15TH INTERNATIONAL CONGRESS OF PROTISTOLOGY

30th July – 4th August 2017
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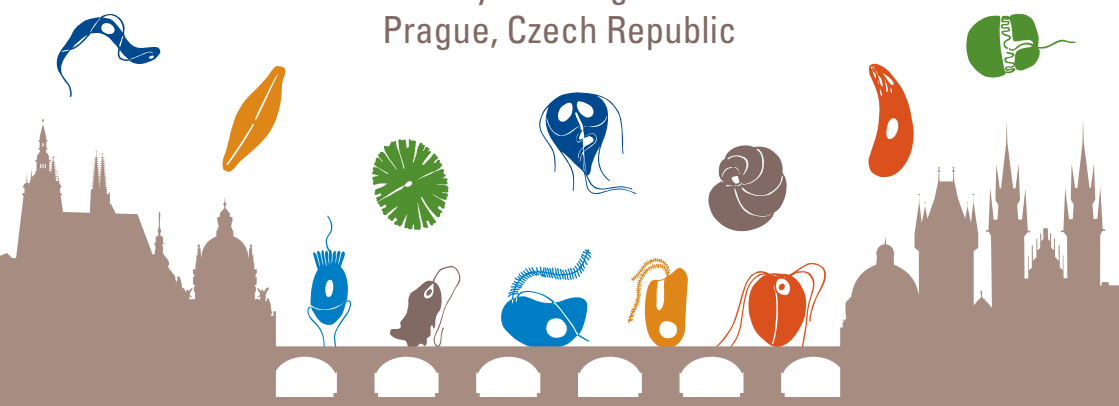

BOOK OF ABSTRACTS

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PLENARY LECTURES

A journey to the past: The first Protozoology Conference in Prague, and a tribute to Otto Jírovec, its spiritual father

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The First Protozoology Conference was held in Prague (August 22–31, 1961), and was attended by 260 participants from 23 countries, bringing together a representative forum of protozoologists from over the world. The conference definitely established protistology as a substantial branch of science and started the tradition that in 4 years interval, protozooology (now protistology) conferences are convened. Thus, 13 meetings have been held between 1961 and 2017. The conference took place thanks to the initiative of parasitology professor Otto Jírovec, the founder of the Czech parasitology and protistology schools, the president of the 1961 conference. His contribution to protistology, especially as the research of *Toxoplasma* and *Pneumocystis* is concerned, will be discussed in more detail. I will also describe how one of former Jírovec's students and the First Protozoology Conference attendee, discovered the source of the so far largest world epidemics of *Naegleria*-caused meningoencephalitis. The First Protozoology Conference in 1961 convened at the time when Europe was divided into two hostile political blocks, when travel and information exchange in many countries was restricted and state-controlled. Still, political issues were of no concern to the participants, which enabled fruitful and friendly meeting of scientists from both sides of the "Iron Curtain", in the spirit of academic freedom.

Keywords: Otto Jírovec, *Toxoplasma*, *Pneumocystis*, *Naegleria fowleri*

Euglenoid pellicle morphogenesis and evolution in light of comparative ultrastructure and trypanosomatid biology: implications for unity of Euglenozoa and evolution of excavate protozoa and chromists

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Uniquely in eukaryotes, euglenoid pellicles comprise longitudinal proteinaceous, epiplasmic strips underlain by microtubules. Previously contradictory interpretations of pellicle microtubule duplication and segregation assumed opposite microtubule polarity from kinetoplastid Euglenozoa and conservative microtubule segregation. I shall discuss examples from *Distigma proteus* that show that new euglenoid pellicle microtubules nucleate posteriorly as in trypanosomatids, unifying euglenoid and kinetoplastid pellicle morphogenesis; and argue that strip-growth is unpolarised. Epiplasmic insertion and cutting make new strip junctions between alternating wide and narrow daughter strips that grow intussusceptively. Nanotubules, overlooked epiplasm-associated components, arguably define strip edges. At strip heel/toe junctions all euglenoids have a morphogenetic centre where dense amorphous material links to the heel a microtubule mt2/3 pair, which I argue segregate semi-conservatively into daughter strips, not conservatively as previously assumed. Arguably, proteolysis, epiplasmic growth, and toe-nanotubule-associated epiplasmic scission initiates daughter strips, separating old mts2/3; new mt2/3/bridge-B assembly, sub-heel scission, nanotubule-bridge-A assembly complete pellicle complex duplication. Only mt2/3 pair fully enters the canal, one master microtubule also the reservoir, other pellicle microtubules terminating near canal rims. A related cytokinesis model involving ciliary attachment zone duplication explains invariably even strip number in spirocute euglenoids (Peranemea plus Euglenophyceae). I consider *Serpenomonas* and *Entosiphon* alternating heteromorphic strips developmental stages of 'strip transformation'; explain intergroup diversity of strip morphology and dorsoventral strip differentiation causally by specific pellicle-complex components; propose centrin-based mechanisms for strip shaping and euglenoid movement; unify pellicle cytokinetic microtubule segregation across Euglenozoa; and discuss origin and diversification of pellicle complexes. Postgaardean (=symbiontid) pellicles are very different from and not derived from those of euglenoids, as sometimes suggested, but may be their sisters. I shall discuss whether any cytoskeletal structures in other protist phyla, especially excavates and chromists may share microtubule polarity with the pellicular microtubules of Euglenozoa, which probably ancestrally had opposite polarity to microtubules of ciliary roots.

Functional ecology and diversity of planktonic protists

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Functional ecology (FE) was established approximately 30 years ago as the branch of ecology that investigates the functions that species have in the community or ecosystem in which they occur. Functional diversity (FD) is the concept inherent in FE, linking species' properties to ecosystem processes via functional traits. Functional diversity has been studied in much detail in terrestrial plant ecology, and trait-based community ecology of phytoplankton is well established. FD research of aquatic protists has gained considerable momentum over the past decade, when the significance of mixotrophic species for biogeochemical cycling in the ocean was detected. However, in spite of their significance as key elements of pelagic food webs, comparatively little is known on FE and FD of heterotrophic planktonic protist. Heterotrophic protists are taxonomically and ecologically extremely diverse organisms; undersampling, difficulties to cultivate many species, and lack of standardization in experimental work hamper their FE and FD analyses. Therefore, I will focus my analysis on planktonic ciliates, a monophyletic and relatively well studied group of single-celled eukaryotes. The co-occurrence of many closely related ciliate species in seemingly homogeneous environments such as the open ocean indicates a wide range of their ecological niches. Variation in space and time may foster co-occurrence and prevent violating the competitive exclusion principle among ciliates using the same resources. Considering that many ciliates may be dormant and/or rare in many habitats, ciliate species diversity must be higher than can be deduced from simple sampling techniques; molecular methods of identification clearly point to this hidden diversity. From a functional point of view, the question is how much of this diversity represents redundancy. A key challenge for future research is to link the ecophysiological performance of naturally co-occurring ciliates to their functional genes. To this end, more experimental research is needed with functionally different species

Keywords: functional ecology, functional diversity, plankton, ciliates

Oxytricha: A cell with 16,000 chromosomes and millions of noncoding RNAs

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The ciliate *Oxytricha trifallax* possesses a dynamic pair of genomes, and massive DNA rearrangements produce a highly fragmented but functional somatic macronucleus from a complex germline micronucleus. This process eliminates nearly all noncoding DNA, including transposons, and rearranges over 225,000 short DNA segments to produce tiny gene-sized "nanochromosomes." In the precursor germline genome, the shattered segments of different genes often interweave with each other, frequently overlap and sometimes combinatorially assemble (Chen et al. 2014 Cell 158:1187). The whole process produces a mature, somatic genome of over 16,000 nanochromosomes (Swart et al., 2013 PLoS Biology 11: e1001473). Noncoding RNAs regulate the entire process of genome rearrangement. Millions of 27nt piRNAs provide the critical information to mark and protect the retained DNA segments of the genome (Fang et al., 2012 Cell 151:1243) and a distinct set of piRNAs mark a subset of deleted regions to assist with their elimination. Maternally-inherited, long, non-coding (lnc) RNAs provide three additional layers of continuity across generations, including serving as templates for genome remodeling and RNA-guided DNA repair (Nowacki et al., 2008 Nature 451:153) while also regulating gene dosage and chromosome copy number (Nowacki et al., 2010 PNAS 107:22140). This illustrates the ability of noncoding RNAs to transmit heritable changes to the next generation. Together, Oxytricha's elaborate epigenome, assembled through complex interacting networks of both long and small non-coding RNAs, encapsulates an RNA-driven world packaged in a modern cell. The mechanism for all of these dynamic actions bypasses the traditional modern pathway of inheritance via DNA, hinting at the power of RNA molecules to sculpt genomic information.

Keywords: ciliate, genomics, noncoding RNA, small RNA, epigenetics

Matters of life and death: a protistan perspective

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Picoeukaryotes represent the smallest free-living eukaryotic cells known and have important roles in marine ecosystems. Many cultured picoeukaryotes are photosynthetic and have relatively broad environmental distributions. At the same time a plethora of uncultured protists exists, and these can best be studied using creative *in situ* sampling methods that maintain the cellular integrity of the organism alongside information about its ecological role. Here, we will discuss methodological innovations that are providing insights into the molecular physiology of photosynthetic picoeukaryotes grown in conditions that reflect changes expected under several future climate scenarios. Additionally, we will explore data on predatory protists that consume these diminutive algae in nature. These studies have revealed novel deep-branching lineages that are widespread in the world oceans as well as interactions between protistan groups and their relationships with other microbes.

Keywords: phytoplankton, climate change, predatory protists, microbial interactions, *in situ* methods

Marine microbial ecology involves three domains of life, not two

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College textbooks almost universally omit protists from discussions of “microorganisms/microbiology/microbial ecology” despite a substantial body of literature describing their amazing diversity and their presence and activity in nearly all environments investigated. This is especially true for textbooks on marine ecology and marine microbiology. No doubt at ICOP XV most attendees recognize these issues, and will advocate for change. To further inspire advocacy, this talk will explore a few of the important ecological roles that protists play in the marine realm and some recent lessons learned.

Small but mighty: marine plankton in a dynamic ocean

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Marine microbes make planet Earth habitable. Plankton represent tremendous biological, physiological and behavioral diversity and may provide the keys to unlocking many environmental, medical and sustainability challenges. Marine protistology has a long and distinguished tradition addressing critical, societal challenges. At the same time, the curious are endlessly supplied with tantalizing puzzles. In great appreciation of the honor of receiving the Hutner award, I will present results from our research on marine, eukaryotic protists. The key challenge motivating my collaborators and myself, is to reconcile the biology of diverse microscopic species with their globally relevant ramifications, such as the cycling of organic carbon in the ocean. These investigations are done with the knowledge that the ocean is changing at a rapid pace. The focus of my presentation will be on predator prey interactions in the micro-plankton and how these microscopic, cell-cell interactions affect large-scale plankton population dynamics and primary production. In turn, these small-scale interactions are highly species-specific, subject to environmental modulation and often variable in space and time. I'll address the challenges of dealing with this variability and will suggest that intra-specific variability in physiology and behavior promotes biodiversity. Like many before us, we observe that protists never cease to amaze in their fabulous capacity to present surprising solutions to ecological challenges. These rewarding discoveries are in and unto themselves motivation to further the study of protists and the contributions they can render to humanity.

Keywords: plankton, predation, primary production, biogeochemical cycling

Physiology, anaerobic mitochondria, endosymbiosis, complexity and too much LGT

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Endosymbiotic theory posits that bacterial genes in eukaryotic genomes entered the eukaryotic lineage via organelle ancestors. It predicts episodic influx of prokaryotic genes into the eukaryotic lineage, with acquisition corresponding to endosymbiotic events. Lateral gene transfer theories predict a constant flux of prokaryotic genes into eukaryotic genomes. Genome data can discriminate. Clustering and phylogenetic analysis of all eukaryotic gene families having prokaryotic homologues shows (1) that gene transfer from bacteria to eukaryotes is episodic and coincides with the origin of chloroplasts and mitochondria, (2) that gene inheritance in eukaryotes is vertical, sparse gene distributions stemming from differential loss, and (3) that continuous, lineage-specific lateral gene transfer does not contribute to long-term gene content evolution in eukaryotic genomes. Moreover, eukaryotic coding sequences that share more than 70% amino acid sequence identity to prokaryotic homologs are typically assembly or annotation artifacts. The origin of eukaryotes and the origin of vertical lineage inheritance coincide. In modern formulations of symbiotic theory, the eukaryotic endomembrane system originated from mitochondria via outer membrane vesicles (OMVs) released within the cytosol of its archaeal host at eukaryote origin. Confined within the host's cytosol, OMVs accumulated naturally, fusing either with each other or with the host's plasma membrane. This matched the host's archaeal secretory pathway for cotranslational protein insertion with outward bound mitochondrial-derived vesicles consisting of bacterial lipids, forging a primordial, secretory endoplasmic reticulum as the cornerstone of the eukaryotic endomembrane system. In terms of cell biology, eukaryotes differ fundamentally from prokaryotes, and mitochondria are why.

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Lateral gene transfer is an important mechanism facilitating evolutionary adaptation in protists

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For several decades we have known that lateral (horizontal) transfer of genes is an important evolutionary mechanism shaping prokaryotic genomes. However, the role of lateral gene transfer (LGT) in eukaryote genome evolution remains contentious. Recent technical advances have made the sequencing of smaller eukaryotic genomes more feasible, leading to a rapid increase in the availiability of transcriptomes and near-complete genomes of protists from all major supergroups of the eukaryote tree. Our transcriptomic and genomic investigations of diverse anaerobic protistan lineages have revealed that many of them have modified mitochondria that function anaerobically. Frequently, ancestral mitochondrial pathways have been modified by the addition of new enzymes/proteins encoded by genes that were acquired laterally from a prokaryote or another eukaryote inhabiting a similar environment. LGT has thus facilitated important shifts in metabolic and biogenetic functions in these organisms that adapt them to low oxygen environments. Although LGT is quantitatively less common in eukaryotes than in prokaryotes, it is an extremely important mechanism by which they adapt to new environments.

Keywords: lateral gene transfer, genomes, mitochondria, anaerobic, protists

How did animals evolve? Don't ask animals, ask protists!

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How animals emerged from their single-celled ancestors, evolving into the highly-variable complex body plans we see nowadays, is a fascinating evolutionary question. We have been addressing this question by performing phylogenomic analyses and by obtaining new genomic and cell biology data from several unicellular relatives of Metazoa. Our data together with data from other labs point to a unicellular ancestor of animals with a complex repertoire of genes involved in multicellularity, as well as a complex gene and genomic regulation. To go a step forward, we are now developing an holozoan functional platform to understand animal origins. I will here revise all these recent findings and provide a perspective of what this means to our understanding of animal origins.

Keywords: genomics, animal origins, epigenetics, phylogenomics, holozoans

Can we build a genetic trap for drug resistant malaria?

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Malaria remains a major global health problem, and drug resistance is severely eroding our ability to control disease. The world has been on a resistance treadmill, using then losing a new antimalarial every ~10 years. Beating drug resistance is crucial to combating malaria.

We identified a commonly used anti-malarial, atovaquone, for which resistance is unable to spread. We showed that resistance mutations incur a delayed fitness cost that eventually manifests itself in the mosquito vector phase when parasites attempt to move from one vertebrate host to another. Importantly, maternal inheritance of the drug target gene (mitochondrion encoded cytB) prevents sexual complementation of the unfit (low catalytic turnover) resistance alleles by wild type alleles during the non-haploid mosquito phases of the life cycle. We likened this phenomenon to a 'genetic trap'. The trap concedes that selection for drug resistance occurs—it is likely inevitable with such selection sieves. However, the trap favours parasites taking the “quick fix” option, an option that gives them drug resistance and hence the ability to survive in-patient but ultimately sacrifices their ability to reproduce sexually and spread geographically. Key attributes of the trap are that parasites can readily acquire resistance whilst under less stringent selection for allele fitness in the vertebrate host, but a later fitness cost in the life cycle eventually makes drug resistance lethal—they are trapped inside the patient. These patients need to be treated with an alternative drug, ideally a partner drug to the 'genetic' trap drug.

We recently confirmed that the trap works for a second drug (ELQ300), which also targets mitochondrial cytB. We are now testing whether the trap concept applies to targets in the relict plastid (apicoplast) of malaria, which are also maternally inherited. Our findings suggest that drugs like atovaquone and ELQ300 (and hopefully anti-apicoplast drugs) are potentially future proof being far less prone to the spread of resistance.

Keywords: malaria, endosymbiosis, mitochondrion, plastid, respiration

Evolution of the eukaryotic endomembrane system: Insights from protist genomics

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Encompassing the endoplasmic reticulum, Golgi body, endosomes, and lysosomes, the organelles of the eukaryotic endomembrane system are crucial to healthy cellular function, whether in animals, plants, or most importantly, protists. These compartments are functionally linked by a set of protein machinery that mediates material transport between them and with the cell surface. Though present in diverse eukaryotic cells, the endomembrane compartments, and the proteins that underlie their function, are best characterized in animal and yeast model systems. This raises two questions of evolutionary cell biological importance: 1) What aspects of membrane-trafficking can be validly applied in a pan-eukaryotic, generalizable cell biological model, and 2) what evolutionary mechanism gave rise to these organelles. A combination of protist genomics and phylogenetics has allowed for the definition of a pan-eukaryotic complement of membrane-trafficking machinery, indicating that the Last Eukaryotic Common Ancestor (LECA) possessed an elaborate endomembrane system. Phylogenetic analyses of Heterotetrameric Adaptor protein Complex-Containing Coats (HTAC-CCs) have been particularly informative in understanding the LECA membrane-trafficking complement and its evolutionary history. This complement includes some ancient protein complexes (eg. AP5), present in opisthokont model organisms, that would still be unknown were it not for studies in protists, and other protein complexes (eg. TSET) that are widely present and may play important roles in eukaryotic membrane trafficking, just not necessarily in opisthokonts. Crucially, when the phylogenetic patterns of HTAC-CCs are viewed together with those of other membrane-trafficking proteins (eg. SNAREs, Rabs), a mechanistic model for evolving complexity of non-endosymbiotic eukaryotic organelles can be derived. This model, termed the Organelle paralogy hypothesis, gives insight into the process that shaped stages of endomembrane system evolution leading up to the LECA and appears still to be generating novel trafficking pathways in eukaryotic lineages today.

Keywords: Phylogeny, membrane-trafficking, adaptin

SYMPOSIA

SYMPOSIUM: Symbiosis and parasitism

(organizers: Patrick Keeling and Julius Lukeš)

How did ancestors of Apicomplexa lose photosynthesis? The case of heme pathway

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Chromerid alga *Chromera velia* is the closest known phototrophic relative to apicomplexan parasites. It possesses a unique heme pathway with the d-aminolevulinate (ALA) synthesized using the heterotrophic C4 pathway by the condensation of succinyl-CoA and glycine in mitochondria, similar to apicomplexan parasites and primary heterotrophic eukaryotes; the remainder of the pathway was predicted to be plastid localized. To experimentally determine the intracellular locations of enzymes catalyzing the entire heme pathway, namely ALA synthase (ALAS), ALA dehydratase (ALAD), porphobilinogen deaminase (syn. hydroxymethylbilane synthase) (PBGD), uroporphyrinogen synthase (UROS), uroporphyrin decarboxylase (UROD), coproporphyrinogen synthase (CPOX), protoporphyrinogen synthase and ferrochelatase (FeCH) in *C. velia*, we used specific antibodies generated against synthetic oligopeptides designed of the heme pathway enzymes from *C. velia*. All antibodies localized to the mitochondria in *C. velia*, suggesting that the heme pathway was re-located from other compartments, supposedly plastid, to the mitochondrion. Based on these data, we propose a new hypothesis to explain how ancestors of apicomplexans lost photosynthesis and became parasites.

Keywords: mitochondrion, heme, *Chromera*, Apicomplexa

Phylogeny and evolution of a new parasitic lineage closely related to Apicomplexa

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The Apicomplexa form a group of obligatory intracellular parasites that cause some of the most debilitating diseases of humans (for example, the malaria parasite *Plasmodium*). As such, they possess several unique adaptations to their parasitic lifestyle, for example the apical complex, which gave the Apicomplexa their name and is involved in invasion of the host. Moreover, they possess a highly reduced non-photosynthetic plastid called the apicoplast, which has lost many of its functions, but still contains a small plastid genome. Although reduced, the apicoplast is an essential organelle as it harbors pathways for heme, isoprenoid, fatty acid and iron-sulfur cluster synthesis. Relatively recently, several new free-living lineages, collectively called chrompodellids, were discovered and were shown to be closely related to the Apicomplexa. The best studied of these new lineages are two photosynthetic algae called *Chromera velia* and *Vitrella brassicaformis* whose genomes were recently fully sequenced.

Piridium sociabile is a poorly studied parasite of a marine gastropod (*Buccinum undatum*) that was originally described in 1936 and classified as an apicomplexan parasite. Here, we present transcriptomic and genomic data from *Piridium sociabile* and show that it is actually a sister lineage to the photosynthetic chrompodellid *Vitrella brassicaformis*. It therefore represents a lineage that is closely related to Apicomplexa but acquired a parasitic lifestyle and lost photosynthesis independently. The complete sequence of the *Piridium* plastid genome is similar in size to apicoplast genomes and also has a very similar gene content. We have used our genomic and transcriptomic data to explore and contrast the evolution of non-photosynthetic plastids and the evolution of parasitism between *Piridium* and Apicomplexa.

Possible hijacking of the host plastids by an intracellular parasite (*Amoebophrya* sp., Syndiniales) of microalgae

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Although little studied, many extremely virulent microeukaryotic parasites infecting microalgae have been detected in the marine plankton. This is the case for *Amoebophrya* spp., a member of Syndiniales (also called Marine Alveolates), which constitute a diverse and highly widespread group. This pathogen is an intracellular parasitoid. After its penetration inside the host, the primary target of several strains is the host nucleus, which is rapidly digested once the parasite is installed. This means that the host cannot produce *de novo* nucleus-encoded proteins for its growth and defense mechanisms. Technically, the host dies long before the release of the parasite out of its host, likely when the nucleus is totally digested. In that sense, the parasite replaces its host and uses its envelop and the available resources to replicate, a strategy very similar to that of viruses. The means of acquisition of the energy necessary to the parasite to mature as a trophont into the host is one of the key process of the parasitic interaction. It is thus worth noting that during the whole maturation processes (2-3 days), the host cell keeps swimming actively until the release of the vermiform stage, suggesting that the host cellular machinery may be pervasively controlled by the parasite. We observed that the host plastids remain astonishingly well preserved during infection. The potential parasitic control of the host thus possibly includes the takeover of the host plastids, which would then be hijacked for the parasite purpose. We will present some evidences towards this hypothesis.

Defining mitochondrial proteome of *Trypanosoma brucei* using genome-wide protein localization approach

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Trypanosoma brucei is not only a serious human parasite but also an important model protist. However, a limit of trypanosomes as model organisms is their evolutionary distance from other well-studied eukaryotes, resulting in a relatively high number of proteins lacking homologs outside of Kinetoplastea. Our study is based on collaboration with TrypTag, an ongoing genome-wide protein localization project in *T. brucei*. From all proteins that are being fused with mNeonGreen fluorescent tag and expressed from an endogenous locus, we have focused on proteins identified by TrypTag as localized to the mitochondrion. Up to now, from a total of 412 such proteins, 203 were tagged with a C-terminal tag, 138 with a N-terminal tag and 71 proteins were tagged on both termini. While 123 proteins have a clear mitochondrial pattern, 252 including those involved in the mitochondrial Fe-S cluster machinery are enriched in and around the kinetoplast, which is a single huge network of mitochondrial DNA, situated close to the basal body of the flagellum. Remarkably, they all appear to be localized into the antipodal sites, which are two specific and defined foci on the periphery of the kinetoplast disk. So far, only a handful of proteins were shown to have this unusual localization. Since a vast majority of non-kinetoplast-enriched proteins contains one or more transmembrane domains, we suppose that those are located in the outer membrane and/or intermembrane space of the organelle. In contrast, the majority of kinetoplast-enriched proteins are soluble and likely belong to the matrix proteins. Further study is needed to understand the hypothetical role of the kinetoplast in protein transport, regulation and/or storage. In addition, our approach may also provide a useful technique to identify soluble proteins present in the mitochondrial intermembrane space.

Keywords: mitochondrion, *T. brucei*, localization

Coral, photosynthesis, and the emergence of parasitism in Apicomplexa

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The discovery over a decade ago that apicomplexan parasites contain a relict plastid organelle prompted many questions about the nature of the parasites' ancestors and the role of photosynthesis and its loss in their evolution. More recently, the discovery of still-photosynthetic relatives of Apicomplexa, *Chromera* and *Vitrella*, living in some association with coral has prompted further speculation on the role of photosynthetic symbioses with corals in that evolution. Resolving these questions is confounded by a poor understanding of the real distribution of photosynthesis in apicomplexans and their relatives, and by a poor understanding of the functional relationship between coral-associated apicomplexan relatives and their putative animal "host". Moreover, two key lineages in this story remain known only as "unidentified environmental clades": Genotype N, known from nuclear SSU rRNA, and Apicomplexan-Related Lineage-V, or ARL-V, known from plastid SSU rRNA. Here, we re-evaluate the environmental distribution of coral-associated apicomplexans, and show that *Chromera* and *Vitrella* are not actually associated with coral per se, but rather with coral reef environments more broadly. ARL-V and GenotypeN are truly coral-associated, but co-occurrence patterns in natural samples suggest they are the same organism. This is confirmed by *in situ* co-localization in lab-cultured corals, which shows both sequences occur in the same cells at the distal edge of the mesenterial filaments of the host coral, altogether suggesting they most likely correspond to a previously described genus of coral apicomplexan, *Gemmocystis*. Using metagenomic data from coral communities, we assembled near-complete plastid genomes from samples positive for ARL-V plastid SSU rRNA, confirming ARL-V to be non-photosynthetic, but interestingly retaining genes involved in chlorophyll biosynthesis.

The expansion of Microsporidia

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Some protists with microsporidian-like cell biological characters have SSU rRNA gene sequences that are much less divergent than canonical Microsporidia. We used phylogenetics to place these lineages within known opisthokont diversity, and showed that they group with other microsporidians to the exclusion of the clade including *Rozella*. These results show that the phylogenetic scope of Microsporidia has been greatly underestimated. We propose that much of the lineage diversity previously thought to be rozellid is microsporidian, offering novel insights into the evolution of the highly specialized parasitism of canonical Microsporidia. This insight is important for accurate interpretation of environmental diversity, and has major implications for our understanding of opisthokont evolution and ecology and the development of therapeutics. Our analyses also demonstrate that many opisthosporidian (aphelid + rozellid + microsporidian) SSU V4 OTUs from forest soils group with the short-branching Microsporidia, consistent with the abundance of their protist hosts in soils.

Keywords: Microsporidia, Cryptomycota, *Rozella*, *Mitosporidium*, *Nucleophaga*

Symbiosis: new perspectives from eukaryotic endosymbionts within pathogenic amoebae

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Endosymbiotic relationships between eukaryotic and prokaryotic cells are common in nature. Endosymbioses between two eukaryotes are also known; on multiple occasions cyanobacterium-derived chloroplasts have spread horizontally when one eukaryote assimilated another. A unique instance of a non-photosynthetic, eukaryotic endosymbiont involves members of the genus *Paramoeba*, disease-causing amoebozoans that infect marine animals such as farmed fish and sea urchins. *Paramoeba* species harbor endosymbionts belonging to the Kinetoplastida, a diverse group of flagellate protists that are themselves often pathogenic. Here I will discuss the genomes and transcriptomes of *Paramoeba pemaquidensis* and its endosymbiont *Perkinsela* sp., sequenced to elucidate the nature of their endosymbiotic association. Mosaic biochemical pathways suggest extensive 'cross-talk' between the two organisms, most notably in heme, ubiquinone, nucleotide, amino acid, and trypanothione metabolism. Electron microscopy shows that the endosymbiont ingests the cytoplasm of its amoeba host, a novel form of endosymbiont-host communication. These data help explain the obligate relationship between *Perkinsela* sp. and its amoeba host in terms of metabolic and cell biological interconnectivity, and provide a launch point for understanding the determinants of pathogenicity in economically important *Paramoeba*.

ISOP SYMPOSIUM: Deciphering the activity and function of protists in the environment using single-cell ecophysiology approaches

(organizers: Johan Decelle and Fabrice Not)

Ecophysiology of plankton photosymbiosis

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Marine plankton play central roles in marine ecosystems' functioning, including the transfer of energy to higher trophic levels (*i.e.* representing the basis of marine food webs) and the carbon export to the deep layers of the oceans (*i.e.* contributing to global biogeochemical cycles). A large fraction of planktonic organisms are protists, encompassing all lineages of eukaryotic life. Nowadays, there is a raising awareness of symbiotic interactions among plankton being a determinant feature structuring the communities and functions of ecosystems. Among them, photosymbiotic relationships are widespread and recent studies demonstrated that Radiolaria, a group of photosymbiotic plankton belonging to the Rhizaria lineage, can contribute significantly to oceanic biomass. Such associations between microalgae and Radiolaria cannot be cultivated and along with the paucity of genomic data available to date, their ecology and physiology is poorly known.

Here we present the rational and first results of an integrated approach to investigate the ecophysiology of photosymbiotic plankton exposed to thermal stress. Following carefully planned experimental procedures carried out with freshly collected photosymbiotic Radiolaria, we used a combination of *1*- photophysiology *2*- transcriptomic *3*- metabolomic approaches. Photophysiology of single cell microalgae in symbiosis was performed using PAM (pulse amplitude modulated) microscopy. Transcriptomic samples were collected throughout the experiment and besides regular comparative analyses, bioinformatic analytical procedures specifically designed for transcriptomic investigation of symbiotic protists were used. Finally, on the same set of samples, phenotypic responses of radiolarians holobiont to thermal stress were evaluated by analysing their endo-metabolomes. Beyond straight acquisition of ecophysiological data on single photosymbiotic cells, which remains challenging because of the low quantity of biological material available for uncultured protists, another concern is to integrate multiple dataset together. We intend to achieve such integration through an ecosystems biology and modelling approach.

Keywords: plankton, symbiosis, transcriptomic, ecophysiology, Radiolaria

Visualizing the chemical landscape of planktonic symbioses using single-cell chemical imaging

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Symbiosis between heterotrophic hosts and microalgae (photosymbiosis) is a widespread and ecologically important phenomenon in the oceanic plankton. The ecological success of photosymbiosis in nutrient-poor waters must rely on the capacity of the partners to intertwine their metabolic networks and on the efficiency with which they take up and recycle nutrients. However, contrary to terrestrial and reef symbioses, the functioning and metabolism of symbioses in plankton remain enigmatic. Here, we visualized the ***in-situ*** distribution of nutrients (N, P, S, Fe) and small metabolites (amino and fatty acids) at subcellular level, using high-resolution chemical imaging techniques (NanoSIMS, ToF-SIMS and synchrotron X-ray fluorescence), in correlation with electron microscopy. Live symbiotic cells (radiolarians with their endosymbiotic algae: the haptophyte *Phaeocystis* or the dinoflagellate *Brandtodinium*) were cryofixed and resin-embedded for sectioning. From ultrathin sections, mapping of elemental composition and stoichiometric ratios of the host and symbionts provide information about their metabolic roles and needs. Compared to the host, chloroplasts of symbionts are rich in nitrogen, reflecting a high concentration of proteins and pigments in photosystems to maximize primary production. By contrast, phosphorous (mainly RNA and phospholipids) is poorly present in symbiont chloroplasts, and tend to be lower in symbiosis vs free-living stage. Several fatty acids were identified and visualized in host vacuoles and symbiont cytoplasm. For sulfur, its subcellular distribution (e.g. high concentration in chloroplasts and vacuoles of symbionts) and expressed genes involved in the S pathway demonstrate the key role of symbiotic microalgae in sulfur metabolism, particularly for the production of antioxidants (e.g. methionine, glutathione and DMSP). Overall, this study demonstrates the high potential of correlated single-cell chemical imaging to better understand the *in situ* metabolism and biogeochemical roles of uncultivable protists in the ocean.

Keywords: symbiosis, nanoSIMS, synchrotron, nutrients, radiolarians

Taxon-specific responses of diatoms to micronutrient gradients in the ocean revealed by single-cell element analysis

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The recognition of fairly constant average nutrient composition of ocean plankton by Redfield has served as an enduring central tenet of marine biogeochemistry. More recently, the concept of a single broadly-representative element stoichiometry has been extended to trace-metal micronutrients in cultured phytoplankton. Such stoichiometries enable testing of high-level assumptions about connections between plankton and ocean nutrient ratios. However, as in genomics, single-cell elemental analyses are now revealing significant intra- and inter-population diversity in elemental composition between co-occurring plankton groups, including between co-occurring species of diatoms. In this talk I will describe field data collected from across natural nutrient gradients in the coastal and open Pacific Ocean using single-cell synchrotron X-ray fluorescence microscopy (SXRF) that demonstrate significant variations in the trace-metal micronutrient content of diatoms. *Chaetoceros* and *Pseudo-nitzschia* diatoms diverged in their response to ambient concentrations and to added luxury metal inputs. Cellular metal quotas will be linked both to transcriptomic data and ambient metal concentrations in an effort to understand the underlying physiological and environmental drivers of the phytoplankton elemental composition and physiology in the ocean.

Deciphering the behavior and physiology of single cells via microfluidics

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Microfluidics has emerged as a key technique for the targeted observation and manipulation of microbial cells. This success is owed, in part, to the great design flexibility of this technique and by the possibility to observe cells using automated microscopy, which together enables the extraction and quantification of parameters relevant to microbial life (e.g. growth rates) at the single cell level. In combination, these methods provide a powerful recipe that has succeeded in addressing fundamental questions in microbial ecology. In this symposium, I will introduce the basic steps in the design, construction, and operation of microfluidic devices, and present instances where microfluidics has been applied in the aquatic sciences, specifically in the observation of microbial cells. Examples will include the use in our lab of microfluidics in (1) monitoring growth in photosynthetic eukaryotes, (2) the study of bacteria–phytoplankton interactions, and (3) the use of microfluidics in conjunction with pulse-amplitude-modulated (PAM) chlorophyll fluorometry to assess the (photo) physiological state of a cell. Using the latter method, we are now able to investigate single-cell physiologies in response to precisely controlled microenvironments, permitting, e.g., the screening for favorable ecophysiological effects and their subsequent selection and refinement for growth purposes. These examples illustrate a few of the diverse possible applications of microfluidics, and highlight the richness of data that would be almost impossible to obtain using regular methods.

Insights into protist ecology and evolution from single cells

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For the past two decades, molecular methods based on the amplification and sequencing of 18S rRNA genes from natural samples has revealed a far wider diversity of microbial eukaryotes than ever imagined. Most of that protist diversity remains uncultured, making it difficult to associate specific morphotypes to phylogenetic groups and precluding the study of individual functions and life history traits. Furthermore, most cultured heterotrophic protists are not axenic, which complicates the reliable reconstruction of full genome sequences but also characterizing interactions with potential endosymbionts or preferred preys. If metagenomics represented a potential solution to access the genomes of prokaryotes in natural communities, it is much less useful in the case of eukaryotes owing to their lower proportion in natural communities combined with their larger genome size, the occurrence of intron and long intergenic regions and higher compositional variability along the genome. Single cell approaches based on the physical separation of individual cells by micromanipulation, fluorescence-activated cell sorting (FACS) or microfluidics followed by genome and/or transcriptome amplification and sequencing are a powerful tool to access sequence-based evolutionary information but also functional information, e.g. metabolic pathways, from these organisms. They also allow to study microbial interactions at the level of single eukaryotic cells, including mutualistic symbiosis, parasitism and predation. With this aim, we have recently implemented a single-cell 'omics' facility, UNICELL. I will present case studies of single-cell analyses devoted to characterize endobionts in anaerobic/microaerophilic amoeba and to contribute solving phylogenetic relationships within the Opisthokonta.

ISOP SYMPOSIUM: The eukaryome, bringing protists into the spotlight of microbiome research

(organizers: Laura Parfrey and Javier del Campo)

Inter-kingdom interactions in the human gut microbiome-the prevalence of the intestinal protist *Blastocystis* is linked to host age, antibiotic use and gut bacterial diversity and composition

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The human gut is host to a complex microbial ecosystem that plays a central role in host health. In addition to bacteria, viruses and archaea, the gut microbiota includes a diversity of fungal and protist species that are collectively referred to as the gut 'eukaryome'. Although research into the gut eukaryome is in its infancy, emerging data indicates that the intestinal protist *Blastocystis* is perhaps the most common member of the human gut eukaryome worldwide. Despite its association with intestinal disease, asymptomatic carriage is common with *Blastocystis* frequently observed in surveys of the healthy adult gut microbiome. Furthermore, *Blastocystis* is less prevalent in chronic diseases such as Irritable Bowel Syndrome compared to healthy controls. Antibiotic administration significantly reduces *Blastocystis* prevalence rates between case and controls groups with the reduction in *Blastocystis* prevalence in the antibiotic treated group possibly due to direct effects on *Blastocystis* and/or secondary loss due to loss of bacteria that *Blastocystis* interacts with. In support of this latter hypothesis, data showing correlations between the presence of *Blastocystis* and specific features of the bacterial component of the gut microbiome (high diversity and a specific bacterial composition) are suggestive of inter-kingdom interactions between bacteria and *Blastocystis* in the gut microbiome. *Blastocystis* is less prevalent in infant populations relative to contemporaneous adult populations indicating that *Blastocystis* is not adapted to the infant gut. Given the difference in microbiome composition and diversity in infants compared to adults perhaps *Blastocystis* requires a more adult-like gut microbiome for successful colonisation. Collectively, emerging data suggests that successful colonisation of the gut by *Blastocystis* is linked to the composition and diversity of the bacterial fraction of human gut microbiome. Consequently, interactions between *Blastocystis* and bacteria in the gut microbiome may account for some of the variation in prevalence rates observed across age, health and geography.

Keywords: *Blastocystis*, gut microbiome, bacteria, antibiotic, prevalence

Diversity of protists and bacteria on seagrass and seaweeds

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Multicellular marine organisms host diverse collections of microbes (bacteria, protists, and others) on their surface and within their tissues. These microbes are integral to the biology of the host, playing key roles in defense, development, and nutrient acquisition. Yet, we do not understand basic parameters of community assembly and dynamics on marine hosts. For example, is there a core microbiota for each species that performs essential functions? Are microbial taxa species-specific, or are they broadly shared across seaweed species? We surveyed the epibiotic communities of bacteria and protists living on 35 sympatric species of seagrass and seaweed in the nearshore environment along the central coast of British Columbia. By sampling multiple replicates for each species and the environmental pool of microbes from the water column and biofilms we are able determine which microbes are 1) characteristic of host species, 2) broadly distributed across seaweeds and seagrass, and 3) transients from the environment. We show that bacteria are much more likely to be specific to a host species, while protists are broadly distributed across seaweeds and seagrasses. These results suggest that community assembly and co-evolutionary history differ for protists and bacteria on marine hosts.

Keywords: host-associated, diatoms, community assembly, microbial ecology, core microbiota

Host-protozoan interactions impacting gut microbial diversity and host immunity

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Numerous protists are considered pathogens of the murine and human intestine, including *Encephalitozoon cuniculi, Entamoeba histolytica, Toxoplasma gondii, Giardia* spp., and *Cryptosporidium* spp. to name just a few. It is increasingly evident that a constitutive protist microbiota exists as an integral part of the vertebrate microbiome. Using WGS phylogenomic methods to survey 18S and 16S diversity within the gastrointestinal tract, and specifically, the colon of naïve rodent C57BL/6J mice, we have identified a commensal population of rodent parabasalids, that we collectively refer to as *Tritrichomonas musculus (T.mu)*. This previously unrecognized protist of the rodent microbial flora is capable of re-shaping mucosal immune responses, and is associated with a mild goblet cell hyperplasia and host epithelial inflammasome activation, including localized release of IL-1β and IL-18. The epithelial derived IL-18 induces maturation of CD103 positive dendritic cells that promote Th1 and Th17 immunity to confer a bystander protection against bacterial and protozoan mucosal infections. Phylogenetic classification of *T. mu* has identified a rich genetic diversity that variously impacts 16S diversity within the mouse colon. How this genetic heterogeneity impacts host mucosal immune responses, at the cost of increased risk of inflammatory diseases, will be discussed. Our work is gleaning new perspective on the etiology of inflammation and immune homeostasis in hosts colonized with rodent and related human (*i.e., Dientamoeba fragilis*) parabasalids.

Keywords: protist, parabasalid, 18S, *T.mu*

The eukaryome. Unveiling the animal associated micro-eukaryotic diversity

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Unlike the study of bacterial microbiomes, the study of the micro-eukaryotes associated with animals has largely been restricted to visual identification or molecular targeting of particular groups. The application of high-throughput sequencing (HTS) approaches, such as those used to look at bacteria, has been restricted because the barcoding gene we use to study protist ecology and distribution in the environment, the 18S rRNA gene, is also present in the host animals. As a result, when host-associated microbial eukaryotes are analyzed by HTS, the results are dominated by host sequences. Stemming from our work on coral-associated protists, we successfully developed an approach that avoids the amplification of metazoan host genes, which allows us to use high-throughput methods to study the microeukaryotic communities of animals. This approach will open the doors to the study of diversity and distribution of protists in myriad environments, from the coral surface to zooplankton or the human gut.

Keywords: microbiome, eukaryome, corals, human gut, zooplankton

A critical comparison of eukaryotic and prokaryotic microbiome research

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Microbiome research is a very popular topic these days. Most of it is prokaryotic and specifically, about bacterial members of particular microbiota. Far less microbiome research is eukaryotic, but efforts in this direction are growing. With my colleague Katarzyna Hooks (University of Bordeaux), I have carried out a bibliometric and conceptual analysis of both eukaryotic and prokaryotic microbiome research. Bibliometry is the statistical analysis of bodies of literature. It aims to find quantified trends and patterns in that literature. My focus as a philosopher is conceptual and methodological trends in microbiome literature. I will show the similarities and differences between the two subfields – prokaryotic and eukaryotic. I will outline some serious conceptual problems and their methodological underpinnings in prokaryotic microbiome research. These problems are particularly concerned with the evolutionary and ecological status of microbiota as a whole, and whether microbiota is in any sense physiologically balanced and working for the good of their hosts. I will show whether or not these issues are occurring to any extent in eukaryotic microbiome research. In part due to the lesser medical emphasis of the latter, the conceptual and methodological issues are different. Even the similar problems are far less developed. I will conclude with some suggestions for how eukaryotic microbiome research might continue to make progress without having to make the same missteps that prokaryotic microbiome research has made.

