is severely altered in almost 100 % of cells ablated for mtHsp70. Sucrose gradient centrifugation revealed that a portion of mtHsp70 co-sediments with kDNA. Other putative functions of this mitochondrial chaperone and interacting proteins are under study.

Functional Analysis of TbFis1 Protein in *T. brucei*

Jiří Ťápal[1,2], Lucie Kafková[1,2], Julius Lukeš[1,2] & Hassan Hashimi[1,2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice

Mitochondrial fission and fusion are important processes because they control mitochondrial distribution and function. Two proteins play essential role in mitochondrial fission, dynamin-related protein (termed DRP), primarily localized into the cytosol and Fission protein (Fis1), integral membrane protein, which is located in outer mitochondrial membrane. These proteins are conserved across eukaryotic tree. Homologues of Fis1 protein, was found in yeast and in mammals respectively, are necessary for targeting DRP to outer mitochondrial membrane for occuring mitochondrial fission. *Trypanosoma brucei* has single large mitochondria in cell and therefore it is suitable model organism for studying mitochondrial fission, which is important for transmission of one complete mitochondria to each daughter cells during cytokinesis. As TbDRP in *T. brucei*, was already characterized. We aim to identify Fis1 in *T. brucei* and describe its function. In our study we identified a putative homologue of Fis1, which we called TbFis1. We made knock-down cell lineages of this gene. Our data shows that in *T. brucei* the TbFis1 protein is not essential for growth of the parasite as well as we did not observe morphological phenotype. This finding is surprising because a lack of Fis1 protein has strong effect on mitochondrial morphology in yeasts and mammals. So it looks like the function of the protein may differ among organisms.

Suf System for Iron Sulphur Cluster Assembly in *Monocercomonoides* (Oxymonads)

Vojtěch Vacek[1], Lukáš Novák[1], Zuzana Zubáčová[1], Miluše Hroudová[2], Čestmír Vlček[2] & Vladimír Hampl[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha
[2]Academy of Sciences of the Czech Republic, Institute of Molecular Genetics, Praha

Oxymonads are the last large group of eukaryotes without confirmed mitochondrion-like organelle. We spent few last years by searching for mitochondrion in *Monocercomonoides* Pa203. During this search we obtained 924,658 EST reads from 454 pyrosequencing (9,772 contigs) and recently more than 48,000,000 reads from Illumina (54,998 unigenes). Among all these sequences we found only 3 genes which are usually associated with mitochondrion-like organelle – pyruvate:ferredoxin oxidoreductase, [FeFe]-hydrogenase and pyridine-nucleotide transhydrogenase. Interestingly, were not able to find any transcript for protein associated with mitochondrial synthesis of iron-sulfur clusters. On the other hand, the data set contains sequences for 5 subunits of Suf system for assembly of iron-sulfur clusters. This system is known in eukaryotes only from plastids and from *Blastocystis hominis*, which probably acquired it by horizotal gene transfer from archaebacteria. The fact that the closest homologues of *Monocercomonoides* Suf proteins were found in the transcriptome of *Trimastix*, the free-living relative, suggests that these transcripts are not derived from bacterial contamination. Furthermore, gene for SufB was localised in the nucleus of *Monocercomonoides* using fluorescence in situ hybridization. Phylogenetic analyses of SufB, SufC and SufS indicate that the Suf system of Preaxostyla was acquired by HGT from CFB group of bacteria.

Isolation of Chloroplasts and Chloroplast Membranes from *Euglena gracilis*

Anna Vanclová[1], Róbert Šuťák[1] & Vladimír Hampl[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Euglenophyta is a group of photosynthetic euglenids belonging to supergroup Excavata. These organisms harbour secondary chloroplasts which had been obtained via endosymbiosis with green alga already possessing a chloroplast as a result these chloroplasts are surrounded by three membranes instead of two. In this poster we present a procedure of isolating intact chloroplasts from *Euglena gracilis* and extracting their membranes from the sample for further usage. This method consists of breaking the cells using glass beads, brief differential centrifugation followed by filtration steps to remove whole cells and cellular debris, and finally high-speed centrifugation on Optiprep gradient which should yield pure chloroplast fraction. The chloroplast membranes with membrane proteins are then isolated via carbonate extraction. Described method is to be used later for obtaining chloroplast membrane proteomes of *Euglena gracilis* and *Eutreptiella gymnastica*, two distantly related euglenophytes. In this future work we will focus on proteins mediating transport of nuclear-encoded chloroplast proteins across the three membranes, which may differ remarkably from transport to primary or other secondary plastids.

Alternative NADH:Ubiquinone Oxidoreductase in Procyclic *Trypanosoma brucei*: an Intermembrane-Space-Oriented Counterpart of Mitochondrial Complex I

Zdeněk Verner[1,2,3], Ingrid Škodová[3], Simona Poláková[4], Vladislava Ďurišová-Benkovičová[3], Anton Horváth[3] & Julius Lukeš[1,2]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice
[3]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Bratislava
[4]DAPHNE ČR – Institut aplikované ekologie, České Budějovice

The respiratory chain of the procyclic stage of *Trypanosoma brucei* contains the standard complexes I through IV, as well as several alternative enzymes contributing to electron flow. In this work, we studied the function of an alternative NADH:ubiquinone oxidoreductase (NDH_2). Depletion of target mRNA was achieved using RNA interference (RNAi). In the non-induced and RNAi-induced cells, growth, membrane potential change, alteration in production of reactive oxygen species, overall respiration, enzymatic activities of complexes I, III and/or IV and distribution of NADH:ubiquinone oxidoreductase activities in glycerol gradient fractions were measured. Finally, respiration using different substrates was tested on digitonin-permeabilized cells. The induced RNAi cell line exhibited slower growth, decreased mitochondrial membrane potential and lower sensitivity of respiration to inhibitors. Mitochondrial glycerol-3-phosphate dehydrogenase was the only enzymatic activity that has significantly changed in the interfered cells. This elevation as well as a decrease of respiration using NADH was confirmed on digitonin-permeabilized cells. The data presented here together with previously published findings on complex I led us to propose that NDH_2 is the major NADH:ubiquinone oxidoreductase responsible for cytosolic and not for mitochondrial NAD^+ regeneration in the mitochondrion of procyclic *T. brucei*.

Protein MIX as a Drug Target in *Leishmania major*

Michaela Veselíková[1,2], Brian Panicucci[1] & Alena Zíková[1]
[1]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice
[2]University of South Bohemia, Faculty of Science, České Budějovice

The trypanosomatid parasites, *Leishmania* and *Trypanosoma*, are the causative agents of leishmaniasis and Human African Trypanosomiasis, respectively. Both diseases are fatal if not treated, thus a deeper understanding of the parasite biology is crucial for the development of new treatments to replace the existing antiquated drugs. The hypothetical mitochondrial inner membrane protein, designated MIX, was first identified as a virulence factor in *L. major*. Using the closely related and more convenient model organism, *T. brucei*, the MIX homologue was found to be associated with cytochrome c oxidase complex (complex IV). Interestingly, it seems to form its own distinct node at the periphery of the complex IV interactome, suggesting that it might form a subcomplex with an additional function besides complex IV activity. To examine all of the possible functions of MIX, we have taken advantage of the infectious bloodform stage (BF) of *T. brucei*, which lacks cytochrome c oxidase activity. While our BF MIX RNAi cell lines did not exhibit a growth phenotype, we determined by QPCR that the RNAi was not very effective in these cells. Therefore, we are creating a BF MIX double knock-out cell line with a regulatable ectopic MIX gene that will allow us to study the function of the gene product even if it proves to be essential in this life cycle stage. Furthermore, a *Leishmania* expression plasmid was created to purify potential binding partners of a TAP tag fused MIX protein. The purification scheme is being optimized and the purified sample will be analyzed by mass spectrometry.