Keywords: microbiome, microbiota

SYMPOSIUM: 70 years of protistology
(organizer: John Dolan)

70 years of protist phylogeny

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It goes without saying that there have been huge changes in our understanding of the phylogenetic tree of protists over the last several decades. During this time broad-scale protist phylogenetics has gone from a peripheral and highly speculative endeavour to one of the central scientific enterprises of protistology. This occurred within the context of increased emphasis on phylogenetics in biology in general, but was particularly striking in our discipline. The progress of protist phylogenetics has been profoundly influenced by the successive deployment of technical innovations, most notably electron microscopy, molecular phylogenetics (especially of ribosomal RNA genes), and the overlapping introductions of genomics/transcriptomics and environmental sequencing approaches. The intellectual innovation represented by the revival and development of endosymbiotic theory (especially for plastids) was also crucial. Nonetheless, in probably all of these cases there was a marked lag between an innovation being introduced, and genuinely new and lasting advances in our understanding of the phylogenetic tree of (protistan) eukaryotes. While our current understanding of the overall tree is likely to be 'substantially correct' there are some regions that have remained extremely difficult to resolve; furthermore, the recent history of organism discovery suggests that 'the tree' may be more skeletal than generally realised. Consequently, protist phylogenetics is likely to remain a central concern of ISoP into its eighth decade and beyond.

Keywords: phylogenetics, phylogenomics, tree-of-life, evolution, ISoP

Research in trypanosomatids revealed unique features and led to fundamental discoveries in biology

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Morbidity and mortality associated with diseases caused by trypanosomatids affect millions of people around the world, causing thousands of deaths and affecting the ability of more people to raise cattle and crops, or earn a living. In the search for targets for drugs, vaccines and diagnostics, research on these protists have revealed unique biological characteristics and uncovered biochemical pathways that later on were found in other organisms. The completion of their genomes and the development of novel molecular tools for their study led to a rapid progress in understanding how they invade, modify and survive within their hosts. The accessibility of some of these parasites to multiple genetic manipulations has converted them in model systems in cell and molecular biology studies that could led to the understanding of basic biological processes, as well as their evolution and pathogenesis. Some peculiarities of these protists described in the last seventy years are the structure of the kinetoplast DNA, the pathway for antigenic variation, the presence of a spliced leader in messenger RNA, novel organelles such as glycosomes, acidocalcisomes, and contractile vacuoles, novel structures in their flagella, and novel enzymes such as those involved in thiol metabolism and the *trans*-sialidases. In addition, their study has also resulted in the discovery of biological processes later found in multicellular organisms, such as *trans*-splicing, RNA editing, and glycosylphosphatidylinositol (GPI) membrane anchor synthesis, or their study was important for the discovery of the molecular nature of important proteins such as the mitochondrial calcium uniporter.

Keywords: trypanosomatids, Kinetoplastida, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania* spp.

70 years of progress: Ecology of marine planktonic ciliates and phagotrophic dinoflagellates

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Elegant observations of diversity and natural history of planktonic ciliates and dinoflagellates were made prior to 1947, but our quantitative understanding of the role of these organisms in ecosystems has developed over the last 70 years. The first step was documentation of their abundance and biomass in the plankton. This was followed by progress in culture of planktonic species and subsequently in description of their numerical and functional responses to prey. The high estimates of microzooplankton grazing on phytoplankton and studies that found that ciliates were important in the diet of zooplankton and fish larvae lead to recognition of their importance. By the early 1980's the "microzooplankton link" in pelagic food webs was recognized. Soon after, field studies led to the hypothesis that protistan grazing largely controls phytoplankton standing stocks and occurrence of blooms. Recognition of the importance of ciliates and heterotrophic dinoflagellates in marine ecosystems has continued to strengthen, but the picture has become more complex. Internal predation within the microzooplankton is common, and hypothesized to influence secondary production and trophic transfer to zooplankton. Many ciliates and dinoflagellates are mixotrophic, contributing to both grazing and primary production. Symbiosis, including parasitism, and organelle sequestration are common among ciliates and dinoflagellates and thought to contribute to their ecological success and diversity. Parasitism of larger zooplankton by ciliates and dinoflagellates is common; the effects on population dynamics of zooplankton are largely unexplored. Genomics and transcriptomics have revealed hidden genetic diversity and functional potential in many planktonic protists. The "complications" and "side flows" are a rule rather than an exception; they challenge use "phyto-" and "zoo-" to categorize plankton and the usefulness of concepts such "trophic level" and "growth efficiency". It is an exciting and challenging time to be a marine (or freshwater) protistologist!

Keywords: ciliate, dinoflagellate, protistan ecology, microzooplankton, mixotrophy

Mixotrophy among planktonic protists: Towards global recognition, characterization and incorporation into global biogeochemical models

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Mixotrophy encompasses a variety of trophic activities and ecological strategies among single-celled eukaryotes (protists) and, collectively, is conducted by a tremendous diversity of taxa spread across many lineages. Broadly defined, mixed nutrition among protists may involve phagotrophy of prey by photosynthetic flagellated protists, the acquisition of functional chloroplasts by heterotrophic protists from their photosynthetic prey, or a myriad of symbiotic associations including numerous apparently-mutualistic relationships between endosymbiotic photosynthetic protists within the cytoplasm of heterotrophic taxa. While awareness of these behaviors has existed for many decades, their implications for the structure and function of aquatic food webs have only slowly gained full recognition among biologists and biogeochemists. Recent field surveys have now begun to demonstrate the widespread occurrence and often predominance of mixotrophy across vast stretches of the ocean and from a rapidly expanding number of freshwater environments. This presentation will attempt to summarize the breadth and distribution of mixotrophic nutrition among protists, and identify some of the processes that are still poorly understood, or present considerable difficulties, for the incorporation of these behaviors into models describing aquatic biogeochemical cycles and processes.

Keywords: mixotrophy, ecology, nutrition, symbiosis

SYMPOSIUM: UniEuk: time to speak a common language in protistology!

(organizers: Colomban de Vargas and Pelin Yilmaz)

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Universal taxonomic frameworks have been critical tools to structure the fields of botany, zoology, mycology, and bacteriology, as well as their large research communities. Animals, plants and fungi have relatively solid, stable morpho-taxonomies built over the last three centuries, while bacteria and viruses have been classified for the last three decades under coherent molecular taxonomic frameworks. By contrast, no such common language exists yet for protists, even though environmental 'omics' surveys suggest that they could make up most of the organismal and genetic complexity of our planet's ecosystems. With the current deluge of eukaryotic molecular data, we urgently need to build up a universal eukaryotic taxonomy bridging the 'protist-omics' age to the fragile, centuries-old body of classical knowledge that has effectively linked protist taxa to morphological, physiological, and ecological information.

UniEuk is an open, inclusive, community-based and expert-driven international initiative to build a flexible, adaptive universal taxonomic framework for eukaryotes, focused primarily on protists. The *UniEuk* system will integrate expert knowledge about morphology and ecology with key molecular information from phylogenetic markers and environmental metabarcoding surveys to capture our total current knowledge on eukaryotic diversity, evolution and ecology. It comprises three complementary modules, *EukRef*, *EukBank*, and *EukMap*, which allow direct community input and will together inform the online universal taxonomic framework. The resulting *UniEuk* taxonomy will be directly implemented into the European Nucleotide Archive at EMBL-EBI, ensuring its broad use and long-term preservation.

During this multi-speaker special symposium, we will present a demonstration of the *UniEuk* system, including the online navigable tree and community interaction module (*EukMap*) and case studies of the *EukRef* and *EukBank* modules. These presentations will be followed by a general roundtable during which *UniEuk* team members will answer questions and welcome feedback from the audience.

Keywords: eukaryotes, taxonomy, diversity, online, community-based

ORAL PRESENTATIONS

Impact of bacterivory by protists on terrestrial biogeochemistry

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We have obtained additional functional response curves for protists grazing on bacteria. The rate of ingestion of bacteria for 20 soil species are compared. The numbers are compared to equivalent data from marine species. We show that details of ingestion preferences can help distinguish between species niches in the environment. We have performed additional calculations to estimate the quantity of biomass transformed daily under various weather scenarios. From these estimates, additional calculations allow predictions about the impact on microbial biomass turnover in different ecozones and under various climatic scenarios. The numbers indicate that protists are the principal consumers of bacteria in the soil, thus a keystone functional group in nutrient cycling in both soils and marine systems. These results lay the ground work for microcosm studies that include plants, to quantify root nutrient uptake under various food web or species community networks.

Keywords: soil ecology, bacterivory, functional response, biogeochemistry, ecosystem

Predation by a vampyrellid amoeba affects the short-time dynamics of its nanoplanktonic diatom prey in a tropical coastal lagoon

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Vampyrellids constitute a worldwide distributed and relatively easily recognizable group of predators feeding on protists and small metazoans in both sediment and pelagic compartments of freshwater and marine systems. Recent molecular surveys revealed a high phylogenetic diversity of these organisms in marine planktonic assemblages, where they are supposed to play important ecological roles. Yet, formally described vampyrelloid species were mainly isolated from metaphytic populations predating large sized microalgae with information on their ecological relevance remaining still strictly qualitative. Here we present the first description of a vampyrellid predating a nanoplanktonic microalgae and their associated predator-prey dynamic. Phytoplankton community was monitored in Rodrigo the Freitas Lagoon (Rio de Janeiro, Brazil) from 2012 to 2016. The undescribed vampyrelloid was observed during a bloom of the diatom *Chaetoceros minimus* from June to August 2013. Although other diatom species were observed during the study, only *C. minimum* was observed to be preyed by this organism both in field samples and cultures. 18S rDNA sequences grouped the vampyrelloid in the Hyalodiscidae clade (former linage B3) whereas very distinctive morphological characters support its status as a new species to science (currently in phase of description). The high sampling frequency (twice a week) allowed the detection of the classic Lotka-Volterra predator-prey dynamic between both organisms, with predation by the vampyrelloid significantly affecting short-term oscillations of the diatom species. Prey availability as well as salinity levels where the main variables determining the short-time window (3 months) in which the vampyrelloid species was observed despite the long term duration of the survey (five years). These results suggest that although vampyrelloids could be indeed very ecologically relevant in marine plankton systems their relative importance to the planktonic dynamics depends strongly on complex interactions between both biotic and abiotic factors.

Keywords: vampire amoebae, plankton ecology, predator-prey dynamics

An interactive eukaryotic map - A comparative tool for the core components of the Exon Junction Complex and intron density in the evolution of eukaryotes

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The exon junction complex (EJC) has a central role in marking splice sites in eukaryotic mRNA transcripts. Previous comparative genomics studies of the four core components of the EJC complex (Magoh, Y14, eIF4AIII and MNL51) have been performed mainly within the Opisthokonta and Archaeplastida eukaryotic super-groups. Many eukaryotic pathogens, such a trypanosomes and *Plasmodium*, fall outside the supergroups containing animals, fungi and plants; trypanosomes are excavates and *Plasmodium* falls in the SAR. I have expanded the analyses to include several trypanosomes and related organisms in the Excavata eukaryotic super-group as well as parasitic apicomplexans and other organisms of the SAR super-group. Identifying unique differences between both trypanosomatid and apicomplexan parasites and their free-living relatives would provide insight into the management of their corresponding diseases. I have demonstrated that a core protein of the exon junction complex, eIF4AIII, is conserved in all eukaryotes and was present in the last eukaryotic common ancestor (LECA). Magoh and Y14 were present in the LECA, but were selectively lost in intron-poor species. Y14 has undergone a founder effect within the trypanosome lineage.

I have designed an interactive model/eukaryotic map illustrating

1. The distribution of the EJC amongst the six eukaryotic super-groups.
2. The correlation of the core components of the EJC to intron density amongst both parasites and non-parasites.

The interactive feature of the model can be used to illustrate the variation of intron densities in comparison to the presence of the different EJC core units amongst different eukaryotic species as well as an instantaneous display of the position of any newly sequenced genome of both parasitic and non-parasitic eukaryotes on the intron density scale.

Keywords: trypanosomes, *Plasmodium*

Contribution of symbiogenetic genes to the evolution of eukaryotes

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Ancestral eukaryotic cells and the first photosynthetic eukaryotes evolved as a result of major evolutionary transitions. They are the products of substantial introgression of intact genes from foreign sources through horizontal and endosymbiotic gene transfer. Consequently, nuclear genomes are phylogenetically mosaic. Interestingly, genetic chimerism in eukaryotes goes even further, and can be observed at the subgenic level. Using protein similarity networks that can disentangle reticulate gene histories, we discovered chimeric nuclear genes (S-genes) that are built from prokaryotic domains likely derived from symbiotic partners. We propose that these S-genes are critical for explaining eukaryotic evolution, with their origins driving the massive leap forward that occurred in cellular complexity. A total of 286 S-gene families contributed solutions to many of the challenges faced by early eukaryotes, including enhancing the informational machinery, processing spliceosomal introns, tackling genotoxicity within the cell, and ensuring functional protein interactions in a larger, more compartmentalized cell. In particular, Bacteria contributed 9-fold more S-genes than Archaea, including a two-fold greater contribution to informational functions. In the genomes of photosynthetic eukaryotes, the "recycling" of genetic information also generated novel composite genes, exclusive to these lineages. These S-genes are comprised of a cyanobacterium-derived domain fused to one of cyanobacterial or other prokaryotic origin. Transcriptome data demonstrate the existence and expression of these S-genes across a wide swath of algae, and functional data indicate their involvement in tolerance to oxidative stress, phototropism, and adaptation to nitrogen limitation. Many of these S-genes have roles in plastid maintenance. Given that S-genes are of functional and evolutionary importance, their presence demonstrates that recycling of genetic material into novel domain combinations was a key feature of organelle evolution, possibly coupled with isostatic constrains.

The plant SNARE proteins NPSN and Syp7 represent ancient eukaryotic proteins, and further elucidate the evolution of the Qb- and Qc-SNARE families

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Membranes and cargo are trafficked through a system of organelles and vesicles in eukaryotic cells, which facilitates internal processing and exchange of material with their environment. Proteins of the Soluble N-ethylmaleimide sensitive factor Attachment protein Receptor (SNARE) superfamily mediate membrane fusion in each trafficking pathway. This superfamily comprises the Qa-, Qb-, Qc-, and R-SNARE families. In plant cells, the Qb-SNARE NPSN and the Qc-SNARE Syp7 are known to function together at the cell plate during cell division, and are considered to be plant-specific. However, through homology searching and phylogenetic analysis, we have confirmed the presence of orthologues in diverse non-plant eukaryotes including the amoebozoan *Dictyostelium discoideum*, with losses in animals and fungi, identifying these SNAREs as ancestral eukaryotic proteins. Furthermore, NPSN shows phylogenetic affinity for the Qb domain of Qbc-SNAREs (which contain both a Qb and Qc SNARE domain). These findings are a key step towards reconstructing the evolution of the Qb- and Qc-SNARE families, and revealing the origin of Qbc-SNAREs, which include the human protein SNAP-25 that functions in exocytosis. To investigate the function of these proteins in non-plant eukaryotes we generated *D. discoideum* strains over-expressing fluorescently tagged reporters. Preliminary results from confocal fluorescence microscopy of live cells reveals that NPSN reporters localize primarily to the plasma membrane, while Syp7 reporters localize primarily to intracellular membranes, which potentially compose the endoplasmic reticulum. Overall, this is comparable with the known localizations of these SNAREs in plant cells, and is consistent with potential ancestral roles in exocytosis at the cell surface.

Keywords: organelle, phylogenetics, *Dictyostelium*

A metatranscriptome workflow and its application to European freshwater ecosystems

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Next generation sequencing (NGS) technologies are increasingly applied to analyse complex microbial ecosystems by mRNA sequencing of whole communities. The approach is currently limited to prokaryotic communities and communities of few known species with sequenced genomes, mainly due to unavailable reference databases to infer the community composition for microeukaryotes.

In this study, we focus on the development of a tool (TaxMapper) for a reliable mapping to a compiled microeukaryotic reference database, reflecting the diversity within all main lineage of the eukaryotic tree of life, and a comprehensive analysis workflow. TaxMapper is used to assign taxonomic information to each read by mapping to the database and filtering of low quality assignment by classification using logistic regression. The complete metatranscriptome workflow effectively removes sequencing adapters and low-quality bases to assign cleaned mRNA reads to genes and functions and taxonomic origins. Subsequently the abundances can be analysed statistically also including environmental data. A Snakemake workflow is provided to empower researchers to easily apply and adapt it to metatranscriptome analyses of any environmental sample.

The methodology was applied to analyze taxon diversity and functional diversity in 21 mainland European freshwater lakes from the Sierra Nevada (Spain) to the Carpathian Mountains (Romania) and from northern Germany to the Apennines (Italy) covering altitudinal ranges from 38 m to 3110 m above sea level. Whereas taxon composition varied considerably between lakes, the relative importance of distinct metabolic pathways was much more stable, indicating that ecosystem functioning is buffered against shifts in community composition through a functional redundancy of taxa.

Keywords: metatransciptome, workflow, freshwater ecosystems, taxon diversity, functional diversity

A molecular survey of genus *Nebela s. str.* diversity, ecology and geographical distribution

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Molecular techniques are revealing a considerable cryptic diversity in many protist groups. These organisms, which are morphologically similar but genetically distinct, have been traditionally pooled together within single species, leading to erroneous diversity estimates, and wrong conclusions on their ecology (e.g. habitat specificity) and geographical distributions. The arcellinid genus *Nebela s. str.* is a typical example of cryptic diversity in protists: more than six genetically different species have been reported from peat bogs in the Swiss Jura Mountains, while only two species were originally described. These species differ in subtle morphological details, but also with respect to their ecological requirements. We surveyed a broad range of potentially favourable environments in order to (1) find more species within this group and (2) determine the respective influences of geographical distance and environmental conditions in generating this diversity. We extracted environmental DNA obtained from *Sphagnum*, forest moss and litter samples from Europe, North and South America, performed genus-specific PCRs on the mitochondrial marker COI (cytochrome oxidase first subunit) and cloned and sequenced the products. Phylogenetic analysis of these data and previously sequenced taxa revealed the existence of several new species in different regions of the world, but also in other environments. Moreover, our phylogenetic tree showed the existence of four clades separated not by continent but rather by ecosystem: *Sphagnum*-dominated peat bog (strongly N-limited), peat bog margins (less N-limited), rainforest and Atlantic (oceanic) peatlands. Our study suggests therefore that environmental conditions (climate and soil organic matter characteristics) are the primary drivers of diversity within *Nebela s. str.* while geographical distance plays a secondary role. Further sampling aiming at obtaining a more balanced ecological and geographical coverage will be needed to obtain a clearer overview of the diversity within this genus and what factors determine it. We welcome possible collaboration to reach this goal!

Keywords: Hyalospheniidae, testate amoeba, taxonomy, phylogeny, biogeography

Seasonal succession of planktonic eu-and prokaryotic communities in three different lakes

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The diversity of freshwater communities is enormous in terms of protists and bacterioplankton species. Aquatic community structures are influenced by factors like habitat size, geographic distance or lake chemistry. In addition, they undergo constant changes due to seasonality. Comparisons of chemical and morphometrically similar lakes often show the same seasonal fluctuations in terms of plankton succession, at least on the level of higher taxonomic levels. So far, such studies are often based on morphological observations and monitoring campaigns. In the present study, amplicon sequencing of the eukaryotic V9 and prokaryotic V4 region was used to study the seasonal succession of bacterioplankton and protists communities in three Austrian lakes, Lake Fuschlsee, Wallersee and Augstsee. Cluster analyses based on OTU composition group together samples according to their originating lake followed by a separation into “spring”, “summer” and “autumn” groups. Showing, that each lake has a unique signature based on OTU composition which is more pronounced then seasonal shifts between lakes. We compared the pro- and eukaryotic datasets via Co-Inertia analyses. The comparison shows, that the clustering of pro- and eukaryotic samples is consistent. The shifts in eukaryotic communities and bacterioplankton occur at the same time and scale.

Distribution pattern and functional differentiation of protists and protistan communities on a European scale

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Using amplicon diversity and metatranscriptome analyses, we analyzed protistan freshwater communities in 280 mainland European freshwater lakes ranging from the Sierra Nevada (Spain) to the Carpathian Mountains (Romania) and from southern Scandinavia to the Apennines (Italy) covering an altitudinal range from 38 m above sea level (a.s.l) to 3110 m a.s.l. The dominant eukaryotic taxa were green algae, ciliates, diatoms and chrysophytes. Dominant bacterial taxa were Actinobacteria, Alphaproteobacteria, Bacteroidetes, Betaproteobacteria, Cyanobacteria and Verrucomicrobia. Our analyses indicate that historical factors as well as environmental factors structure protist community composition. However, the presence of distinct species and groups of organisms does not necessarily reflect their effective functional role in their respective ecosystems. Nevertheless, microbial community composition is a good first proxy for the analysis of ecosystem functions. However, whereas taxon composition varies considerably between lakes, the relative importance of distinct metabolic pathways is much more stable, indicating that ecosystem functioning is buffered against shifts in community composition through a functional redundancy of taxa.

Keywords: amplicon diversity, metatranscriptomics, molecular diversity, biogeography

A framework for theory evaluation in historical sciences – the case of Archezoa

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What explains the rejection of hypotheses? I assume these philosophical questions are of wide interest for scientists. In the context of historical sciences, this requires an exploration of the inferential link between a set of *traces* collected in the present and a set of *claims* that are made about the past.

There are many ways in which claims about the past can be reinforced or undermined. Examples include the finding of new evidence, changes in our conceptual understanding, but also improvements in our understanding of related evolutionary events. In this talk, I propose a framework that captures how these changes can impact on the strength of hypotheses. I will present and explain a simple but complete terminology of *warrant, backing*, *rebuttal* and *qualifiers* used for this task.

I will then apply this framework to the case of the 'Archezoa hypothesis': an initially supported, and then collectively rejected hypothesis arguing for the past and present existence of a set of primitively amitochondrial eukaryotes. By capturing the subtle interplay of theories and evidence that can lead to hypothesis rejection, I believe this conceptual framework can be useful for the everyday practice of evolutionary biologists confronted with the hard task of evaluating or defending competing hypotheses about the past.

Keywords: evolutionary biology, philosophy of science, Archezoa, origin of eukaryotes

Replaying the tape: genome evolution in multiple origins of intracellular symbionts

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Endosymbiosis plays an important role in both ecology and evolution, but fundamental aspects of the origin of intracellular symbionts remain unclear. The extreme age of many relationships, lack of data on free-living ancestors, and uniqueness of each event hinder investigations. However, the symbiosis between the bacterium *Polynucleobacter* and the ciliate *Euplotes* may constitute an exception to all three rules. The bacteria evolved independently and under similar conditions from closely-related, free-living ancestors to become obligate endosymbionts of closely-related hosts: as close to "replaying the tape of evolution" as a natural system can be. The origin of the relationship appears to be much more recent than in most well-known symbiotic systems, and probably involved repeated symbiont replacements. We have sequenced the complete genomes of multiple *Polynucleobacter* strains, reduced in parallel from similar starting states, to provide unique glimpses into the mechanisms underlying genome reduction. We find gene loss is contingently lineage-specific, with no evidence for an ordered streamlining. However, some genes in otherwise disrupted pathways are retained, possibly reflecting cryptic genetic network complexity. We also measured substitution rates between many endosymbiotic and free-living pairs for hundreds of genes, showing that genetic drift, and not mutation pressure, is the main non-selective factor driving molecular evolution in endosymbionts. The unique features of this system and the amount of data now available make the *Polynucleobacter-Euplotes* symbiosis an excellent model for symbiosis research.

Keywords: ciliates, symbiosis, genome reduction

Pathogen-related and *Paramecium*-specific genes are preferentially upregulated during early stages of infection of *Paramecium caudatum* by *Holospora undulata*

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Many strains of *Paramecium caudatum* are susceptible to infection by the *Rickettsial*-like endosymbiont *Holospora undulata*. Susceptible strains of *Paramecium* are infected when a *H. undulata* infectious form is ingested through phagocytosis, breaks out of the maturing food vacuole, and then makes its way specifically to take up residence in the micronucleus of the cell.

Interestingly, resistant strains of *P. caudatum* exist, including some that appear to block infection very early, indicating that there are genetic differences between strains that control infection. With the larger goal of uncovering the genetic factors that determine host susceptibility and resistance in this interaction, we set out to determine specific genes that are upregulated very early in infection in a susceptible host *P. caudatum* strain. We tracked early infection of a naive K8 strain of *P. caudatum* microscopically, extracted mRNA from the host, and conducted whole-genome RNA sequencing on the host mRNA at early timepoints. We found that a small number of genes were upregulated at 10 and 30 minutes post-inoculation, and that the earliest-responding genes are much more likely to be *Paramecium*-specific. This is consistent with faster-evolving *Paramecium*-specific proteins involved in the initial signaling and trafficking events associated with infection. Intriguingly, a few genes of interest that have emerged include homologs to pathogen- and defense-related genes in other species, indicating that conserved genes as well may be involved in the infection process.

Keywords: *Paramecium*, *Holospora*, endosymbiont, whole-genome, RNAseq

Between a pod and a hard test: the deep evolution of Amoebozoa

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Amoebozoa is the eukaryotic supergroup sister to Obazoa, which is a lineage that contains the animals and Fungi, as well as their protistan relatives, and the breviate and apusomonad flagellates. Amoebozoa is extraordinarily diverse, encompassing important model organisms and significant pathogens. Although amoebozoans are integral to global nutrient cycles and present in nearly all environments, they remain vastly understudied. We present a robust phylogeny of Amoebozoa based on broad representative set of taxa in a phylogenomic framework (325 genes). By sampling 61 taxa using culture-based and single-cell transcriptomics, our analyses show three major clades of Amoebozoa. This phylogeny refutes previous studies in major respects. Our results support the hypothesis that the last common ancestor of Amoebozoa was sexual and flagellated, it also may have had the ability to disperse propagules from a sporocarp-type fruiting body. Overall, the main macroevolutionary patterns in Amoebozoa appear to result from the parallel losses of homologous characters of a multiphase life cycle (flagella, sex, and sporocarps) rather than independent acquisition of convergent features.

Keywords: phylogenomics, transcriptomes, reductive evolution, phylotranscriptomics, amoeba

Seasonality in ciliate communities characterized by morphotype and genotype

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Ciliates play important roles in marine food webs, but many aspects of their diversity and variations in community structure remain unexplored. While morphology is a standard method for identifying many ciliates, DNA sequencing has provided new insight into diversity, predator-prey interactions and discrepancies between morphologically defined species and genotypes. A 10-year time series at the Martha's Vineyard Coastal Observatory (MVCO) provides a unique opportunity to explore ciliate genotype and morphotype variations over time. At MVCO, an autonomous, submersible imaging-in-flow cytometer, Imaging FlowCytobot (IFCB), allows for the observation and morphological identification of live herbivorous ciliates (chlorophyll-containing) in situ without the need for culture or preservation. We also used high-throughput sequencing (HTS) of water samples collected approximately monthly over 2.5 years to explore the genetic seasonal community change of the class Spirotrichea, including both chlorophyll-containing and non-chlorophyll-containing taxa. In particular, tintinnids, which have distinct lorica characteristics, allowed a direct comparison between IFCB and HTS detection in this study. IFCB image concentrations of the total tintinnid population exhibited repeated elevations in the spring and fall, but at higher taxonomic resolution, tintinnid groups exhibited distinct seasonal patterns and fine structuring of niches; particularly for size classes of the genus *Tintinnopsis*. We report on species and genera of ciliates for which morphotype and genotype displayed high congruency (i.e. *Stenosemella pacifica., Tintinnopsis* spp. size classes, *Eutintinnus* spp.). In comparing how well temporal aspects of genotypes and morphotypes correspond, we found that HTS was critical to detect and identify tintinnid genera not efficiently captured with the IFCB, thus allowing further understanding into the seasonality of these ciliates at MVCO. We further showed that when these types of analyses are combined with IFCB results, they can provide hypotheses about food preferences.

Keywords: tintinnids, automated imaging, high-throughput sequencing, time series, seasonality

To eat or absorb? Predicting phagocytosis from genomes

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Phagocytosis, the active internalization of particles, is a eukaryotic hallmark, and is often considered a pivotal process in the origins of eukaryotic cells. It is inferred to have already been present in the Last Eukaryotic Common Ancestor (LECA). Recent evidence from new archaeal groups suggests that the archaea have some of the proteins and complexes used in phagocytosis, previously thought to only occur in eukaryotes, raising the possibility that some archaea also internalize particles. Core proteins in phagocytosis, are however, poorly defined. Using the genomes of a diverse set of organisms that complete phagocytosis, from mammals to green algae, we found 940 proteins enriched among phagocyte genomes that are relatively depleted among non-phagocyte genomes. We grouped those proteins by function and derived a predictive model of whether an organism completes phagocytosis based on the information in its genome. The model was applied to members of the Asgard archaea, a group of uncultured microorganisms that show close affinity to the eukaryotes, and they were shown to look like typical, non-phagocytotic prokaryotes based on the proteins in the model. A subset of the proteins enriched among phagocytes show affinity to bacteria, particularly those proteins involved in calcium signaling, important effectors during phagocytosis. The results suggest that the process of phagocytosis was derived from both archaeal and bacterial contributions, along with a set of eukaryote innovations, all of which are present in the LECA.

The cytoskeleton architecture of algivorous protoplast feeders (Viridiraptoridae, Rhizaria) in free-living and trophic states

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Several distantly related, phagotrophic microeukaryotes share the remarkable ability to perforate foreign cell walls in a well-defined pattern to acquire protoplast material as food. The underlying cellular processes, especially the local application of cell wall degrading agents, are still unexplored. We examined the distribution of F-actin and alpha-tubulin in the algivorous, viridiraptorid amoeboflagellates *Orciraptor agilis* and *Viridiraptor invadens* over their life histories using phalloidin conjugates and immunolocalization. During attack, both species form distinctive, F-actin-rich structures at the contact zone to the algal prey cell, which exactly match the species-specific cell wall perforations and resemble invadopodia and podosome rosettes of mammalian cells to a certain extent. Furthermore, F-actin is involved in the extraction of plastid material by *Orciraptor* and in prey cell invasion by *Viridiraptor* (here, F-actin localizes to a characteristic hyaline channel, which surrounds the streaming cytoplasm). The digestive-reproductive stages of viridiraptorids display a highly ordered microtubular cytoskeleton, whereas distinct phalloidin-positive actin structures could not be detected.

Evolution of metabolic capabilities in Euglenozoa revealed by a comparative transcriptomic analysis of diplonemids, kinetoplastids and euglenids

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Euglenozoa (Excavata) is a diverse group of protists incorporating i/ kinetoplastids, widely known as important pathogens of humans, livestock and plants; ii/ mostly photosynthetic euglenids, and iii/ understudied heterotrophic flagellates called diplonemids. Diplonemids are abundant in the deep ocean and, surprisingly, represent the most speciose clade of pelagic eukaryotes. We sequenced the transcriptomes of *Hemistasia phaeocysticola*, a recently isolated diplonemid that feeds on diatoms and dinoflagellates, and *Trypanoplasma borreli*, a haematozoic endoparasite of fish. *De novo* transcriptome assemblies were generated for *H. phaeocysticola*, *T. borreli, Diplonema papillatum*, *Rhabdomonas costata*, *Euglena gracilis*, *Neobodo designis*, *Bodo saltans* and two trypanosomatid species, *Trypanosoma brucei* and *Leishmania major*, using the Trinity transcriptome assembler. The transcriptome sequences were processed through the following pipeline: prediction of coding regions with Transdecoder; automatic annotation of the predicted coding regions using BlastKoala; reconstruction of metabolic pathways with KEGG mapper. A comparison of active metabolic pathways in diplonemids and other euglenozoans may help to decipher the biology of diplonemids, which represent a significant component of the marine plankton, and yet are virtually not studied.

Our preliminary analyses indicate that diplonemids and euglenids are metabolically more versatile than free-living kinetoplastids. Several important metabolic pathways, including branched and aromatic amino acid biosynthesis, folate and purine biosynthesis, were apparently lost in a lineage leading to the free-living *B. saltans* and parasitic trypanosomatids. Additionally, we investigated the evolution of the kinetochore system and DNA replication origin recognition complex in Euglenozoa.

Percolozoan revisited with newly discovered species from marine and saline inland waters

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Percolomonas cosmopolitus is a free-living flagellate, widespread in surface coastal and oceanic waters, placed within the Heterolobosea based on ultrastructure studies. We sequenced 18S rDNA from different *Percolomonas* species found in marine and saline inland waters, to clarify their evolutionary position among known reference taxa. Our analyses demonstrated that our 5 newly found species branch between two different tetraflagellated lineages, both named "*Percolomonas cosmopolitus*". The long branch-length that separates the two sequences of *P. cosmopolitus* also indicates that they represent two very different lineages. Based on paraphyletic association to morphologically described species and the morphological as well as molecular knowledge we added to expand this tree, we propose that the name of one of the two *P. cosmopolitus* sequences should be changed to a new genus. This study aims to describe new species of *Percolomonas*, and clarify the taxonomy of existing data. We add molecular reference data which resolves the taxonomy based on both morphological and molecular data.

Multidipliscinary investigations on lethal epibiotic bacteria hosted by the ciliate *Paramecium primaurelia*

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A novel epibiotic bacterium was previously retrieved in association to a newly isolated strain of the ciliate *Paramecium primaurelia*. Such finding represented the first record of epibiotic bacteria for the genus *Paramecium.* Several investigations were conducted, including live observations, electron microscopy, molecular characterisation, and FISH with a species-specific probe. The epibionts were found to cover partially or completely the host cells, forming a dense layer. The overall observed impact on the host was dramatic. On the host surface areas where the epibionts were present the ciliature was highly reduced or even absent, and some alterations were found in the alveoli. Moreover, infected host cells were smaller and eventually died, possibly due to impaired feeding behaviour. The spread of the infection within the host strain highly compromised and finally prevented its long-term laboratory maintenance. The infectious capability of the epibiotic bacteria was confirmed also in specific experiments, in which they were able to colonise both aposymbiotic cells derived from the original host strain and other strains of the *Paramecium aurelia* group. Newly infected cells displayed the same symptoms as in natural host cells. The analysis based on the 16S rRNA gene sequence indicated a phylogenetic relationship with members of *Rickettsiales* (*Alphaproteobacteria*), an order of obligately intracellular bacteria, living in association with various multicellular and unicellular eukaryotic hosts, including ciliates. Within *Rickettsiales*, a number of human and vertebrate pathogens are known (e.g. *Rickettsia prowazekii*, *Ehrlichia chaffeensis*), although in several other cases the host-bacterium interactions were not determined precisely. In order to investigate more clearly the novel epibiont, in particular its phylogenetic origin and its metabolic and physiologic interactions with the host, in comparison with other symbiotic and free-living *Alphaproteobacteria*, we sequenced its complete genome (1205153 bp; 32.9 GC%). Genome analyses are currently on the way.

Molecular phylogenetic reconstructions suggest that the "helmet-shape" body in Entodiniomorphida (Litostomatea, Trichostomatia) do not reflect evolutionary divergence

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The genus *Triadinium* was described by Fiorentini to include *Triadinium caudatum*, entodiniomorphid ciliate with "helmet-shape" body. Four other species, with the same body shape, were described posteriorly: *T. minimum*, *T. galea*, *T. elongatum* and *T. magnum*. However, according to Wolska and Grain the classification of *T. minimum* and *T. galea* in *Triadinium* was realized based only in superficial aspects of morphology. In this way, the authors propose two new genera to include them: *Circodinium* and *Gassovskiella*, respectively. Although there are some recent molecular studies that investigate the phylogenetic relationships in order Entodiniomorphida, in none of them it is discussed whether the "helmet-shape" body in entodiniomorphid ciliates reflect evolutionary divergence. The present study discusses this issue using molecular phylogenetic analyses based on 18S rDNA sequences. According to our phylogenetic reconstructions the "helmet-shape" body in entodiniomorphid ciliates is a homoplastic character and do not reflect evolutionary divergence, since ciliates with "helmet-shape" body do not constitute a monophyletic group. Wolska described the infraciliature and the ultrastructure of *Circodinium minimum* and concluded that this species is morphologically similar to species belonging to the family Blepharocorythidae and classified it into this family. This hypothesis was recovered in our analyses, since the species *C. minimum* emerged in a monophyletic clade with *Blepharocorys* spp. and *Ochoterenaia appendiculata* (Blepharocorythidae) (99 ML/ 1.0 BI). Additionally, Wolska and Grain described the infraciliary pattern and ultrastructure of *T. caudatum* and *G. galea* and pointed out great similarity to Spirodiniidae species. In our analyses, *T. minimum* was grouped with spirodiniid ciliates (72 ML/ 1.0 BI), supporting Wolska's hypothesis. *G. galea*, however, contradicting Grain's hypothesis, was positioned as sister-group of *Polydiniella mysorea* (Polydiniellidae) (80 ML/0.85 BI). Data on infraciliature and ultrastructure of *P. mysorea* will be indispensable for better understand this issue.

Was the chlamydial adaptative strategy to tryptophan starvation an early determinant of plastid endosymbiosis?

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Chlamydiales were recently proposed to have sheltered the future cyanobacterial ancestor of plastids in a common inclusion. The intracellular pathogens are thought to have donated those critical transporters that triggered the efflux of photosynthetic carbon and the consequent onset of symbiosis. Chlamydiales are also suspected to have encoded glycogen metabolism TTS (Type Three Secretion) effectors responsible for photosynthetic carbon assimilation in the eukaryotic cytosol. We now turn our attention to the reasons underlying other chlamydial lateral gene transfers evidenced in the descendants of plastid endosymbiosis. In particular, we show that half of the genes encoding enzymes of tryptophan synthesis in Archaeplastida are of chlamydial origin. Tryptophan concentration is an essential cue triggering two alternative modes of replication in Chlamydiales. In addition, sophisticated tryptophan starvation mechanisms are known to act as antibacterial defenses in animal hosts. We propose that Chlamydiales have donated their tryptophan operon to the emerging plastid to ensure increased synthesis of tryptophan by the plastid ancestor. This would have allowed massive expression of the tryptophan rich chlamydial transporters responsible for symbiosis. It would also have allowed possible export of this valuable amino acid in the inclusion of the tryptophan hungry pathogens. Free-living single cell cyanobacteria are devoid of proteins able to transport this amino acid. We therefore investigated the phylogeny of the Tyr/Trp transporters homologous to *E. coli* TyrP/Mre and found yet another LGT from Chlamydiales to Archaeplastida thereby considerably strengthening our proposal.

Keywords: Chlamydiales, tryptophan, plastid

Perkinsela sp., the amoeba-dwelling kinetoplastid endosymbiont

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Secondary endosymbiosis is a phenomenon commonly associated with plastids, which typically involves extensive reduction of the eukaryotic symbiont. The example of *Paramoeba/Neoparamoeba* (Amoebozoa, Dactylopodida) demonstrates that endosymbiosis between two eukaryotic cells is not restricted to photosynthesizing organisms, as it harbors a heterotrophic protist, *Perkinsela* sp. (Excavata, Kinetoplastida). We have investigated *Paramoeba pemaquidensis* and *Paramoeba invadens* by microscopy and comparative genomics, to investigate the impact of endosymbiosis on originally independent heterotrophic organisms with parasitic tendencies. Our results show that the obligate perinuclear endosymbiont *Perkinsela* has lost several cellular and metabolic features such as flagella and complex I of electron transport chain, reflected in reduction of cytoplasmic volume, nucleus and mitochondrially encoded proteins. The endosymbiont is dominated by an enormous DNA-rich mitochondrion, unlike secondarily derived plastids, and rich vesicular trafficking including predicted glycosome. Endosymbiotic gene transfer in this system appears to be minimal, underlining the contributions of *Perkinsela* sp. and its host to our understanding of endosymbiosis and the biology of eukaryotic cells.

Keywords: *Perkinsela*, *Paramoeba*, endosymbiosis, mitochondria, evolution

Cytoskeletal elements of two morphologically distinct coelomic eugregarines *Urospora travisiae* and *Urospora ovalis* from marine polychaete *Travisia forbesii*

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The genus *Urospora* unites eugregarines of considerable morphological plasticity, inhabiting body cavity and tissues of a wide range of marine and freshwater invertebrates. Only few members were investigated from ultrastructural and molecular phylogenetic viewpoint. Combining electron and confocal laser scanning microscopy, here we present a morphological study of *Urospora travisiae* and *Urospora ovalis*.

The trophozoites of *U. travisiae* are of V-like shape with two narrowing branches converging at a point with attachment organelle. Detached trophozoites exhibit a gliding motility. Their pellicle forms longitudinal epicytic folds (EF) along the axis of each branch. In contrast, solitary ovoid trophozoites of *U. ovalis* float freely in the coelom and exhibit metabolic activity. Their surface is covered with EF; additional longitudinal superfolds bearing a number of EF form in the region of contractions during metaboly.

The phalloidin labelling showed that the distribution of filamentous actin fits to the pattern of EF. In *U. travisiae*, tiny filaments of actin lay superficially and parallel to each other along the longitudinal axis of the branch. In solitary *U. ovalis*, individual filaments are not clearly seen, however, general pattern of their distribution corresponds to that in *U. travisiae*. The α-tubulin immunolabeling showed that subpellicular microtubules in *U. travisiae* and *U. ovalis* distribute circumferentially along the cell axis, with abundant cytoplasmic clusters. Phalloidin staining of *U. ovalis* syzygy demonstrated that filamentous actin distributes in bands along with superfolds, and individual longitudinal filaments pass along EF that run in-between superfolds. The microtubules in *U. ovalis* syzygy orient not circumferentially as in solitary trophozoites, but along longitudinal cell axis and repeat the pattern of the superfolds organisation. Numerous clusters of α-tubulin can be also seen in cytoplasm. Despite considerable differences in their morphology and motility mode, both species show similarities in cytoskeleton organisation.

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Keywords: cytoskeleton, F-actin, tubulin, *Urospora* sp., confocal laser scanning microscopy

Recent contributions to the morphology, ecology and molecular phylogeny of peritrichs (Ciliophora, Peritrichia) from Brazil

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The peritrichs are classified into two assemblages: the free-living Sessilida and the parasitic Mobilida. Although a comprehensive recent review is not available, these ciliates comprise at least 800 described species. Peritrichs are commonly found in freshwater and marine ecosystems, colonizing living or non-living substrates, playing an important role in the control of bacterial populations. Recently, molecular information from the 18S-rDNA sequences, has been used to re-evaluate phylogenetic relationships among peritrichs, however, these molecular analyses have yielded different results from morphologically-based taxonomic studies. In this work, we studied the morphology, ecology, and 18S-rDNA phylogeny of some peritrich ciliates from marine (plankton), freshwater (streams and bromeliads) and terrestrial (lichens) Brazilian ecosystems. The morphological study of twenty nine species of peritrich ciliates was carried out using *in vivo*, protargol-stained and SEM observations, with six new species described (*Epistylis* sp. nov., *Lagenophrys* sp. nov., *Paravorticella* sp. nov., *Pseudovorticella* sp. nov., *Rhabdostyla* sp. nov. 1 and *Rhabdostyla* sp. nov. 2). We analyzed the influence of organic pollution on the composition and structure of peritrichs community from tributary streams of a river in southeast Brazil, which receive variable levels of domestic sewage (oligotrophic, mesotrophic and eutrophic). The results showed that changes in the water quality produced quantitative and qualitative changes in the structure of the peritrichs community. To shed more light into the evolutionary relationships within peritrichs, 18S-rDNA sequences of nineteen sessilids species were used to construct phylogenetic trees. Our results show that (1) the alpha-taxonomic information on peritrichs is expected to improve the knowledge about geographic distribution and diversity of these ciliates in the neotropics; (2) peritrich ciliates respond to gradients of organic pollution and are useful bioindicators of water quality in neotropical streams; (3) and many morphological characters used to define the families that compose the subclass Peritrichia do not reflect evolutionary divergence.

Keywords: Peritrichia, Neotropical area, taxonomy, ecology, 18S-rDNA phylogeny

Differential binding of mitochondrial transcripts by MRB8170 and MRB4160 regulates distinct RNA processing fates in trypanosomes

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A dozen mRNAs are edited by multiple insertions and/or deletions of uridine residues in the mitochondrion of *Trypanosoma brucei*. Several protein complexes have been implicated to perform this type of RNA editing, including the mitochondrial RNA binding complex 1 (MRB1). Two paralogous novel RNA-binding proteins, MRB8170 and MRB4160 are loosely associated with the core MRB1 complex. Their roles in RNA editing and effects on target mRNAs are so far not well understood. In this study, individual-nucleotide resolution UV-crosslinking and affinity purification (iCLAP) revealed a preferential binding of both proteins to mitochondrial mRNAs, which was positively correlated with their extent of editing. Integrating additional *in vivo* and *in vitro* data, we propose that binding of MRB8170 and/or MRB4160 onto pre-mRNA marks them for the initiation of editing and that initial binding of both proteins may facilitate the recruitment of other components of the RNA editing/processing machinery to ensure efficient editing. Surprisingly, MRB8170 also binds never-edited mRNAs, suggesting that at least this paralog has an additional role outside of RNA editing to shape the mitochondrial transcriptome.

Keywords: RNA editing, RNA biology

Protists in the deep dark sea

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Sampling at a deep water site in the N. W. Mediterranean was employed to examine the composition and variability of protistan microzooplankton in the mesopelagic through periods of different water column structure (stratified -> mixed -> stratification). The focus was on tintinind ciliates and phaeogromid radiolarians (challengerids) as species identifications can be made based on gross morphology. The working hypothesis was that deep water populations would increase with decreasing stratification and species composition would remain stable. At weekly intervals large volumes of water from 250 m depth in the mesopelagic and 30 m depth, in the surface layer for comparison, were filtered through a 20 µm net; small aliquots of Lugol's-fixed net material were examined with an inverted microscope. Distinct assemblages of species were found in the mesopelagic and surface layer. Under stratified conditions the mesopelagic populations were dominated by forms found largely only in deep waters and some taxa found appear to be new to science. As the water column shifted to mixis, surface water forms were found in the deep waters and deep water forms decreased in relative and absolute abundances. Nonetheless the forms dominant in the surface layer differed from those dominant at 250 m. With stratification the deep water forms again dominated the population at depth. Contradicting the working hypothesis, during the period of deep water formation in the Mediterranean (winter mixis) the mesopelagic appears to be invaded by certain surface water taxa.

Keywords: tintinnid ciliates, phaeodarian radiolarian, plankton, microzooplankton, mesopelagic

Biogenesis of mitochondrial organelles in anaerobic protists

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Anaerobic protists carry whole range of metabolically streamlined mitochondrial forms sometimes classified as hydrogenosomes and mitosomes. The metabolic adaptation has been accompanied by customization of the pathways controlling the organelle biogenesis such as the protein transport, organelle division and their overall dynamics. We have studied these processes in the mitosomes of *Giardia intestinalis* and have shown that in addition to the minimalist protein import machinery, the mitosomes have harnessed their dynamics to the cell- and life-cycle of the parasite. It will be discussed whether these characteristics represent ancient or derived traits of the eukaryotic organisms.

Keywords: mitochondria, mitosome, dynamics, protein import, mitochondrial division

Chimeric origins of ochrophytes and haptophytes revealed through an ancient plastid proteome

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Plastids are supported by a wide range of proteins encoded within the nucleus, that are imported from the cytoplasm. These plastid-targeted proteins may originate from the endosymbiont, the host, or other sources entirely. Here, we identify and characterise 770 plastid-targeted proteins that are conserved across the ochrophytes, a major group of algae including diatoms, pelagophytes and kelps, that possess plastids derived from red algae. We show that the ancestral ochrophyte plastid proteome was an evolutionary chimera, with 25% of its phylogenetically tractable proteins deriving from green algae. We additionally show that functional mixing of host and plastid proteomes, such as through dual targeting, is an ancestral feature of plastid evolution. Finally, we detect a clear phylogenetic signal from one ochrophyte subgroup, the lineage containing pelagophytes and dictyochophytes, in plastid-targeted proteins from another major algal lineage, the haptophytes. This may represent a possible serial endosymbiosis event deep in eukaryotic evolutionary history.

Keywords: chloroplast, MMETSP, endosymbiosis, shopping bag hypothesis, protein import

A new species of folliculinid bearing endosymbiotic dinoflagellates

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A previously undescribed folliculinid has been found in the temperate near shore coastal sea-grass beds of the Gulf Saint Vincent South Australia. It contains endosymbiotic dinoflagellates. Such dinoflagellates are found in coral hydroids, in Foraminifera, Radiolaria and heterotrich ciliates. This is the first record of a *Symbiodinium*-like organism being found in the Folliculinidae. The trophont of the folliculinid contains between 500–600 dinoflagellate cells and 150–300 in its swarmers. Mulisch (1987) suggested that the Folliculinidae evolved from the ancestors of recent Stentoridae (i.e. *Stentor*). More recent DNA studies propose that *Stentor* spp. are more closely related to *Blepharisma*, while it is *Maristentor* that is closest to folliculinids. Folliculinids are almost exclusively marine, as is *Maristentor dinoferus,* both tend to cluster and both have pigmented granules in the cortex. The folliculinid was collected from a number of sites and was always attached to *Zostera* or *Amphibolis* sea-grass. Collections of sea-grass with the ciliates were placed in dishes of filtered sea water to remove any planktonic dinoflagellates. Two generations of trophonts and swarmers were cultured, all contained symbionts. This paper presents the results of detailed taxonomic and genomic studies of this new species, and explores the phylogenetic connections between folliculinid protists, coral hydroids and endosymbiotic dinoflagellates.

Keywords: *Eufolliculina symbiodinia*, *Symbiodinium*, new species of Folliculinidae

The phenotypic masquerade in centrohelid heliozoan *Raphidiophrys heterophryoidea*

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Centrohelids are protists usually having a peculiar cell coverings consisting of organic spicules, siliceous scales or their combination. Siliceous scales have a complex morphology and are broadly used for species identification. However, our studies involving establishing and regular observation of cultures have shown that cells of one and the same clone can have considerably different scale morphology. Particularly, *Raphidiophrys heterophryoidea*, initially described as a species combining organic and siliceous elements, which is itself a unique feature among centrohelids, demonstrate a complex life cycle with an alteration of different morphologies. Firstly, it produces another type of scales when forming a cyst; after excystment they persist on the cell surface, changing the cell appearance. Secondly, the spontaneously forming colonies were observed in cultures. Colonial cells were connected with cytoplasmic bridges, the whole colony sometimes produced the stalk, composed of several axopodia attached to the substratum and covered with scales. Colonial individuals dramatically increased in size in comparison to solitary cells and their scale morphology underwent considerable changes. Organic spicules were absent, while scales, surrounding the cells were different in form and size from those of solitary cells. Transitional forms combining both “individual” and ”colonial” scales were observed. Individuals with a contrasting morphology were shown to have identical 18S rDNA sequences, which confirmed their co-specificity. Finally, colonial cells are also capable of forming cysts with unique scale morphology. Thus, *R. heterophryoidea* can produce at least five types of skeletal elements in different combinations during its life cycle. As it was shown in other studies, centrohelids bearing organic spicules only (HLO, heterophrys-like organisms) can be found in different unrelated clades, basal members of which possess siliceous scales. Taking into account *R. heterophryoidea*, this phenomenon also can be explained by the presence of the complex life cycle.

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Keywords: ultrastructure, Heliozoa, Centrohelida, scales, life cycle

Where does the *Kraken* belong?

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In this talk, we highlight the question "Where does the *Kraken* belong?" under two different lights: Taxonomy and Dispersal.

The genus *Kraken* represents a morphologically unique lineage of filose amoebae within Cercozoa. Currently only one species, *Kraken carinae* of which light microscopy and molecular data was given, has been described. SSU phylogeny showed an affiliation with the Cercomonadida, branching with weak support at its base. In this talk, ultrastructure of *K. carinae* will be presented, this data conjoined with a concatenated SSU and LSU phylogeny was used to give more insight into *Kraken* taxonomy. The data confirmed the absence of flagella, but also showed novel characteristics, like the presence of extrusomes, osmiophilic bodies, mitochondria with flat cristae and, surprisingly, the presence of scales. The scales being single-tiered and oval, resemble much the expected scales of the last common ancestor of the class Imbricatea. The phylogenetic analyses however confirmed previous results, indicating *Kraken* as a sister group to *Paracercomonas* within the Sarcomonadea with an increased but still weak support of 0.98/63. Based on the unique features of the *Kraken* and contradicting results in morphology and phylogeny the *Kraken* remains *incertae sedis*, Cercozoa.

Furthermore we conducted (and reinvestigated) Illumina sequencing studies of soils and litter samples from Europe and South America to gain insight into the dispersal of the *Kraken*. In every screened site we were able to find up to two (mean) percent of total Cercozoa sequences representing *Kraken*-related OTUs with up to 100% similarity. The results indicate a high dispersal and ubiquitous occurrence of the *Kraken*, raising the question why it has been discovered only very recently.

Keywords: Sarcomonadea, Imbricatea, scales, naked amoebae, electron microscopy

Meiotic genes in colpodean ciliates support secretive sexuality

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Colpodean ciliates potentially pose a problem to macro-organismic theories of evolution: they are putatively asexual and extremely ancient, and yet there is one apparently derived sexual species. If macro-organismic theories of evolution also broadly apply to microbial eukaryotes, though, then most or all of the colpodean ciliates should merely be secretively sexual. Here we show using de novo genome sequencing, that colpodean ciliates have the meiotic genes required for sex and these genes are under functional constraint. Along with these genomic data, we argue that these ciliates are sexual given the cytological observations of both micronuclei and macronuclei within their cells, and the behavioral observations of brief fusions as if the cells were mating. The challenge that colpodean ciliates pose is therefore not to evolutionary theory, but to our ability to induce microbial eukaryotic sex in the laboratory.

Endosymbiosis, origins and gene expression in the photosynthetic protist *Euglena gracilis*

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The photosynthetic flagellate *Euglena gracili*s harbours a secondary endosymbiotic plastid and is a distant relative of pathogenic trypanosomatids, a major component of global aquatic ecosystems and of considerable biotechnological potential with resistance to harsh conditions. Here we report genome, transcriptome and proteome drafts for *E. gracilis*. The genome is over 2Gb and has a coding potential of 36,526 predicted ORFs. Less than 25% of the genome is single copy sequence, indicating extensive repeat elements. Several gene families likely associated with the cell surface and signal transduction possess very large numbers of lineage-specific paralogs, suggesting great flexibility in environmental monitoring and, together with divergent mechanisms for metabolic control, novel solutions to adaptation to extreme environments. There are clear contributions from photosynthetic eukaryotes to the nuclear genome with red, green and brown algal genes evident, together with orthogroups shared with only trypanosomes and also with other excavates. Furthermore, we demonstrate that the majority of control of protein expression level is post-transcriptional despite the presence of conventional introns, that mRNA metabolism is highly unusual in transcriptional and nuclear export mechanisms and which differentiate euglenids from the trypanosomatids. These data are a major advance in the understanding of the nuclear genome of euglenids and provide a platform for investigation of the contributions of *Euglena gracilis* and relatives to the biosphere.

Keywords: *Euglena*, genome assembly, genome architecture, splicing, secondary endosymbiosis

Meteora sporadica represents a new major lineage of eukaryotes

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Meteora sporadica is an extraordinary gliding nanoprotist. It is characterised by long anterior and posterior extensions that form its long axis, and (usually) two shorter lateral 'appendages' that swing through large arcs on either side of the cell. It was seen once from the deep sea by Hausmann et al. (2002), but no permanent culture was established nor molecular data ever obtained; consequently, its basic cell structure and phylogenetic affinities have been mysterious. We report two new cultivated isolates of *Meteora* from intertidal sediment in Cuba and port sediment in Japan. Electron microscopy demonstrates that the extensions and appendages are microtubule-supported, but neither of them are axonemes. This invites comparisons with axopodial amoebae in Rhizaria and Centrohelida (amongst others), and opens the door to understanding the cell-biological basis of the motility of the lateral appendages. SSU rDNA sequences do not resolve the phylogenetic position of *Meteora*, and we have sequenced transcriptomes for both strains, from which we recruited a phylogenomic gene dataset. The resulting phylogenomic analyses revealed that *Meteora* does not branch within any established major group of eukaryotes (and is not closely related to either Rhizaria or Centrohelida, for example), and instead represents a very deep-branching 'orphan' lineage. The phylogenetic position of *Meteora* is therefore of importance in resolving the tree of eukaryote life at the 'supergroup' level.

Keywords: heterotrophs, phylogenomics, ultrastructure, deep eukaryote phylogeny

A phylogenetically broad analysis of protist genomes unveils the ancestral eukaryotic complexity of the Ras superfamily of GTPases and novel aspects of eukaryotic cell biology

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The highly diversified Ras superfamily of GTPases is one of the central components of the molecular pathways underpinning the basic logistics in the eukaryotic cell. Different eukaryotic groups may differ substantially in the complexity of their complements of Ras superfamily paralogs due to lineage-specific duplications and losses, but it is clear this diversity stems from a certain number of ancestral paralogs that define the core cell biology of a prototypical eukaryotic cell. I have been engaged in a long-term project to reconstruct the evolutionary history of the Ras superfamily in eukaryotes, with a particular aim to define the actual set of paralogs that can be traced to the last eukaryotic common ancestor (LECA). The accumulation of genomic and transcriptomic data from phylogenetically diverse eukaryotes, particularly protists, has now enabled to draw a picture of the LECA's complement of Ras superfamily paralogs with an unprecedented accuracy. The LECA seems to have been endowed with up to around 60 different proteins of the Ras superfamily, which is a number substantially exceeding previous estimates. Whereas some of these ancestral paralogs have ever since remained an essential component of the eukaryotic cell, others have experienced more or less frequent losses. A notable category are paralogs correlated in their distribution with the capability of the organism to build a cilium. It includes not only well established cilium-associated GTPases, but also some paralogs hitherto lacking a clear functional assignment. Our analyses for the first time show wider taxonomic occurrence and apparent ancestral origin of some GTPases so far reported only from metazoans, and unveil novel, functionally uncharacterized ancestral paralogs with a sporadic distribution avoiding standard model organisms. These GTPases presumably indicate the existence of unknown functional pathways in the prototypical eukaryotic cell, making their study one of the priorities of evolutionary cell biology.

The archaeal roots of eukaryotes

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Woese and Fox' 1977 seminal paper on the discovery of Archaea lifted a revolution in evolutionary biology. It showed that life was not only divided in prokaryotic and eukaryotic cells, but that prokaryotes themselves actually comprised two very different types of organisms – Bacteria and Archaea. Soon after, it became evident that eukaryotes emerged either sister to or within Archaea. In fact, eukaryotic cells represent hybrid organisms: in addition to eukaryotic-specific complex cellular features, they exhibit a mixture of archaeal and bacterial ones. While it has been accepted for some time that mitochondrial organelles descend from endosymbiotic alphaproteobacteria, the exact nature of the evolutionary relationship between eukaryotes and Archaea has long been debated. Here, we propose a brief history of our understanding of the tree of life before focusing on the recent discovery of myriad archaeal lineages. Their exploration led to unprecedented insights into the relationships between the three domains of life, and into the origin of eukaryotes. We revisit the main questions encompassed in the quest for understanding the process of eukaryogenesis, from FECA (the first eukaryotic common ancestor) to LECA (the last eukaryotic common ancestor). Finally, we examine how studies of newly discovered archaeal lineages allowed us to tackle some of them, and what issues remain to be addressed.

Keywords: eukaryogenesis, Asgard Archaea, FECA, LECA

Identification and phylogenetics of Photosystem I subunits in *Chromera velia* and *Vitrella brassicaformis*

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The light-dependent reactions of photosynthesis take place in a dynamic system of protein complexes, including photosystems I and II (PSI and PSII), embedded in the thylakoid membrane. Although the overall structure and function of these complexes is conserved throughout photosynthetic organisms, their molecular dynamics differ between taxa and are influenced by environmental conditions. The subunits comprising the photosystems interact with light-harvesting pigments and other molecules to provide structural integrity and to ensure that electron transport (and, by extension, carbon fixation) is carried out properly. The coral symbiont, *Chromera velia*, possesses a plastid descended from a red alga and is able to maintain high levels of photosynthetic efficiency and carbon fixation. In an attempt to understand the structural and evolutionary basis for this efficiency, we queried the genomes of *Chromera* and its close relative, *Vitrella brassicaformis*, to identify photosystem subunits and compare them to those of other photosynthetic eukaryotes. Additionally, isolated PSI from *Chromera* and *Vitrella* were separated using SDS-PAGE and their components identified using mass spectrometry. Both *Chromera* and *Vitrella* lacked several PSI subunits present in other algae with red plastids, but possessed novel subunits with no apparent homologs. Moreover, in both organisms PSI was associated with one (*Vitrella*) or two (*Chromera*) iron superoxide dismutases (FeSODs), suggesting that the water-water cycle plays a major role in dealing with reactive oxygen species produced as by-products of electron transport; the precise evolutionary origin of these FeSODs, however, could not be determined. Fucoxanthin-chlorophyll-binding proteins (FCPs) were also associated with PSI in *Chromera* and *Vitrella*; phylogenetic analyses of light harvesting complex (LHC) proteins indicated that PSI-associated FCPs in *Chromera* were mostly related to PSI-associated LHCs in other (primary and secondary) red plastid lineages, whereas PSI-associated FCPs in *Vitrella* were recovered in a separate clade, suggesting divergent light harvesting strategies in the two algae.

Keywords: photosynthesis, *Chromera velia*, *Vitrella brassicaformis*, photosystem I

Transition boundaries for protistan plankton community turnover in hypersaline waters of different biogeographic regions

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Hypersaline habitats belong to the least understood ecosystems on our planet. Especially research about halophilic and halotolerant microbial eukaryotes (protists) lacks far behind the prokaryotic counterpart, although protists are key players in aquatic ecosystems. We, therefore, collected and analyzed protistan genetic signatures of 24 salt ponds with salinities between 4 and 44% of different geographic regions (Europe and South America). With a sequencing depth of 4.5 Mio. high-quality V4 fragments of the SSU rDNA, we reached sample saturation even in the sample with the lowest number of reads. Partitioning of diversity revealed a niche differentiation, suggesting three environmental transition boundaries for protistan species turnover. Regardless of their geographic origin, protistan communities in these four salinity classes had different taxonomic memberships and different degrees of diversity. Although salinity seemed to superpose the distance effect, our data provided evidence for biogeographic structuring. The strength of salinity versus biogeographic structuring emerged as a function of distance. Local protistan diversity appeared to be much lower than in previous intra-ecosystem studies suggesting a higher degree of endemism for salt ponds compared to less isolated ecosystem such as the open ocean. Our thus far unique sample collection enabled us to investigate basic concepts on the ecology of protistan communities thriving in high-salt environments. The results highlight several important gaps in our knowledge, such as salt tolerances of halophilic/halotolerant protists and their ecophysiology. Our findings pinpoint the necessity of (mechanistic) experiments, which reveal cellular strategies enabling some protists to cross salt transition boundaries and confining others within.

Keywords: protist, halophiles, eDNA, salinity, biogeography

Dispersal of ciliated protozoa: lessons from a 4-year-experiment with environmental micro-and mesocosms

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Still, there is a widespread believe that microscopic organisms have cosmopolitan distribution because they are easily spread by, e.g., air and water. Presently, two dispersal models are discussed, viz., cosmopolitanism vs. moderate endemicity (~ 1/3 of species). Current research clearly favours the moderate endemicity model ranging from bacteria to rotifers. However, the reasons for the restricted distribution remain unclear. Several have been discussed, e.g., dispersal by air, water, animals and low cyst weight. Thus, I performed a 4-year-experiment using a microcosm with 1.5 l water and two mesocosms with 6 and 12 litre water. Each container was investigated monthly. As the early data showed few species and considerable extinction, I performed two kinds of controls: centrifuged container water was used as culture medium for a variety of ciliates and a chemical water analysis at end of the experiment. Altogether, only 20 ciliate species were observed at the 25 sampling occasions: 14 in the 1.5 l microcosm, 11 in the 6 l, and 9 in 12 l mesocosms. Most of the species found are terricole or semiterricole, and all can produce resting cysts. The observations show repeated extinction and recolonization of the ciliate fauna. Air dispersal was dominant. As many cyanobacteria developed, I supposed that they produced substances restricting ciliate growth. However, this was disproved by the controls mentioned above. Dispersal by animals was also observed, viz., once I saw a raven cleaning a piece of bread in the 6 l container followed by a ciliate bloom. Masses of cyanobacteria, various algae, rotifers (*Phialina roseola*), and biting midges developed frequently; especially, the insect larvae fed on the accumulating mud destroying cyst reservoirs. Island biogeography suggests the larger the area the more species. The opposite occurred in my experiment.

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Keywords: diversity, dispersal, ciliates, microcosms

The genus *Frontonia* Ehrenberg, 1833. Unfinished story

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The genus *Frontonia* is one of the most numerous taxon in Peniculia and it is in urgent need of revision. In many descriptions and redescriptions made for frontiniids during last decades, unfortunately, some authors often ignored important morphological and biological characteristics such as number and type of micronuclei; structure of contractile vacuole and mode of ciliates rotation. Moreover, it happened that the old species descriptions have not been checked and compared carefully enough before generating novel species attributions. Subsequently some recently described *Frontonia* spp. to be synomyms of ciliates described before. One example is "*F. ocularis"*, which was described by Bullington (1939) from USA and redescribed from China (Pan et al., 2013), although it should be attributed to the European *F. fusca* (Quennerstedt, 1869; Kahl, 1931; Fokin, 2008). Another misidentification regarding frontoniids could be addressed to "*F. vernalis"*. This freshwater ciliate bearing *Chlorella*-like cytoplasmic symbionts (Berninget et al., 1986; Esteban et al., 2010) does not fit at all to the *F. vernalis* as it was described by Bullington (1939). In its own turn the description made by Bullington was obviously not fitted with previously known *F. vernalis* (Ehrenberg, 1838; Kahl, 1931). Apparently green frontoniids form a set of different species. The most distinctive morphological and biological features for species' discrimination are presently discussed, together with phylogenetical analysis of the genus. From our study the genus *Frontonia* turned out to be not monophyletic (Andrioli et al., 2007), then it was proved (Gao et al., 2008). The set of 19 species of the genus used for the analysis, formed three distinguishable groups which are composed namely by 1) *F. canadensis, F. salmastra, F. subtropica, F. magna, F. sinica, F. tchibisovae, F. mengi*; 2) *F. paramagna, "F. vernalis"* set*, F. leucas*; 3) *F. didieris, "F. ocularis"(F. fusca), F. elegans, F. pusilla* (and some other).

Keywords: *Frontonia*, biodiversity, misidentification, phylogenetical analysis

Novel protist diversity inferred from network analyses: a case study using colpodellids

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The order Colpodellida is as a sister group to the parasitic Perkinsidae and comprises free-living heterotrophic flagellates that prey on other protists. Our knowledge on colpodellid diversity is scarce at best. Ten different species have been described in the literature, molecular data exist for only four of them. In 2013, an environmental sequencing study reported colpodellids among the dominant eukaryotic organisms in a hypersaline lake in Australia, which was a first indication that their diversity had previously been underestimated.

In our approach, we screened V4 SSU rDNA environmental high-throughput sequencing datasets from various sampling events for other records of colpodellids. At 45 different sampling sites, which ranged in salinity from seawater (3,9%) to hypersaline habitats (45%), this search returned sequences whose closest taxonomical match was affiliated to the Colpodellida. For further analyses, we combined the molecular data of these sampling events with all other publicly available sequence data of colpodellids. Using the Swarm sequence clustering algorithm, we examined this dataset as a case study for evaluating swarm OTUs by means of network analyses with a special emphasis on finding novel diversity.

The environmental OTUs of our screening approach were strictly separated from all previously known colpodellid data in the swarm network. Thus, pointing towards a high degree of undescribed diversity of these organisms, most of which occurred at salinities between 29-38%. Besides laying the foundation for the targeted recovery of the underexplored colpodellid diversity, this study shows how network analyses may be successfully adapted to evaluate swarm clustering results.

Keywords: colpodellids, hypersaline habitats, novel diversity, network analyses, swarm clustering algorithm

Life cycles of apicomplexan-related lineages as adaptation to trophic strategies

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Current environmental sequence data suggest that core apicomplexans, represented by strict parasites, are nested within a plethora of lineages with various trophic strategies, i.e. parasitic, predatory, and even photoautotrophic. We have identified sexual behavior in an algal relative of apicomplexans, *Vitrella brassicaformis,* exhibiting a fusion of motile cells. In contrast, the alga *Chromera velia* does not seem to perform cell fusion in any stage of its life cycle. On the other hand, *C. velia* possesses an apical complex-like structure, possibly to invade corals, while *V. brassicaformis* lacks this apical structure. Based on life cycle complexity in apicomplexans (and dinoflagellates) we suppose that the common ancestor of *Vitrella*, *Chromera*, and the apicomplexans had a complex life cycle. Some of the extant species underwent a reduction of this cycle to better suit their trophic strategies. At the same time, a complex ancestral life cycle might have enabled apicomplexans to establish dixenous parasite-host interactions. In this talk we discuss how life cycles could have reflected feeding preferences in core Apicomplexa and related lineages.

Keywords: life cycle, meiosis, Apicomplexa

Endogenous mitochondrial ATPase inhibitor: twist from menace to salvation in *Trypanosoma brucei* life cycle

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During hypoxia aerobic eukaryotes protect themselves against deleterious ATP depletion caused by the reversal of mitochondrial (mt) ATP synthase activity to ATP hydrolysis, which is inevitable consequence of the mt membrane potential ($\Delta\Psi_m$) collapse in the lack of oxygen. The protection involves IF1, a protein inhibiting ATP hydrolysis by reversible binding to F_1-ATPase, the catalytic sector of the enzyme.

IF1 has been studied in organisms representing opisthokonts and Archaeplastida. Using structural homology tools we identified IF1 homolog in *Trypanosoma brucei*, a digenetic mammalian parasite belonging to Excavata. TbIF1 inhibits F_1-ATPase *in vivo* and *in vitro*, but has no effect on the mammalian enzyme. TbIF1 is not expressed in the *T. brucei* bloodstream form (BF), wherein ATP hydrolysis coupled to H^+ pumping into the intermembrane space is essential physiological process maintaining $\Delta\Psi_m$. The ectopic TbIF1 expression in these cells is lethal due to severe mt membrane depolarization. In the insect procyclic form (PF), where the ATP synthase generates ATP, TbIF1 localizes to mitochondria, but is dispensable under standard *in vitro* conditions. However, when hypoxia is simulated by inhibition of respiration, it acts against the restoration of $\Delta\Psi_m$, ensuring ATP conservation.

Due to its detrimental effect for the BF cells TbIF1 expression has to be tightly controlled during the progression of the parasite's life cycle. RNA half-life measurement and reporter assays revealed that TbIF1 expression is regulated by its mRNA stability and translation efficiency. The gene's regulatory 3'UTR region contains recognition motifs for RNA-binding proteins Rbp10 and Rbp6. Rbp10 is expressed exclusively in BF, where it destabilizes PF specific transcripts. Rbp6 triggers PF to BF differentiation cascade, to which TbIF1 might contribute.

We provide evidence that *T. brucei* harbors a natural, tightly post-transcriptionally regulated, inhibitor of the F_1-ATPase activity that can be exploited for future structure-based drug design.

Disentangling sources of variation in SSU rDNA sequences from single cell analyses of ciliates: impacts of copy number variation and experimental errors

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Small subunit ribosomal DNA (SSU rDNA) is widely used for phylogenetic inference, barcoding, and other taxonomy-based analyses. Recent studies indicate that SSU rDNA of ciliates may have high sequence variations within a single cell, which impacts interpretation of rDNA-based surveys. However, sequence variation can come from a variety of sources including experimental errors, especially the misreading of polymerase in PCR. In the present study, we explored the impact of four DNA polymerases on sequence variations and found that low-fidelity polymerases exaggerate estimates of single-cell sequence variation. Therefore, using of a polymerase with high fidelity is necessary for surveys of sequence variation. Another source of variation can result from errors during amplification of SSU rDNA within the polyploidy somatic macronuclei of ciliates. To further investigate the impact of SSU rDNA copy number variation, we examined the intra-individual SSU rDNA polymorphisms in ciliates with varying levels of SSU rDNA amplification: *Halteria grandinella*, *Blepharisma americanum* and *Strombidium stylifer* using high-fidelity polymerase. We estimated the rDNA copy numbers of these three species by single-cell quantitative PCR. The results indicate that (1) sequence variation of SSU rDNA within a single cell is authentic in ciliates, but the level of intra-individual SSU rDNA polymorphism varies greatly among species; (2) rDNA copy numbers vary greatly among species, even those within the same class; (3) the average rDNA copy number of *Halteria grandinella* is about 567,893 (SD=165,481), which is the highest record of rDNA copy number in ciliates to date; (4) based on our data and the records from previous studies, it is not always true in ciliates that rDNA copy numbers are positive correlated with cell or genome size.

Keywords: Ciliophora, polymerase fidelity, rDNA polymorphism, rDNA copy number, quantitative PCR

N6-adenine DNA methylation is associated with H2A.Z-containing well-positioned nucleosomes in Pol II-transcribed genes in *Tetrahymena*

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DNA N^6-methyladenine (6mA) is newly rediscovered as a potential epigenetic mark across a more diverse range of eukaryotes than previously realized. As a unicellular model organism, *Tetrahymena* is among the first eukaryotes reported to contain 6mA modification. However, lack of comprehensive information about 6mA distribution hinders further investigations into its function and regulatory mechanism. In this study, we provided the first genome-wide, base pair-resolution map of 6mA in *Tetrahymena* by applying single-molecule real-time (SMRT) sequencing. We provide evidence that 6mA occurs mostly in the AT motif of the linker DNA regions. More importantly, we found that distribution of 6mA is strongly correlated with nucleosome positioning and H2A.Z levels in Pol II-transcribed genes. These results indicate that 6mA is an integral part of the chromatin landscape shaped by ATP-dependent chromatin remodeling and transcription.

Keywords: DNA methylation, N6-methyladenine (6mA), *Tetrahymena*, nucleosome positioning, H2A.Z

Phylogenetic characterisation of oomycetes infecting toxic species of the marine diatom genus *Pseudo-nitzschia*

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In aquatic ecosystems parasitic interactions between protists have been shown to shape host species dynamics. At sea, sampling problems, the limited number of culturable species, and the dearth of morphological information in protist parasites hampered the assessment of their diversity, phylogenetic position and ecological importance. According to morphological observations, a parasitoid of the marine toxigenic diatom *Pseudo-nitzschia pungens* has been tentatively affiliated to the oomycete genus *Ectrogella*, reported to infect both marine and freshwater diatoms. No phylogenetic affiliation was provided, opening the debate about its systematic position. By single-cell genetic analyses, we obtained 18S rDNA sequences of six distinct intracellular eukaryotic parasitoids, infecting four toxic *Pseudo-nitzschia* and one *Melosira* species in the North Atlantic coastal waters. Robust phylogenetic analyses demonstrate that our sequences cluster into two separate clades within the phylum Oomycota, being related to the seaweed parasites *Anisolpidium* and *Olpidiopsis*. Morphological features of our specimens were not sufficient to unambiguously attribute these parasites to any described *Ectrogella* species. Therefore, we named our two Oomycota clades OOM-1 and OOM-2, awaiting additional morphological and genetic information. A screening of global databases of the regions V4 and V9 of the 18S rDNA, demonstrated the presence of our parasites beyond the North Atlantic coastal regions. In a biweekly metabarcoding survey of the diatom communities in the Concarneau Bay (France, Brittany), high abundances of OOM-2 coincided once with the decline of *Pseudo-nitzschia* spp. and then with that of *Cerataulina pelagica*. This finding, together with the genetic identification of the same oomycete infecting both *Pseudo-nitzschia australis* and *Melosira* sp. supports the hypothesis of a lack of host specificity of the studied parasites. Our finding highlights a complex and still unexplored genetic diversity within oomycete parasitoids of diatoms and calls for new biological evidences to unveil the evolutionary history and ecological role of these marine protists.

Keywords: marine plankton, molecular taxonomy, phylogeny, biogeography, metabarcoding

Soil Protist Initiative: Let's make this field great again!

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Protists are the most diverse and abundant eukaryotes in all ecosystems. In soils, their high abundances, their role in food webs as predators of bacteria and their role as plant-growth promoters is widely acknowledged. However, both the taxonomic and functional diversity of soil protists remains largely ignored and are only starting to be better known with the advent of high throughput sequencing combined with experimental approaches. Indeed, work on soil protists lags behind that on aquatic protists and other soil organisms.

To increase the awareness and importance of soil protists, we started the soil protist initiative that aims at bridging soil and aquatic protistologists, but even more importantly at promoting soil protist research among soil ecologists and microbiologists who only too frequently ignore protists.

We will highlight some examples of the previously unknown taxonomic and functional diversity of soil protists. These results help make the case that soil protists are by far the most diverse eukaryotes, potentially even organisms in general, but that also their functions expand widely beyond their role as bacterivores.

We are involved in some broader soil initiatives (e.g. global soil biodiversity initiative), fundamental and applied research, and have led a joint publication in a top soil journal in which we list the main open questions in soil protistology. With these actions we hope to further increase the awareness of both protistologists and soil ecologists that soil protists are a major Eldorado of opportunities for discovery, and soil protistology a rising field for future studies! To further strengthen this field, we need as many people to join our uneven path to make soil protistology a key avenue of future research!

Keywords: soils, functional diversity, taxonomic diversity, global initiative

Evolution and transmission of termite hindgut symbiotic protists

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The cockroach lineage that includes termites and *Cryptocercus* is probably the first insect lineage to evolve wood-digestion capability. This capability was enabled by symbiosis with cellulolytic protozoa, which in turn enabled the evolution of true sociality in termites. The protist/termite symbiosis was already well established before the split of *Cryptocercus* and termites, roughly 175 million years ago. Since that time, the protists have broadly co-speciated with their termite and *Cryptocercus* hosts, being vertically inherited through the mechanism of proctodeal feeding that ensures inoculation of the brood. Today there are between 500 and 1000 “lower” termite species and between a few and several *Cryptocercus* species, each of which harbors its own characteristic set of symbiotic hindgut protists.

Termite and *Cryptocercus*-specific symbiotic protists belong to the major groups Parabasalia and Oxymonadida. These groups consist mainly of endobionts, mostly of termites but also inhabiting a broad range of other vertebrates and invertebrates. Parabasalia includes a handful of free-living protists. By far the most speciose and morphologically complex lineages occur solely within hindguts of lower termites and *Cryptocercus*. These protists, in turn, form symbioses with diverse bacteria and archaea such that the termite wood digestion process is a multi-domain collaboration.

We will present progress toward understanding the evolution and transmission of termite symbiotic protists. We ask what the complex distribution of protists across the termite phylogeny can tell us about the maintenance and function of protist communities. We use morphological and molecular approaches, including single cell isolation, microscopy, and custom 18S amplicon sequencing of hindgut community DNA.

Correlated evolution of alternative splicing and genome architecture in eukaryotes

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Alternative splicing (AS) is a major mechanism of transcriptome regulation in eukaryotes that can facilitate the diversification of the proteome, give rise to key evolutionary innovations and serve as an additional and responsive layer of gene expression control. We here explore the landscape of AS across 60 eukaryotes from all major lineages using high-coverage transcriptomic data. We uncover a consistent correlation between interspecific changes in the frequency of different modes of AS (exon skipping and intron retention) and the evolution of 21 parameters describing gene architecture and sequence composition. For example, exon skipping-rich AS profiles, typical of multicellular animals and plants, are also a readily evolvable feature in protistan lineages with conducive genome architectures, *i.e.* intron-dense, with short exons and heterogeneous splice sites. This is the case of the colonial chlorophyte *Volvox carteri* or the ichthyosporean *Sphaeroforma arctica*. Furthermore, we confirm previous reports that intron retention is the dominant AS mode in the vast majority of eukaryotes.

By analyzing the effect of these 21 structural and compositional *cis*-features on AS, we establish a pan-eukaryotic code that determines the relative frequency of exon skipping and intron retention in extant eukaryotes. This AS code can be then combined with ancestral reconstructions of genome architecture in order to infer the AS profile of ancestral eukaryotes. Thus, we apply a predictive model of exon skipping and intron retention frequencies to the genomes of key eukaryotic ancestors, from the LECA to animals and their unicellular holozoan relatives. This approach reveals that, as in extant eukaryotes, ancestral transcriptomes were dominated by intron retention; and confirms the increase in exon skipping associated with animal multicellularity.

Overall, our results emphasize the effect of long-term genome evolution in shaping AS, a fast-changing transcriptome regulatory layer. Thus, the study of genome architecture is key to understand the role of AS as a source of evolutionary innovations in eukaryotes.

Keywords: alternative splicing, genome architecture, ancestral reconstruction, Holozoa, *Sphaeroforma arctica*

Cryptophyta as the major freshwater flagellate bacterivores in natural plankton samples manipulated with different bacterial prey

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The diversity of small eukaryotes (heterotrophic and mixotrophic nanoflagellates) and their taxonomic classification are currently hot topics in aquatic microbial ecology. Despite the increasing interest in their diversity, core questions regarding predator-prey specificity remain largely unanswered, e.g. which heterotrophic nanonflagellates (HNFs) taxa are the main bacterivores in freshwater systems and what bacterial groups are rapidly decimated by small HNFs. To answer these questions we fed natural communities of HNFs from Římov reservoir (Czech Republic) with 5 distinct bacterial strains (belonging to genera *Polynuclebacter* and *Limnohabitans*). We employed a combination of amplicon sequencing and CARD-FISH analysis to track specific responses of the natural HNF community to the bacterial prey amendments. Using the amplicon-sequence data two new CARD-FISH probes were designed and used to compare the results from amplicon sequencing and CARD-FISH. We show that community composition of HNFs is strongly dependent upon the prey food quality. Moreover, a quantification based solely on numbers of reads by amplicon-sequencing is insufficient to accurately estimate abundance of certain groups. We also developed a new "double-hybridization" technique that allows simultaneous phylogenetic identification of both predator and prey. Surprisingly, flagellates targeted by a general Cryptophyta probe were the most abundant bacterivores and the growth of the CRY1 clade of Cryptophyta was strongly stimulated by one *Limnohabitans* strain in our experiment. To our best knowledge the CRY1 group is not considered heterotrophic. Thus, our study clearly shows that colorless Cryptophyta are major bacterivores in summer plankton samples facilitating carbon flow to the grazer food chain.

Keywords: flagellate bacterivory, flagellate growth responses, next-generation sequencing, "double-hybridization" technique, bacterial prey food quality

The order of intron removal during splicing of α-tubulin (tubA) pre-mRNA in *Euglena gracilis*

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Nonconventional introns found in nuclear genomes of euglenids (Euglenida), phytoflagellated excavate protozoans, possess a set of unusual traits, such as (I) variable non-canonical borders, (II) ability to form a stable, slightly conserved RNA secondary structure, which brings together intron ends, placing adjacent exons in close proximity. Despite many years of study, these intragenic regions remain the only type of introns with yet undiscovered mechanism of removal.

To gain insight into this topic, we have investigated the order of splicing of six introns in α-tubulin (*tubA*) gene in model organism, *Euglena gracilis*. Studied gene contains (I) three conventional spliceosomal introns, which obey the GT-AG rule, (II) two nonconventional introns and (III) one intron combining features of both aforementioned types, i.e. an intermediate intron.

We have used a strategy based on reverse transcription and the polymerase chain reaction. The chosen method involves a pairwise comparison of molecules that retain one intron and have either retained or spliced another intron(s). A highly preferred (relative) order of intron removal do not represent a linear progression from the beginning of the transcript to the end. On the contrary to the suggestions proposed in previously published reports, our data indicate that conventional spliceosomal introns are excised before nonconventional ones. It seems that nonconventional introns are removed very rapidly (perhaps simultaneously), as determination of exact order of their excision was not possible. Based on our data, it may be supposed, that splicing of conventional and nonconventional introns may not only require different molecular machinery, but also occurs in different cellular compartments.