Parasitic Trypanosomatids and the Phototroph *Euglena gracilis* Possess Common Motifs in Mitochondrial Targeting Presequences

Matej Vesteg[1,2], Katarína Krnáčová[3], Vladimír Hampl[4], Čestmír Vlček[5] & Anton Horváth[3]

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

[2]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Bratislava

[3]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Biochemistry, Bratislava

[4]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

[5]Academy of Sciences of the Czech Republic, Institute of Molecular Genetics, Praha

Parasitic trypanosomatids *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*, and the phototrophic euglenid *Euglena gracilis* possessing chloroplasts of secondary green algal origin belong to the protist phylum Euglenozoa, which might be among the earliest eukaryotic branches. The predicted mitochondrial presequences of *E. gracilis* and these trypanosomatids seem to be highly variable in sequence length (5–118 aa), but they share statistically significant similarities. The common (M/L)RR motif is usually present at the N-terminus mitochondrial preproteins and it is probably responsible for recognition via import apparatus of mitochondrial outer membrane. In some cases, this motif is present inside the predicted presequence region. This motif is generally followed by a hydrophobic region rich in alanine, leucine and valine. It is proposed that in Euglenozoa, either RR motif or arginine-rich region within hydrophobic aa-s present at the N-terminus of a preprotein can be sufficient signals for mitochondrial import irrespective of presequence length.

Trichomonads in Cats and Dogs

Pavlína Vobořilová[1], Jaroslav Kulda[1], Ivan Čepička[2] & Jan Tachezy[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha
[2]Charles University in Prague, Faculty of Science, Department of Zoology, Praha

Trichomonads are frequent commensals or parasites inhabiting digestive, respiratory, and reproductive tracts of vertebrates, including domestic cats and dogs. In these hosts, four trichomonad species has been described: *Trichomonas canistomae* and *Trichomonas felistomae* that are commensals of the host oral cavity; *Pentatrichomonas hominis*, a commensal of intestinal tract that could be found in dogs and cats but also in other mammals; and pathogenic *Tritrichomonas foetus* that causes feline intestinal trichomonosis. Although trichomonads in dogs and cats are probably of cosmopolitan distribution we have no information about their presence in Czech Republic.

The aim of this study is an analysis of prevalence of trichomonads in different populations of cats and dogs in Czech Republic, and associations between the presence of trichomonads and potential host management, health and demographic risk factors. A cross-sectional study is conducted involving cats and dogs from a veterinary clinics, shelters, breeders and exhibitions. Risk factor information is assessed through a questionnaire. For trichomonad identification, oral and rectal swabs are collected and placed to Dobell and Leidlaw's biphasic medium (oral trichomonads) and TYM medium (intestinal trichomonads) for culture. The same specimens are used for nested PCR to amplify the ITS region. Examination of specimens from oral cavity of 21 dogs and 35 cats resulted in isolation of 7 and 4 trichomonad strains, respectively. Analysis of ITS1-5.8rRNA-ITS2 sequences revealed a presence of two types of oral strains. The first type includes organisms isolated from dogs as well as from cats for which we obtained identical sequences that were previously assigned to *T. canistomae*. The second type that was isolated from cats is identical with a commensal of the human oral cavity *Trichomonas tenax*. The rectal swabs were taken from 38 cats and 8 dogs. However, all were negative for trichomonads.

Occurrence, Prevalence and Progression of Microsporidial Infection in Horses and Ponies from Czech Republic

Pavla Wagnerová[1,2], Bohumil Sak[2], Dana Květoňová[2], Martin Kváč[1,2] & Iva Langrová[3]

[1]University of South Bohemia, Faculty of Agriculture, České Budějovice

[2]Biology Centre ASCR, v. v. i., Institute of Parasitology, České Budějovice

[3]Czech University of Life Sciences in Prague, Faculty of Agrobiology, Food and Natural Resources