Keywords: intron, splicing, Euglena, nonconventional, α-tubulin

Phytoplankton community structure in the West Antarctic

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The highly productive coastal waters of the West Antarctic Peninsula (WAP) are among the most climate-sensitive regions on the planet. We characterized the molecular diversity and abundance of phytoplankton in this region by combining 16S rRNA V1-V2 amplicon (Illumina) sequencing and flow cytometric analysis from two expeditions. Samples were collected from the Andvord Bay, Antarctica during the austral spring and fall of 2015-2016 and analyzed using phylogenetic placement methods against the Baselines project reference trees. Over 99% of the plastid-derived amplicons were from stramenopile, cryptophyte, green alga, and prymnesiophtye groups. Cryptophytes were found to have the highest relative amplicon abundances (up to 90% of plastid-derived amplicons) during the spring at stations in the inner bay, and the most abundant amplicons were close to *Geminigera cryophila*. The stramenopiles (consisting primarily of diatoms, dictyochophytes, pelagophytes, and bolidophytes) made up the bulk of the phytoplankton amplicons during the fall and at stations just outside of the bay. The contribution of diatoms, which included high abundances of amplicons close to *Lauderia borealis*, *Detonula confervacea*, and *Chaetoceros*, to the total community ranged from 1.4-91.2% (mean 39.5%, s.d. 29%) and was smallest at stations dominated by cryptophyte amplicons. The green algae had lower relative amplicon abundances and consisted primarily of prasinophyte Class I (the Pyramimonadales) and Class II (specifically *Micromonas* Clade E2), and *Bathycoccous*. Our size-fractionated Chl *a* data showed that the picoeukaryotes (<3 micrometer size fraction) were often the primary contributors to phytoplankton biomass. This is additionally supported by our amplicon data, which revealed the presence of representative picoeukaryote groups, including the bolidophytes, pelagophytes, and prasinophytes, indicating the importance of taxa within this size range in the Antarctic. Furthermore, the higher cryptophyte amplicon abundance relative to the typically larger-sized diatoms at multiple stations during the spring may have significant ecological and biogeochemical implications.

Keywords: Antarctica, phytoplankton

Studying the evolutionary history of the Stramenopiles using novel organelle genomes from four classes, Pinguiophyceae, Dictyochophyceae, Synchromophyceae, and Pelagophyceae (Stramenopiles)

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The photosynthetic Stramenopiles (heterokont algae) form a monophyletic group of algae that have been extensively studied since the 19th century. With more than 10,000 described species and an estimated one million species, it represents one of the largest and diverse eukaryote groups. Taxonomically, 17 classes are recognized within the Stramenopiles that were recently further grouped in three large clades: SI, SII, SIII clades. Those classes differ greatly in morphology and physiology. For example, the class Phaeophyceae is composed of true multicellular algae whereas the class Bacillariophyceae regroups the infamous diatoms micro-algae. To this day organelle genomic information is restricted to only six of the 17 classes of the Stramenopiles. This lack of information makes it difficult to understand the evolutionary history of the organelles in the Stramenopiles. Furthermore, phylogenetic relationships among these classes remain partially unresolved. Therefore, acquiring organelle genome sequences for the missing classes would prove useful in order to study the evolution of the Stramenopiles and be a valuable source of data for a phylogenomic study of the Stramenopiles. In this study, we sequenced chloroplast and mitochondrial genomes from five micro-algal species belonging to four different classes of the Stramenopiles that were never studied before. They were the *Pinguiococcus pyrenoidosus* (Pinguiophyceae), *Rhizochromulina marina* and *Dictyocha speculum* (Dictyochophyceae), *Chlamydomyxa montana* (Synchromophyceae), and *Sarcinochrysis* sp. CCMP770 (Pelagophyceae). Along with publicly available organelle genome data, we were able to cover most of the classes of the photosynthetic Stramenopiles. Using this new data we discuss the evolution of organelle in Stramenopiles. Furthermore, we conducted phylogenomic studies in an attempt to fully resolve the relationships among the classes in the Stramenopiles.

‘Dactylomonas’ – a novel deep-branching lineage of Heterolobosea

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We have established laboratory cultures of three strains of morphologically unique heterolobosean flagellates (‘Dactylomonas’) obtained from freshwater sediments in Canada, Czech Republic, and Cambodia. The cells were spindle-shaped with a distinctive anterolateral rostrum and had one anterior and one recurrent flagellum. The proximal part of the recurrent flagellum bore a conspicuous finger-like projection. On the basis of the cell shape and diameters and SSU rRNA gene sequences, we distinguished two species of ‘Dactylomonas’. Interestingly, ‘Dactylomonas’ was closely related to the amoeba *Selenaion koniopes*, the basal lineage of Tetramitia (a major group of Heterolobosea). A detailed ultrastructural study of one ‘Dactylomonas’ strain showed an unexpected organization of the flagellar apparatus, which resembled Pharyngomonada (the second lineage of Heterolobosea) instead of Tetramitia: basal bodies were orthogonal to each other and a putative root R1 was present in the mastigont. On the other hand, ‘Dactylomonas’ possessed several highly derived features: the absence of a ventral groove, presence of a complete microtubular corset, massive pinocytosis on the whole cell surface, a distinctive rostrum supported by the main part of the right microtubular root (R2), and the presence of a finger-like projection on the proximal part of the recurrent flagellum. Moreover, because of successful long-term cultivation under anoxic atmosphere and the absence of mitochondrial cristae, we assume that the cells were anaerobic (or microaerotolerant). ‘Dactylomonas’ is a novel anaerobic freshwater lineage with an important phylogenetic position and intermediate morphology between Pharyngomonada and Tetramitia.

Unicellular origin of the Microprocessor and microRNAs

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In the past decade we have learned that the unicellular sister lineages of animals have surprisingly complex genetic machineries, and share many of the gene pathways and regulatory networks that are key to animal development. One group of genes that is believed to be key to the acquisition of metazoan complexity is the miRNAs. However, the protein machinery essential for biogenesis of miRNAs in animals, the so-called Microprocessor complex, has not been identified outside animals and could therefore be a genetic innovation specific to animals. We have therefore investigated the presence of genes involved in miRNA biogenesis, as well as the miRNA genes themselves, in unicellular relatives of animals by transcriptome and small RNA sequencing. We find homologues of the entire animal miRNA biogenesis machinery, including Drosha and Pasha (the components of the Microprocessor), as well as miRNA genes, in a wide range of ichthyosporeans. This implies that miRNAs and the Microprocessor complex evolved before the metazoan last common ancestor. This is consistent with the view that most of the animal developmental toolkit evolved prior to animals. Furthermore, it implies that absence of the miRNA biogenesis pathway in other lineages of Choanozoa, Ctenophores and Placozoa are result of secondary loss, which also seems to correspond to the absence of miRNA genes.

Investigation of an Antarctic dinoflagellate harboring kleptoplastids of remarkable longevity

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Plastid evolution in dinoflagellates is characterized by its remarkable complexity, reflecting multiple endosymbiotic events. The majority of dinoflagellates harbour a secondary plastid of red-algal origin, but there are a number of exceptions: for example, some dinoflagellates have substituted this plastid with a tertiary plastid of haptophyte origin, another lineage has taken up a plastid of chlorophyte origin in a serial secondary endosymbiosis while others again possess transient plastids harvested from their prey (kleptoplasts).

In this context we are investigating the Ross Sea dinoflagellate (RSD), a genus of dinoflagellate that was found to be highly abundant in Antarctic seawater and meltwater habitats. Like several other dinoflagellate lineages, the RSD retains kleptoplasts sequestered from its prey (the haptophyte *Phaeocystis antarctica*), however this association is unique in its extreme longevity of the plastid maintenance (at least 29.5 months) and the high specificity of the host for its prey and kleptoplast source. These observations indicate a prolonged co-evolution between RSD and *P. antarctica*; additionally the relationship of the RSD to dinoflagellates with permanent tertiary haptophyte plastids raises the possibility that the RSD is in the process of replacing a lost permanent haptophyte plastid.

We are testing this hypothesis by searching RSD transcriptome data for host-encoded haptophyte-associated genes putatively transferred from a lost haptophyte plastid. To date, we identified several candidates for such genes, the majority of them plastid-associated. Additionally, investigation of the plastid targeting sequences of these genes and other genes associated with plastid function indicates that targeting takes place not only to the kleptoplasts but also to the original plastid of red-algal origin, suggesting in turn that RSD is still retaining its ancestral secondary plastid.

Keywords: kleptoplast, dinoflagellate, endosymbiosis, Antarctic

Morphological and molecular description of a marine relative of malawimonads

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Excavata is a 'supergroup' of eukaryotes comprising three lineages, basal members in each of which share several ultrastructural traits. Two of the groups, Discoba and Metamonada, are large and diverse, and each of these includes both morphologically 'typical excavates' and morphologically derived forms. The third lineage of excavates is represented only by the single described species *Malawimonas jakobiformis* and a handful of closely related undescribed strains. All malawimonads are freshwater organisms, and are extremely similar under both light and electron microscopy, exhibiting all of the 'typical excavate' characteristics. In spite of this shared morphology, the three component lineages of excavates rarely form a clade in phylogenetic analyses, and support for such a clade is always limited. More often, Discoba and Metamonada group together, or are only separated by a few weakly supported nodes, while malawimonads branch more basally, often with another enigmatic eukaryote group, the collodictyonids, with varying statistical support. One commonly acknowledged limitation of these analyses is the lack of broader representation of the malawimonad lineage. We have isolated a new strain of eukaryote that may address this problem. Our new strain, "Slancy", is a marine isolate from the Solomon Islands. It exhibits "typical excavate" morphology in most investigated respects. However, it also exhibits two novel features: a "pom-pom" on the anterior flagellum (probably an internal coiling of the axoneme) and the capacity to produce broad pseudopodia from the posterior end of the cell. The latter characteristic appears to function in anchoring the organism to the substrate. We present SSU and LSU phylogenies, and light and scanning electron microscopy, and compare these data to both malawimonads in particular and to eukaryotes in general.

Keywords: *Malawimonas*, Excavata, phylogeny, microscopy, novel organism

Transcriptomic analysis of highly pathogenic, mouse-passaged *Naegleria fowleri* reveals the cellular pathways and novel genes associated with infection

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Naegleria fowleri is an amoeboflagellate in the supergroup Excavata. It is found around the world in warm freshwater, and as a free-living amoeba, causes severe meningoencephalitis in humans and other animals. More than 95% of patients infected die, usually within two weeks. Infection occurs when contaminated water enters the nose (e.g. when swimming), from where *N. fowleri* aggressively migrates through host tissue to reach the brain. In addition to causing destruction by cytolysis and trogocytosis, its presence induces substantial inflammation, and without rapid amoebicidal and supportive treatment, quickly leads to death. As infection is relatively uncommon, misdiagnosis further increases the mortality rate.

Several proteomic studies have identified individual genes potentially involved in pathogenesis, but the overall picture of gene regulation during infection remains unclear. In order to assess the transcriptional landscape during infection, we performed a comparative transcriptomic analysis of highly pathogenic *N. fowleri* MP (ATCC30894) (continuously passaged through mice) and *N. fowleri* grown in axenic culture. We identified approximately 200 up-regulated and 100 down-regulated genes in mouse-passaged versus cultured *N. fowleri*, ~1/3 of which are of unknown function and found only in the *Naegleria* genus. Of the genes that are up-regulated in highly pathogenic *N. fowleri* (ATCC30894), many are involved in lysosomal function and proteolysis, actin-based cell motility, protein synthesis, and metabolism. Proteases alone make up ~15% of the up-regulated genes, therefore we undertook comparative genomics and phylogenetics analyses of proteases in three sequenced strains of *N. fowleri*, and the related non-pathogenic organism *Naegleria gruberi*. Our transcriptomic analyses have elucidated the cellular pathways related to *N. fowleri* pathogenesis, and generated a list of novel targets that could be exploited for both detection and therapeutics.

The algivorous UFO (Unknown Flagellate Organism) reveals a novel mode of swimming locomotion for eukaryotic microbes

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Eukaryotic microbes living in the aquatic environment developed a diversity of swimming modes, which generally involve the action of thread-like appendages, namely flagella. In contrast to bacterial swimmers, which have a molecular rotary motor attached to the bacterial flagellum, eukaryotes swim by running waves over the (much more complex) eukaryotic flagellum or by a periodic beating motility (power and recovery stroke). The accidental discovery of an algivorous UFO (Unknown Flagellate Organism) parasitising zygnematophycean green algae revealed not only a flagellated amoebozoon with a peculiar set of cellular characteristics, but also a fascinating swimming mode of a eukaryotic cell, which has been unknown in the world of biology until today. The talk will present the UFO with phenotypic and phylogenomic data, and furthermore explain its swimming mode with slow motion video analysis.

Keywords: flagellates, algae, swimming, electron microscopy

The evolution of meiosis and sex in Amoebozoa

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Sex and reproduction are treated as a single phenomenon in animals and plants, because in such organisms reproduction implies sex (*mixis*) and meiosis. However, in most eukaryotes, sex and reproduction are independent biological phenomena that may or may not be linked. Regardless of organism, meiosis is part of any *bona fide* sexual process involving haploid and diploid forms. The evolutionary advent of a process as complex as meiosis is remarkable and clearly evolved a single time. It is difficult to explain both how it arose in the first place, as well as its evolutionary persistence given the costs implied in the process. Every major eukaryotic group present significant evidence for the presence of meiosis suggesting that the process is a synapomorphy ancestral to all extant eukaryotic life. However, genetic recombination occurs in prokaryotic organisms and viruses. The enzymatic machinery responsible for homologous recombination is highly conserved and may be detected in so divergent groups as Eubacteria, Archaea, Eukarya and viruses. One way to approach the evolution of meiosis is to evaluate the evolution or presence of a group of genes that are meiosis specific (Spo11, Dmc1, Msh4, Msh5, Mer3, Hop1, Hop2, Mnd1, Rec8). Traditionally considered asexual, amoebozoans are a group of flagellated and amoeboid organisms. Using a set of genomic-level data from the breadth of amoebozoan lineages, we applied a bioinformatic approach to study the occurrence of sexual processes in the group investigating the presence of meiosis toolkit. Our results suggest the occurrence of meiotic processes in the amoebozoan ancestor. Given the wide presence of meiotic genes in diverse extant lineages pertaining to the group, most amoebozoans could perform ploidy reduction and recombination through meiosis. The existence of most forms of the meiotic toolkit grants the possibility of sexual cycles to several organisms long thought to be asexual.

Keywords: Amoebozoa, meiosis, sex

Nutrient intakes of thraustochytrids (Labyrinthulea) by their ectoplasmic nets

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The Labyrinthulea is characterized by an ectoplasmic net system. Thraustochytrids inhabit the ocean all over the world and have been recognized as important eukaryotic decomposers in the marine ecosystem. The ectoplasmic nets are superficially similar to narrow pseudopods of many protists but different in the following points: their origin from a unique organelle named the bothrosome, membrane invagination along ectoplasmic net, and absence of mitochondria and ribosomes. We revealed that Golgi body is related to the formation of the bothrosome and that actin is present in the ectoplasmic nets and in the cytoplasm around the bothrosome. When the ectoplasmic nets attached the baits, the ectoplasmic nets became massive and the structure of membrane invagination in the ectoplasmic nets were developed complexly. This suggested that the ectoplasmic nets recognized the organic matter and efficiently secrete digestive enzyme and intake nutrients at the attached area. On the other hand, the vegetative cells of *Aplanochytrium* extended the ectoplasmic nets to the diatom cells, and then chloroplasts of the diatoms shrank and bleached. It was suggested that the thraustochytrids play the role as not only decomposers but also “predators”. *Aplanochytrium* probably intake the nutrients from the ecologically important primary producers, diatoms, which located in the wide sphere of radially developed ectoplasmic nets.

Keywords: stramenopiles, Labyrinthulomycetes, ultrastructure, food chain, assimilation

Progress in morphogenetic studies on hypotrichous ciliates (Protista, Ciliophora)

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Ontogenetic data help to reconstructing ciliated phylogeny, so it becomes one of the hotspots in ciliatology for a long time. During the last four years, 30 species of hypotrichous ciliates were morphogenetically studied in our group. Ontogenetic modes of 6 genera and 20 species were revealed for the first time. Results demonstrate that many hypotrichous congeners possess similar morphogenetic patterns, which can thus be used for generic assignment. Some new findings are available, e.g. the additional cirri between the left fontal cirrus and buccal cirrus is derived from undulating membrane anlage in *Amphisiella milnei*; midventral rows are confirmed to be present in *Apoholosticha* and *Heterokeronopsis*, both should be placed in the Pseudokeronopsidae; unique dorsal morphogenesis supports the transfer of *Holosticha bradburyae* to the genus *Uncinata*; two frontoventral rows are originated independently in *Bistichella*; *Trichototaxis* represents an intermediate form between the Pseudourostylidae and the Pseudokeronopsidae; frontoterminal cirri are apparently lacking in *Parabistichella*, *Apobakuella* and *Heterokeronopsis*. However, compared with species description, morphogenetic studies are extremely insufficient in the Hypotrichia, so much more work are needed in the future. A greater diversity of morphogenesis can be anticipated in this group of ciliates.

Keywords: ciliate, Hypotrichia, morphogenesis, phylogeny

Parallel genome reduction in pedinophyte-derived plastids in green-colored dinoflagellates

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The transformation of the endosymbiotic cyanobacterium into the plastid (primary endosymbiosis) should have associated with severe reduction of the endosymbiont genome. Some of us previously proposed the plastid genome in the pedinophyte endosymbiont in the dinoflagellate *Lepidodinium chlorophorum* seemingly underwent an extra reduction. However, it remains unsure whether similar reductive trends can be observed in the plastid genomes of two undescribed dinoflagellate strains TRD-132 and MRD-151, of which current 'green-colored' plastids were acquired from pedinophyte endosymbionts. In this work, we compared the plastid genomes of three free-living pedinophytes to those of three 'green-colored' dinoflagellates, namely *L. chlorophorum*, TRD-132 and MRD-151. As the three dinoflagellates most likely integrated pedinophyte endosymbionts as the plastids separately, the impact of the endosymbiosis in a dinoflagellate cell on the plastid genome of a pedinophyte endosymbiont can be evaluated by the three independent lineages in our comparison. The three pedinophyte-derived plastid genomes appeared to harbor similar numbers (60-68) of functionally assignable open reading frames (ORFs), although the size varies from ~66 to ~102 Kb. We here propose that similar reductive pressures worked on the plastid genomes of pedinophyte endosymbionts in the separate dinoflagellate cells. Firstly, the ORF repertories in the three pedinophyte-derived plastid genomes appeared to be similar subsets of those in the pedinophyte plastid genomes. Secondly, the pedinophyte-derived plastid genomes commonly but separately lost the inverted repeats after being engulfed by the dinoflagellate hosts, as all the plastid genomes of the three free-living pedinophytes share the corresponding structure.

Keywords: dinoflagellates, plastid genome, serial endosymbiosis, genome reduction, pedinophytes

Phylogenomic testing of Archaeplastida to illuminate plastid origins

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Despite being a key event in eukaryotic evolution, the origin and spread of plastids remain poorly understood. In particular, the origin of primary plastids in glaucophytes, rhodophytes, and chlorophytes (collectively known as Archaeplastida) is still controversial due to inconsistencies between plastid- and nuclear-based evidence. The strong signal for monophyly recovered in plastid phylogenies and shared molecular and cell biological characters have favoured the parsimonious scenario of a single acquisition of plastids in an ancestor of Archaeplastida. However, a single endosymbiotic event ultimately requires monophyly from the side of both plastid and host (nuclear) lineages and alternative (more complex) scenarios can in principle explain plastid monophyly without requiring the nuclear monophyly of Archaeplastida. Previous nuclear phylogenomic analyses have not strongly supported the monophyletic origin of Archaeplastida, nor have they reached an alternative consensus. Since resolving the relationships among Archaeplastida lineages is inextricably linked to reconstructing robust deep eukaryotic relationships, we set out to tackle this issue. We review the phylogenomic evidence for Archaeplastida by reanalysing four relevant nuclear phylogenomic datasets assembled by independent labs. We study the effect of gene and taxon sampling, which partially overlap in these four datasets, in recovering deep eukaryotic relationships. Common sets of genes or taxa fail to converge to a stable tree topology, revealing the existence of complex interactions between both factors. We explore the performance of both site-homogeneous and site-heterogeneous (mixture) substitution models, which are key to correct for systematic error. Finally, we present a phylogeny of eukaryotes built with a novel mixture model that uses site-specific amino acid frequencies.

Keywords: phylogenomics, plastid, Archaeplastida, systematic error, Tree of Life

How to withstand oxygen when you don't breathe it? Evolution of oxygen defenses in diplomonads

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Oxygen and reactive species of oxygen (ROS) are considered important stress factors. These molecules can react with different compounds of a cell and disrupt its normal functioning. To avoid this situation, all organisms present barriers against ROS. These barriers are especially important in anaerobic species because many of their key enzymes in the energy metabolism are O_2-labile. An example of this situation is the group diplomonads. They have an anaerobic metabolism but at the same time they can withstand fluctuating levels of oxygen. Previous studies have showed the role of different enzymes independently. Here, we identified 23 protein families in diplomonads putatively in the antioxidant defenses, and propose how these enzymes interact to create a system well-adapted for coping with the effect of changing O_2-levels. These antioxidant defenses can be divided in oxygen detoxification system and accessories pathways for the synthesis of non-protein thiols that play a role in the oxygen detoxification system. For its part, the oxygen detoxification system can be divided in direct systems that detoxify ROS before they cause damage, including a first defense acting on the cell surface, and indirect systems that repair protein damage by ROS in cysteine and methionine residues. We used a phylogenetic approach to investigate the evolutionary origin of the individual components of this oxygen detoxification system and the accessory pathways. Our results show the existence of a central eukaryotic core in the oxygen detoxification system to which different enzymes were added or replaced via lateral gene transfer (LGT) during the evolution of the group. Regarding to accessory pathways, our results show differences between lineages due to gene losses and acquisition of pathways via LGT. These processes most likely have played an important role in the adaptation process of diplomonads to parasitic lifestyle in association with an aerobic host organism.

Keywords: reactive species of oxygen, Diplomonadida, evolution, lateral gene transfer, gene loss

Breaking through the silence of limited amoebozoan genomes

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Amoebozoa is the eukaryotic supergroup sister to Obazoa, the group containing Opisthokonta and several protist lineages (i.e., the breviate and apusomonad flagellates). Although Amoebozoa represents the closest outgroup to Obazoa, little genomic-level data and attention has been given to the supergroup. Amoebozoa appears to have split from the last common ancestor of Obazoa+Amoebozoa somewhere around 1.5 billion years ago, and the genomic complement that was present in this ancestor is a mystery. To examine the evolutionary history of the genomic repertories within this ancestor and the ancestral trajectory of amoebozoan genomes and morphological variation observed within the group, we have deeply sampled roughly 100 transcriptomes. These robust data sampled from the either breadth of known amoebozoan clades, have led to massive rearrangements of our understanding of the evolutionary histories of some of the most well-known protein complexes, once believed to be either animal or fungal specific. We show the presence of an ancestral complex of adhesion proteins which predate the evolution of the Amoebozoa, which is present in Tubulinea and Evosea, but absent in Discosea. In addition, we highlight the evolutionary histories of other important protein families associated in with cell signaling and differentiations. Our results highlight that many of these proteins appear to have evolved earlier in eukaryote evolution than previously thought.

Keywords: Amoebozoa, transcriptome, amoeba, evolution

Reduction and expansion in the genome of parasitic rhizarian, *Mikrocytos mackini*

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Mikrocytos mackini is an intracellular protistan parasite of oysters. It has been only recently shown, based on phylogenomic analyses, that it belongs to the super-group Rhizaria. To better understand *M. mackini* biology and evolution, we sequenced, assembled and annotated its genome.

Reconstruction of the metabolic pathways revealed the absence of many of the essential eukaryotic pathways. A similar pattern has been observed in other unicellular parasites and reflects strong dependence on the host metabolism. For example, carbohydrate metabolism in *M. mackini* is restricted to glycolysis and trehalose metabolism. Interestingly, the trehalose metabolism in *M. mackini* might be linked with the host metabolism, because trehalose in some mollusks is an important storage carbohydrate.

Genome annotation also revealed the lack of introns. Reduction of number and length of introns is often observed in the compact genomes of parasites (*Giardia* or Microsporidia), and intron-poor state is a result of extensive, lineage-specific intron loss. In contrast, we observed the expansion of transposable elements, which led to the expansion of the genome size. We annotated almost 65% of the genome as repetitive elements and a half of them we were able to classify to the known groups of transposable elements. Strikingly, all known classes of TEs are present in *M. mackini* genome, and their similarity to the transposable elements from other organisms is higher than the similarity to the eukaryotic conserved genes. That might suggest relatively recent acquisition and the burst of transposable elements in *M. mackini*.

In summary, our preliminary data suggest bizarre genome dynamics in *M. mackini*, with expanded transposable elements in an otherwise compact genome.

Keywords: parasite, Rhizaria, transposable elements, whole genome sequencing

Predation of picocyanobacteria *Prochlorococcus* by pelagic nano-scaled protists and their catabolism on divinylchlorophylls

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It has become understood that *Prochlorococcus* are the sole important primary producer in population density as well as biomass in the vast oligotrophic ocean of low to middle latitudes. Importantly, because they are conspicuously minute coccoids down to < 0.6 µm in diameter, a classical paradigm on the marine food web is unlikely to be applied where phytoplankton would have to be grazed by multicellular filter-feeders; instead, their direct consumer should very likely be phagotrophic protists, if not viral lysis feeding to the microbial loop. Here, we demonstrate that wide varieties of nano-scaled protists (mostly < 10 µm) are indeed potential predators of *Prochlorococcus*, based on a proxy study of their unique chlorophyll catabolites as well as culture experiments. Protists of diverse lineages have been demonstrated to catabolize chlorophylls (Chls) into cyclopheophorbide enols (CPEs), non-phototoxic pigments; on one hand, *Prochlorococcus* are characterized by their unique pigmentation where divinylchlorohylls (DV-Chls) instead of ordinary Chls are exclusively produced. We thus hypothesized that predation of *Prochlorococcus* by protists should result in production of divinyl-formed CPEs (DV-CPEs). Using a synthesized authentic standard, we identified a DV-Chl-*a*-derived DV-CPE from the total suspended particles of pelagic seawaters in oligotrophic region of the Pacific, concentrated by ultrafiltration membrane on an on-ship tangential flow filtration system. Together with tentative identification of a DV-Chl-*b*-derived DV-CPE, we interpreted that at least a portion of *Prochlorococcus* are consumed by those protists. We also designed on-ship culture experiments where axenic culture of *Prochlorococcus* was added to raw seawater samples. Responding specifically to additions of *Prochlorococcus*, a variety of nano-scaled protists including biflagellates, amoeba and flagellated amoeba became proliferated; some of the isolated strains were sequenced for molecular phylogenetic identification. Significantly, many of the isolated strains produced DV-CPEs after fed on *Prochlorococcus*, supporting the above interpretation on the natural occurrences of DV-CPEs.

Keywords: pelagic nanoflagellates, *Prochlorococcus*, cyclopheophorbide enols, divinylchlorophylls, chlorophyll catabolism

The kinetoplastid MICOS complex

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Cristae are invaginations of the mitochondrial inner membrane that protrude into the matrix and its membrane is enriched for respiratory chain complexes in aerobic organisms. Cristae junctions (CJs) form the boundary between the cristae and inner boundary membranes. A protein complex called the Mitochondrial Contact Site and Cristae Organization System (MICOS) that was initially discovered in *Saccharomyces cerevisiae* is responsible for the formation of CJs. There are six subunits of MICOS in budding yeast, most of which are conserved in animals. Of these only three subunits have been shown to have widespread distribution among eukaryotic supergroups, namely Mic10, Mic19 and Mic60. The last protein even has an ortholog identified in α-proteobacteria, a testament to the ancient origin of MICOS and endosymbiotic origin of the mitochondria. To expand our knowledge of MICOS outside of opisthokonts, we have undertaken an investigation of MICOS in the model kinetoplastid, *Trypanosoma brucei*, an organism that is amenable to genetic manipulation and phenotypic analysis by RNA interference. In kinetoplastids, only the presence of Mic10 has been detected by phylogenetic analysis, and trypanosomatids such as *T. brucei* have two paralogs of the protein, which we call TbMic10-1 and TbMic10-2. We have allele tagged both paralogs and have confirmed that both are mitochondrial and associate with mitochondrial cristae. Immunoprecipitation of both TbMic10 paralogs pulls down the same set of proteins that appear to assemble into a macromolecular complex of the same size. The conserved and unique features of kinetoplast MICOS in comparison to the opisthokonts complex will be discussed.

Keywords: mitochondria, cristae, Kinetoplastida, *Trypanosoma brucei*

Tintinnid fingerprints in a plankton time series

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Tintinnid plankton in species-specific sensitivities to preferred hydrographic conditions has been monitored to link the temporal variations to climate change since 2008. Temporal changes of tintinnids were weekly investigated for 8 years at a coastal site in South Korea as part of an ongoing regional monitoring program. Seasonal changes of water temperature and salinity at the monitoring site displayed a typical pattern in the northern temperate coast: higher temperatures over 20 ℃ and salinity drop in summer. Recurrent seasonal patterns were observed for dominant species. *Amphorellopsis acuta*, *Rhizodomus tagazi*, *Tintinnopsis beroidea*, *T. radix*, *T. tocantinensis* were fingerprints for summer while *T. baltica* for winter. *Codonellopsis nipponca* and *Schmidingerella arcuata* occurred mainly in moderate seasons of spring and fall. The occurrence of *Helicostomella subulata* (at < 20℃) and *H. longa* (at >20℃) was separated by a temperature boundary of 20℃. Rare species such as *Protorhabdonella curta*, *Metacylis mereschkowskii*, *Metacylis* sp., and *Acanthostomella conicoides* were also detected in a sporadic spike. As these rare species have been recorded from warm oceanic waters, intrusion of warm water mass into Korean coastal waters can be monitored using this indicator species. An approach for detecting interannual changes in observed tintinnid time series is also introduced in this study.

Keywords: tintinnids, temporal changes, interannual changes, indicator species, fingerprints

Towards a systematic understanding of the apicomplexan membrane-trafficking system: reductions, expansions, and novel features

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Apicomplexa are a phylum of obligate intracellular parasites with global distribution and a wide range of hosts and cell types. Much of apicomplexan cell biology, including gliding and invasion, relies on the membrane-trafficking system (MTS), which is responsible for moving protein and lipid components between organelles. Despite that the underlying machinery of this system is conserved across eukaryotes, there is significant plasticity, both in terms of gene complement and organelle organization, in many organisms. Little is known about the organization of the MTS in Apicomplexa, with only a handful of genes characterized in model organisms *Toxoplasma gondii* and *Plasmodium falciparum* and little knowledge of the precise organization of organelles. Previous comparative genomic work revealed reduction of MTS machinery in ESCRT, adaptor protein, and tethering complex families, and suggested that almost all components were lost following the split of apicomplexan parasites from their free-living photosynthetic chromerid relatives. However, these families represent a fraction of MTS machinery, and many families lack any associated cell biological data in Apicomplexa, leaving their roles in trafficking uncertain. To this end, we have expanded comparative genomic and phylogenetic analyses across all MTS machinery in Apicomplexa and close relatives. Overall, the apicomplexan MTS is comparable, or slightly reduced, compared to that hypothetically encoded by the last eukaryotic common ancestor. Detailed phylogenetic analyses revealed a number of novel paralogues in some MTS families restricted to Apicomplexa and close relatives, suggesting that expansion also played a role in the evolution of trafficking in these organisms. Endogenous gene tagging and genetic disruption of a number of these novel factors suggest they are *bona fide* MTS components. Our results shed new light on the evolution and function of a ubiquitous eukaryotic system in some of the world's most successful parasites.

Keywords: Apicomplexa, parasites, membrane trafficking, evolution, phylogenetics

Seasonal dynamics of high-latitude benthic foraminifera, Kongsfjorden, Svalbard

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High-latitude ecosystems function under unordinary light regime, the long polar day changes for months of winter darkness. Abrupt shifts in illumination result in pronounced seasonal dynamics of the phytoplankton and, consequently, the flux of fresh organic detritus to the seafloor. The local benthic community – including foraminiferans presented here mostly by herbivorous species – in turn should react to such dynamics with fluctuations in abundance and, possibly, species composition.

To tests this hypothesis, bottom sediment was collected during different months (January, September 2015 and January, June 2016) near the tidewater glacier in Kongsfjorden and stained with Cell Tracker Green fluorescent probe for living foraminiferans. Apart from the phytoplankton dynamics the fjord is characterized by notorious shifts in Arctic and Atlantic water masses prevailing here in different seasons and possibly also influencing local foraminiferal community.

Our data show that benthic foraminiferal community in Kongsfjorden is characterized by pronounced seasonal dynamics. The abundance of living foraminifera in summer is one and a half times higher than in winter. According to SIMPER analysis such increase is mostly due to the dominant *Elphidium excavatum* and *Cassidulina reniforme* which, apparently, either reproduce in spring months or react with a rapid growth to the influx of fresh organic matter to the seafloor following the bloom. There are seasonal variations in species composition but they are not dramatic. *Stainforthia fusiformis*, *Nonionellina labradorica* and *Spiroplectammina biformis* are more frequent in summer whereas *Quinqueloculina stalkeri* and *Robertina arctica* occur in winter.

The seasonal change in the high-latitude foraminiferal assemblage, thus, is most evident in the abundance of the dominant taxa, and this change is largely driven by the spring influx of food. Therefore, abundancies of the dominant species in the Late Quaternary record from the Arctic shelves characterize mainly the spring bloom, not the long months of food deficiency.

Keywords: the Arctic, benthic foraminifera, seasonal dynamics

Protein transport machinery for import into complex plastids was horizontally transferred during tertiary plastid endosymbiosis in dinoflagellates

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The endosymbiotic origin of plastids accompanied by extensive horizontal gene transfer from endosymbionts to host nuclei necessitated the development of means targeting proteins back to plastids. In Archaeplastida the TIC/TOC complex facilitates the passage of proteins through the two membranes of the primary plastids. However, all other eukaryotic algae acquired plastids *via* secondary or tertiary endosymbioses that entailed further gain of plastid membranes and therefore required additional translocons to allow protein import. The Sec61 complex for ER transport has been universally employed in the outermost membrane in all known cases but many such plastids have at least one further membrane. The symbiont's ERAD machinery, which otherwise serves for export of misfolded proteins from the ER for degradation, has been repurposed for trafficking plastid proteins inward across the second outermost membrane. This symbiont-specific ERAD-like machinery (SELMA) is present in most plastids of red-algal origin. The only exception is the peridinin plastid of dinoflagellates, which is surrounded by only three membranes. The commonalities in the plastid targeting offered support to the 'Chromalveolate Hypothesis' that argues that one endosymbiotic event could explain plastid presence in five major phyla. However, this hypothesis has been challenged by recent phylogenies and multiple endosymbiotic events are now favored. This presumes either independent origins or horizontal transfers of SELMA machinery between lineages. Dinoflagellates *Karlodinium* and *Karenia* provide a well-supported case of the gain of a plastid from SELMA-utilizing haptophytes and allowed us to test the possibility of SELMA being transferred and reemployed for plastid import during a tertiary endosymbiosis. We found all SELMA sub-complexes, which have clear phylogenetic affinities with haptophytes, and have bipartite plastid-targeting sequences that are otherwise lacking in the cytosolic ERAD paralogues. Thus, these haptophyte-derived plastids show that multiple elements of targeting machineries are transmissible during the establishment of new complex plastids.

Keywords: SELMA, chromalveolate hypothesis, plastid evolution

Genome of *Pandoraea novymonadis*, a recently acquired endosymbiotic bacterium of the trypanosomatid *Novymonas esmeraldas*

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The family Trypanosomatidae is one of iconic groups of protists, known mostly for medically important *Trypanosoma* and *Leishmania*. However, this group also contains extensively studied subfamily Strigomonadinae, the members of which bear intracellular bacteria *Kinetoplastibacterium* spp. The long-term evolution of this symbiosis resulted in metrabolic integration of both partners and synchronization of their cell cycles. Recently we described another trypanosomatid – *Novymonas esmeraldas* bearing an endosymbiotic bacterium *Pandoraea novymonadis*. This association seems to be recently established since i) close relatives of both microbes are not involved in such relationships, ii) bacteria preserve cell wall, iii) division of the partners is not coordinated iv) trypanosomatid employs lysosomes to control endosymbionts and get nutrients. To better understand these relationships we sequenced *P. novymonadis* genome. It revealed typical features for endosymbionts: size reduction, massive gene losses, decreased GC content and lower codon usage bias (as compared to other *Pandoraea* spp.). Meanwhile the genome preserves main metabolic pathways, including synthesis of vitamins and heme, which may be important for the trypanosomatid. However, the bacterium does not produce some amino acids, which are apparently available from the trypanosomatid or insect host, but preserved ability to synthesize many of those for which trypanosomatids are known to be auxotrophic. The comparison of *P. novymonadis* with other symbiotic bacteria suggests that it is a recently acquired endosymbiont whose genome experienced a fast adaptive evolution.

Unexpected diversity of parabasalian symbionts of non-termite cockroaches

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The large, complex, and visually attractive hypermasitigids inhabiting the hindgut of termites and cockroaches of the family Cryptocercidae have attracted most of the interest focused on the cockroach parabasalians for decades. However, little attention has been paid to endosymbionts of "normal" cockroaches, although these insects show a great diversity both in morphology and lifestyles, inhabiting much more diverse habitats than the xylophagous groups. As the complex hypermastigid forms of parabasalians have arisen multiple times independently, an extensive survey of the diversity of parabasalid symbionts of cockroaches in general is crucial for understanding the origin of hypermastigids from "small trichomonads" of cockroaches. We dissected cockroaches from long-term laboratory cultures as well as from freshly brought natural populations. The cockroaches collected from the wild were obtained from several European countries, South America, Africa, Southeast Asia, and Madagascar. We have examined 354 cockroaches belonging to 130 species and 22 subfamilies and sequenced SSU rRNA gene of 120 strains of parabasalids. Majority of the obtained trichomonads nest either within Honigbergiellida, where they form several clades around the recently discovered hypermastigote *Cthulhu*, or within the genus *Hypotrichomonas*. Surprisingly, our strains form multiple novel clades of *Hypotrichomonas* indicating that this genus originated in cockroaches. Moreover, some of the new clades are specific for particular cockroach lineages, the Madagascar hisser cockroach hypotrichomonads being the most striking example.

Keywords: Parabasalia, cockroach, host specificity

Cytoskeletal elements and motility in the archigregarine *Selenidium* sp.: observations on native and drug-treated parasites

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Since apicomplexans represent exclusively parasitic unicellular organisms with medical and economic impact, the principles of their motility and host cell invasion have been studied intensively. Ancestral apicomplexan lineages, including gregarines, represent organisms suitable for research on motility evolution and diversification. Archigregarines are restricted to a marine environment and, based on their plesiomorphic characters, represent basal group of gregarines and perhaps apicomplexans as whole. The vermiform trophozoites of *Selenidium* archigregarines possess apical complex and exhibit pendular, rolling or coiling movement. The presented study revealed the fundamental role of cytoskeletal proteins, such as actin and α-tubulin, in archigregarines motility, and allowed us to compare the mechanism of their movement to the gliding machinery (= the so called glideosome concept) described in apicomplexan zoites. Motility of *Selenidium* sp. parasitising the intestine of polychaete *Pygospio elegans* (Spionidae) was investigated using probes influencing polymerisation of cytoskeletal proteins. To verify the role of actin filaments, jasplakinolide (induces actin polymerisation) and cytochalasin D (blocks polymerisation of actin microfilaments) were applied. The effect of disruption of subpellicular microtubules on motility was monitored after treatment with oryzalin and colchicine (both probes destroy existing microtubules and inhibit tubulin polymerization). Despite we succeed to completely block the motility of archigregarines, our experiments revealed the tolerance of organisms to cytoskeletal drugs' concentrations routinely used in other apicomplexans and their prolonged survival in extreme conditions. Evaluation of *Selenidium* sp. surface observation did not show any damages or abnormalities of pellicle after drugs' applications. Though, evident changes in distribution of cytoskeletal proteins after confocal laser scanning microscopic analysis were revealed. Oryzalin and colchicine showed the highest impact on archigregarine movement, suggesting that subpellicular microtubules play the main role among motor components, facilitating the pendular motility of trophozoites.

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Keywords: Apicomplexa, gregarine, motility, actin, α-tubulin

Exploring 10 years of marine microbial interactions

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The oceans are populated by a multitude of microbes, both eukaryotes and prokaryotes, but our knowledge of their ecological interactions is still limited. To help shed light on important long-term interactions we have constructed microbial association networks from marine plankton samples taken every month for 10-years in the North-Western Mediterranean (Blanes Bay Microbial Observatory). From the 120 samples we sequenced 18S and 16S rDNA amplicons from two different size fractions (pico-plankton [0.2-3mm] and nano-plankton [3-20mm]) and recorded 18 environmental variables. We calculated positive and negative correlations between Operational Taxonomic Units (OTUs) from the amplicons with eLSA (extended Local Similarity Analysis). The association network from OTUs present in at least 10% of the samples (2826 eukaryotes and 2856 bacteria) was highly connected with 2 947 606 significant edges ($p<0.0001$; fdr corrected), a mean number of node neighbours of 1066.4 and average clustering coefficient (C) 0.77. The clustering coefficient is considerable larger than for a random network of the same size (C=0.19). Almost all edges were positive showing co-occurrences between OTUs, while only 1 % was negative indicating mutual exclusions. In order to find OTUs involved in direct symbiotic interactions and not only covarying with ecological changes we are identifying OTUs that are most likely occurring together due to shared environmental preferences by calculating the mutual information between all triplets of OTUs and environmental variables in the network. In addition, we are building a database of known microbial interactions based on published literature. The database will be used to identify edges of known interactions, validating co-occurrences as symbiotic interactions, as well as help us uncover novel interactions. Further work on network deconvolution will let us predict important long-term ecological interactions and increase our knowledge of the marine microbial interactome.

Keywords: interactions, networks, symbiosis, time-series, databases

Thermal adaptation and evolutionary rescue of *Paramecium* microcosms

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Environmental change is probably the most common cause of biological extinctions. At the same time, it forces organisms to adapt to novel environments thereby preventing them from extinction. Evolutionary Rescue (ER) provides the framework to study the capacity of populations to recover from an initial population decline through genetic adaptation. While such evolutionary adaptations can occur very rapidly within few tens of generations, the success of ER and the evolutionary trajectory of the population strongly depend on the rate of environmental change. The freshwater ciliate *Paramecium caudatum* seems to hold the ability for temperature adaptation as we can find thermal generalist and specialist populations in nature. Within the presented study we investigated how different rates of temperature increase (from 23 °C to 32 °C) affect population persistence and evolutionary change in experimental microcosms of *P. caudatum*. Here we found that those populations experiencing the slowest rate of temperature increase were the least likely to become extinct and tended to be the best adapted to the new temperature environment. After about 200 asexual generations, all high-temperature populations had superior growth rates at optimum temperatures and were more tolerant to severe heat stress (35 °C, 37 °C), but showed reduced growth at low temperatures (5–9 °C). This shift and alteration of the temperature niche indicates a trade-off between high- and low-temperature tolerance and can only partly be explained by selection from standing variation of six initial founder genotypes. While we detected complete genetic divergence between control and high-temperature populations, the observed increased resistance to lethal heat stress beyond the maximum selection temperature (32 °C) is indicative for adaptive evolution. Our results support theories of thermal adaptation and confirm basic predictions of ER by illustrating how adaptation to an extreme local environment can produce positive as well as negative correlated responses to selection.

Keywords: experimental evolution, local adaptation, phenotypic change, selection, standing variation

A revision of the Himatismenida (Amoebozoa): cell coat evolution and scale structure paradoxes in *Cochliopodium*

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The order Himatismenida (families Cochliopodiidae with *Cochliopodium* and *Ovalopodium*; and Parvamoebidae with *Parvamoeba*) comprises unusual discoid amoebozoans enclosed in an organic cell coat located only on the dorsal surface of the cell when it adheres to the substratum. The genera of Himatismenida are distinguished by the structure of their dorsal cell coat which is most complex in *Cochliopodium* where it is represented by a layer of complex scales (tectum). *Cochliopodium* is currently the most diverse himatismenid genus. In this communication we summarize recent findings on taxonomy and phylogeny of the Himatismenida based on the light microscopy, scanning and transmission electron microscopy together with molecular phylogenetic analysis of SSU rRNA and COI genes. Description of several novel phylogenetic lineages of *Cochliopodium* and related genera provides new insights into the cell coat evolution and taxonomy of Himatismenida. In particular, discovery of more *Cochliopodium* species requires a more complex model of scale evolution than the simple idea that different scales correspond to different morphospecies. Our evidence suggests that completely different scale types may be present in pairs of strains showing almost no differences in gene sequences (like *C. minus* and *pentatrifurcatum*). Some lineages show a shift from one scale type to another within a single clonal culture. Finally, in at least one new lineage two completely different scale types are simultaneously present in the same clone and sometimes in the same cell. This is not a culturing artifact as this phenomenon has been recorded in at least two independently isolated strains and in amoebae freshly picked from nature. In addition, recently isolated strains closely related to *Ovalopodium desertum* and not belonging to *Cochliopodium* have a dorsal layer of flat, oval scales indicating that the scaly cell coat has evolved at least twice in Himatismenida.

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Keywords: Amoebozoa, *Cochliopodium*, scale structure, evolution, taxonomy

Phylogenomics and ancestral state reconstruction of arcellinid shells illuminates eukaryotic evolution in the Neoproterozoic

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Shelled microbes are central tools in understanding the evolution of deep eukaryotic lineages, because these organisms leave a consistent and well-documented fossil record. However, thorough analysis of these fossil taxa is hampered by a lack of robust phylogenetic frameworks. The Arcellinida are a species-rich lineage in Amoebozoa, and many Neoproterozoic fossils can be attributed to it. Here we combine modern phylogenomic methods to generate a robust phylogenetic framework for the Arcellinida with ancestral state reconstruction methods to make inferences on the early evolution of shell bearing eukaryotes. We have generated transcriptome sequences from 20 arcellinid taxa, that represent the whole sum of known major lineages in the group. These data were analyzed through a phylogenomic pipeline to construct a robust deeply sampled tree based on ~350 genes and 100,000 amino acid sites. The resulting tree reveals a monophyletic Arcellinida, with nine well-defined deep lineages, of which only two had been previously identified. Ancestral state reconstruction for the hypothetical ancestors of these nine lineages yields morphologies that are strikingly similar to the microfossils described in the Neoproterozoic for the Chuar Group (~750mya) and the Urucum Formation (889 ± 44 – 587 ± 7mya). These findings demonstrate that arcellinids rapidly diversified after the major global atmospheric event of ocean oxygenation (~850mya). These data illustrate that the major lineages of Arcellinida evolved before and were well established before the great glaciations of the Cryogenian period (710mya).

Keywords: Arcellinida, phylogenomics, ancestral state reconstruction, vase shaped microfossils, Neoproterozoic

Microbial diversity on Heron Island Reef, Australia – impacts of coral bleaching on benthic ciliates

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Field and laboratory studies conducted at the Heron Island Research Station (HIRS) located in the Capricorn region of the Great Barrier Reef revealed complex protistan communities. Heron Reef, home to about two-thirds of the coral species found on the GBR, has experienced repeated bleaching episodes in the past decade. This study, with comparisons to similar observations in 2006, emphasizes the diversity of ciliates and diatoms found in reef sediments and examines ecological changes. Observations included the interactive role of the protistan component of a coral reef, in particular the dynamics of opportunistic and/or potentially pathogenic species associated with damaged corals undergoing disease conditions such as Brown Band Disease, including *Porpostoma* sp. These altered communities are characterized by rapid tissue breakdown in hard corals, coupled with the release of high levels of dissolved organic matter. Benthic samples were taken by direct capture, observed with phase contrast and epifluorescence microscopy, recorded by video and photomicrography, and fixed for further identification and genomic assessment. The relative abundance of diatoms/flagellates/ciliates appeared to show a higher proportion of photosynthetic species as compared to previous observations. Macroalgae were also examined for epiphytic protista. The Brown Band ciliate, *Porpostoma* sp., was not found on the corals, in the sediments, on algal surfaces, or in the plankton prior to bleaching in the 2016 samples in late January, posing the question of their location when not acting as opportunistic pathogens. Photographic and video images are being assembled into online reference libraries that will be available for future HIRS researchers and other coral reef scientists, accompanied by field notes and locational information.

Keywords: marine ciliates, microbial diversity, Brown Band Disease, Great Barrier Reef, *Porpostoma* sp.

Diversity, environmental distribution, and molecular variability of the bacterial endosymbiont "*Candidatus* Megaira" widespread in ciliates and other protists

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In the last years, the recently described bacterial genus "*Candidatus* Megaira" (*Rickettsiales*) caught the attention of many researchers from different fields. This genus comprises obligatory intracellular bacteria, which are closely phylogenetically related to the pathogen *Rickettsia*. Many diverse organisms have been colonized by bacteria of the genus "*Candidatus* Megaira", both unicellular (e.g. ciliates, amoebae, and algae), but also multicellular (cnidarians, porifers, plants, and worms).

Herein, we report the multidisciplinary description of a novel "*Candidatus* Megaira" species inhabiting the cytoplasm of *Paramecium bursaria* and *Paramecium nephridiatum*, together with the characterization of several new isolates of the type species *"Candidatus* Megaira polyxenophila" in different ciliate species. Our ultrastructural investigations showed that these bacteria display variations in dimensions like other members of *Rickettsiaceae*; and the novel species presents polymorphic features such as the presence of flagella, and the predilection of being closely associated to endosymbiontic algae of *Paramecium bursaria*. Moreover, we performed several trans-infection experiments in order to understand how this bacterium is spread in the aquatic environment, how it influences the host fitness within the same population of ciliates, and how it interacts with other symbiotic bacteria already present or invading the same host cell.

In addition, we also screened 16S rDNA amplicon databases to see host and environmental distribution of members of the *"Candidatus* Megaira" genus. We propose an updated phylogeny of the genus based both on phylogenetic analysis and 16S rRNA gene diversity, thus subdividing the genus in four distinct and separated species.

All together our findings give support to the hypothesis that this poorly investigated bacterium may be transmitted by protists in aquatic ecosystems.

3D structural analysis of a colony-forming choanoflagellate

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Choanoflagellates are the closest unicellular relatives of the animals (Metazoa). The marine choanoflagellate *Salpingoeca rosetta* is capable of transitioning through colonial life stages, and is therefore used as a model organism to study the origin of metazoan multicellularity. It is currently unclear as to whether cellular ultrastructure differs across *S. rosetta* cell types. In the present study, we used fluorescent vital staining and serial transmission electron microscopy (TEM) sectioning to visualise and 3D reconstruct the microanatomy of unicellular and colonial *S. rosetta* cells. These structomic reconstructions enabled the enumeration and volumetric quantification of the choanoflagellate ultrastructure. Fluorescent vital staining was congruent with serial TEM reconstructions, providing good evidence that 3D models presented herein are biologically representative. Colonial cells exhibit more pseudopodia and phagosomes than observed in single cells, resulting in a more amoeboid morphology. Colonial cells also display asymmetric and disconnected intercellular bridges, suggesting inheritance of the electron-dense septum. In addition, *S. rosetta* possesses morphologically-distinct intracellular vesicle populations. We have also identified extracellular vesicles associated with the microvillar collar. The implications of these findings in relation to the origin and evolution of metazoan multicellularity will be discussed. These data represent the first 3D structural analysis of any choanoflagellate species, which will help to better illuminate the physical constraints and modifications of metazoan multicellularity.

The obscure group Hemimastigophora is a 'novel' super-kingdom-level lineage of eukaryotes

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Our understanding of the diversity and phylogeny of eukaryotes has been transformed in the last 15 years. Almost all 'candidate major lineages' known at the turn of the century have now been placed within a handful of 'supergroups' using molecular phylogenetics. Hemimastigophora is probably the most distinct known lineage still awaiting molecular phylogenetic analysis. These cells are free-living, with two rows of flagella and a peculiar cytoskeletal ultrastructure. Given this, Foissner and colleagues (1988, 1993) ranked Hemimastigophora as a phylum when defining the group. 'Hemimastigotes' have been rarely observed and are eukaryotrophs, which complicates cultivation. We instead used single-cell methods to obtain high-quality transcriptomes of *Hemimastix* and *Spironema* species from soil. Unexpectedly, we were later able to culture the *Hemimastix* species. We detected hemimastigotes in existing soil, freshwater and marine environmental SSU sequence datasets, but they do not correspond to a well known clade of environmental sequences. A 351-gene phylogenomic analyses placed hemimastigotes outside all established eukaryote supergroups; they instead formed an extremely deep branch in the 'Diaphoretickes' half of the eukaryote tree. Additional analyses with increased taxon sampling, removal of fast-evolving taxa, and fast-evolving sites confirmed this deep position. The 'Phylum' rank of Hemimastigophora actually understates their phylogenetic distinctiveness, and they are a crucial lineage for understanding the deep-level evolutionary history of eukaryotes, potentially down to the 'root' of the eukaryote tree.

Keyword: phylogenomics

Unicellular holozoans shed light on the evolution of programmed cell death machinery during the emergence of multicellularity

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Programmed cell death (PCD) involves the death of specific cells in a controlled manner, in response to extrinsic or intrinsic triggers. It is an essential function in animals, where it regulates normal development, responses to cell damage, and cell turnover. Accordingly, the PCD machinery present in the unicellular ancestors of animals was likely critical to the appearance of complex multicellularity in animals. PCD appears to be widespread in eukaryotes, including *Chlamydomonas*, *Thalassiosira*, *Emiliania*, *Tetrahymena* and *Dictyostelium*; however, its molecular basis in these organisms is poorly understood.

Filastereans, ichthyosporeans, corallochytrids and choanoflagellates are the closest unicellular relatives of animals; they exhibit simple multicellularity during some stages of their life cycle. As a result, they present an excellent opportunity to understand the elements of the PCD repertoire that were present in the unicellular ancestors of Metazoa, and the functional and regulatory changes that they might have undergone during the emergence of animal multicellularity. Using a comparative genomics approach, we have examined known PCD-related proteins and protein domains across Opisthokonta. We show that the best-known animal PCD pathway, apoptosis, is a true animal innovation, but that both animal-specific and more widely conserved elements of PCD machinery were already present prior to the emergence of animals.

Keywords: programmed cell death, apoptosis, multicellularity, Holozoa

Foraminiferal diversity and their environmental implications in the Yellow Sea

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Diversity and the environmental implications of benthic Foraminifera in the continental shelf of the Yellow Sea were studied. The temporal dynamics of foraminifera from tidal flat and spatial distributions of Foraminifera from the Yellow Sea were investigated. Temporally study revealed a unimodal-type seasonal dynamics of Foraminifera in the tidal flat. Spatial study indicated that the community composition and distribution of continental Foraminifera were significant controlled by environmental temperature, salinity and depth. Four typical functional groups were recognized including intertidal fauna, offshore shallow water fauna, cold-water-mass fauna and Yangtze estuary fauna. Based on the temporal and spatial studies and statistical analysis results, in comparison with the season, habitat was supposed the more significant contributory factor in regulating benthic foraminiferal faunas in the Yellow Sea.

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Keywords: Foraminifera, diversity, environment, seasonal variation, ecology

Persistent biogeographic patterns of tropical free-living and parasitic protists

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Animal and plant communities in tropical rainforests are known to change quickly over relatively short distances, but the species turnover remains relatively low over larger scale. To evaluate if protists have similar biogeographic patterns, soils were sampled in three Neotropical rainforests and sequenced for the V4 region of 18S-rRNA. Taxa-area relationship (TAR) and distance-decay relationship (DDR) were estimated by fitting linear models in a logarithmic space. Both biogeographic relationships were compared to null model expectations in order to assess their non-randomness. TAR slopes were significant within and between forests for both OTU- and phylogeny-based diversity indexes, with significant steepest slopes within forests. However, most slopes within forests were not different from the null model expectations. DDR slopes were only significant and non-random between forests. These TAR and DDR patterns were largely persistent across the dominant protist taxa (Apicomplexa, Cercozoa and Ciliophora) and their most abundant and widely distributed subtaxa, suggesting that free-living and parasitic protists have similar biogeographies. These results showed that protists within Neotropical rainforests are so hyperdiverse that their community dissimilarity is extremely high regardless if they are sampled a hundred meters or a few kilometers apart. The comparison to null models revealed that there were more forest specific OTUs and clades than expected by chance, thus highlighting that macroecological processes such as dispersal limitation and speciation shaped the Neotropical forest protists communities at the regional scale, just as observed for animals and plants.

Keywords: biogeography, tropical forest, parasite, free-living, metabarcoding

Evolutionary tendency in chromatophore genome of *Paulinella* during endosymbiotic organogenesis

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The thecate filose amoeba *Paulinella chromatophora* is a good model organism for understanding plastid organellogenesis because it has two sausage-shaped plastids termed chromatophores that were derived from an alpha-cyanobacterium. The primary endosymbiosis of *P. chromatophora* occurred relatively recently (90-140 Mya), whereas the primary endosymbiosis that gave rise to the Archaeplastida occurred before 1,500 Mya. This event was completely distinct from the major plastid organellogenesis, or primary plastid endosymbiosis. Up to date, three different photosynthetic *Paulinella* has been reported; *P. chromatophora, P. micropora,* and *P. longichromatophora.* In this study, we sequenced chromatophore genome of *P. longichromatophora;* the genome was 979,356 bp in total length, the GC content was 38.8%, and 915 genes were annotated. Comparative genomic analysis with *Synechococcus* species and other photosynthetic *Paulinella* showed several genomic changes during endosymbiosis. Genome size shrank from ~2.5 Mb to ~1 Mb and A+T content has been elevated. Functional category of gene contents showed that the portion of environmental information processing and unclassified genes were lower in chromatophore genome. Differential gene loss was documented in chromatophore genomes. Orthologous gene set of *Synechococcus* species were compared with chromatophore genome of *Paulinella* in functional group category, and similar patterns were found in the GC content and dNdS values. These results could provide genetic clues concerning early plastid establishment.

Keywords: *Paulinella longichromatophora*, genome, endosymbiosis, chromatophore, organellogenesis

Transfection and expression by cell-penetrating peptide in choanoflagellate

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Hitherto no reliable and efficient method for transfection of choanoflagellates, the closest relatives of metazoans, was available. Here we present cell-penetrating peptides (CPPs) as an alternative. CPPs have been applied as a useful carrier to deliver cargos into metazoan cells over the last decade. In a series of experiments with the choanoflagellate we proofed for the first time that transfection of both, plasmid DNA and siRNA, with CPP is a reliable method. The uptake of labeled CPP into choanoflagellate and the expression of a fluorescent protein plasmid were monitored by fluorescence microscopy. In addition, we were able to silence a transporter gene and hence manipulate the morphological cell, which was proved by electron microscope. High gene expression efficiency was determined by fluorescence microscopy. In addition, no cytotoxic effects of CPP were detected using propidium iodide staining. Our new method allows the reliable and efficient transfection of choanoflagellates, finally enabling us to verify the function of genes. E.g., we would be interested to study gene function involved in colony formation by silencing them via siRNA as a step stone for the research on the origin of multicellularity in metazoans.

Phylogenomics reveals new insights into the pre-metazoan genetic tool-kit by using single-cell amplified genomes of uncultured choanoflagellates

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Holozoa is the eukaryotic group composed by animals and their protist relatives. The genomes of the unicellular holozoans so far sequenced have revealed an unexpected repertoire of genes related in multicellular functions on those taxa. Additionally, new transcriptomic data from choanoflagellates, have increased such repertoire. However, in environmental studies based in the sequencing of the 18S gene, choanoflagellates, the closest unicellular relatives to animals, appear as the most diverse unicellular holozoan clade. Thus, there is still a lot of choanoflagellate genomic information awaiting to be discovered; which might help to better reconstruct the genetic toolkit of the animal unicellular ancestor. Therefore, in this work, we shed new light into this choanoflagellate gene repertoire by sequencing 4 single-cell amplified genomes of uncultured choanoflagellates, one of them being the third most abundant choanoflagellate in TARA oceans database. Two of the SAGs (UC2, UC3) presented extremely low genome completeness, although for one of them (UC2) we recovered its mitochondrial genome. For the two remaining SAGs we recovered 20–30% of genome completeness. We, first, aimed to improve the holozoan tree of life, especially the relationships within choanoflagellates, using a taxon-rich phylogenomic dataset that included 39 unicellular holozoan genomes and transcriptomes. We recovered a well-supported choanoflagellate phylogeny, with *Codosiga hollandica* as the earliest branching choanoflagellate, being SAGs UC4, UC1 the earliest branching lineage of Acanthoecida and Craspedida clade I, respectively. The other SAGs UC2 and UC3 fall within Acanthoecida. Moreover, our phylogenomic analysis supports the monophyly of Teretosporea as the earliest-branching Holozoa clade. Finally, SAGs revealed protein domains thought to be metazoan specific, such as NPM1-C, C-terminal of the Nucleophosmin TF, which is related to apoptosis and gametogenesis in animals. Overall, our results have allowed to have a clearer picture of the holozoan phylogeny as well as to infer a more complete premetazoan genetic toolkit.

Arctic perils: Northern Baffin Bay as a Janus gateway

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Northern Baffin Bay, between Greenland and Ellesmere Island (Canada) is considered an oceanographic gateway with net flow from the Arctic to the North Atlantic influencing North Atlantic nutrient cycles, and timing and magnitude of the spring bloom. The increasing northern penetration of Atlantic origin waters along the West Coast of Greenland could alter the nature of this gateway, which has been regarded as a benign net flow of fresher phosphate rich waters into the North Atlantic system. Recent synoptic trends indicate changes in the timing and areal extent of surface chlorophyll concentrations, but little is known about the associated species composition. Here we amplified the V4 region of the 18SrRNA gene and 18SrRNA using high throughput sequencing (HTS) to compare mid-August marine microbial eukaryote populations at bottom of the Polar mixed layer (20 m) at the same latitude from two sides of Northern Baffin Bay. There were significant differences in species composition, with high species variability on the Canada side and mostly *Pseudo-nitzschia* spp. abundant on the Greenland side. Network analysis of diatom interactions with other microbial eukaryotes showed the predominance of positive interactions on the Canadian side and a mix of interactions on the Greenland side including potential antagonism between some ciliates and diatoms. Overall the differences between the two sides show that Baffin Bay is not a single eco-region, with the eastern side of Baffin Bay being the more benign. The other side of the gateway with potentially toxic *Pseudo-nitzschia* spp., suggest a need for increased monitoring of species trends, in light of the large marine mammal and bird populations that migrate in and out of the area. These two faces of the Northern Baffin Bay suggest a *Janus* Gateway with the two sides having vastly different characteristic species and ecological networks.

Keywords: Arctic ocean, ciliates, microbial networks, microbial-biogeography, *Pseudo-nitzschia*

Comparative analysis of stramenopile genomes reveals processes of genomic reduction in *Blastocystis hominis*

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Blastocystis hominis is an intestinal pathogen of humans associated with Irritable Bowel Syndrome, with the smallest known stramenopile genome. To understand the evolutionary causes for its size and to examine potential adaptations for pathogenicity, we conducted a comparative analysis of stramenopile genomes.

In the absence of effective out-groups for comparison, we produced a genome containing 28,067 genes for *Proteromonas lacertae*, the closest known relative of *Blastocystis* and an intestinal commensal, and a transcriptome containing 18,439 transcripts for *Cafeteria roenbergensis*, a free-living stramenopile, which allowed us to establish character states in the ancestor of *Blastocystis* spp.

Our analyses show that *B. hominis* has lost all flagellum components otherwise conserved in Stramenopiles including dyneins and kinesins. Besides motility, we observed no other losses of major cell function. Rather, we show reduced diversity of genes associated with adhesion (EGF domains), protein interactions (WD domains) and metabolism.

Overall, evolution of the *B. hominis* genome has involved a streamlining of genomic complexity. Resulting in a reduction in the number of genes compared to other Stramenopiles. We analysed the phylodiversity of conserved gene families of diverse functions and found that *B. hominis* routinely retains fewer ancestral gene lineages than other Stramenopiles. We suggest that this gene loss, combined with an expansion of *Blastocystis* specific proteins, reflects a history of increasing dependency on the gut mucosa.

The molecular basis of *Euplotes* cell-cell union in mating pairs: insights from pheromone crystal structures

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Euplotes synthesizes and constitutively secretes species-specific families of protein pheromones, each encoded by one of a series of co-dominant alleles at the *mat* genetic locus and acting as both autocrine signal for the cell vegetative growth and paracrine inducer of cell-cell union in mating pairs. The structures of a number of *E. raikovi* pheromones have been determined by solution NMR spectroscopy, and the structure of the pheromone E*r*-1 was solved also by X-ray crystallography. Since the soluble pheromone of each cell type finds a structural counterpart with its membrane binding site—both the molecules being encoded by the same gene—the protein-protein interactions in the crystal have been assumed to mimic those which cells utilize as trigger to unite with one another in mating pairs. We have now re-determined the crystal structure of E*r*-1, confirming the previous results, and determined the crystal structure of a new pheromone, E*r*-13. In both cases, we applied non-conventional *de novo* structure determination methods that exploit the high-resolution of the collected diffraction data (0.7 Å, E*r*-1; 1.4 Å, E*r*-13). The E*r*-13 structure has the same up-down-up three-helix fold as the E*r* pheromone family, including E*r*-1. In spite of this and 45% sequence identity between E*r*-1 and E*r*-13, the arrangement of the molecules in the crystals and the resulting intermolecular contacts differ markedly, which is reflected in the different crystallographic space groups C2 for E*r*-1 and $P4_1$ for E*r*-13. These differences in the crystallization patterns between structurally homologous pheromones suggest that the determination of other E*r* crystal structures is necessary for a general rationalization of the molecular mechanism that *E. raikovi* cells utilize to unite in mating pairs.

Keywords: cell-cell signalling, protein pheromones, crystal structure, ciliate molecular biology

Functional analysis of an atypical Translocase of the Outer Membrane (TOM complex) of the hydrogenosomes of *Trichomonas vaginalis*

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All forms of mitochondria evolved from a common bacterial endosymbiont and the origin of protein import is tightly linked to the transition of the endosymbiont to an organelle. Despite the presence of a universal protein import pathway, the molecular machines mediating the translocation exhibit remarkable diversity. Here, we have identified and analyzed the components of the Translocase of the Outer Membrane (TOM complex) of the hydrogenosomes, an anaerobic form of mitochondria in *Trichomonas vaginalis*. TvTom40, a β-barrel protein that forms the core translocation-channel is present in a high molecular weight complex of ~500-600kDa. As revealed by electron microscopy studies, the translocase has a diameter of around 140-150Å and can form three channels. The fact that this feature is conserved in both classical mitochondria and hydrogenosomes indicates that the Tom40 of the protomitochondrion in the early eukaryote was trimeric in nature. Proteomic analysis of the immunoprecipitated TOM complex revealed the presence of four TvTom40-associated proteins - TvHomp36, TvHomp46, TvHomp38 and TvHomp19. The former three have a cytosolic N-terminal Hsp20-like domain and three tetratricopeptide repeat (TPR)-like domains and a C-terminal transmembrane helix. Their domain architecture resembles the recently reported TOM receptor, ATOM69 in *Trypanosoma brucei*. Investigation via *in vitro* preprotein import assay and co-immunoprecipitation revealed the interaction of a translocation-arrested hydrogenosomal matrix protein with TvTom40 along with TvHomp36 indicating that these two proteins are involved in protein import and perhaps, TvHomp36 may serve as a receptor. Our results show that hydrogenosomal TOM complex in *T. vaginalis* consists of multiple isoforms of conserved Tom40 porins, unique composition of putative receptors and other Tom40-associated proteins.

Keywords: TOM complex, protein import/translocation, hydrogenosomes, *Trichomonas vaginalis*, mitochondrial biogenesis and evolution

Protist metagenomics in the mega city of New York

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Protists are important components of terrestrial and aquatic environments, as well as animal and human microbiomes. Despite their ubiquity, they receive scant attention in high-throughput environmental surveys compared to other microbes. With 8 million inhabitants, New York City (NYC) is the most populous and cosmopolitan metropolis, and a major international gateway city into the USA. It operates the most extensive mass transit system, has a world-renown unfiltered water supply, and pumps 1.5 billion gallons of wastewater through 7,400-miles of sewer pipes daily. This vast ecosystem is full of microbes, and an ideal locale for urban microbiome studies. As a part of our "Mapping the New York City Microbiome" project, we have used environmental metagenomics (shotgun metagenomic and amplicon sequencing of the 18S rRNA marker gene) to characterize and map microbial communities across NYC including those on circulating paper currency, ATM buttons, and soil of contaminated community gardens. Most recently we have examined protists in raw sewage. Sewage samples were collected over a 12-month period from 14 treatment plants in all five NYC boroughs, and compared with samples from other NYC environments including: soil from parks and green spaces, storm water, and sediment. Sewage contained a diverse protist community dominated by free-living clades, and communities were highly differentiated across environmental samples. Protists typically associated with human and animal guts or feces, for example multiple species of *Entamoeba*, *Blastocystis* and trichomonads, were frequently detected underscoring sewage as a valuable biomarker for monitoring urban microbes. We are currently expanding our study to investigate parasites in human pets (cats and dogs) and pests (rats, pigeons, cockroaches) in NYC, with the eventual goal of establishing a baseline protist microbiome of the city.

Keywords: New York City, urban microbiome, sewage, microbial ecology, environmental sequencing

An obligate kleptoplastic phototrophy of a euglenoid *Rapaza viridis*

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Rapaza viridis Yamaguchi *et al*. 2012 was originally described as a mixotrophic alga that preys exclusively on a specific strain *Tetraselmis* sp. We were primarily interested in *R. viridis* for their CPE catabolism, the process catabolizing chlorophylls into non-phototoxic cyclopheophorbide enols (CPEs). Unexpectedly, however, *R. viridis* exhibited only trace amounts of CPE productions along with "phycophagy" on *Tetraselmis* cells, and no substantial generation of degradation products of chlorophylls were evident in any stage after predation. In addition, unlike typical phycophagic euglenoids, degradation of chloroplasts was not observed within cells of *R. viridis*. In a quantitative experiment, total chlorophylls were not significantly changed after the predation and only slightly reduced in the later stage; furthermore, total chlorophylls per cells of *R. viridis* during the stationary phase were unchanged through time, and so was chlorophyll *a*/*b* ratio. These evidences indicated that chlorophylls of ingested *Tetraselmis* had not substantially been altered in cells of *R. viridis*, hence implying retention of photosynthetic machinery of *Tetraselmis* intact. In optical and electron microscopic observations, interestingly, the ingested chloroplasts were shown to become subdivided into many pieces 8-14 hours after predation, which was followed by cell divisions of *R. viridis* where the subdivided chloroplasts were distributed to the daughter cells. Along with the division event, serial losses of typical features of the chloroplast of *Tetraselmis* were apparent, such as dispersant and eventual loss of intraplastidial eyespot globules, reduction of starch grains, and diminishment of *Tetraselmis*-type pyrenoids, thus the *Tetraselmis*-type chloroplast being indistinguishable from the *Rapaza*-type unlike the original description. We infer absence of proper chloroplast of *R. viridis* and kleptoplastic origin of all chloroplasts retained therein. Furthermore, we measured photosynthetic activity of *R. viridis* and compared with that of *Tetraselmis*; the results indicated significant level of photosynthetic capacity of kleptochloroplasts, if so, was sustained in cells of *R. viridis*.

Keywords: kleptochloroplast, phagocytosis, chlorophyll catabolism, photosynthesis

Contribution of chlorarachniophytes to the chlorophyll a synthesis in green-colored dinoflagellates

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The plastid proteome of dinoflagellates experienced plastid replacement comprises proteins with diverse origins, namely those (i) acquired from the endosymbiont ('endosymbiont-derived proteins'), (ii) inherited from the ancestral dinoflagellate beyond plastid replacement ('vertically-inherited proteins'), and (iii) gained from organisms related to neither host nor endosymbiont lineage ('laterally-acquired proteins'). In this study, we investigated the degree of the chimerism in the chlorophyll (Chl) *a* synthetic pathway in *Lepidodinium chlorophorum*, and two undescribed dinoflagellate strains TRD-132 and MRD-151, which experienced plastid replacement initiated by green algal endosymbiosis. We here surveyed the transcriptomic data of these species to identify genes encoding the proteins involved in the Chl *a* synthesis, and identified all the proteins required to convert protoporphyrinogen IX to Chl *a*, except Mg-protoporphyrin IX monomethyl ester (oxidative) cyclase, in the three green-colored dinoflagellates. As expected, our phylogenetic analyses detected all of the three protein types, i.e. endosymbiont-derived, vertically-inherited, and laterally-acquired proteins, in the pathway of interest. However, to our surprise, the proteins most likely acquired from chlorarachniophytes appeared to involve in four out of the five steps in the Chl *a* synthesis examined here. Such gene transfer from a specific lineage, which is closely related to neither host nor plastid lineage of the green-colored dinoflagellates, was not observed in plastid-localized pathways for the isopentenyl diphosphate and heme syntheses. We need to expand our analysis to other metabolic pathways localized in the plastids to judge whether the contribution of chlorarachniophyte-derived genes is restricted to the Chl *a* synthesis.

Keywords: plastid replacement, chlorophyll a synthesis, lateral gene transfer

Experimental evolution of a photosynthetic endosymbiosis

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Photosynthetic endosymbioses are widespread in aquatic microbial communities, yet their evolution is poorly understood. Symbiotic interactions between heterotrophic protists and algae range from loose facultative associations to mutually dependent obligate partnerships, but we know little of the transitions between these two extremes. Here we report on experiments using the model protozoan *Paramecium bursaria* and its algal endosymbiont *Chlorella*. By examining intra-specific variation in host-symbiont reaction norms in response to light, we show that some host strains are more obligately symbiotic and that this may be associated with a significant fitness cost. We show that host-control of symbionts is important for hosts to exploit the benefits of symbiosis. During experimental evolution, we found that varying horizontal versus vertical transmission drove evolution of host-symbiont reaction norms, and suggested again that for *Paramecium*, transitions towards obligate symbiosis may be detrimental to host fitness. These results have implications for understanding the evolution of photosynthetic endosymbiosis, and may help to explain the prevalence of facultative endosymbionts found in nature.

Keywords: endosymbiosis, experimental evolution

Rethinking how temperature affects the growth rate of free-living protists

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Environmental temperature is possibly the most important abiotic driver of protistan growth, and thus has a major influence on their role in ecosystems. Likewise, in applied research, temperature is an easily controlled variable in productivity optimisation. Accurately constructing temperature-growth responses has, therefore, been and continues to be the focus of a large body of protistan research. However, underlying much of these fields of research is a tacit assumption, based on enzyme thermo-kinetics, that *per capita* growth rate increases exponentially with temperature (over a defined range), following an Arrhenius (or Q_{10}) response. This assumption is applied, even in the face of a large body of contradictory evidence. We see that it is timely to revisit this issue. Using a meta-analysis approach, we explore the appropriateness of assuming an exponential function. To do so, we examine data from ~30 studies, covering ~100 strains or species of ciliates, flagellates, and amoebae. Using non-linear curve fitting methods to fit functions, we evaluate the shape of responses and assess which models best represent the data using AIC methods. In doing so, we offer alternative, mechanistic explanations for the temperature-growth responses of protists, ones that may be generally applicable to ecological and industrial studies.

Keywords: temperature, ecophysiology, thermal response, growth rate, population biology

Surface ocean metabarcoding confirms limited diversity in planktonic foraminifera but reveals unknown hyper-abundant lineages

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Since the advent of DNA metabarcoding surveys, the planktonic realm is considered a treasure trove of diversity, inhabited by a small number of abundant taxa, and a hugely diverse and taxonomically uncharacterized consortium of rare species. Here we assess if the apparent underestimation of plankton diversity applies universally. We target planktonic foraminifera, a group of protists whose known morphological diversity is limited, taxonomically resolved and linked to ribosomal DNA barcodes. We generated a pyrosequencing dataset of ~100,000 partial 18S rRNA foraminiferal sequences from 32 size fractioned photic-zone plankton samples collected at 8 stations in the Indian and Atlantic Oceans during the *Tara* Oceans expedition (2009–2012). We identified 69 genetic types belonging to 41 morphotaxa in our metabarcoding dataset. The diversity saturated at local and regional scale as well as in the three size fractions and the two depths sampled indicating that the diversity of foraminifera is modest and finite. The large majority of the newly discovered lineages occur in small size fraction, neglected by classical taxonomy. These unknown lineages dominate the bulk [>0.8 µm] size fraction, implying that a considerable part of the planktonic foraminifera community biomass is produced by unknown lineages.

Keywords: metabarcoding, planktonic foraminifera, MOTUs, molecular nomenclature

The new red algal subphylum Proteorhodophytina comprises the largest and most divergent plastid genomes known

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Red algal plastid genomes are often considered ancestral and evolutionarily stable, thus more closely resembling the last common ancestral plastid genome of all photosynthetic eukaryotes. However, sampling of red algal diversity is still quite limited. We aimed to remedy this problem. To this end, we sequenced six new plastid genomes from four undersampled and phylogenetically disparate red algal classes (Porpyridiophyceae, Styloneamtophyceae, Compsopogonophyceae and Rhodellophyceae) and discovered an unprecedented degree of genomic diversity among them. These genomes are rich in introns, enlarged intergenic regions and transposable elements (in the rhodellophycean *Bulboplastis apyrenoidosa*), and include the largest and most intron-rich plastid genomes ever sequenced (that of the rhodellophycean *Corynoplastis japonica* with a size of 1.13 Mbp). Sophisticated phylogenetic analyses accounting for compositional heterogeneity show that these four 'basal' red algal classes form a larger monophyletic group, Proteorhodophytina *subphylum nov.*, and confidently resolve the large-scale relationships in the Rhodophyta. Our analyses also suggest that secondary red plastids originated before the diversification of all mesophilic red algae. Our genomic survey has challenged the current paradigmatic view of red algal plastid genomes as 'living-fossils' by revealing an astonishing degree of divergence in size, organization, and non-coding DNA content. A closer look at red algae shows that they comprise the most ancestral, as well some of the most divergent plastid genomes known.

Keywords: red algae, ptDNA, deep phylogeny, secondary plastids, group II introns

Cell differentiation in the unicellular holozoan *Capsaspora owczarzaki* analyzed by single-cell RNA-seq

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Cell differentiation is a fundamental attribute of complex multicellular organisms, underpinning the functional specialization of cells and tissues during embryonic development. It has been proposed that animal multicellularity originated by a transition from temporal to spatial cell differentiation. Different types of simple multicellularity can be found during the life cycles of unicellular holozoans, the closest relatives of animals. There is the clonal development of colonial choanoflagellates, the aggregative behavior of *Capsaspora owczarzaki*, and the coenocytic development of ichthyosporeans. Those colonies and aggregates are assumed to be without cell differentiation. However, there is no molecular data proving that all cells within those colonies or aggregates or coenocytes are identical. Here, we show our advances in developing single-cell transcriptomics methodology in *C. owczarzaki* to molecularly characterize its cell types. We used the microfluidics-based single-cell RNAseq methodology called inDrops to analyze transcriptomes from a few thousands of single-cells from *C. owczarzaki* aggregates, as well as from adherent cultures. The possibility of analyzing differential gene expression at the single-cell level in *C. owczarzaki* aggregates will allow us to better understand the molecular mechanisms underlying programs of cell differentiation in the origin of animals.

Keywords: cell differentiation, origin of multicellularity, unicellular holozans, *Capsaspora owczarzaki*

Metchnikovellids, an evolutionary important yet poorly studied group at the root of microsporidian tree

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Metchnikovellids are a deviated group, which is very important for untangling the early evolution of Microsporidia. All known metchnikovellids are hyperparasites of gregarines inhabiting the gut of polychaetes and some other invertebrates. Metchnikovellidean spores are devoid of the most components of canonical microsporidian extrusion apparatus; instead they possess a structure called “manubrium” and a complex of associated organelles. The life cycle of metchnikovellids is also atypical. It misses merogony and includes a division by internal budding resulted in formation of cyst-bound spores. The metchnikovellids form a robust monophyletic clade at the root of microsporidian SSU rRNA tree. Recent phylogenomic analyses confirm the early divergence of metchnikovellids. They are the most basal group that shows typical microsporidian characters, like a high rate of sequence evolution, genome reduction and an absence of mitochondrial genome. The study of metchnikovellids is hindered by hyperparasitic life style, small size and occasional occurrence in the environment. Up to now, less than 30 species of these organisms are described, most of them are known only from old descriptions. Only eight species were investigated by TEM. During the last thirty years the isolation of only five metchnikovellidean species was reported; three of them were found during our extensive screenings in the populations of the polychaete *Pygospio elegans* in the White Sea, North-West Russia. We discovered two new species – *Metchnikovella spiralis*, *M. dogieli* and reisolated *M. incurvata*, a species described hundred years ago from other locality. The biodiversity of this group is likely to be largely unexplored. To expand our yet very limited knowledge on the diversity and distribution of these hyperparasites and to sample more taxa for phylogenetic studies the long-term screenings of the populations of potential hosts combined with molecular ecological approaches are desirable.

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Keywords: microsporidia, parasites, diversity, phylogeny, evolution

Blastocrithidia, a trypanosomatid with all three stop codons reassigned

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Recently, two groups of protists (a trypanosomatid *Blastocrithidia* and ciliates *Condylostoma* and *Parduczia*) were shown to reassign all three stop codons to code amino acids. *Blastocrithidia* represents an ideal model system for studying this phenomenon. It belongs to the order Trypanosomatida, a well-studied protist group, which includes model species *Trypanosoma* and *Leishmania,* with available complete genomes and established methods of forward and reverse genetics. Unlike ciliates, which are well-known for genetic code reassignments, all known trypanosomatids except *Blastocrithidia* have a canonical nuclear genetic code. Therefore, the reassignment happened in this group relatively late on the evolutionary scale. Hence, it may be possible to trace key steps leading to the emergence of such system. We have sequenced and analyzed the genomes and transcriptomes of two cultivable *Blastocrithidia* species and also the genome of *Leptomonas jaculum*, a close relative of *Blastocrithidia* with a canonical genetic code. Besides, we have performed mass-spectrometry analysis of *Blastocrithidia* proteins to verify the predicted amino acid specified by the in-frame stop codons. We provide evidence that UGA codes for tryptophan, UAG and UAA code for glutamate, while only UAA is also used as a genuine stop codon in a context-dependent manner. We show the unique prerequisites of *Blastocrithidia*, which likely made the reassignment of all stop codons possible, and speculate about translation termination in this system.

Keywords: genetic code, stop codons, translation termination, *Blastocrithidia*, trypanosomatids

Understanding phytomyxid-host interactions by combining transcriptomics with *in-situ* transcript visualisation

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Phytomyxea are obligate biotrophic protist belonging to the Rhizaria. They are parasites of land plants, diatoms, oomycetes and brown algae. Some members cause economically important diseases of land plants with the most prominent example of clubroot disease accounting for approximately 10% loss of the global brassica crop. Despite this economic importance, the genetic and physiological basis of the interaction are still not understood. This is because phytomyxids cannot be grown without a living host and because usually different stage of the 6-stage life cycle are present in the host at the same time. To address this lack of knowledge we recently generate transcriptomes of the clubroot pathogen *Plasmodiophora brassicae* and the brown algal parasite *Maullinia ectocarpii*. These transcriptomes are compared to the available genomes of *P. brassicae* and we aim to identify highly expressed genes, of which promising candidates were selected and different single molecule FISH methods tested to validate (i) the expression of the gene in the parasite and (ii) to link the transcripts to specific stages of the life cycle. Using a Methyltransferase which is produced by *P. brassicae* (PbBSMT) and which is to date the best studied gene in *P. brassicae* we were able to show that the mRNAs of the PbBSMT are present mainly in older plasmodia. We also tested our method on actin as a control (present in all stages) and a chitin synthase of *P. brassicae* (present when the chitin containing spores are formed) and are currently in the process of analysing a set of selected host genes. Overall we show that the combination of transcriptome analysis with single molecule transcript visualisation is a powerful tool to study the interaction of parasite and their host along both spatial and temporal gradients.

Keywords: Phytomyxea, parasites, FISH, gene expression

Single cell transcriptomes of the symbiotic protists in the wood-feeding termite gut suggest that chitin degradation is assigned to a symbiotic species

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Wood-feeding termites harbor a complex symbiotic system comprising various bacteria, archaea and protists in their hindgut. The symbiotic system is known to be indispensable for termites to thrive solely on recalcitrant wood. However, the detail of each function of microbial species is almost unknown due to their uncultivability. In the recent studies, the genomes of symbiotic bacteria were sequenced through DNA amplification method from single or hundreds of cells and uncovered their role in nitrogen fixation. In contrast, the sequence data of symbiotic protists are still poor despite their abundance in the termite gut.