Prevalence of microsporidia was observed on 23 farms with various management systems in the Czech Republic. *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* was identified in 17.3 % and 6.9 % of horses, respectively, on 16 farms. The prevalence of *E. cuniculi* in horses over 3 years of age was significantly higher compared to younger horses. Significantly higher infection rates of *E. bieneusi* and *E. cuniculi* were recorded in horses kept in stables than those on pasture. Two genotypes of *E. cuniculi* (I and II) and 15 genotypes of *E. bieneusi* including six previously described and nine novel genotypes were detected. To determine the course of infection, 9 one year old ponies were used for the experimental infection with *E. cuniculi* genotype II (107 spores per animal). Individual horses were on a weekly basis and tissues samples were processed for the histology and molecular diagnostic. ELISA was used for determination of humoral immune response. Although no clinical signs of microsporidiosis were observed, dissemination of microsporidia into almost all organs and significant increase of concentration of specific antibodies in blood were observed from 28 to 42 DPI. After this acute stage, microsporidia remain detectable in kidney till the experiment termination. No pathological changes were observed with exception of one mare's brain, where *E. cuniculi*-positive abscess cavity formed in the lobus piriformis.

Exploring the Possibilities of Using Chitosan as Antiprotozoal Agent and Modulator of Rumen Fermentation

Monika Wencelová[1], Zora Váradyová[1], Katarína Mihaliková[1], Svetlana Kišidayová[1] & Dušan Jalč[1]
[1]Slovak Academy of Sciences, Institute of Animal Physiology, Košice

The influence of chitosan (CH, natural substance derived from deacetylation of chitin with antimicrobial properties) on rumen fermentation and ciliate protozoan population has been investigated. The rumen inocula were mixed with McDougall's buffer at a ratio 1:1 and 35 ml doses were dispensed by automatic pump into preheated 120 ml serum bottles containing 0.25 g of diet and incubated for 72 h at $39 \pm 0.5\,^{\circ}$C. The following three diets were used: high fiber (HF, meadow hay and barley, 800:200 w/w), high concentrate (HC, meadow hay and barley, 500:500 w/w) and maize silage (MS) alone or supplemented with CH (100 mg/l) and sunflower oil (SO, 35.0 g/kg) or rapeseed oil (RO, 35.0 g/kg). In HF and HC-diets, the number of rumen ciliate protozoa *Ophryoscolex caudatus tricoronatus*, *Isotricha* spp., *Enoploplastron triloricatum*, *Polyplastron multivesiculatum* and *Ophryoscolex c. tricoronatus* with SOCH or ROCH was significantly lower. In MS-diet, the number of rumen ciliate protozoa *Dasytricha ruminantium*, *Enoploplastron triloricatum*, *Polyplastron multivesiculatum* and *Ophryoscolex c. tricoronatus* by ROCH was decreased ($P < 0.001$), whereas the number of *Isotricha* spp. and *Enoploplastron triloricatum* by SOCH was increased ($P < 0.001$). Chitosan can be used as antiprotozoal agent and fermentation modulator depending on diets, oil composition and rumen microbial activity.

A Small Portion of Plastid Transcripts is Polyadenylated in the Flagellate *Euglena gracilis*

Kristína Záhonová[1,2], Matej Vesteg[1,2] & Juraj Krajčovič[2]
[1]University of Ostrava, Faculty of Science, Department of Biology and Ecology, Ostrava
[2]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Genetics, Bratislava

Euglena gracilis, a fresh-water flagellate possessing secondary plastids of a green-algal origin, belongs to the phylum Euglenozoa. A common euglenozoan feature is the processing of nuclear transcripts by trans-splicing, which includes replacement of the 5'-end of pre-mRNA by the 5'-end of the so-called spliced leader (SL) RNA. The presence of the SL sequence on the 5'-end of mRNA indicates that the transcript is nuclear rather than organellar. We searched expressed sequence tag (EST) data available for *E. gracilis*, which were presumably derived from polyA-selected mRNA molecules, and found several EST sequences corresponding to genes know to be located on the plastid genome, raising the possibility that nuclear copies of these genes giving rise to polyadenylated transcripts exist in *E. gracilis*. However, our PCR-based experiments failed to detect the presence of the SL sequence in any of the plastid-like transcripts examined, suggesting that these transcripts indeed originate in the plastid yet may be to some extent polyadenylated. Subsequent Real-Time RT-PCR experiments indeed revealed the presence of polyadenylated plastid transcripts, although the ratio of total to polyadenylated RNA variants ranged from 10^3 to 10^5, depending on the gene tested. The amount of mRNA for individual plastid genes was 1.5–7-fold higher in light-grown strains in comparison to dark-grown strains, and was generally higher than the amount of mRNA for individual nuclear genes used for comparison. Our results thus bring new important insights into the plastid transcription in a model secondary plastid, including the first reported evidence for polyadenylation.