Here, we applied single cell transcriptome for all the three symbiotic protists species (*Pseudodrichonympha grassii*, *Holomastigotoides mirabile* and *Spirotrichonympha leidyi*) in the wood-feeding termite *Coptotermes formosanus* to reveal their roles in the hindgut metabolism. The obtained data indicated high expression of chitinase genes in *S. leidyi*. Furthermore, only *S. leidyi* has gene sets to convert degradation product by chitinase to fructose 6-phosphate and ammonium, the source of ATP and proteins, respectively. *In situ* hybridizations showed these genes are actually transcribed in *S. leidyi* cells. We also confirmed the significant higher activity of chitinase in a *S. leidyi*- and *H. mirabile*-enriched fraction than that of three-species mixed fraction. These results suggest that *S. leidyi* actively degrades chitin in the hindgut. We infer that *S. leidyi* decomposes moulting skin of the nestmate and/or cell wall of fungi taken by the host glooming, and that *S. leidyi* contributes to recycling of nitrogen sources which lack in wood and/or to defence against fungal infection. Interestingly, the phylogenetic analyses suggested the genes converting chitin to ammonium have close affinity to those from distantly related organisms such as fungi and bacteria. We will discuss the evolutionarily history of the genes involving chitin decomposition and its implication.

Keywords: termite, symbiosis, single cell transcriptome, evolution, chitin degradation

Bridging the gap between morphological species and molecular barcodes – Exemplified by loricate choanoflagellates

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Translating the vast amounts of molecular barcodes from global surveys of microbial eukaryotes into ecological insight depends critically on a well-curated reference database with adequate taxonomic coverage. In this respect, the choanoflagellates resemble other eukaryotic lineages: reasonable coverage at higher taxonomic levels, but missing diversity at the species level. The acanthoecid (loricate) choanoflagellates are well-characterized morphologically, with over 115 species described, but less than 10% with any sequence data. Because lorica shape is species-specific, the acanthoecids represent an opportunity to link morphological with molecular data within a lineage of eukaryotes. To match morphospecies to sequences, we sampled the Kattegat and the Isefjord in Denmark in September 2014 and February 2015. We identified 45 morphospecies and sequenced ribosomal DNA of nine previously unsequenced species, roughly doubling the number of acanthoecid species with sequence data, including the first data representing five genera: *Bicosta, Calliacantha, Cosmoeca, Crinolina* and *Pleurasiga*. Our phylogenetic analysis is mainly congruent with morphology-based systematics. Five of the newly sequenced species match a previously unidentified barcode from Tara Oceans, providing access to the global distribution of species isolated from Danish waters. One species, *Calliacantha natans*, is the second most globally abundant choanoflagellate present in Tara Oceans. Our project translating new ribosomal DNA sequences to distributions of described species on a global scale supports the approach linking morphology to molecular barcodes for microbial eukaryote ecology.

Keywords: choanoflagellates, acanthoecids, phylogeny, NGS data mapping

Genomics and cell biology of the free living preaxostylan flagellate *Paratrimastix pyriformis*

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The understudied metamonad lineage Preaxostyla has recently attracted the attention of the protistological community when *Monocercomonoides* sp. was identified as the first known completely amitochondriate eukaryote. *Monocercomonoides* belongs to a divergent, highly diversified endobiotic crown group of Preaxostyla, the oxymonads. We believe the evolutionary processes leading to the complete loss of mitochondrion can only be understood in broader phylogenetic context. Therefore, we focus on more plesiomorphic, free-living relatives of *Monocercomonoides* from the paraphyletic assemblage of trimastigids, namely *Paratrimastix pyriformis*, which harbors, already partially characterized, hydrogenosome-like organelle. We present a preliminary annotation of the approximately 40-megabase draft genome of *Paratrimastix pyriformis* with emphasis on the energy metabolism, amino acid metabolism, iron-sulfur cluster assembly pathways and other cellular systems crucial for the reductive evolution of mitochondria.

Keywords: Preaxostyla, mitochondrion, evolution, hydrogenosome, *Paratrimastix*

Amebic trogocytosis: discovery of specific kinase that differentiates trogocytosis from phagocytosis

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The protozoan parasite *Entamoeba histolytica* is the etiologic agent of amoebiasis, an endemic infection in developing countries with considerable morbidity and mortality. Motility and phagocytic properties of *E. histolytica* play important roles in pathogenicity. Recently trogocytosis has been recognized as the key step in amoebic cytolysis and invasion, a paradigm shift in understanding pathogenicity in this organism. Only live host cells undergo trogocytosis while dead cells are taken up by phagocytosis. In this study, we show that AGC family kinase1 is specifically involved in trogocytosis of live human cells and does not participate in phagocytosis of dead cells. These conclusions are based on imaging of this kinase during trogocytosis and phagocytosis and properties of *E. histolytica* cells that have been silenced for expression of the kinase. This kinase localizes in the long and thin tunnels formed during trogocytosis but not in the trogosomes (endosomes formed after trogocytosis). The silencing of the gene led to a defect in the CHO cells destruction and trogocytosis while other endocytic processes remained unaffected. The results suggest that trogocytic pathway is likely to be clearly different from phagocytosis though many of the steps and molecules involved may be common.

Keywords: trogocytosis, phagocytosis, kinase, pathogenesis, Amoebozoa

Key regeneration genes in *Stentor* uncovered with single-cell RNA sequencing

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Ciliates of the genus *Stentor* are known for their ability to regenerate when the cell is wounded. To identify genes involved in regeneration, RNA sequencing of single *Stentor polymorphus* fragments was done. The fragments were produced by splitting the cell over the anterior-posterior axis. After the split, the posterior part needs to regenerate the oral apparatus while the anterior part needs to regenerate the hold fast. For both fragments, four different time points post-split of the cell were analyzed, leading to a total of eight conditions included in the experiment. The differential expression analysis identified over 10 000 upregulated genes, highlighting processes such as signaling, microtubule-based movement and cell cycle to be important during regeneration. Nine times as many posterior specific upregulated genes were identified, when comparing regeneration of the anterior and posterior fragments, indicating that regeneration of the oral apparatus is a more complex process. Two groups of genes were identified as key regulators of regeneration: Aurora and Polo-like kinases. The differential expression analysis confirmed observations from earlier morphological studies, which suggests that regeneration and vegetative division share many similarities.

Isolation and symbionts: Co-dependent community formation in the genus *Nephromyces*

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Nephromyces, a genus in the phylum Apicomplexa, has a mutualistic relationship with its host *Molgula* tunicates. The dynamics of this relationship are complicated by multiple *Nephromyces* species co-infecting a single host animal. Multi-species infections are common with other apicomplexans, however unlike other apicomplexans where co-infection is coincidental, co-infection appears to be mandatory for *Nephromyces* as no *Nephromyces* species has ever been found in isolation. A combination of cloning, multi-gene amplicon sequencing, genomic sequencing, laboratory culturing, and single cell isolation have uncovered a highly complex, interdependent, endosymbiont community comprised of a single genus. This single genus community has evolved in relative isolation from competition and has developed into a complex mosaic of inter-dependent closely related species. The community structure, diversity, relationships, and genomic consequences of community dependence will be discussed.

Diversity of free-living marine ciliates (Alveolata, Ciliophora): faunal studies in coastal waters of China during the years 2011–2016

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In the period 2011–2016, a series of investigations were carried out on the marine and brackish free-living ciliate fauna of the temperate-tropical coastal waters of China. About 210 morphotypes including over 100 new species within six groups (cyrtophorians, hypotrichs *s.l.*, karyorelicteans, oligotrichs, pleurostomatids, and scuticociliates) were isolated and described in detail from observations of live cells and silver-stained specimens. Based on their morphology, morphogenesis and molecular phylogeny, three new families (Wilbertomorphidae, Kentrophyllidae, Protolitonotidae) and 22 new genera (*Apotrachelocerca*, *Wilbertomorpha*, *Protolitonotus*, *Paracyrtophoron*, *Heterohartmannula*, *Aporthotrochilia*, *Falcicyclidium*, *Paramesanophrys*, *Pseudodiophrys*, *Monocoronella*, *Neourostylopsis*, *Apobakuella*, *Parabistichella*, *Heterokeronopsis*, *Heterotachysoma*, *Antiokeronopsis*, *Apoholosticha*, *Pseudogastrostyla*, *Antestrombidium*, *Sinistrostrombidium*, *Williophrya*, and *Varistrombidium*) were established. In the present review, we summarize these studies which show there is a large, undiscovered diversity of ciliates, especially in undersampled habitats, such as subtropical/tropical coastal waters, mangrove wetlands, estuaries and aquaculture ponds. We also highlight the importance of integrative approaches, combining morphology, morphogenesis and molecular phylogeny, in order to understand ciliate systematics and ecosystem function.

Keywords: free-living ciliate, diversity, taxonomy

New transcriptomic and genomic data reveal the internal phylogeny of the Heterolobosea and evolution of the anaerobic lifestyle within the group

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Heterolobosea (Discoba: Discicristata) is a group of ~150 described species of heterotrophic protists that exhibits an extraordinary ecological breadth and morphological diversity. The typical life cycle of heteroloboseans includes distinct flagellate, amoeboid, and cyst stages, although some of the stages may be missing in some lineages. Terrestrial amoebae of Acrasida, the only known multicellular lineage of Discoba, are able to aggregate to form stalked fruiting bodies. Most heterolobosean species are marine or freshwater aerobes, but the group also includes many thermophiles, several clades of halophiles, and obligate anaerobes. Thus, Heterolobosea is a promising model group for comparative studies focused on adaptation to unusual environments. However, studies of its internal phylogeny are based almost exclusively on 18S rDNA with very little transcriptomic or genomic data available. Consequently, deep level relationships amongst the main heterolobosean groups remain unclear.

To clarify the internal phylogeny of the group, we carried out a phylogenomic analysis based on publicly available sequence data from Heterolobosea together with newly obtained transcriptomic data from 12 strategically sampled species and genomic data from *Neovahlkampfia damariscottae*. Our analyses include all main lineages of Heterolobosea defined by previous 18S rDNA analyses. This phylogeny together with newly obtained sequence data provide a foundation for future comparative ecological, morphological, and evolutionary studies of the Heterolobosea. We present results of our on-going comparative genomic and transcriptomic analyses highlighting interesting aspects of the heterolobosean biology, including the evolution of the anaerobic metabolism within the group and the loss of ancestral stages (i.e., flagellates).

Plastid transcript editing across dinoflagellates: a non-synonymous-oriented mechanism shows lineage-specific application but conserved trends

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Dinoflagellates are a group of unicellular protists with immense ecological and evolutionary significance as well as incredible cell biological diversity. Photosynthetic dinoflagellates contribute to oxygen fixation and primary production in aquatic ecosystems, some dinoflagellates are causative agents of harmful algal blooms and others, such as *Symbiodinium* spp., are essential symbionts of coral reefs and other marine organisms.

Of the photosynthetic dinoflagellates, the majority possess a plastid containing the pigment peridinin, while some lineages have replaced this plastid by serial endosymbiosis with plastids of distinct evolutionary affiliations, including a fucoxanthin pigment-containing plastid of haptophyte origin. Previous studies have described the presence of widespread and promiscuous substitutional transcript editing in both peridinin and fucoxanthin plastid-encoded genes. However, because reports of this process have been limited to manual assessment of individual lineages, global trends concerning the nature of transcript editing and its effect on the biological function of the plastid is largely unknown.

Here we employ bioinformatic methods to examine the evolutionary dynamics of transcript editing over a large multispecies dinoflagellate dataset and shed light on characteristics of the underlying mechanism. Our results are consistent with a post-endosymbiosis transfer from the peridinin-containing ancestor into fucoxanthin plastids of a shared editing mechanism, with remarkably conserved functional consequences in the new lineage in spite of the lineage-specific application of individual editing events. Our results support a model for non-synonymous-oriented editing relying on codon positional preference and mutation rates, subsequently shaped by purifying selection that generally mitigates against deleterious mutations at the genomic level.

Phycospheric native bacteria *Pelagibaca bermudensis* and *Stappia* sp. improve biomass productivity of *Tetraselmis striata* (KCTC1432BP) in co-cultivation system through mutualistic interaction

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Effective sustainable algal cultivation techniques are essential for mass production of the marine microalga *Tetraselmis* for biofuel and array of co-products. The phycospheric communities affect the microalgal growth and metabolism through various allelochemical and nutrient interactions; hence, their potential to affect the quantity and quality of both biomass and bioproducts is significant. In the present study, we have screened the phycospheric communities of biofuel producing *Tetraselmis striata* (KCTC1432BP). A total of 26 bacterial strains were isolated and identified from the phycosphere of *Tetraselmis striata* mass culture. Then, each bacterial strain was tested in co-cultivation conditions with *Tetraselmis striata* for evaluating its growth promoting and inhibitory effects. Among these all strains, two promising strains (*Pelagibaca bermudensis* KCTC 13073BP and *Stappia* sp. KCTC 13072BP) were selected because of their maximum growth promoting effects and mutualistic interactions. The growth rate, biomass productivity, lipid contents and fatty acids were analyzed during their combined growth in O3 media and compared with axenic growth of *Tetraselmis striata*. Later, growth promoting mechanisms in the co-cultivation environment were investigated for these promising bacterial strains under replete and limited conditions of nutrients (nitrate, phosphate and vitamin B_{12}). The growth promoting potential of *P. bermudensis* was illustrated by the two fold enhancement in biomass productivity. These bacteria are promising for microalgal cultivation without any negative effects on the native seawater bacterial communities, as revealed by NGS analysis. This study represents, to date, the first report highlighting the role of phycospheric growth promoting bacteria of promising biofuel feedstock *Tetraselmis striata*.

Keywords: microalgae, phosphate, phycosphere, growth promoting bacteria, biomass

Capsaspora owczarzaki as a unicellular model to study co-option of the ancestral integrin adhesome

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Adhesion systems and signaling networks are both essential in multicellular organisms. A well known example of this is the integrin adhesome of metazoans. Many of the elements of this adhesome are conserved in the closest unicellular relatives of animals, indicating these proteins already existed in the unicellular ancestor of metazoans and that co-option could have been essential to multicellular emergence. To determine whether co-option of the adhesome elements may have played a major role at the origin of metazoans, we aim to unravel the function of the integrin adhesome in the filasterean *Capsaspora owczarzaki.* This protist is the closest unicellular relative to metazoans that contains in its genome the basic set of components that constitutes the integrin adhesome. As a first approach into understanding its role, we performed immunostaining with antibodies raised against several of these proteins to localize them in the adherent filopodiated stage of *C. owczarzaki*. Moreover, we assessed the level of conservation between *Capsaspora* elements and the animals ones. To this mean, we evaluated the localization of *C. owczarzaki* integrin adhesome proteins in different tissues of the *Drosophila melanogaster* embryos. Results and the implications will be further discussed.

Keywords: *Capsaspora*, integrin adhesome, co-option

Single cell high-throughput sequencing unveils different patterns of intragenomic polymorphism in ribosomal RNA genes of Foraminifera

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Nowadays, the assessment of microbial eukaryotes diversity is commonly done using metabarcoding approach based on high-throughput amplicon sequencing. In metabarcoding studies, the species or Operational Taxonomic Units (OTUs) are usually distinguished based on more or less fixed thresholds that define the level of intraspecific variations. However, the analyses of metabarcoding data rarely take into account the intragenomic variations, often considered as result of technical errors. Here, we use single-cell high-throughput sequencing approach to evaluate the level of intragenomic polymorphism (IGP) in 18S rRNA gene of 130 specimens of benthic Foraminifera, representing different taxa and living in different habitats. Our study confirms previously shown widespread occurrence of IGP in Foraminifera. We report different patterns of IGP, including the single-nucleotide polymorphism (SNP) and expansion segments polymorphism (ESP), resulting in occurrence of numerous haplotypes, which divergence may reach up to 5%. Interestingly, while SNPs are present in all examined species, the ESPs are found only in multi-chambered calcareous species that generally show much higher IGP level than the single-chambered species. We also observe a significant difference of IGP level between shallow-water coastal and deep-sea taxa; the later showing surprisingly low level of intra-individual sequence divergence. These taxonomic and ecological patterns indicate that at least a part of the IGP (mainly ESP) have biological origins. The majority of SNPs are probably technical errors. We estimate their rate at about one error per 1000-2000 sequenced bases. Whatever are their origins, the IGPs drastically increase the number of OTUs assigned to the same species using traditional clustering methods, up to one level of magnitude in some cases. Therefore, we recommend single-cell HTS testing of IGP level to avoid overestimation of environmental diversity in metabarcoding studies.

Keywords: metabarcoding, high-throughput sequencing, intragenomic polymorphism, mutation rate, Foraminifera

Biodiversity of *Rickettsiales* and *Holosporales* symbionts in ciliates: state of the art and future trends

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The description of novel taxa of bacterial symbionts of ciliates boosted with the turning of the century. Molecular biology techniques integrated in the full cycle rRNA approach represented the technical basis of this renaissance of study on symbionts of ciliates. In this talk I will focus on recently discovered biodiversity within two related groups of obligatory symbionts: *Rickettsiales* and *Holosporales*, a group of organisms encompassing also mild to severe human and vertebrate pathogens. Especially for what concerns *Rickettsiales*, results from the last ten years dramatically changed our view on this bacterial order. Once considered as typical endosymbionts/pathogens of arthropods and vertebrates, it is now known that the vast majority of the biodiversity of *Rickettsiales* is hosted by aquatic organisms, among which many are represented by protists, in general, and ciliates, in particular. Moreover, the first example of epibiotic *Rickettsiales* has been recently described, see accompanying presentation by Castelli et al., additionally increasing the spectrum of biodiversity present in this order. In the very last years, also genomic data on bacterial symbionts of ciliates started to be produced. Genomic study on symbionts will surely represent the future of the field, and will help shedding light also on functional interaction between host and symbiont, a topic that, probably due to the recent focus on molecular characterizations, received less attention in the last years. In conclusion, present data suggest that we know only the tip of ciliate symbionts' diversity and many years will be necessary to properly describe it also in functional terms.

Keywords: Ciliophora, endosymbiont, *Rickettsia*, *Holospora*

Searching for a role of TatC, component of twin-arginine translocase, in the mitochondrion of *Naegleria gruberi*

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In the past, ancestor of eukaryotic cell adopted alphaproteobacteria, which subsequently became mitochondrion. During the development of endosymbiosis, the protein transport between the host and the endosymbiont had to be evolved. "Classical" aerobic mitochondria contain components of bacterial origin (TOM and SAM complex of the outer mitochondrial membrane, Oxa1 in the inner mitochondrial membrane) and eukaryotic innovations such as TIM complex in the inner mitochondrial membrane. The last eukaryotic common ancestor (LECA) probably already contained these four main protein translocation machines. Special group of eukaryotes carries additional bacterial genes for protein transport – SecY or subunits of twin arginine translocase (TAT) in their mitochondrial genomes. In bacteria, TAT complex transports substrates from cytosol to periplasm and is composed of three main subunits – TatA which is responsible for creating the transport channel and TatB, TatC which recognize the signal peptide of the substrate. A similar situation also exists in the chloroplast thylakoid. We show that mitochondrial TAT translocase was independently lost over twenty times during the evolution of eukaryotes. The loss of TatC from the mitochondrial genome was never accompanied by the transfer of the gene to host nucleus. We have investigated the function of TatC encoded in the mitochondrial genome of *Naegleria gruberi*. Without the apparent presence of TatA subunit, mitochondrial TatC forms a membrane complex larger than the entire bacterial TAT translocase. However, TatC was not capable of functionally replacing the bacterial orthologues. Our data question the role of mitochondrial TAT translocase in the translocation of Rieske protein across the inner mitochondrial membrane.

Hide and seek: The complex evolutionary history of green secondary plastids

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Photosynthesis in eukaryotes arose from the endosymbiosis between a cyanobacterium and a heterotrophic host. This primary endosymbiotic event gave rise to the Archaeplastida, a supergroup composed of Glaucophyta, Viridiplantae (green algae and land plants) and Rhodophyta (red algae). In their turn, red and green algae established secondary endosymbioses spreading the ability to photosynthesize to other eukaryotic groups.

During endosymbiosis, plastid genes from the algal endosymbiont were relocated to the host nucleus, a process known as Endosymbiotic Gene Transfer (EGT).

Through the phylogenetic analyses of EGT genes in extant phyla with green-alga derived plastids (chlorarachniophytes and euglenids), we showed that approximately half of the identified EGT's seem to have been transferred from red algae. These unexpected results suggest that chlorarachniophytes and euglenids likely carried a red plastid that was later replaced by a green one. We propose that the former red plastid might have helped to establish the secondary endosymbiosis with a green alga.

Keywords: endosymbiosis, plastid, red algae

Identification of candidate genes involved in shell formation in *Arcella intermedia* (Amoebozoa: Arcellinida) through comparative transcriptomic analyses

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Shells (tests) are rigid structures that cover the cells of diverse unicellular eukaryotic lineages. Tests have evolved multiple times in various eukaryotic lineages, one of these is the Arcellinida, who present a wide variety of test shapes and compositions: the test can be secreted, agglutinated, and have diverse chemical compositions. These organisms reproduce by binary fission, and before each division a new test is formed. Shells in Amoebozoa evolved once in Arcellinida, a lineage that shares a common exclusive ancestor with non-shelled Amoebozoa lineages. However, the genes involved in shell biogenesis remain unknown, and so is the molecular evolution related to the shell origin. With transcriptomic sequencing, we can identify active genes and transcripts during different cell conditions and processes. We obtained transcriptomes from individuals of *Arcella intermedia* (Amoebozoa: Arcellinida) in two distinct moments: individuals before the beginning of test formation and individuals forming a new test. We analyzed and compared the transcriptomes obtained, determining qualitative, and quantitatively gene expression differences. We are currently identifying the highly expressed genes during test formation, which will be the candidate set of transcripts involved in the test formation. We are going to determine whether these genes are exclusive to Arcellinida or present in other relatively closely related eukaryotes (other Amoebozoa, fungi, and animals). We will annotate candidate genes that are homologous to well described model amoebozoan, fungi, and animals genes, inferring functions. This study is the first attempt to identify candidate genes involved in the test biogenesis in Arcellinida.

Keywords: testate amoeba, differential gene expression, molecular evolution, evolutionary novelty, shell biogenesis

The green *Tetrahymena utriculariae* n. sp. (Ciliophora, Oligohymenophorea) with its endosymbiotic algae (*Micractinium* sp.), viving in traps of a carnivorous aquatic plant

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The genus *Tetrahymena* (Ciliophora, Oligohymenophorea) probably represents the best studied ciliate genus. At present, more than forty species have been described. All are colorless, i.e. they do not harbor symbiotic algae, and as aerobes they need at least microaerobic habitats. Here we present the morphological and molecular description of the first green representative, *Tetrahymena utriculariae* n. sp., living in symbiosis with endosymbiotic algae identified as *Micractinium* sp. (Chlorophyta). The full life cycle of the ciliate species is documented, including trophonts and theronts, conjugating cells, resting cysts and dividers. This species has been discovered in an exotic habitat, namely in traps of the carnivorous aquatic plant *Utricularia reflexa* (originating from the Okavango Delta, Botswana). Green ciliates live as commensals of the plant in this anoxic habitat. Ciliates are bacterivorous, however, symbiosis with algae is needed to satisfy cell metabolism but also to gain oxygen from symbionts. When ciliates are cultivated outside their natural habitat under aerobic conditions and fed with saturating bacterial food, they gradually become aposymbiotic. Based on phylogenetic analyses of 18S rRNA and mitochondrial *cox1* genes *T. utriculariae* forms a sister group to *T. thermophila*.

Keywords: mixotrophic ciliates, symbiotic algae, *Tetrahymena*, *Utricularia*

Metagenomic analysis of microbiomes associated with ciliates: each cell is a world!

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The analysis of microbiomes of metazoan organisms became one of the most rapidly developing research fields in microbiology as well as in physiology. However, contribution of bacteria in survival and environmental adaptation of protists, as well as role of protists in maintenance of bacterial consortia associated with them, has never been investigated. We studied for the first time bacterial communities associated with several species of ciliates, in particular the sessile ciliate *Stentor coeruleus*, and a number of *Paramecium* strains of different origin. The ciliates were isolated from wastewaters or taken from laboratory collections. 16S rDNA metagenomic analysis of single cell ciliate microbiomes was performed, and it allowed to assess diversity of prokaryotes associated with each ciliate specimen. In the ciliate microbiomes, five prokaryotic phyla were identified as major with average presence ≥ 1 % per sample; all OTUs were classified until genus level in each sample, when possible. The most abundant OTUs (top ten) of each sample had at least 0.8 % presence. Each retrieved set of 16S rDNA amplicons associated with a certain ciliate was compared to the bacterial community of its maintenance medium. We conclude that microbial consortia associated with a ciliate cell are highly different from respective environmental prokaryotic communities. Ciliate microbiomes are unique for each genus and show specificity for the strains of different origin. Moreover, a number of potentially pathogenic bacteria were detected in ciliate microbiomes, thus opening a new and interesting possibility to consider ciliates as potential reservoirs of human pathogens.

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Keywords: microbiome, ciliates, 16S rDNA, bacterial consortia, pathogenic bacteria

New flagellate genera from the termites *Glossotermes oculatus* and *Serritermes serrifer* (Serritermitidae)

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Lower termites depend on their symbiotic gut flagellates for the digestion of the lignocellulosic food. The flagellates are characteristic for a termite species and mostly coevolved with their host. The symbionts of the serritermitid termites *Glossotermes oculatus* and *Serritermes serrifer* are yet unknown to science. We found three genera of parabasalid flagellates in both termite species, which resemble known genera. Oxymonads were not present. The large *Pseudotrichonympha*-like species (around 200 µm) were completely covered by longitudinal rows of flagella. *Leptospironympha*-like cells were of medium size (25-70 µm) and their flagella were arranged in two clockwise spiralled bands. The bands contained numerous short rows of basal bodies which lay oblique to the path of the bands. The *Hexamastix*-like flagellates were quite small (around 10 µm) and possessed a bundle of 5 anterior flagella plus one recurrent flagellum. Despite their morphological similarity to known species, phylogenetic analysis of their SSU rRNA genes revealed that these flagellates are only distantly related to their morphological counterparts in other termite families. In fact, the phylotypes assigned to *Pseudotrichonympha*-like and *Leptospironympha*-like species do not cluster with other eucomonymphids and spirotrichosomids, suggesting that they represent new lineages beyond the genus level that clearly differentiate *Glossotermes* and *Serritermes* from related rhinotermitid termites. We present a detailed characterization of the *Leptospironympha*-like flagellate from *G. oculatus* by light microscopy, scanning and transmission electron microscopy.

Keywords: flagellate, Parabasalia, symbionts, termite

Protist functional stability in pico-nanoplanktonic marine coastal communities

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Seeking for general patterns of protist community structuring in the marine coastal ecosystem, we tried to synthesise the genetic diversity of marine planktonic protists by a functional diversity approach. We sampled pico-, nano-, and micro-plankton communities in marine coastal ecosystems submitted to different physical forcings, at different spatial and time scales. By means of a metabacording approach based on the high-throughput sequencing of the V4 domain of the 18S rDNA, we identified and taxonomically annotated 111089 Operational Taxonomic Units (OTUs). We established a conceptual framework of 30 protist biological traits which reflect the variety of morphological forms, trophic strategies, physiology, and mode of life of both autotrophic and heterotrophic protists. A rigorous bibliographical work allowed annotating 13 out of 30 traits to at least half of our taxonomically annotated OTUs. Despite an evident lack of knowledge of protist biological traits, a combination of multivariate and clustering statistics allowed the identification of trade-off between well-annotated traits and the establishment of 6 distinct functional groups. The relative abundances of these functional groups have been correlated with environmental variables and compared among group of samples similar for their genetic diversity composition, and across size fractions. Some environmental preferences of the functional groups and variations of their relative contributions among the different sample groups were highlighted. These variations were less important in the smallest planktonic size fractions, where the 6 functional groups showed comparable contributions across sample groups. Taking into account the whole biological diversity of marine protists, this study proposes new developments in the field of protist functional ecology, which was so far assessed only for the photoautotrophic compartment. It suggests that the functional and the genetic diversity of the coastal protist communities covary, and that the pico-nano-plankton is more stable than the micro-plankton both from a functional and genetic diversity perspective.

Keywords: molecular ecology, functional diversity, biological traits, metabarcoding, statistics

Analysis of kinases in the large ciliate *Stentor* reveals a role for cdc2 in completion of oral development during regeneration and division

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The giant ciliate *Stentor coeruleus* has the ability to fully regenerate after being cut in half, in a way that perfectly preserves the original polarity and structure. This ability has made *Stentor* a classical model system for studying regeneration of a single cell. So far, however, the molecular details behind this incredible phenomenon have remained largely unstudied. We wish to understand how the regeneration process is coordinated at the molecular level. In particular, as some of the details of the regeneration process parallel the events of cell division, we wish to understand whether the cell co-opts certain cell division signalling pathways to initiate and control regeneration. Our laboratory has developed a system for RNAi knockdown of *Stentor* genes, and has recently sequenced the *S. coeruleus* genome. To identify candidates for RNAi knockdown we analyzed the kinome of *Stentor*. *Stentor* was found to encode over 2000 kinases, making up 6% of the total protein coding genes. Many of these consist of expansions in mitotic kinase families such as CDKs, PLKs, NDRs, and NEKs, as well as very large expansions in CDPK and DYRK families. To begin to investigate the role of the cell cycle in regeneration, we performed RNAi knockdown of a cdc2 homolog in *Stentor*. Similar to other organisms, loss of cdc2 results in a cytokinesis defect. However, in *Stentor* it also impairs the cell's ability to complete oral development, both prior to division and during regeneration. This suggests that the regeneration process, particularly oral development, employs cell cycle signalling pathways.

Keywords: ciliates, cell cycle, kinases, regeneration

Glaucopyhte plastid comparative genomics: ancient divergence between genera

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The plastid genomes (ptDNAs) of the glaucophytes *Gloeochaete wittrockiana, Cyanoptyche gloeocystis, Glaucocystis incresata* and *Glaucocystis* sp. strain BBH were sequenced using Illumina and PacBio technology. The sequences presented complete the collection of plastomes from at least one species of each of the four different glaucophyte genera deposited in scientific collections. The glaucophyte plastid coding capacity (~150 protein-coding genes) is highly conserved, including 121 genes commonly present in the four genera and 18 orthologous sequences encoded in at least three representatives. In contrast, the gene order shows little conservation, indicating that major plastome rearrangement have occurred during glaucophyte diversification. The inter-genus genetic distances (Kimura 2-P) and non-synonymous substitutions between glaucophyte plastid genes are similar to sequence divergence values estimated between representatives of different red algae or viridiplant families, respectively. These genetic divergence values suggest that the known glaucophyte genera represent lineages of ancient divergence. Gene synteny in the two *Glaucocystis* ptDNAs is conserved (the gene order is identical) and the coding regions present high (>90%) average identity at nucleotide level. However, the ptDNA of *G. incresata* presents a ~7.6 kb insertion not detected in *Glaucocystis* sp. BBH and other glaucophyte plastomes. This unusual insertion, experimentally corroborated by PCR, contains 10 open reading frames (ORFs), including sequences with similarity (BLAST e value < 10^{-10}) to bacterial primases (type virE), recombinases and N-acetylmuramoyl-L-alanine amidases. None of the predicted ORFs in the *G. incresata* ptDNA insertion is of obvious cyanobacterial origin, or similar to regions from other algal or plant plastid genomes. We discuss the possibility that the insertion was acquired via HGT solely in *G. incresata.* Further comparative studies of additional glaucophyte species will be important to untangle the evolutionary history of this rare but important archaeplastidian lineage.

Keywords: glaucophytes, *Cyanophora*, plastid, plastome, Archaeplastida

Gene expression profile during growth of the testate amoeba *Arcella intermedia*

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Typical microbial growth curves have four phases: lag (adaptation to environment, no growth), log (exponential growth), stationary (equilibrium between growth and death) and decline (death). These phases are a response to environmental limitations to growth. The ability to sense the environment and regulate cellular functions determines the fitness of the organism. Most of our knowledge about these processes is a result of laboratory studies using bacteria as models. For this model, gene expression changes through time in population growth. For bacteria, this regulation may vary between organisms in clonal cultures, leading to multiple distinct fitness levels in clones. Here we seek to describe the genetic machinery related to the development of a clonal population of the testate amoeba *Arcella intermedia.* We generated growth curves in three microenvironments, removing individuals for molecular analysis throughout. We separated nine individual cells at each growth phase from each microenvironment. With high throughput sequencing of each of these cell's transcriptome, we identify genes expressed in each treatment. With these data, we are building a gene expression profile for each of the four growth phases. Our goal is to determine differences in the presence/absence of expressed gene families between growth phases as well as differences in expression quantities between individuals. This study is the first attempt to molecularly understand population growth in free-living testate amoebae.

Keywords: molecular biology, gene expression profile, Amoebozoa, growth curves

Identifying adaptations in the membrane trafficking system across the diversity of ciliates

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Ciliates are an extremely diverse protist phylum, notable for both their extreme morphological heterogeneity and ability to colonise a variety of ecological niches. They are essential in a range of habitats for nutrient cycling, as bacteriovores and as a prey source for metazoan predators. Ciliates are notably present in highly contaminated environments; however, there is evidence that contamination with hydrocarbons affects the membrane trafficking system (MTS) of ciliates. Membrane trafficking is an extremely diverse aspect of cell biology, particularly in ciliates; the phylum shows multiple specialisations of the MTS, such as trichocysts and mucocysts. Though well characterised in specific model species such as *Tetrahymena thermophila* and *Paramecium tetraurelia,* the distribution of MTS genes is not well known across the diversity of ciliates. We have used additional genome data from the published ciliate genomes *Stentor coeruleus, Stylonychia lemnae, Oxytricha trifallax* and *Ichthyopthirius multifilis*, as well as 25 published transcriptomes sampled from across the ciliate phylum, to determine the evolution of MTS components in the radiation of ciliate diversity. We have determined the presence of multiple ancestral duplications as well as lineage-specific expansions in ciliates. In particular, our analysis of the evolutionary history of the *VPS8* gene in the HOPS/CORVET complex suggests an ancestral loss of essential HOPS components concurrent with an expansion in CORVET. Future studies of the effects of contamination in ciliates will have to take this unique cell biology into account.

Diversity-dependent diversification: but do modern planktonic foraminifera actually compete?

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Studies of diversification dynamics of planktonic foraminifera (PF) fossil record concluded that PF diversification rates are regulated by the number of coexisting species in the group (diversity-dependent diversification, DDD). The main mechanism proposed to explain this pattern is competition among species along the Cenozoic Era. However, if interspecific competition is an important driver of planktonic foraminifera evolution, we expect it to be also a strong structuring force of modern planktonic foraminiferal communities. Species may be eliminated from a habitat by competition from individuals of other species, resulting in a pattern of non-overlapping species' ranges (i.e. allopatry). On the other hand, if competing species coexist, individuals of at least one of them suffer reductions in abundance due to the presence of the other, leading to a pattern of negative correlation of abundances through time. To test these two predictions of interspecific competition, we used 4193 worldwide core-top PF assemblages to assess living species' ranges, plus 37 PF populational time-series collected globally from sediment traps to assess species coexistence. Most species co-occur, rejecting allopatric species ranges. When analysing the community time-series, species' abundances correlated more positively than negatively through time. Our results suggest that the abiotic environment (and possibly phylogenetically distant species) drive populational dynamics of PF. Thus there seems to be a mismatch between the processes inferred from macroevolutionary patterns over deep time and those inferred from ecological patterns observed in a shorter time scale.

Keywords: planktonic foraminifera, community ecology, macroevolution

Novel marine lineages of anaerobic ciliates hosting prokaryotic symbionts

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Anaerobiosis has independently arisen several times in Ciliophora, which are generally well known for their diverse symbiotic relationships, particularly within the anaerobic lineages. However, almost nothing is known about the nature of these relationships, such as host specificity and metabolic pathways. The true diversity of anaerobic ciliates is still far from fully described. While mapping the diversity of free-living anaerobic ciliates within the SAL group (Spirotrichea, Armophorea, and Litostomatea), including newly discovered deep marine lineages, we observed that many host various ecto- and endosymbionts that are noticeable in scanning and transmission electron microscopy, protargol preparations, or even light microscopy. The fact that the symbionts persist in our cultures on a long-term basis gives us a great opportunity to shed light on their identity and relationships. Using fluorescence microscopy, we confirmed that at least some of the endosymbionts are methanogenic Archaea. Surprisingly, one species of the novel deep marine lineage hosts methanogens on their cell surface as ectosymbionts. Accordingly, we used FISH and CARD-FISH methods to detect and identify the symbionts of several representatives of these ciliate lineages. We also analyzed symbiont 16S rDNA in one of the ciliate species. Among other things, our results confirmed the presence of a group of sulphate reducing bacteria in the novel marine anaerobic lineages.

Keywords: anaerobic ciliates, diversity, symbiosis, methanogenic Archaea, sulphate reducing bacteria

Evolutionary and natural history of introns and splicing in eukaryotes

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Comparative genomics has revealed that spliceosomes and spliceosomal introns are ancestral and nearly universal to eukaryotes, however, knowledge of spliceosomal systems remains largely concentrated in a small number of well-studied lineages. The explosion of genomic sequencing of less well-studied eukaryotic lineages has allowed for a much better understanding of the similarities and differences of spliceosomal systems across eukaryotes, and has produced a wide variety of intriguing surprises. In this talk, I will first discuss the general outlines of the history of spliceosome and spliceosomal introns in eukaryotes, including the evolutionary history of intron types, numbers, sequences and functions, and emphasizing the high degree of convergent evolution observed. I will then turn to a variety of intriguing peculiarities observed in various protists, including trans-splicing of coding sequences in *Giardia*, *de novo* emergence of separate classes of introns in *Trichomonas*, co-evolution of introns and core spliceosomal machinery in *Entamoeba*, massive proliferation of minor spliceosomal introns in *Physarum*, origins of the tiny exons of *Symbiodinium*, and simplification of the spliceosome in red algae.

Keywords: genome complexity, genome evolution, splicing

Gregarines (Apicomplexa, Gregarinasina) in psocids (Insecta, Psocoptera).

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Gregarine apicomplexans are unicellular organisms that infect invertebrate hosts in marine, freshwater and terrestrial habitats. The largest group of invertebrates infested on land is the insects. The insect order Psocoptera (booklice) has recently gained wider interest due to specimens occurring in stored food products and therefore being considered pest organisms. Biological control agents are often used to eliminate pest organisms. In this study we examined the psocid *Dorypteryx domestica*, an invasive psocid species that is spreading all over the world. We were able to isolate and describe a new gregarine species (*Enterocystis dorypterygis* sp. n.) infecting *D. domestica*. The trophozoites are panduri- or pyriform and their association/syzygy is caudo-frontal. The surface is inscribed by longitudinal epicytic folds covering the complete cell. Phylogenetic analyses of the SSU rDNA gene revealed an only weakly supported relationship with two *Gregarina* species *G. ormieri* and *G. basiconstrictonea*, both from tenebrionid beetles. Gregarines have been proposed to have some potential as biological control agents for several insects. Identifying the gregarine species infecting pest organisms like psocids is a first step and prerequisite for the probable utilization of these parasites as biological control agents in the future.

Keywords: apicomplexan parasite, Enterocystidae, biological control agent

A novel microsporidia-like eukaryotic parasite of *Paramecium*

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Ciliates often harbor prokaryotic or eukaryotic microorganisms in their cytoplasm. The intruders can be commensals, mutualists, or parasites, harmful for their host. Microsporidia are obligate intracellular parasites with a wide range of hosts. Nevertheless, only a few cases of microsporidian infection have been registered in ciliates so far, and none of them has ever been found in *Paramecium* genus. This is the first report on a *Paramecium* bearing a Microsporidia-like parasite in the cytoplasm. The strain SpM 5-3 (*P. aurelia* group of species) was isolated from a natural population in Spain in 2015. Living cell observations revealed conspicuous spheric microorganisms (diameter ca 5 µm). Fluorescence *in situ* hybridization with the universal eubacterial probe was negative, while distinct nuclei of numerous parasites were easily visualized in the host cytoplasm by DAPI staining. In electron micrographs, the parasite demonstrated morphological features typical of Microsporidia. The spore shaped like a regular sphere possessed an electron dense exospore and an electron lucent endospore, an anchoring disk, a short isofilar polar filament, a posterior vacuole, a granular polaroplast and a cell nucleus. Various life cycle stages of irregular shape were abundant in the host cytoplasm. No diplokaryotic stages were noticed. A dividing four-nuclear plasmodium at the transition stage from merogony to sporogony was observed. Sporonts contained numerous ribosomes, a well-developed endoplasmic reticulum and had patches of the primordial exospore on their plasma membrane. In experimental infections using the culture medium cleared of the infected paramecia, only 3 of 8 tested strains of *P. aurelia* group of species were infected. Successful experimental infection and maintainability of the newly infected culture in the laboratory makes it a promising model for further studies of the host-parasite relationship. Sequencing of the SSU rRNA gene of the parasite and phylogenetic analysis is under way.

Supported by RFBR grant 15-04-06410.

Keywords: Microsporidia, intracellular parasites, *Paramecium*, electron microscopy, experimental infection

An automated Search Pipeline for Orthologs of Components of Key Molecular systems (SPOCK) applied to the draft genome of *Carpediemonas membranifera*, a free-living relative of metamonad parasites

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Accurate identification of gene orthologs and their manual curation during functional annotation of genomes are difficult and time-consuming tasks. To achieve this, we have combined state-of-the-art tools and designed decision-making-algorithms that provide the most relevant information for a set of query proteins in a scientifically rigorous, time efficient and unsupervised manner. We benchmarked and validated our tool against eggnog-mapper using the well-studied RNA decay pathway in the model protistan parasite *Trypanosoma brucei* and, a widely occurring and diverse protein family (i.e. helicases) in Candidate Thorarchaeota archaeon SMTZ1-83. Here, we showcase the utility of our tool for comparative genomic analysis using three metamonads genomes. A quick retrieval of evidence for pathways/molecular systems was carried out for *Carpediemonas membranifera* (free-living flagellate), *Giardia intestinalis* and *Spironucleus salmonicida* (parasites). The pipeline enabled the identification of several gene family expansions and slightly different versions of the RNA decay pathway, transcription factors, cell cycle and meiosis pathways in *C. membranifera* in relation to diplomonad parasites. This suggests that there have been some secondary losses and modifications in diplomonads as a result of their parasitic lifestyle. We are currently exploring other pathways that are relevant for comparative analyses among free living and parasitic metamonads including the spliceosome, cytoskeleton, oxidative stress systems, variant-specific surface proteins and encystation.

Red algal parasite evolution is shaped by plastid origin

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Red algal parasites are predominately unpigmented and appear as erumpent pustules or irregular growths on their free-living red algal hosts. Despite their diminutive nature, parasitic red algae share morphological characteristics with other close relatives, allowing them to be assigned to tribes or families from their initial discovery. Historically parasites that have infected close relatives have been considered 'adelphoparasites', while those more distantly related to their hosts are called 'alloparasites'. Molecular data has suggested that a gradient exists between adelphoparasites and alloparasites and therefore, these terms may no longer be suitable. Recent research has focused on the role of plastids in red algal parasite evolution. It is logical that selective pressure on genes involved in plastid functions will change as a parasite relies on its host for the products of photosynthesis. Accordingly, all 'adelphoparasites' that have been thoroughly investigated appear to have lost their native plastid and instead hijack a copy of the host plastid when packaging their spores. Furthermore, a highly reduced native plastid was recently sequenced from the 'alloparasite' *Choreocolax polysiphoniae*. Here we present data supporting a single evolutionary event that gave rise to a clade of alloparasites in the Rhodomelaceae, which all retain their native plastids. Additionally, we discuss transcriptomic and genomic sequence data from nuclear-encoded plastid-targeted genes of both red algal adelpho- and alloparasites. We demonstrate that differences in plastid origin impacts expression and conservation of genes involved in the carotenoid biosynthesis pathway, which produces pigments necessary for photosynthesis. Finally, we propose that developmental differences may be a more appropriate characteristic for distinguishing and defining types of red algal parasites.

Keywords: Rhodophyta, alloparasite, adelphoparasite

Combined effects of global and local stressors (high temperature and low light) on symbiont-bearing foraminifera from Okinawa, Japan

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Larger benthic foraminifera (LBF) are calcareous protists. They act as "living" sediments and stabilize small reef islands in the Indo-Pacific. Under elevated temperatures due to global warming, foraminifera lose their photo-synthetic algae, a process called bleaching. Here we examine the impacts of global warming effects in combination with a local stressor "low-light" caused by increased sediment run-off on two LBF species, *Amphistegina lobifera* and *Amphisorus hemprichii.* Living specimens were collected on a beach lagoon in Sesoko Island and exposed in the laboratory for three weeks to ambient temperature (26°) and three elevated temperatures (30, 32 and 34°C) and two light levels (~20 and ~80 µmol photons m^{-2} s^{-1}). We measured survivorship, photosynthetic yields, as well as bleaching rate using color values (Ciel*a*L color space). Survivorship was high in *A. hemprichii* in all treatments whilst in *A. lobifera* survivorship was reduced in the 30 and 32°C treatments compared to the control but not in the 34°C treatment. Photosynthetic yields and growth rates declined with increasing temperatures. Growth rates were reduced in the low-light treatment indicating that a reduction of light due to runoff reduces growth and likely impacts calcification. We identify a species-specific bleaching pattern based on the whiteness value (L*) measured at two positions of the foraminiferal shell. During bleaching endo-symbiotic microalgae inside the protists are moved from their original position towards the apertures (shell openings) before being expelled. *Amphisorus hemprichii* is more tolerant to thermal stress than *A. lobifera*. *Amphisorus hemprichii* was stronger negatively affected by low-light than *A. lobifera.* We conclude that *A. hemprichii* will not grow well at turbid and run-off impacted locations. We conclude that global warming in combination with low-light due to enhanced sediment run-off will stress the physiology of benthic foraminifera due to changes to the the coastal sediment production on these reef islands.

Keywords: Foraminifera, global warming, bleaching, sedimentation, photosynthesis

Phylogeny and classification of the family Sainouroidea (kingdom: Cercozoa, supergroup: Rhizaria) reveals a highly diverse and divergent clade with similar morphology

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Sainouroidea is a family of Cercozoan flagellates and naked amoebae in the supergroup Rhizaria. The 18S rRNA gene of Sainouroids is very divergent and does not amplify using universal eukaryotic PCR primers. Previous environmental sequencing of fecal samples and globally collected soils with primers designed for the Sainouroid clade revealed great diversity and high sequence divergence in the family Sainouroidea. The two described genera of amoebae in this clade are *Guttulinopsis* Olive 1901, which also displays aggregative multicellularity, and *Rosculus* Hawes 1963. Although the identity of *Guttulinopsis* is straightforward, the same in not true for *Rosculus*, and the actual identity of Hawes' isolate is unclear. Here we isolated and analyzed cultures of amoebae with morphology descriptive of *Guttulinopsis* and *Rosculus* from many environments. We describe 4 novel genera and 10 novel species of naked amoebae in the family Sainouroidea based on 18S rDNA sequencing, differential interference contrast microscopy, and transmission electron microscopy. Identification of these amoebae is problematic and potentially unresolvable without the 18S rDNA sequence. Moreover, aggregative fruiting has only been observed in the genus *Guttulinopsis* and is only found in small clade of several *Guttulinopsis* isolates. Most genera have flat mitochondrial cristae, but tubular mitochondrial cristae have been found in basal branching groups.

Keywords: Rhizaria, Cercozoa, phylogenetics, amoeba

Biodiversity of Foraminifera (Rhizaria) in intertidal sites of the French Atlantic coast: comparison between individual specimen sampling and environmental DNA with Next Generation Sequencing

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Traditionally, benthic foraminifera have been studied on the basis of morphological criteria. This strategy has led to much discussion about the range of variability that can be admitted in a single species, and largely different species concepts have been developed. Moreover, morphology-based taxonomy has been more or less restricted to fossilisable taxa with hard shells, whereas soft-shelled forams have often been neglected.

Since 20 years, molecular identification or DNA barcoding of single foraminifers has been developed. Foraminifers are usually collected alive one by one, their morphology is documented and they are individually extracted for DNA. This method allows linking the morphology-based taxonomy with DNA barcoding in one individual, but is very time consuming. Compared to traditional morphologically-based studies, it usually lacks a quantitative dimension and is rather qualitative.

Next Generation Sequencing (NGS) applied to environmental DNA (eDNA) has increasingly developed in the recent years and allows obtaining millions of sequences for a very low cost compared to older methods with cloning and Sanger sequencing. However, to connect eDNA biodiversity with former knowledge based on morphological taxonomy, extended databases bridging DNA and morphological data are needed.

Here, we will compare single foraminifer DNA barcoding and eDNA NGS results for four site situated on the French Atlantic coast: the Bay of Bourgneuf, the Brillantes mudflat in the Loire estuary, the island of Bailleron and the Bono river, both situated in the Gulf of Morbihan. Two of these sites, Brillantes and Bourgneuf, are also routinely studied with quantitative morphologically based methods for their foraminiferal biodiversity since several years, allowing further comparisons with morphology-based methods.

Keywords: Foraminifera, biodiversity, intertidal area, next generation sequencing

Phylogeny of the rare freshwater ciliate *Neobursaridium gigas* (Oligohymenophorea, Ciliophora) and other peniculids from India

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Ciliates belonging to Subclass Peniculia (class Oligohymenophorea) are represented by genera *Paramecium, Frontonia, Apofrontonia, Stokesia, Lembadion*, *Paranassula*, together with some more rare ciliates such as the giant ciliate *Neobursarium gigas*. Phylogenetic relations among genera inside the subclass are far from being resolved. Indeed, one the most representative genus, *Frontonia*, resulted paraphyletic in several previous studies as well as in the present one. In the present work, we provided for the first time a molecular characterization of the rare *Neobursaridium gigas.* Moreover, we performed a phylogenetic analysis of Peniculia, including several 18S rDNA gene sequences we characterized from different *Paramecium* and *Frontonia* species from India, in addition to the sequence of *Neobursaridium gigas*. Quite unexpectedly, the inclusion of the 18S rDNA gene sequence of *Neobursaridium* did not help in resolving the complex phylogenetic relations inside the clade. Conversely, it contributed to create new and intriguing questions in *Paramecium* phylogeny and evolution.

Multicellularity in the Excavata, the genome sequence of *Acrasis kona*

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Acrasids (Heterolobosea, Excavata) are soil amoebae that undergo aggregative multicellularity when food becomes scarce to form small elevated sorocarps on the soil surface. They were long considered the primitive sister group to the well studied sorocarpic amoebae, *Dictyostelium* spp. (Amoebozoa) but are now known to be very distant relatives. Thus acrasids represent independent evolution of aggregative multicellularity and the only example of multicellularity in Excavata. This makes *Acrasis* a unique model system to study aspects of multicellularity such as cell-cell communication, differentiation and kin recognition. We have recently completed sequencing and annotation of the nuclear genome of *Acrasis kona*, one of five recognized species of *Acrasis* with distinct sorocarp morphology. The genome is ~44 Mb in size with 15,868 predicted proteins, of which 4,987 (~31%) are novel. Amongst the remaining 10,881 *A. kona* genes, many are uniquely shared with various groups of non-excavate organisms, suggesting recent acquisition by horizontal gene transfer. This includes genes apparently acquired from bacteria and expanded into multigene families in *A. kona*, some of which appear to be targeted to the cell membrane. The *A. kona* genome is rich in signaling proteins, particularly histidine kinases and G-protein signaling domains. The genome also encodes more than 80% of the genes predicted to be involved in flagellar motility and nearly all genes essential for sexual recombination, although flagella and a sexual stage are unknown for the organism.

Keywords: aggregative multicellularity, sorocarp, Excavata, horizontal gene transfer, multigene families

The interaction between *Tetrahymena pyriformis* and *Escherichia coli* as a model for initiation of endosymbiosis

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Endosymbiosis is a common phenomenon in nature and occurs among almost all groups of organisms. Especially within protists these associations are found in considerable frequency and variety. Even though numerous studies characterised a lot of these interactions in detail, little is known about their mechanistic origins. With few exceptions, it remains unclear how endosymbionts enter their hosts. Therefore, a laboratory model is required for studying these initial steps in endosymbiosis establishment. This model is provided by the interaction between *Tetrahymena pyriformis* and *Escherichia coli*. By fluorescence and transmission electron microscopy we show that *E. coli* is capable of escaping from food vacuoles of *T. pyriformis* without further manipulation. Furthermore, after coculturing with bacterial transformant strains expressing a neomycin resistance gene, the ciliate is more resistant against paromomycin compared to axenic cultures or cocultures with untransformed bacteria and, consequently, benefits from the intracellular persistence of resistant *E. coli* cells. Intracellular bacteria are detected up to two weeks after exposure to the antibiotic, which additionally kills all external bacteria.

To understand how *E. coli* manages to escape from food vacuoles of *T. pyriformis*, their surfaces were chemically modified to create defined biochemical and biophysical surface traits. By means of a carbodiimide any substance carrying an amino or carboxyl group can be covalently bound to the bacterial surface. *Escherichia coli* with increased surface hydrophobicity or alkalinity exhibit higher chances to escape from food vacuoles of *T. pyriformis*. In addition, an artificial oligopeptide designed for mediating membrane transport, enhances the frequency of bacterial escape events. Also F-pili seem to contribute to these processes. Therefore, we conclude that bacterial surface traits play an important role in these very initial steps of establishing endosymbiosis.

Keywords: ciliates, endosymbiosis, model system, *Tetrahymena*

Temporal patterns of soil micro-eukaryotic diversity beneath decomposing pig cadavers as assessed by high throughput sequencing

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Cadavers represent a natural perturbation that influences abruptly environmental parameters in a soil for up to several years. A major objective in forensic sciences is dating death, which is relatively straightforward when less than one month has elapsed, and still very challenging for longer periods. We hypothesized that micro-eukaryotic communities should change underneath a corpse, and that characteristic species will thrive in this very particular habitat. Furthermore, we hypothesize also that these species will follow a succession. We conducted a field experiment using pig cadavers decomposing in scavenger-proof cages in a mixed forest above Neuchâtel, Switzerland, and followed the changes in soil micro-eukaryotic communities by Illumina sequencing of the V9 region of the rDNA SSU gene. We identified indicator OTUs responding positively ("cadaver lovers") or negatively ("cadaver haters") to the presence of cadavers using the IndVal approach. Micro-eukaryotic communities changed significantly beneath decomposing cadavers, and diversity decreased. However, this relatively low diversity of "Cadaver lovers" included rarely reported taxa such as the cellular slime mold *Fonticula alba,* many parasites of other protists such as basal alveolates like colpodellids and approximately half of these OTUs had a low match (< 80%) to any GenBank sequence. Altogether, this suggests that our hypothesis that the communities associated to cadavers are highly specific is confirmed. Furthermore, we witnessed a clear succession between early colonizers (small bacterivores, characteristic of the early decomposition stages) and late settlers arriving after about two months. These results open the way for developing a new tool in criminal investigations, but also to the discovery of an understudied side of soil eukaryotic diversity.

Keywords: cadavers, forensic, high throughput sequencing, V9 region of the rDNA SSU gene, diversity

Generating the diversity – uncovering the speciation mechanisms in symbiotic protists

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More than 150 years since Darwin published his ideas on the origin of species, evolutionary biologists try to untangle processes which influence the speciation. Although a great number of speciation mechanisms have been proposed, the attention was almost exclusively focused on animals and higher plants. The microorganisms, on the other hand, are largely understudied by evolutionary biologists. Strangely, the protists are even much more neglected than the other microbes, such as bacteria, Archaea, and viruses, despite their considerable role in shaping the global ecosystem. We performed a complex study to improve our understanding of putative speciation mechanisms in protists, taking into account the role of evolutionary dynamics. We aimed to differentiate between the processes shaping the current distribution patterns and those driving speciation. The study was focused on a common green algal genus *Asterochloris*, living in a facultative symbiotic association with lichenized fungi. Such living strategy allowed us to study all putative drivers of diversity, including the biotic interactions. We performed a variation partitioning analysis to evaluate the relative contribution of geography, climate, substrate, and a host partner to the algal genetic diversity. Next, we carried out a set of phylogenetic comparative methods to find out what are the predominant speciation mechanisms in this taxon. In general, ecological speciation seems to have a major role in species diversification, yet more data are still needed to fully corroborate this assumption.

Keywords: speciation, ecology, algae, symbiosis

Phylogeny, evolution and systematics of Amoebozoa

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Amoebozoa is a supergroup of eukaryotes unifying naked and testate lobose amoebae as well as archamoebeans, mycetozoans, a diverse assemblage of organisms now known as Variosea and a small but probably high-ranked lineage called Cutosea. Recent findings in the fields of molecular phylogeny and increasing number of phylogenomic data changed some of our views on the relationships between various lineages of Amoebozoa. Moreover, description of new lineages as well as finding of new representatives belonging to already known lineages warranted numerous revisions and clarifications of the current system. Among the most remarkable changes that happened in the recent years it is possible to note shaping and drastic size increment of the Variosea clade, finding of sporocarpic fruiting in various amoebozoan branches, erection of Cutosea clade, new view on the relationships between the main lineages within Discosea, finding of the first representatives of clades that were known from environmental sequences only, and many other findings. In the same time, some of the basic concepts, persisting since the beginning of the molecular systematics of Amoebozoa, like the concept of Tubulinea, still are valid and seem to be already well-proved. The talk is aimed to provide a brief review of the recent changes and proposes the first outlines of an improved system of Amoebozoa that incorporates modern findings.

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Keywords: Amoebozoa, phylogeny, systematics

A novel brackish tintinnid with dual-ended lorica collapsibility

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A tintinnid ciliate isolated in waters of the Thames River (Connecticut, USA) is described through combined behavioral, morphological and phylogenetic information. The novel genus and its type species are distinct from established tintinnid taxa by 1) a partially pleated lorica that collapses on both anterior and posterior ends, 2) a ciliary pattern characterized by a single dorsal kinety and low level of complexity, and 3) its genetic distance with respect to all other sequenced genera. The new genus is placed in the family Eutintinnidae based on a cylindrical lorica opened on the anterior and posterior ends, the presence of a dorsal kinety (but absence of lateral ciliary field and posterior kinety), and a basal (though divergent) phylogenetic position with respect to *Eutintinnus*. Prevalence in mesohaline waters of both the Thames River and the Black Sea (where very similar specimens were first detected) may explain the delay in the discovery of these tintinnids. This work exemplifies integrated approaches for description of tintinnid taxa, and the potential for diversity discovery, even in protists that have historically been well-studied in marine waters.

Keywords: Ciliophora, Tintinnida, diversity, collapsibility, mesohaline

Ciliate protists from the sediment-water interface in the Northeastern Gulf of Mexico

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Benthic marine protists have been well documented from shallow marine benthic habitats but remain understudied in deeper habitats on continental shelves and slopes, particularly in the Northeastern Gulf of Mexico (NEGOM). In the past, marine muds have been considered to be depauperate in interstitial fauna, and methodological restrictions have missed sampling of the nepahloid layer in deeper waters. We hypothesized that distinct ciliate community assemblages would be present in this region, not as interstitial fauna as found in shallow marine sands, but as epibenthic organisms in the nephaloid zone of the sediment-water interface over clay and silt sediments. Further, this community of ciliates would be comprised of a mix of benthic and planktonic forms associated with benthic microbial processes. Water column and nephaloid layer samples were obtained via Niskin bottles and a multicorer at stations across the NEGOM from 50 to 1600m depths. Phylum specific primers were utilized to construct clone libraries of ciliate assemblages from the deep water column and the sediment-surface interface. BLAST searches in the NCBI database indicated that a majority (~75%) of the clone sequences corresponded (94-100% similarity) with listed, yet unclassified sequences. Several species were common at most site locations and depths, and many were unique to the nephaloid layer. Known benthic ciliates such as *Uronychia transfuga*, *Uronychia setigera*, and *Spirotrachelostyla tani*, were common in the nephaloid layer samples, and sequences of planktonic forms were recovered that were unique to the near bottom water samples, indicating a diverse and active multi-trophic level community of microbes associated with benthic biogeochemical processes in the deeper waters of the Gulf of Mexico.

Keywords: ciliate, marine, benthos, Gulf of Mexico, oil spill

From single species to co-occurrence networks: the importance of ciliate plankton in lake ecology

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Planktonic ciliates are key organisms in lakes and oceans. Although species identification is standard in any phytoplankton or zooplankton investigation, ciliates are commonly neglected in lake surveys despite their importance in the trophic transfers in aquatic food webs and their good indicator value. In lake plankton, around 150 ciliate species can be found during the course of a year and each one of them has different demands on its environment. In this respect, ciliates have to be identified to the species level because ecologically different, i.e., mixotrophic and heterotrophic species are affiliated to one genus. Well-known examples are species of the genera *Urotricha, Halteria* or *Rimostrombidium*. In our studies, we generally follow a 'bottom-up process' starting with the morphological identification of a species followed by sequence analyses and feeding of public databases which in turn form the basis for massive parallel sequencing approaches. I here present some case studies of ciliate plankton including the autecology of single species up to co-occurrence networks to show why ecological ciliate plankton analyses need to be based on a combination of morphology, molecular sequences and environmental data. Moreover, I will point to the necessity of microscopic investigations in the understanding of co-occurrence networks and why this is important in identifying species-specific dependencies not only among predators and prey but also among parasitic ciliate-ciliate relationships.

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Keywords: ciliate plankton, ciliate ecology, co-occurrence networks, ciliate diversity

Gene transfer accompanying the secondary endosymbiosis of euglenid plastid

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Autotrophic euglenids (Euglenophyta) form a monophyletic group with secondary green plastids, which were most probably acquired by their common ancestor. However, the acquisition of the plastid earlier in the evolution of euglenids (plastid-early hypothesis) cannot be ruled out. The process of organelle acquisition is accompanied by the transfer of genes from the endosymbiont to host (EGT), the presence of such genes indicates past endosymbiosis. To test the plastid-early hypothesis and to learn more about the contribution of EGT to euglenid genome, we have analysed transcriptomes of 5 euglenids (2 osmotrophic, 3 autotrophic) using a pipeline, which enabled us to select genes related to algae. The contribution of algal genes in autotrophic euglenids (around 2 % of genes) is higher than in primary osmotrophs (around 0.07 %) supporting the plastid-late hypothesis. Surprisingly, we observed a high number of genes related to other algal groups than green algae.

The evolution of morphology in Amoebozoa. Are there useful, phylogenetically informative morphological characters?

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The idea that protists have too few morphological characters to be of use for reconstructing phylogeny is widely promulgated in the field of eukaryote systematics. Among the protists, the amoebae are presented as the “poster children” for this point of view. The alternative to morphology, of course, is the use of molecular systematics. However, I will present a case where it appears that, until the very most recent molecular results on the systematics of Amoebozoa, a formal phylogenetic analysis of amoebozoans that was based on morphology was in several ways superior to molecular results. While there are certainly limits to the use of morphology, its characters can and should be used in conjunction with molecular characters. We will present a particular case that told us a lot about the new taxon Evosea before it was even recognized, and we will point out how to use morphological data in the proper context in future phylogenetic studies.

Keywords: phylogenetic analysis, morphology, amoebae

Transcriptome profiling of the fish pathogen *Spironucleus salmonicida* in response to oxidative stress

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Spironucleus salmonicida is an anaerobic metamonad parasite (related to the human pathogen *Giardia intestinalis*) that infects multiple species of fish including Atlantic salmon. *Spironucleus* typically colonizes the anoxic intestines of fish but, unlike *Giardia*, has also been found in oxic environments such as on surface lesions and in the blood. Many proteins of *S. salmonicida* are sensitive to oxygen, particularly within the anaerobiosis-adapted mitochondria (e.g. hydrogenosomes). Therefore, we aim to explore how these parasites cope with oxygen stress during different stages of infection.

To investigate gene expression-level changes specifically related to oxygen stress, we performed transcriptomic analyses of cells grown in presence of oxygen or in media depleted of antioxidants (cysteine and ascorbic acid). We identified 161 genes that are differentially expressed in both of these conditions and an additional 387 and 161 genes that are uniquely regulated in oxygen and antioxidant-depleted conditions, respectively. These differentially regulated transcripts encode proteins related to oxidative stress defense, signal transduction, hydrogen production, and FeS cluster biosynthesis as well as large number of proteins of unknown function. Finally, we compared the gene expression profiles of *Spironucleus* with *Giardia* (grown under similar stress conditions) and uncovered candidate genes related to the unique ability of *Spironucleus* to invade oxygenated host tissues.

Altogether, this study has provided a glimpse into the intriguing biology of the 'salmon killer' *Spironucleus salmonicida*. Understanding how the parasite has evolved the ability to occupy different host environments will provide the foundational knowledge for the development of novel therapeutics to combat *Spironucleus* infections.

Keywords: *Spironucleus*, anaerobic metabolism, oxidative stress, transcriptomics, evolution

Functional genomics of genome development in *Paramecium*

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Paramecium species are classical protist models for studies of developmental, cellular and molecular biology. Nuclear and genomic dimorphism of ciliates, including *Paramecium*, are intriguing features of these organisms that are actively being researched. During development, *Paramecium*'s new somatic nucleus is generated from a germline nuclear copy by large scale elimination of superfluous, widely interspersed DNA. To date a handful of molecules and molecular modifications that are essential for proper DNA elimination have been verified by reverse genetic techniques. These include: (i) a domesticated transposase, which serves as a DNA excisase; (ii) small RNAs, which guide some DNA elimination; (iii) certain histone modifications, which alter chromatin state. Currently one of the key challenges is determining what the relationships are between these molecules and modifications, and the DNA removal taking place during ciliate somatic genome development. By taking advantage of the ability to precisely measure and correlate the effects of disruption of genes that affect DNA elimination, via high-throughput sequencing, we show that there is now a general approach to identify and analyze relationships among the molecules involved in this process. We provide specific examples of the relationships we have established, including those for which corroborating experimental evidence has subsequently been found. This approach provides a way to systematically identify and investigate the essential components required for genome development in *Paramecium* and other ciliates.

Diversity of free-living Preaxostyla

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Preaxostyla (Excavata: Metamonada) is a group of anaerobic protists important for understanding the evolution of mitochondrion. It is divided into two major assemblages: trimastigids and oxymonads. Trimastigids are free-living and morphologically plesiomorphic; they are also paraphyletic in respect to oxymonads and species-poor, including only four species in two genera (*Trimastix* and *Paratrimastix*). Oxymonads, on the other hand, are endobiotic, species-rich, and morphologically diverse and highly apomorphic. In order to fully understand the diversity of free-living Preaxostyla, we have established 52 cultures of trimastigids, mostly obtained from freshwater sediments. Based on the light-microscopy and SSU rRNA gene analysis, we identified 7 new *Paratrimastix* freshwater species, 2 new *Trimastix* marine species, and a new freshwater genus with two species. Most *Paratrimastix* species are morphologically very similar to the described species *P. pyriformis*. Interestingly, one new species has miniaturized cells with only three flagella in contrast to four flagella found in all other trimastigids. Our data show that the free-living Preaxostyla are much more diverse than previously thought, similarly to some other anaerobic lineages, such as psalteriomonadid heteroloboseans, stygiellid jakobids, and *Carpediemonas*-like organisms.

Sand harbours distinct communities of attached and interstitial protists and bacteria

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Sandy beaches dominate coastlines world wide and serve as buffers between land and sea. These are dynamic environments composed of shifting sediments and variable mixtures of marine and freshwater due to tidal and seasonal storm cycles. To better understand the contributions of the microbial community to sandy beach ecosystems, we investigated the diversity of protists and bacteria from five sandy pocket beaches on the northwest corner of Calvert Island, located in the central coast of British Columbia, Canada by sequencing variable regions of the small subunit ribosomal RNA gene (V4 and V9 18S rRNA for protists and V4 16S rRNA for bacteria). Sand was sampled from different elevations along transect lines from the high tide line to the swash zone. As expected, we uncovered rich microbial communities that likely contribute to biogeochemical processes and support benthic and interstitial meiofauna or other organisms of the ecosystem. Communities of protists and bacteria attached to the sand and interstitially were distinct compared to each other and to seawater. The communities attached to the sand were relatively consistent along the transects and among beaches. In contrast, the interstitial communities were more variable along the transects, with the most variation from mid-tide samples which may be related to variations in salinity and moisture content. These results suggest that the distribution and dispersal of microbes from sandy beaches is likely dependent on whether they have attached or interstitial habitats, as well as their moisture and nutrient requirements.

Spatial and disturbance related variation in protist communities of old- world tropical rainforests

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Tropical rainforests are some of the most biodiverse ecosystems on the planet. Typically, the insects are thought of as the most diverse eukaryotic group within the rainforest. Recent work in neotropical rainforests has shown that it may be protist diversity that far exceeds insect diversity, particularly when considering insect parasites. In this study we looked at protist diversity in an old-world pristine South-East Asian tropical rainforest in the Danum Valley region, Borneo, Malaysia. Using high-throughput sequencing of the 18S rRNA gene we characterized the total protist community at fine to meso-scale spatial variations (6cm -~200m) from partitioned root, soil and leaf litter samples. We then compared these communities to another old growth forest at Maliau Basin, Borneo as well as two other adjacent sites that have had a history of logging to look at the impact of this disturbance on protist diversity. We found that several groups dominated a large majority of libraries the Apicomplexa, Cercozoa, Amoebozoa and Ciliophora. We saw a high diversity and abundance of the gregarine Apicomplexa within both soils and leaf litter, as had been observed in the neo-tropical rainforest soils and also a high diversity within the Amoebozoa. There was a significant difference in community composition between Root, Soil and Litter communities and significant variation in the communities over larger meso scales 200-300m and between sites >100 km. We saw significantly lower diversity of protists in logged forests compared to pristine rainforests, showing that logging may impact protists too. To our knowledge this the first study to look at protist communities in old world tropical rainforests in depth and certainly the first to look at the impacts of logging on protists. As the micro-eukaryotes exhibit high functional diversity their importance in rainforest ecosystems should not be overlooked compared to the macro-eukaryotes.

Keywords: rainforest, diversity, high-throughput sequencing, protist, ecology

Acanthoecid choanoflagellates from the Atlantic Arctic Region – a baseline study

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The examination and statistical analysis of loricate choanoflagellate material collected from Greenland waters during the period 1988-1998 represents a de facto baseline study of heterotrophic nanoflagellates from the Atlantic Arctic Region. The geographic sites sampled are Disko Bay (West Greenland) and the high-arctic North-East Water (NEW) and North Water (NOW) polynya. The analyses encompass close to 50 taxa. Some of these are described as new species, i.e. *Acanthocorbis glacialis*, *A. reticulata* and *Diaphanoeca dilatanda*. Two distinct clusters of species that are separated in time and space occur at all three sampling sites. A PCA analysis of NEW and NOW data points to that one community is linked to e.g. an early season high nutrient and low phytoplankton biomass scenario, whereas the other is predominant when nutrient levels are exhausted and the phytoplankton biomass high or declining. The material additionally allows for a comprehensive examination of e.g. the *Cosmoeca ventricosa* morphological variability encountered, as well as puts on record bimodal size variability within a number of species.

Discovery of the novel deep-branching unicellular holozoans and their evolutionary importance

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Investigation of ancestral to Metazoa phylogenetic lineages is of primary importance to resolving the fundamental problem of the origin of multicellular animals and understanding the genome and morphological transitions, leading to the multicellularity. To understand those key evolutionary transitions, we need to know true diversity and biology of unicellular opisthokonts, especially organisms, which occupy the ancestral position to Metazoa; estimate the evolutionary position of each taxonomic group within the opisthokont and holozoan phylogenetic tree; reveal the main molecular and morphological precursors within the basal holozoans to the origin of multicellularity. Here we report the morphological, ultrastructural and phylogenomic analyses of three novel unicellular holozoans, which are very similar in morphology and lifestyle (the main stage in the life cycle is typical opisthokont swimming flagellate cell which remains sperm of most animals and zoospore of the chytrid fungi) but not closely related. *Pigoraptor vietnamica* and *Pigoraptor* Chilean are distantly related to filastereans, and *Syssomonas multiformis* forms a new phylogenetic lineage with the previously enigmatic *Corallochytrium*. Both new genera of unicellular holozoans are shortest and slowly evolving branches on the tree. All three species are predatory flagellates feeding on large eukaryotic prey, which is very unusual for basal Holozoa. All three species also appear to exhibit complex life histories with several distinct stages, including multicellular structures and possess some important precursors to multicellularity, both morphological and molecular.

Keywords: Opisthokonta, Holozoa, flagellates, phylogenomic, multicellularity

Africa: The geographical origin of *Giardia intestinalis* assemblage B, but not assemblage A

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The different genetic characteristics of assemblage (ass.) A and ass. B of *Giardia intestinalis* are well known, though the evolutionary backgrounds of such features are uncertain. To understand the diversity formations of those genotypes, the geographic distributions of the haplotypes were assessed by phylogenetic network analyses using the DNA sequence data-set of three loci—glutamate dehydrogenase gene (GDH), triosephosphate isomerase gene (TPI), and beta giardin gene (Bg). The total numbers of collected sequences for ass. A and ass. B were 696 (GDH:311/TPI:143/Bg:242) and 1273 (GDH:665/TPI:345/Bg:263), respectively. The data were divided into nine regional groups based on the collected geographical areas, and the nucleotide diversity index (π) was calculated for each group. In ass. B analysis, the highest diversity in Africa (π=GDH:0.0216/TPI:0.0156/Bg:0.0137) and comparatively lower diversities in other areas were confirmed. Although no apparent region-specific cluster was observed in the phylogeny, the African haplotypes were found to be distributed over almost all of the haplotype sub-clusters, and the same trend was observed in all loci of ass. B. In ass. A, no such obvious feature was seen. Taken together, the low genetic diversity in non-African haplotypes may reflect a certain bottleneck effect during the global spread of ass. B. Africa seems to be the geographical origin of ass. B. These findings also provide confirmatory evidence of the different evolutionary backgrounds of ass. A and ass. B in *G. intestinalis*.

Keywords: *Giardia intestinalis*, geographical distribution, haplotype, phylogeny, diperse

Phylogenomic analysis of *Paraphelidium tribonemae* (Aphelida, Opisthosporidia, Opisthokonta)

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Aphelids are a poorly known group of parasitoids of algae that have raised considerable interest due to their pivotal phylogenetic position in the holomycotan branch of the opisthokonts. Based on 18S rRNA genes, they form a monophyletic group with Rozellosporidia (Cryptomycota) and the highly derived Microsporidia. This clade has been re-classified as the Opisthosporidia, which constitute the sister group to the fungi. Despite their huge diversity, as revealed by molecular environmental studies, only four genera have been described and we still lack genome or transcriptome data. Here we present the first transcriptome for one aphelid representative, the recently described *Paraphelidium tribonemae*.

Aphelids cannot be cultured axenically. Therefore, the transcriptomic data from *Paraphelidium tribonemae* had to be cleaned from host and bacterial contamination using strict criteria. Our phylogenomic analyses using a concatenated supermatrix approach show that aphelids represent the earliest-branching lineage within the Opisthosporidia. We have carried out a comparative gene-content analysis with *Rozella allomycis* (Cryptomycota), microsporidian and other opisthokont genomes. We have detected genes involved in chitin cell-wall synthesis, suggesting this is an ancestral character to Opisthosporidia and Fungi. Aphelids also contain genes involved in the degradation of algal cellulose, attesting at the molecular level for an active mechanism of algal wall perforation. Also, the presence of electron transport chain complex I genes and a standard metabolism gene repertoire, which are missing in *Rozella*, argue for a less specialized parasitic lifestyle in aphelids, in agreement with their more basal phylogenetic position. Finally, the characterization of its repertoire of myosin molecular motors reveals that aphelids and nucleariids have similartiries with both holozoans and fungi, thus retaining many similarities with the ancestral opisthokont set. The availability of *P. tribonemae* transcriptomic data will be useful to address broader questions related to the evolution of parasitism in the Opisthosporidia and osmotrophy in Fungi.

Keywords: phylogenomics, aphelid, opisthokont, transcriptome, gene content

An individual look at the oxymonad *Streblomastix strix* and its bacterial symbionts using single cell genomics

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Oxymonads are a group of poorly studied protists which live as intestinal endosymbionts in the gut of insects and vertebrates. Most of their representatives are found in termites and wood eating cockroaches where they are closely associated with prokaryotes located inside or on the surface of their cells. Since most representatives of oxymonads are not culturable *in vitro* it is difficult to study the relationships between oxymonads and their bacterial symbionts. Our work is focused on *Streblomastix strix*, an oxymonad living in the gut of the termite *Zootermopsis angusticollis* being a highly-adapted cell to harbour bacterial symbionts. Using single cell picking and whole genome amplification we managed to amplify the genomic DNA of *Streblomastix strix* and its bacterial symbionts, and we sequenced it using next generation sequencing. Our preliminary results show the presence of at least 10 bacterial SSU sequences together with the eukaryotic SSU in our genomic data. Using rRNA FISH we managed to confirm that at least 4 of the bacterial SSU sequences originate from symbiotic bacteria of *Streblomastix strix.* We are working on recovery of individual genomes from our dataset by trying to bin the data using metagenomic binners, to annotate the bacterial genomes and to see if we will be able to predict any metabolic interaction between the bacteria and *Streblomastix strix*.

Keywords: symbionts, *Streblomastix strix*, oxymonad, single cell genome

A new long-term cell culturing system for *Cryptosporidium* and new tools for investigating its parasitic life style

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Cryptosporidiosis is a worldwide disease, second only to rotavirus as the leading cause of childhood diarrhoeal mortality. The causative agents, *Cryptosporidium* species, are parasites belonging to apicomplexans and like many of them lack sufficient treatments, both preventative and curative. Similarly, experimentally proven knowledge of *Cryptosporidium* is also thin on the ground.

A significant reason for this lack of treatment and data is the difficulties faced with obtaining the parasite and the lack of sufficient and long-term *in vitro* models of infection to study it. Currently existing methods either rely on the maintenance of infected livestock which is expensive, time consuming and increasingly unpopular with national governments, or infections of cell cultures, namely HCT-8, with life spans insufficient for large scale production or reliable modelling of the infection. Although recent investigations have led to advances in this field, they remain focused on improving the previous generation of techniques and have yet to resolve the issues of short life spans, cost effectiveness or usability. We present, a new *in vitro* cell culture for the propagation of *Cryptosporidium parvum* that significantly exceeds the production and longevity of the previous gold-standard cell type of HCT-8 in all fields.

We will show the new cell culture infected with *C. parvum* produces between 5-30x more oocysts during their life span than HCT-8, displaying significantly longer life spans until total culture senescence and as a result of this are significantly easier to handle, more robust and cost-effective. By utilising a broad, multi-disciplined approach, including atomic force microscopy, fluorescence microscopy, proteomics and lipidomics, we conclusively show that the oocysts produced by these cell cultures are both morphologically and biochemically identical to those provided by animal models. We will present preliminary data using this new methodology in our attempt to understand the biology of *Cryptosporidium* and its parasitic adaptations.

Keywords: *Cryptosporidium*, culturing, parasitic life-style, host-parasite interactions

Evolution of scales and genetic structure of a morphological species in amoeba genus *Korotnevella* (Amoebozoa, Discosea, Dactylopodida)

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Naked lobose amoebae have little amount of morphological characters available for species discrimination. Amoebae of the genus *Korotnevella* contain complex scales in their cell coat, which are considered to be species-specific. This genus is among a few gymnamoebae genera, which have clear morphological character allowing us to distinguish species.

In the course of our study 11 new species of *Korotnevella* were found, four of them are already described. Phylogenetic analysis based on 18S rDNA show that the genus is probably monophyletic, though the branching at the base of *Korotnevella* tree is mostly unsupported. A possible evolutionary scenario of scales evolution within the genus was proposed. Two monophyletic groups (one with sombrero-like scales and other with latticework basket in large scales) inside the genus were revealed.

The phylogenetic analysis based on COX1 gene sequences (12 *Korotnevella* morphospecies) show strong correlation between scale morphology structure and molecular data. The lowest level of inter-specific sequence divergence between different morphological species of *Korotnevella* reached 8%, while the highest level is 23%. Our study shown that each of two *Korotnevella* species, *K. stella* and *K. heteracantha*, consists of several MOTUs (molecular operational taxonomic units). Two of four MOTUs of *K. stella* include COX1 sequences of amoebae isolated from distant habitats, while all MOTUs of *K. heteracantha* were found to be endemic for a single habitat.

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Keywords: *Korotnevella*, species structure, phylogeny, 18S rDNA, COX1

Characterization of ciliate diversity in bromeliad tank waters from the Brazilian Atlantic Forest

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Bromeliads are a diverse group of plants that includes many species whose individuals are capable of retaining water, forming habitats called phytotelmata. These habitats harbor a diversity of organisms including prokaryotes, unicellular eukaryotes, metazoans, and fungi. Among single-celled eukaryotic organisms, ciliates are generally the most abundant. In the present study, we used massively parallel Illumina DNA sequencing to survey the eukaryotic communities, especially ciliates, inhabiting the tanks of the bromeliads *Aechmea gamosepala* and *Vriesea platynema* at an Atlantic Forest site in southern Brazil. Filtered sequencing reads were clustered into distinct OTUs using a 99% identity threshold, and then assigned to phylum and genus using a BLAST-based approach (implemented in QIIME) and the SILVA reference database. Both bromeliad species harbored a very diverse eukaryotic community, with Arthropoda and Ciliophora showing the highest abundance/biomass (as estimated by the number of sequence reads). The ciliate genus *Tetrahymena* was the most abundant among single-celled organisms, followed by the apicomplexan group gregarine, and the ciliate genus *Glaucoma*. The results presented here demonstrate a hidden diversity of eukaryotic organisms that occur in bromeliad tank waters, and opens up new avenues for their in-depth characterization.

Keywords: ciliates, bromeliads, metabarcoding, ecology, Atlantic forest

Iron sulfur cluster assembly in oxymonad *Monocercomonoides*

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Oxymonads are a group of anaerobic or microaerophilic protists living in guts of insects and vertebrates. Currently they are the only known group of eukaryotes without mitochondrion. In their closest free-living relative *Paratrimastix pyriformis* have been found organelles, which resemble hydrogenosomes. In contrast to most other eukaryotes, oxymonad *Monocercomonoides* Pa203 lacks mitochondrial ISC system for the synthesis of Fe-S clusters, yet the bioinformatic prediction of Fe-S containing proteins revealed that *Monocercomonoides* contains about 60 Fe-S cluster-containing proteins, a number comparable with other studied eukaryotes.

Instead of ISC, genes for proteins of SUF system for the Fe-S clusters synthesis were found in its genome and transcriptome: SufB, SufC, SufS and SufU. Thorough search for genomic and transcriptomic data indicate that this pathway has been acquired already in the common ancestor of oxymonads and trimastigids. Heterologous localization of SufB and SufC in *Trichomonas vaginalis* and yeast expression systems showed cytosolic localization. Experiments on *E. coli* have shown that SufB of *Monocercomonoides* can substitute the function of *E. coli* SufB in the synthesis of Fe-S clusters.

Five subunits of CIA, a pathway specific to the eukaryotic lineage, have been identified in the genome of *Monocercomonoides*: Cia1, Nar1, Nbp35, and two Cia2 copies. This composition is similar to the reduced CIA pathways of *G. intestinalis* or *T. vaginalis*. We have performed complementation experiments in yeast to prove functionality of the CIA pathway of *Monocercomonoides*, but these experiments have not been successful so far. Our results indicate that *Monocercomonoides* and possibly whole Preaxostyla are the first known group, which assemble Fe-S clusters in the cytosol by concerted action of SUF and CIA pathways.

Proteome of *Euglena gracilis* plastid – implications of the secondary endosymbiosis and traces of its even more colorful history

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Euglena gracilis is a representative of phototrophic euglenids harboring secondary plastids acquired horizontally from green algae. These organisms are very interesting not only from the applied research point of view, as they are able to synthesize various compounds potentially usable in biotechnology or pharmacology, but mainly from the basic research and evolutionary perspective. They are members of Exavata, a supergroup whose study is crucial to understanding the eukaryotic phylogeny as a whole. Their plastids were established *de novo* in a previously heterotrophic organism in possibly not so distant past via secondary endosymbiosis, an event with far-reaching molecular and evolutionary implications. Moreover, a substantial number of euglenid plastid-associated genes was shown to be related neither to the plastid ancestor, nor other Euglenozoa, which suggests even more complex evolutionary past of these organisms. *E. gracilis* is a well-established laboratory model that has been studied thoroughly regarding its metabolism, physiology and ultrastructure, however, its nuclear genome has not been sequenced until recently because of various challenges posed by its unusual and complex organization and relatively great size. As a part of the EuglenaDB genome sequencing project (https://sites.dundee.ac.uk/euglenadb/), plastid proteins of *E. gracilis* were identified via liquid chromatography tandem mass spectrometry and quantified by MaxQuant. Three replicates and three comparisons against mitochondrial protein fraction were performed to minimize contamination from other cell fractions and to maximize the credibility of the plastid localization of the predicted proteins. The set of 1201 putative plastidal proteins was annotated, sorted and used for metabolism reconstruction, phylogenetic analysis of their evolutionary origin and analysis of plastid-targeting domains.