Evolution of Peroxisomes: Anything Can Happen

Vojtěch Žárský[1], Dušan Hurtoň[1] & Jan Tachezy[1]
[1]Charles University in Prague, Faculty of Science, Department of Parasitology, Praha

Peroxisomes are ubiquitous eukaryotic organelles that compartmentalize a variety of metabolic pathways mainly related to oxidative metabolism of lipids. Because of this, it is believed that the presence of peroxisomes is tightly connected to aerobic mitochondria. By examining the components of the peroxisomal protein import machinery and biogenesis, we discovered a varying mosaic of peroxisomal markers in the genomes of different anaerobic protists of the group Archamoebae. We currently characterize the peroxisomes of *Mastigamoeba balamuthi*, a free-living relative of the pathogenic *Entamoeba histolytica*. Surprisingly we also discovered a complete loss of peroxisomal markers in the genomes of several parasitic platyhelminths, a parasitic nematode *Trichinella spiralis* and a free-living tunicate *Oikopleura dioica*.

Abstracts are sorted by alphabetical order of surnames of presenting authors. Presenting authors are underlined.

Partners of Conference

Vaše Představy, Naše Budoucnost

LI-COR C-DiGit™ Blot Scanner

C-DiGit je první skutečně plnohodnotná a cenově dostupná alternativa za klasický film. Tento skener kombinuje citlivost filmu s pohodlím, flexibilitou a jednoduchostí digitálního zobrazování. Neztratíte tak žádnou výhodu filmu, není třeba dalších investic na jeho nákup a odpadá i čas trávený ve fotokomoře.

MOŽNOST ZAPŮJČENÍ PŘÍSTROJE ZDARMA

Váš klíč k pohodlnému snímání

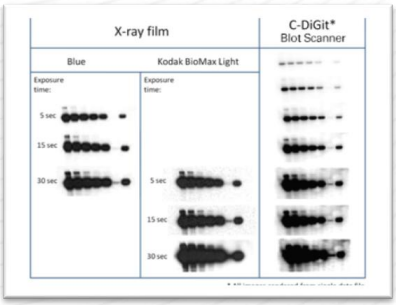
Při stále rostoucích nákladech na film je toto jediný plnohodnotný nástupce. Práci s ním si oblíbíte díky jeho malým rozměrům a skvělým výsledkům, které pořídíte za několik minut. LI-COR C-DiGit umožňuje pořizovat, analyzovat a ukládat kdekoli si jen přejete. Uživatelsky přívětivý program Image Studio pro C-DiGit skener je určen pro chemiluminiscenční Western bloty. Data lze snímat ve standardní a vysoké kvalitě. Jeden datový soubor obsahuje širokou škálu expozic, jež by byly dostupné s filmem. Image Studio nabízí výkonné a snadno použitelné obrazové analýzy.

Vše, co máte rádi na filmu, bez potíží!

- Žádné expozice navíc
- Žádná saturace
- Žádná vývojka
- Žádná fotokomora
- Žádný nepořádek
- Žádná samostatná analýza
- Nižší náklady

Využijte zaváděcí ceny 129000,- Kč při objednání LI-COR C-DiGit™ Blot Scanner do 30. 6. 2013.

Soubor obrázků pořízených C-DiGitem, zobrazuje celý rozsah chemiluminiscenčních dat pro daný blot.



pEGFR v A431 buněčném lyzátu (SuperSignal® West Dura Substrate).

Uvedená cena je bez DPH, na přístroj se vztahuje záruka 1 rok.

KRD – obchodní společnost s.r.o., Pekařská 12, Praha 5, 155 00
Tel.: 257 013 400, Fax: 257 013 405, Email: krd@krd.cz, www.krd.cz

KRD molecular technologies

biomapping

PCR Purification in Just One Minute

Remove Impurities from PCR Reactions in a Single Step – Diffinity RapidTip® Technology

Features and Benefits

- **One minute, one step** – Extremely fast and efficient process to rapidly recover clean DNA
- **Effective purification** – Removes impurities for downstream applications
- **Cost effective** – No capital equipments, extra plastic or liquids required
- **Environmentally friendly** – Waste is limited to the functional pipette tip

Available in Two Formats

Diffinity RapidTip

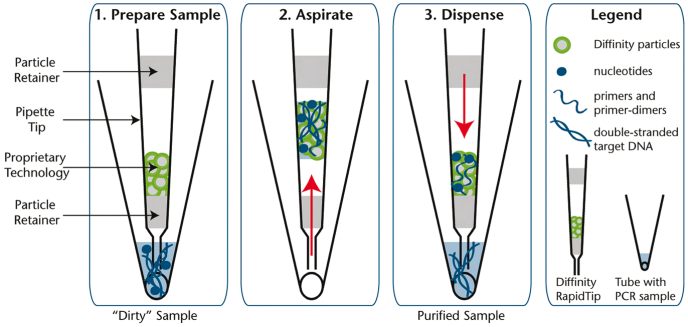
- Removes 90% of primers, ssDNA and primer-dimers
- Application: standard (Sanger) sequencing

Diffinity RapidTip®2

- Removes 90% of primers, ssDNA, primer-dimers and DNA polymerase
- Application: Restriction digest, T-A cloning and standard (Sanger) sequencing

For more information, contact your Sigma-Aldrich Representative:

Diffinity RapidTip Workflow



NOTE: For optimal performance, aspirate and dispense slowly (approximately 15 aspirate/dispense cycles per 60 seconds).

Limited Time Only – Save 15%

Reference promo code SFC when ordering. *Offer valid through December 31, 2012.*

Cat No.	Product Description
D1947-8RXN D1947-48RXN D1947-96RXN	Diffinity RapidTip for PCR Purification
D1947L-8RXN D1947L-48RXN D1947L-96RXN	Diffinity RapidTip for PCR Purification, Compatible with Rainin LTS™ pipettes
D2947-8RXN D2947-48RXN D2947-96RXN	Diffinity RapidTip2 for PCR Purification with Polymerase Removal
D2947L-8RXN D2947L-48RXN D2947L-96RXN	Diffinity RapidTip2 for PCR Purification with Polymerase Removal, Compatible with Rainin LTS™ pipettes

For more information, visit
sigma.com/rapidtip

SIGMA-ALDRICH®

SIGMA® Life Science Where bio begins™

JC201210101016
1102

Lucie Jedličková draw the picture on the title page.

Title: 43rd Jírovec's Protozoological Days
Subtitle: Conference Proceedings
Redaction: Jiří Týč (Biology Centre of the Academy of Sciences of the Czech Republic, v. v. i., České Budějovice)
Editor: Petr Soukal (Charles University in Prague, Faculty of Science)
Publisher: Biology Centre of the Academy of Sciences of the Czech Republic, v. v. i., Institute of Parasitology
Place and Year of Publication: České Budějovice, 2013
First Edition
Number of Pages: 104
Circulation: 111
Exposure and Print: Tribun EU s.r.o., Cejl 892/32, Brno 602 00
This publication did not undergone language editing.
Not for sale.
ISBN 978-80-260-4294-5


ČESKÁ PARAZITOLOGICKÁ SPOLEČNOST
CZECH SOCIETY FOR PARASITOLOGY