Keywords: proteomics, Euglenophyta, secondary endosymbiosis, secondary plastid, *Euglena*

Diversification dynamics of rhynchostomatian ciliates: impact of seven intrinsic traits on speciation and extinction

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Ciliates are a suitable microbial model to investigate trait-dependent diversification because of their comparatively complex morphology. We examined impact of seven intrinsic traits on speciation, extinction, and net-diversification of rhynchostomatians, a group of comparatively large, predatory ciliates with proboscis carrying a dorsal brush (sensoric structure) and batteries of toxicysts (organelles used to kill the prey). Bayesian estimates under the binary-state speciation and extinction model indicate that two types of extrusomes and two-rowed dorsal brush raise diversification through decreasing extinction. On the other hand, the higher number of contractile vacuoles and their dorsal location likely increase diversification via elevating speciation rate. Particular nuclear characteristics, however, do not significantly differ in their diversification rates and hence lineages with various macronuclear patterns and number of micronuclei have similar probabilities to generate new species. Likelihood-based quantitative state diversification analyses suggest that rhynchostomatians conform Cope's rule in that their diversity linearly grows with increasing body length and relative length of the proboscis. Comparison with other litostomatean ciliates indicates that rhynchostomatians are not among the cladogenically most successful lineages and their survival over several hundred million years could be associated with their comparatively large and complex bodies that reduce the risk of extinction.

Keywords: body length, Litostomatea, net diversification, nuclear pattern, proboscis

Discrepancies between molecular and morphological data bases of soil ciliates studied for temperate grasslands of central Europe

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By measuring the change in species communities, the effect that human land use has on grasslands can be monitored to promote sustainable ecosystem functioning. Protists form the active link in the rhizosphere between the plant roots and higher trophic organisms; however, morphological techniques fail to detect cryptic species and strains with very different ecological values may form the morphological species. High-throughput sequencing (HTS) has proven to be a useful tool in biomonitoring, but this technology is database dependent. This is one of the few HTS studies to take a large sample number (N=150) of soil samples representative of central Europe (mesoscale) aimed to analyze the ciliate diversity in grassland soil. Using georeferenced plots the effect of land-use intensity could be studied. To study database discrepancies, we made an inventory of genotypes and compared database entries and related morphospecies identified. Of the 2,404 HTS unique individual reads (UIRs), only 58 operational taxonomic units (OTUs) could be identified up to species level (99.7% sequence similarity). OTUs from all major ciliophoran classes were present. Community trends were visible at class taxonomic level, but taxa with mainly low frequency were responsible for gradients (e.g., land-use intensity, soil water). Dominant taxa like Spirotrichea and Colpodea indicated that gene variants play a pivotal role in species richness detection. Given cases like the genus *Aspidisca*, it is possible that many of the so called novel lineages and hidden diversity pointed out in phylogenetic analyses of environmental data could very well be nothing more than evidence for the severe lack of molecular data for already known and morphologically described species, present in databases.

Keywords: ciliates, protist, soil, grassland, next-generation sequencing

Coevolution of marine amoebae of genera *Paramoeba* and *Neoparamoeba* (Amoebozoa, Dactylopodida) and their kinetoplastid symbiont 'Perkinsela-like organism'

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Two genera of the dactylopodid family Paramoebidae, *Paramoeba* and *Neoparamoeba*, comprise free-living and amphizoic marine and estuarine amoebae which possess an intracellular eukaryotic symbiont related to Kinetoplastida (*Perkinsela*-like organism, PLO). Deep understanding of all aspects of biology of these taxa is not only of theoretic importance. Some of their members are pathogens of fishes, sea urchins and other invertebrates which may cause massive epizootics in mariculture and local populations. PLO is the key feature of these amoebae, forming a unique symbiotic system of two unicellular eukaryotes both of which are heterotrophic. The nature of relationships within this system and co-evolution between the symbiont and the host amoebae are still poorly studied. Today, there are a number of studies on molecular phylogenetic relationships of *Neoparamoeb*a and PLO based on 18S rRNA gene sequences only showing a monophyletic origin of this symbiosis in the last common ancestor of this clade. Comparison of the molecular phylogenetic trees of amoebae and PLOs shows that they co-evolve in the majority of lineages, with some exceptions. With this communication we report the first finding of two PLO-free strains branching deeply within the genus *Paramoeba*. Based on the molecular phylogenetic analysis we conclude that these strains demonstrate a secondary loss of PLO. This makes up a model system to investigate the genomes of PLO-free strains, and will further help us to clarify whether the horizontal gene transfer occurs between the host and the symbiont. The presented study also expands the taxonomic dataset further clarifying coevolutionary pattern of *Neoparamoeba* and *Paramoeba* amoebae and their symbionts. In addition, we update the available set of molecular tools for investigating the evolutionary relationships of these amoebae by adding the mitochondrial cytochrome c oxidase as a phylogenetic marker for these amoebae. Support: grant 15-29-02749 from the Russian Foundation for Basic Research.

Keywords: phylogeny, *Neoparamoeba*, *Paramoeba*, PLO

Single-cell 'omics' of rhizarian amoebae

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Rhizaria, one of the most recently proposed major clades of eukaryotes, contains a tremendous diversity of organisms that are abundant in both aquatic and terrestrial environments. Despite this diversity, Rhizaria is among the least studied of the major eukaryotic clades. To help fill this void, we are using single-cell transcriptomics to investigate two clades of rhizarian amoebae, Foraminifera and Euglyphida. The Foraminifera that we studied are all benthic, isolated either from salt marshes on the Atlantic coast of New England, or the icy waters of Antarctica. The euglyphid amoebae used in this study are from freshwater, low-pH bogs, also in New England. This sampling not only spans a broad diversity of habitats but the taxa represent a large phylogenetic diversity among each clade together providing a unique opportunity to investigate genome content in these understudied Rhizaria. The key findings from high-throughput sequencing of single cell transcriptomes and subsequent analyses with our taxon-rich phylogenomic pipeline reveal insights into the biology of these rhizarian lineages and reveal the power of single-cell 'omics' for studying uncultivable species.

Free-living amoebae as hosts for bacterial pathogens

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Owing to their ubiquity, their resilience and their partly extremely robust cysts, the so-called free-living amoebae (FLA) are of particular importance as hosts and vehicles for viruses, bacteria and fungi. Also several potential pathogens have been demonstrated to survive or even multiply within FLA, including among others *Burkholderia* spp., *Chlamydia* spp., *Legionella* spp., *Listeria monocytogenes*, *Mycobacterium* spp. and *Vibrio cholerae*. Over the past years, in several projects with numerous collaborators, we screened water and soil samples from various habitats for FLA and further screened the isolated amoebae for intracellular bacteria, both by culture and molecular techniques.

We have demonstrated that *Acanthamoeba* is the predominant amoebozoan genus in Austrian engineered waters, its presence and viability not being affected by regular disinfection and correlating with the occurrence of pseudomonads and legionellae. Also, we could prove that starved legionellae remain infectious not only to FLA but also to human macrophages for an extremely long period of time. Further, we detected an unusually high abundance and diversity of *Acanthamoeba* spp. in soil from African regions endemic for melioidosis. Moreover, we provided the first confirmation for a natural infection of *Acanthamoeba* with *Burkholderia pseudomallei,* the causative agent of melioidosis. Altogether, we have isolated numerous environmental and also clinical strains of FLA that naturally harboured diverse bacteria, occasionally also of more than one species at the same time.

Keywords: free-living amoebae, *Acanthamoeba*, *Legionella*, Trojan horse

Beyond the "Code": A guide to the description and documentation of biodiversity in ciliated protists (Alveolata, Ciliophora)

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Recent advances in molecular technology have revolutionized research on all aspects of the biology of organisms, including ciliates, and created unprecedented opportunities for pursuing a more integrative approach to investigations of biodiversity. However, this goal is complicated by large gaps and inconsistencies that still exist in the foundation of basic information about biodiversity of ciliates. In this talk we will briefly review the issues relating to the taxonomy of ciliates and present recommended standards for generating the foundation of data needed for the integrative investigations of their biodiversity. These will be categorized as ones that MUST be done to satisfy basic requirements for taxonomic identification or description of ciliates, ones that SHOULD be done to ensure that the highest quality of taxonomic data are presented, and ones that COULD be done to maximize the value of data to integrative investigations. This effort stems from a workshop held in the UK in 2014 that explored ways to implement six Grand Challenges proposed by the International Research Coordination Network for Biodiversity of Ciliates (IRCN-BC). As part of its commitment to strengthening the knowledge base that supports research on biodiversity of ciliates, the IRCN-BC proposes to populate The Ciliate Guide, an online database, with biodiversity-related data and metadata to create a resource that will facilitate accurate taxonomic identifications and promote sharing of data. Full details of these recommended standards can be found in the recently published paper by Warren et al. (2017): J. Eukaryot. Microbiol. doi:10.1111/jeu.12391.

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Keywords: biodiversity, nomenclature, phylogenetics, systematics, taxonomy

Haloadaptations of heterotrophic protists using ciliates as models

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Investigations of haloadaptation strategies in unicellular organisms have so far focused on prokaryotes. Protists were largely ignored in this respect, mainly due to methodological shortcomings but also due to the misconception that protists play only minor roles in hypersaline environments. Recent molecular diversity studies revealed high protistan diversities even in saturated brines and pointed to the existence of salinity-dependent environmental dispersal barriers. The latter raised questions regarding haloadaptations in protists, which are directly affecting the observed partitioning of diversity. Therefore, we have investigated haloadaptations in the halophilic heterotrophic ciliates *Euplotes* sp., *Platynematum* sp., *Pseudocohnilembus persalinus* and *Schmidingerothrix salinarum*. To this aim, we established two experimental approaches: (i) ^{1}H-NMR spectroscopy as a simple technique for the detection, identification and quantification in intracellular compatible solutes and (ii) ion-imaging with ion-specific fluorescent dyes as a tool to analyze the intracellular ion concentrations in single cells *in vivo*. ^{1}H-NMR spectroscopy identified glycine betaine and ectoine as the major compatible solutes in the investigated ciliates. The intracellular concentrations of both organic solutes increased significantly with increasing external salinity.

Detailed investigations of haloadaptation strategies in heterotrophic protists require an appropriate model organism. Therefore we proposed *S. salinarum* as an optimal candidate, a true halophile with a wide geographic distribution. Exogenous provided choline (a precursor of glycine betaine) stimulated the growth of *S. salinarum* significantly, indicating that the organism synthesized glycine betaine by the oxidation of choline. Uptake-experiments with labeled $^{13}C_2$-choline confirmed this results, as ^{1}H-NMR identified converted $^{13}C_2$-glycine betaine in *S. salinarum* after incubation. Despite an increase of salinity in the exterior medium up to 21% salt, ion-imaging did not show a cytoplasmic accumulation of Na^+ ions.

This first data significantly advanced the existing knowledge about protistan life under high-salt conditions and the established methods paved the way for further research in this field of biology.

Keywords: ciliates, hypersaline, osmoregulation, glycine betaine, ectoine

Single cell genomics of heterotrophic flagellates reveals mitochondrial diversity in phylogenetically important lineages

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Single-cell genomics has offered great insight into diverse microbial interactions. However, it is a relatively under-developed molecular tool – especially for investigating uncultured eukaryotic microbes. The inherent limitations of single-cell genomic analysis of eukaryotes have therefore prevented widespread use of the technique. We used single-cell genomics (SCG) to explore the genomics of understudied eukaryotic lineages by targeting heterotrophic flagellates by isolating non-photosynthetic cells with fluorescent-stained flagellar proteins.

Using this approach, we demonstrated that complete mitochondrial genomes can be assembled in single amplified genomes (SAGs) from heterotrophic flagellates. This approach was corroborated by investigating previously published data from SAGs and successfully assembling additional complete mitochondrial genomes.

Mitochondrial genomes recovered using this new approach encompass nine novel sequences from distinct deep-branching uncultured lineages from across the eukaryotic tree of life. These newly sampled mitochondrial genomes contained several rare mitochondrial proteins leading us to re-evaluate the mitochondrial genomic complexity of ancestral eukaryotes and demonstrating the ancestral mitochondrial genome showed a wider gene repertoire than most derived extant lineages.

This work also uncovered several unique lineage-specific mitochondrial features, including the first eukaryotic-encoded restriction-mediated selfish element in a mitochondrial genome, as well as a novel mitochondrial genetic code in which both major stop codons have been recoded to tyrosine. This study has provided the foundation for future SCG investigations into the diversity and evolution of organelle genomes in diverse and uncultured unicellular eukaryotes.

Keywords: single cell genomics, heterotrophic flagellates, mitochondrial genomes

Evolution of a rare eukaryotic denitrification pathway in foraminifera

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Benthic foraminifera are unicellular eukaryotes populating sediments of aquatic environments. Several species were shown to perform complete denitrification, a rare metabolic pathway among eukaryotes. Due to this ability and their high density in certain marine habitats, foraminifera are thought to play an important role in the marine nitrogen cycle. However, the foraminiferal denitrification pathway is yet unknown and a prokaryotic contribution has been considered. Here we present evidence for a foraminiferal denitrification pathway encoded in their nuclear genomes. Using transcriptomics and metagenomics we searched for denitrification genes in the foraminifera *Globobulimina turgida* sampled from low oxygen environment. Our analysis reveals homologs of nitrite reductase, nitric oxide reductase and nitrate-nitrite porters that are expressed and encoded in the *G. turgida* genome. A phylogenetic analysis indicates that these genes are of prokaryotic ancestry. Additionally, our metagenomic analysis yielded 17 novel genomes of associated bacteria, all of which encode a full or partial denitrification pathway. Our results thus imply that *G. turgida* denitrification is performed, at least in part, by foraminifera-encoded proteins. The denitrification pathway of *G. turgida* constitutes a rare eukaryotic pathway for nitrate respiration.

Keywords: Foraminifera, denitrification, Rhizaria, nitrate, evolution

A new giant virus isolated from its natural host *Saccamoeba* sp.

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During last years, giant viruses have been found in some protists like *Cafeteria* and *Acanthamoeba*. The latter genus belonging to Amoebozoa, Discosea was often used as a bait to extract giant viruses from different habitats. Here, we present a natural host-parasite system isolated directly from the environment. A *Saccamoeba* sp. was isolated from the bark of a sycamore tree and a culture was established (strain SL-5). Inside the amoebae, a *Mimivirus*-like endoparasite (KSL-5) was found via transmission electron microscopic (TEM) investigations. KSL-5 is a polyhedral virus with average capsid size of 290 nm. The length of the fibrils is about 140 nm, resulting in a virion size of 430-450 nm. Replication of the virus has a long lag phase of at least 12 h. First viruses and a developing virus factory were detected after 18 h with a peak at 30 h. After 24 h, an additional small icosahedral virus (about 50-60 nm) became visible within the virus factory. This satellite virus seemed to interfere with the replication of KSL-5. A preliminary sequence analysis of KSL-5 showed the highest similarity of KSL-5 to *Megavirus chilensis* (~75%, Mimiviridae lineage C) while the sequencing analysis of the satellite virus is still ongoing. In conclusion, this is the first giant virus reported in a *Saccamoeba* species (Amoebozoa, Tubulinea). Interestingly, all attempts to transfer KLS-5 to other amoeba strains including *Acanthamoeba* sp. have failed.

Keywords: giant virus, *Saccamoeba*, natural host, virophage

Phylogenetic, morphological and host range diversity of three parasitic chytrids, infecting colonial volvocacean algae

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Chytrids are zoosporic true fungi that belong to the early diverging fungal phylum Chytridiomycota. In the pelagic zone of both freshwater and marine ecosystems, many chytrid species have been morphologically described as parasites on almost all major groups of phytoplankton. However, the majority of these parasitic chytrids has rarely been isolated and studied in detail. Consequently, DNA sequence data for parasitic chytrids is lacking, resulting in a large proportion of "dark taxa" in databases. In this study we isolated and cultivated three parasitic chytrid species on the green algal host *Yamagishiella unicocca,* and characterized their morphology, infection strategy and life cycle. The 18S rDNA and 28S rDNA genes of the chytrids were sequenced for phylogenetic analysis. We isolated 23 additional host colonies and sequenced the rbcL (ribulose-bisphosphate carboxylase) and ITS (internal transcribed spacer) genes. Cross infection assays were performed with all isolates, including the desmid *Staurastrum* sp., for exploring the host range of the chytrids. The three chytrid species display diverse infection (e.g. single versus multiple infections) and development structures (e.g. inside versus outside the host colony) on the same host species. Phylogenetic analysis indicate that they are relatively distantly related to each other and to other chytrid species, i.e. two of the chytrids could not be assigned to any existing order within the phylum Chytridiomycota. Finally, the chytrids also differed in their host specificity. Molecular analysis of the host isolates, revealed the presence of two "cryptic" host species *Yamagishiella unicocca* and *Eudorina* sp., which differed in their susceptibility to the chytrids. The ecological and evolutionary implications of the different chytrid infection strategies and host specificity are discussed.

Keywords: chytrids, parasites, volvocacean algae

Diversity and distribution of ciliates in sediments from intertidal flats to deep-sea floors: a molecular view

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The estimation of the diversity and distribution of protists has long been an ongoing debate and attracted considerable interest. Marine protists have long been thought to be widespread or even cosmopolitan and thus their diversity may not be very high. However, recent molecular investigations have revealed extremely high diversity of marine protists, in which ciliates are one of the most diverse groups. Here we evaluated the diversity and distribution of ciliates in sediments from intertidal flats through continental shelf to deep-sea floors, using high-throughput DNA sequencing. A total of 1,396,860 sequences were obtained from 33 sites and were clustered into 676 different OTUs based on a 97% similarity. The number of OTUs in sediments from the intertidal flats and continental shelf was higher than that from a deep-sea hydrothermal vent, a deep-sea seamount and a deep-sea plain. UPGMA clustering based on the total OTUs table showed that the samples of each habitat clustered together and formed five major groups: the intertidal flats, continental shelf, hydrothermal vent, seamount and deep-sea plain clusters. SIMPROF analysis based on the total OTUs showed that the ciliate communities of the five clusters were significantly different (p = 0.001). The data indicate a restricted distribution for overall ciliates as well as abundant taxa in marine sediments, but contrasting patterns of the abundant and rare taxa. The proportion of abundant OTUs increased from less than 15% in the shallow sea floors to about 30% in the deep-sea areas, while that of rare OTUs was more than 60% and less than 40%, respectively. Abundant taxa usually can disperse in a wider range than rare taxa, though their distribution is restricted to some extent. Rare taxa are potentially more sensitive to changing environments, occur randomly in very low abundance and thus no clear pattern could be observed.

Keywords: benthic ciliates, biodiversity, biogeography, rare OTUs, abundant OTUs

Genome variation and evolution in diplomonads

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Diplomonads consist a group of single cell heterotrophic protists. Majority of the group members have a mirrored cell structure with two nuclei, two sets of four flagella and two cytostomal apparatuses. Genomic studies on diplomonads could bring insights into eukaryotic evolution and diversity. So far there are a few diplomonad genomes available, including a few different *Giardia intestinalis* assemblies and *Spironucleus salmonicida* genome. We are currently working on improving some of the existing genomes as well as sequencing new genomes with Pacbio sequencing technology. The new genomes are mostly complete at chromosomal level when the genomes have low heterozygosity. This applies to the new *G. intestinalis* WB genome in 5 chromosomes, the new *S. salmonicida* in 9 chromosomes and the *G. muris* genome in 5 chromosomes. The draft genomes are very fragmented for *Spironucleus vortens*, *Spironucleus barkhanus* as well as the free-living *Hexamita*, because of the high heterozygosity and large tandem repeats. Currently, we are generating BioNano optical maps for the *S. vortens* and *S. barkhanus* in an attempt to improve the genome assemblies. All diplomonad genomes sequenced so far share a large number of core genes, however they possess their own gene families and differ largely in genomic structures. It will be interesting to investigate the similarity and difference within the diplomonad genomes, as well as to have insights into how the genomes were shaped and evolved.

Keywords: diplomonads, genome, evolution, PacBio

Extension and contraction mechanism of the proboscis of a ciliate, *Lacrymaria olor*

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The free-living ciliate protozoa, *Lacrymaria olor* has an extendable proboscis, which extraordinarily extends up to 10 times longer than its original length. Previous analyses proposed that the extension is elicited by the movement of the oral cilia with contractile forces potentially being produced by contraction of striated bundle structures located under the cell membrane. However, such a hypothesis has not been fully proved yet. Here, we conducted detailed analyses of the extension–contraction of the proboscis and the ciliary movement with a high-speed camera. The results indicate that the oral ciliary movement mainly contributes to the extension power of the proboscis, and a contractile tension constantly exists in the proboscis even during its extension. In addition, high-speed imaging of the movement of the fluorescent microspheres attached on the cell surface revealed that only distance between microspheres attached on the proboscis was changed while that of microspheres on the cell body was unchanged. Furthermore, changes of microsphere distance on the part of the proboscis near the tip were larger than those on the part of the proboscis near the cell body. It is supposed that, when extending, the proboscis has a property like a nonlinear spring that gradually becomes softer toward the distal end. On the other hand, changes of distance between microspheres during contracting periods indicate that each distance between two adjacent microspheres on the proboscis reduces almost at the same speed, suggesting that the proboscis has a property like a linear spring rather than nonlinear spring during contracting periods. This may suggest that some contractile factor which works only during contracting periods exists in the proboscis. Our study confirmed contribution of the oral ciliary movement for the extension of the proboscis and revealed characteristic physical properties of the proboscis of *Lacrymaria olor*.

Keywords: free-living protozoa, ciliate, cell motility, ciliary movement, high-speed imaging

Naegleria gruberi: The journey of discovering the Golgi almighty

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Naegleria gruberi is a free-living, heterotrophic, microbial eukaryote. It is a non-pathogenic member of the excavate supergroup, which contains key pathogens such as kinetoplastids (*Trypanosoma, Leishmania*), *Giardia* and *Trichomonas*, and as such is distantly related to animals, fungi and plants. Thus, *Naegleria* is an important comparative point for understanding cell biology in current model systems of agricultural, medical and industrial importance. It possesses a relatively canonical set of eukaryotic organelles, with the notable exception of an identifiable Golgi body. The genome of *N. gruberi,* published in 2011, has revealed a highly-complicated collection of cytoskeletal, signalling and metabolic mechanisms as well as membrane trafficking systems. The existence of Golgi-associated proteins in *N. gruberi* was used as evidence to predict the presence of a Golgi organelle. However, until now this has never been validated by molecular cell biological data leaving open the question of the existence of the organelle, and should it exist, its morphology and relation to other endomembrane organelles in *Naegleria*. To examine the distribution of Golgi and its morphology in *N. gruberi*, we have employed several techniques. Initially we focused in the generation of antibodies against Golgi's markers in which we later used for the localisation of Golgi across *N. gruberi*. Then we used immunofluorescence microscopy as well as electron microscopy to evaluate Golgi's expression across the organelle landscape of *N. gruberi*. This work has shown the first experimental evidence for the presence and localisation of Golgi-associated proteins in *N. gruberi*. Having markers for Golgi, and other endomembrane organelles, is a critical first step in the study of membrane-trafficking properties and cell biology in this emerging model eukaryote.

Keywords: *Naegleria gruberi*, Golgi

Unravelling the origin(s) of the euglenid plastid: the genome and transcriptome of *Rapaza*

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A large and diverse body of evidence supports the primary and secondary endosymbiotic origins of plastids. It is universally accepted that the common ancestor of both green plants and red algae acquired chloroplasts from free-living cyanobacteria by primary endosymbiosis. Subsequently, different lineages of heterotrophic eukaryotes engulfed either green algae or red algae, facilitating the independent evolution of secondary plastids. The Euglenophyceae is a group of phototrophic microalgae with secondary plastids originating from *Pyramimonas* green alga; however, many details about the origin and early evolution of euglenophyte plastids remain unclear.

In 2012, we described a novel mixotrophic euglenophycean, *Rapaza viridis*, that contained functional chloroplasts and consumed the green alga, *Tetraselmis* sp. Behavioral data, ultrastructural data, and molecular phylogenetic analyses of *Rapaza* demonstrated that this species had several intermediate traits between those in phototrophic species and phagotrophic species. *Rapaza* is the only mixotrophic euglenid known and branches as the sister lineage to all other euglenophyceans. Therefore, *Rapaza* is the best candidate to provide insights into the origin(s) of euglenophycean plastids.

In order to study the evolutionary history of secondary plastid endosymbiosis in euglenids, we sequenced plastid genome and transcriptome from *Rapaza viridis.* Our genome analyses provided the first evidence of kleptoplasty in the Euglenophyceae. Kleptochloroplasts are not inherited vertically and have to be re-established in each generation. Based on automatic annotations and further manual analyses of the transcriptome, we hitherto identified 229 sequences encoding putative plastid-targeted proteins in the *Rapaza* nucleus. Although kleptochloroplasts have been reported in several different lineages of eukaryotes (e.g., sea slugs, dinoflagellates, ciliates and foraminiferans), our data provides crucial insights into the origin and subsequent evolution of plastids not only in the Euglenophyceae but also photosynthetic eukaryotes as a whole.

Keywords: euglenids, chloroplast, genome, transcriptome, evolution

Diversity of RNA viruses in trypanosomatids

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Viruses in general and RNA viruses in particular are capable of infecting virtually any cellular life form on Earth accounting for their immense diversity. Since present-day research is mostly focused on viruses pathogenic to vertebrates and economically relevant plants, the real diversity of viruses is unknown and largely underexplored. The study of RNA viruses in protists is an emerging field, with studies ranging from mere reports of the virus-like particles to full descriptions including nucleotide sequences.

Virus-like particles in trypanosomatids were first documented over four decades ago in *Leishmania hertigi*, with their characterization mostly limited to ultrastructural studies. The first virus characterized in molecular terms was the *Leishmania RNA virus 1* (LRV1) of the family *Totiviridae*. Its host, *Leishmania guyanensis*, is a causative agent of the highly aggressive mucocutaneous form of leishmaniasis. The LRV1 contributes to the pathogenicity through the increased survival and metastatic potential of the parasite.

In this work we investigated diversity, stability, and evolution of RNA viruses in monoxenous trypanosomatids within the subfamily Leishmaniinae and *Phytomonas* spp. In addition to discovering a number of novel viruses belonging to different supergroups (*Narnaviridae*, *Bunyaviridae* and uncharacterized family of *Tombus*-like viruses), for the first time we documented the presence of NERVE (Non-retroviral Endogenous RNA Viral Element) in trypanosomatids. We attempted to infer scenarios of evolution of all these divergent viruses and concluded that they could have independently originated from viruses of fungi, insects, and non-insect terrestrial invertebrates.

Keywords: RNA viruses, Trypanosomatidae

Candidatus Phycorickettsia trachydisci, a novel lineage of Rickettsiaceae engaged in a long-term partnership with eustigmatophyte algae

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Rickettsiales are a group of obligate intracellular bacteria originally found in terrestrial animals, but more recently recognized as widespread endosymbionts of various aquatic hosts, particularly diverse protists. One rickettsial species (*Candidatus* Megaira polyxenophila) was detected also in several green algae, but reports on rickettsial endosymbionts in other algal groups are missing. Here we show that some eustigmatophytes (coccoid algae belonging to Ochrophyta, Stramenopiles) are infected by a novel bacterium denoted *Candidatus* Phycoricktettsia trachydisci. Phylogenetic analyses of the 16S rRNA gene revealed that it constitutes a new genus-level lineage in the family Rickettsiaceae, and phylogenomic analyses of a supermatrix comprising >100 proteins exhibiting one-to-one orthology in the order Rickettsiales confirmed *Ca.* Phycorickettsia trachydisci as a deep lineage presumably sister to Rickettsiaceae members sequenced so far. Fluorescence *in situ* hybridization and transmission electron microscopy demonstrated the occurrence of *Ca.* Phycorickettsia trachydisci as multiple endosymbionts in the cytoplasm of vegetative cells of several distantly related eustigmatophyte species. We sequenced the genome of the endosymbiont in *Trachydiscus minutus* CCALA 838 and found that its size (1.47 Mbp), GC content (34%), and number of predicted genes (~1260) are in the range typical for related bacteria. About 250 genes, mostly encoding hypothetical proteins, lack orthologs in other Rickettsiaceae. However, six of these genes represent the *ebo* operon of incompletely understood function we recently reported from the plastid genomes of two eustigmatophytes and various bacteria. Strikingly, the operons from *Ca.* Phycorickettsia trachydisci and eustigmatophyte plastomes are specifically related, indicating genetic exchange between the endosymbiont and host lineages. We hypothesize that *Ca.* Phycorickettsia trachydisci is a long-term partner of eustigmatophytes and that their interaction is partly dependent on the function of the *ebo* operon.

Keywords: eustigmatophytes, Rickettsiales, endosymbiont, ebo operon

Molecular tinkering in the evolution of the membrane attachment mechanisms of the Rheb GTPase

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Rheb is a highly conserved Ras-like GTPase involved in the cell growth and division regulation. A standard Rheb protein consists of a GTPase domain and a hypervariable tail with a C-terminal motif governing prenylation of a conserved cysteine residue. We conducted a detailed analysis of Rheb sequences based on a dense sampling of the eukaryote phylogeny, including minor lineages of key evolutionary importance. The analysis revealed that the canonical and apparently ancestral Rheb protein structure has been modified in multiple lineages in a way that affects the mode of membrane attachment of the protein. First, Rheb in Cryptista (including *Palpitomonas bilix* as the basal lineage) exhibits an N-terminal extension comprising the conserved phosphoinositide-binding PX (Phox) domain. This protein structure is the first defined candidate synapomorphy of the Cryptista clade. Rheb proteins in Euglenozoa and its sister lineage represented by the novel undescribed protist SRT308 share at the N-terminus an unrelated phosphoinositide-binding domain, FYVE. The C-terminal prenylation motif is retained in the Rheb protein of SRT308, but it was lost before euglenozoan radiation. In Euglenoidea, a second Rheb paralog emerged, lacking the FYVE domain but characterized by an N-terminal amphipathic helix that we demonstrated is myristoylated. All three lineages of the SAR clade (Stramenopiles, Alveolata, Rhizaria) exhibit a novel Rheb form with a long C-terminal extension including four transmembrane segments. This is the only Rheb variant in alveolates and rhizarians, whereas the canonical Rheb is widespread in stramenopiles, indicating Rheb duplication and modification of one paralog in the SAR stem followed by the loss of the standard form from some SAR lineages. Finally, Rheb proteins in several unrelated groups (ancyromonads, labyrinthulids) possess an N-terminal extension of varying length with multiple cysteine residues that might be palmitoylated. Rheb-membrane interaction is thus unexpectedly evolutionarily dynamic and represents an intriguing case of molecular tinkering.

Distinct depth stratification of planktonic ciliate communities from the surface to the abyssopelagic zone in the western Pacific Ocean

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The deep sea is one of the least understood ecosystems on Earth. Knowledge about the patterns of microbial eukaryotic diversity along the water column, in particular below the euphotic zone, is still far from being complete. In this study, we, for the first time, investigated the community composition and structure of planktonic ciliates, the main group of microeukaryotes, from the surface to the abyssopelagic zone at nine sites within the scale of 1300 km in the western Pacific Ocean using high throughput DNA and cDNA sequencing. Analyzing 768,793 DNA and 202,116 cDNA V4 reads, respectively, from the surface, the subsurface chlorophyll a maximum (SCM) zone, 200 m, 1000 m, 2000 m and 4500 m depth, we could detect a distinct stratification within the ciliate communities. Alpha diversities of the ciliate communities of the SCM and the 200 m layer were significantly higher compared to the other layers. The ciliate community of the 200 m layer appeared to be more similar to communities of deeper layers (≥ 1000 m) than to surface and SCM ciliate communities. Key species of the bathypelagic and the abyssopelagic zone, in particular some parasites, were also detected in the 200 m layer, but were almost absent in the surface layers. The 200 m layer, therefore, seems to be an important "species bank" for deep ocean layers. We could not detect horizontal changes in ciliate community composition and structure. Ciliate communities of same layers, but different sites were more similar to each other and the distance decay relationship of planktonic ciliates was not significant within the scale of 1300 km. Statistical analyses furthermore revealed significant effects of temperature and Chlorophyll a on the partitioning of ciliate diversity, indicating that environmental factors are a stronger force in shaping ciliate communities than geographic distance.

Keywords: stratification, pelagic ciliate, high throughput sequencing, the western Pacific Ocean, abyssopelagic zone

Secretome of *Trichomonas vaginalis*

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During infection of the urogenital tract, the parasite *Trichomonas vaginalis* is challenged by factors such as nutrient limitation, the host immune response and coexistence with other members of the microbial fauna. Thus establishment of trichomonad infection depends on multifactorial host-parasite interactions, that involve both contact-dependent mechanisms, such as adherence to vaginal epithelial cells, contact-dependent extracellular killing of host cells, and active phagocytosis of host cells and bacteria, as well as contact independent mechanisms that include secretion of soluble biologically active molecules. To get more insight into the secreted proteome of *T. vaginalis* we detected and quantified released proteins at consecutive time points. Out of 2385 detected proteins, 240 were estimated to significantly increase in time. These include various peptidases and a number of proteins involved in sugar metabolism, most notably beta-amylases which are involved in utilization of host-cell derived glycogen. The prominent secreted proteins belong to the metalloproteinase protein family (GP63) and a family of hypothetical proteins with similarity to immunoglobulin-like domains. We hypothesize that these families cooperate to recognize and modulate the extracellular matrix and vaginal epithelial cells to promote the trichomonas infection.

Keywords: *Trichomonas vaginalis*, secretion, proteome

New perspectives on the evolution of the genetic code in eukaryotes

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Most translation systems employ the same genetic code to interpret the nucleotide sequence in mRNA, but a growing list of exceptions described from various organisms revealed an unexpected evolutionary flexibility of this fundamental molecular mechanism. A series of groundbreaking discoveries published in 2016 and this year has fundamentally expanded the known diversity of genetic codes in eukaryotic nuclear genomes and unveiled new aspects of the evolutionary processes behind the origin of non-standard code. Our lab substantially contributed to this progress by discovering three novel genetic code variants. One, reported from a clade of trypanosomatid, is striking in that the codons UAG and UAA have a context-dependent meaning as sense or stop codons, depending on their position in mRNA. Two other codes, found in the fornicate *Iotanema spirale* and in a novel elusive rhizarian from the Sainouroidea group, share the unique property of having reassigned the UAG codon as a sense one while keeping UAA as the termination codon. This finding for the first time shows that the eukaryotic translation apparatus can evolve to discriminate between the UAG and UAA codons. The first part of the presentation will provide a review of these and other already published exciting discoveries. The second part will be dedicated to presenting our new findings in the field of alternative genetic codes. Thanks to obtaining genomic data from two representatives of the ciliate order Mobilida, we could demonstrate that this group employs the same genetic code as previously demonstrated for their sister order Sessilida, i.e. that the UAG and UAA (i.e. UAR) codons mean glutamate. We thus provide additional support for the monophyly of the subclass Peritrichia. In addition, we have now discovered three additional organisms from three different taxonomic groups (Ciliophora, Stramenopiles, Heterolobosea) with the same genetic code as we previously described in *Iotanema spirale.*

POSTER PRESENTATIONS

POSTER SESSION A (1–80)

Poster No. 1

In vitro modelling of the *Toxoplasma* cellular interconversion in the ocular pathogenesis and potential therapeutic effect of *Curcuma longa*

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The most frequent toxoplasmosis outcome in the immunocompetent subject is the ocular damage. Many years later the congenital infection, the asymptomatic patient can suffer from retinochoroiditis. Acquired toxoplasmosis also may result in ocular toxoplasmosis. The ocular damage is caused by the parasite cyst formation and inflammation and necrosis process occurring. Therefore, cystogen strains of *Toxoplasma gondii* are the main responsible of this pathogenesis. We have developed a new *in vitro* model for ocular toxoplasmosis carring out experimental infection of primary cultures of embryonic retina. This culturing procedure yields a mixed cell population, where all the principal cell types of the retina are present, including neuronal cells. We have observed that this kind of culture induces *T. gondii* cystogen strain to cysts formation, without addition of exogenous substances. This is the first kind of culture in which the parasite switch by free living to cystic stage without experimental stimulation. Since the parasite stage switch *in vivo* is due to the cytokines mechanism induction we have tested this primary retina culture for TNFα production and we have observed high titre of this cytokine in the culture medium where cysts have been produced. Therefore we used this *in vitro* model to test new drugs to prevent the ocular pathogenesis of toxoplasmosis. We have tested a natural active ingredient, the curcumin, isolated from the rhizomes of the plant *Curcuma longa* which ability to inhibit the production of proinflammatory cytochines such as TNFα and anti-inflammatory activities has been well recognized. Retinal cells were infected by cystogen (ME49) and virulent (RH) strain of *T. gondii* and treated with two concentrations of curcumin. Results show a decrease of TNFα concentration in the culture medium where cysts have been produced and treated by curcumin and a parasiticide activity. Possible future applications in the pathogenesis pathways are discussed.

Keywords: ocular toxoplasmosis, cyst, TNFα, curcumin

Poster No. 2

The structure variability of resting cysts of ciliates: light and electron microscopy observations

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The presented study is dedicated to the structure description of resting cysts of ciliates. The terestrial and limnic samples were taken from the different localities of Slovakia (Central Europe). All species were cultivated on lettuce or cereal medium under laboratory conditions. We have observed surface and surface ornamentation of resting cysts of 18 species from 6 different classes (Colpodea: *Colpoda cucullus*, *C. inflata*, *Bresslauides terricola*; Spirotrichea: *Stentor roeseli*, *Gonostomum kuehnelti*, *Oxytricha lanceolata*, *Parentocirrus hortualis*, *Euplotopsis muscicola*, *Phacodinium metchnikoffi*, *Halteria grandinella*, *Tetmemena pustulata*, *Rigidohymena quadrinucleata*; Protostomatea: *Holophrya teres*; Heterotrichea: *Blepharisma lateritium*; Oligohymenophorea: *Vorticellides astyliformis*, *Frontonia terricola*, *Campanella* sp. and, Litostomatea: *Epispathidium papilliferum*). Beside standard observations (e.g. spherical/oval shape, smooth surface, fine granular and mucous layer on surface), some specifics of the surface structures (e.g. unusual surface; unique colour; wide mucous coat; conical-shaped plug; protuberances and other specific ornamentation) were observed. Resting cysts of *P. hortualis* and *R. quadrinucleata* were also investigated, using scanning and transmission electron microscopy. In addition to surface structures, we described the ultrastructure of resting cysts of these two oxytrichids species for the first time. As typical for oxytrichids, the resting cysts of *P. hortualis* and *R. quadrinucleata* are kinetosome-resorbing type and their cystic wall is made of four cystic layers (metacyst, endocyst, mesocyst and ectocyst). However, this study has also revealed two different morphology of ectocyst, typical for family Oxytrichidae (spine-like protuberances and conspicuous wrinkled or reticulate pattern).

Keywords: ciliates, resting cysts, surface, ornamentation, ultrastructure

Poster No. 3

Occurrence of *Blastocystis* among woodland animals in a wildlife park

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Blastocystis is a widespread, anaerobic protist commonly inhabiting the intestinal tract of both humans and animals. At the genetic level, *Blastocystis* is extremely diverse comprising 17 genetically distinct subtypes (ST). Pathogenicity of this enteric microbe is currently disputed and knowledge regarding its distribution, diversity and zoonotic potential is fragmentary.

In this study, we investigated the prevalence and distribution of *Blastocystis* in UK woodland animals housed in a conservation park in Kent. A total of 118 samples were collected from 27 vertebrate species across the park. A combination of cell culturing techniques, microscopy and molecular biology were used to positively identify *Blastocystis*. The barcoding region of the small-subunit ribosomal RNA (SSU rRNA) was used for molecular identification and subtyping.

Eighty four per cent of the samples were positive for *Blastocystis* indicating a wide distribution among the animals in the park. Moreover, the majority of animals positively identified as carriers were asymptomatic reinforcing the idea of its questionable pathogenicity. Interestingly, we identified novel hosts for some *Blastocystis* subtypes (e.g. ST 4), which were considered to have narrower animal host specificity, as well as common trends in subtype distribution with ST 10 being identified in all sequenced samples.

This study provides the first thorough investigation of *Blastocystis* prevalence in a wildlife park in UK, which can be used as a platform for further investigations on the distribution of other eukaryotic gut microbes.

Keywords: *Blastocystis*, diversity, subtypes, conservation park

Poster No. 4

Biofuel from marine microalgae

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Combustion of fossil fuels is the main anthropogenic source of greenhouse gas (GHG) emissions. Carbon dioxide is one of the main GHG, which causes not only global warming, but, also other environmental problems. Agriculture and deforestation associated with it is the second major source of GHG emissions. In this scenario, the need to find clean and renewable energy with minimum footprint is one of the most challenging problems. There is a need to replace fossil fuels with biofuels to prevent further deterioration to the environment. The major challenge in the production of biofuels from microalgae is the need to generate sufficient quantities of microalgal biomass and an environmentally friendly and cost-effective method for extraction of oil from the biomass.

Marine microalgae are the potential third generation energy feedstock with environmental benefits. Saline tolerant microalgae isolated from west coast of India found potential for generating microalgal biomass for preparing microalgal biodiesel. One of the best strain of *Chlorella* isolated from west coast of India which is halotolerant as well as thermotolerant with autosettling capacity. In order to reduce the overall cost of production, a saline tolerant isolate CSIR-CSMCRI's *Chlorella variabilis* (ATCC PTA 12198) grown in open solar salt pans using sea water during the summer season at a temperature of 45 ± 3 °C. In the present scenario with an ability to proliferate in hypersaline environment (3–8 °Be'), C. variabilis is being cultivated in a vast area of 772 m^2 with a total cultivation volume of 360 m^3 with an average biomass productivity of 34.59 $g/m^2/d$. The selected strain (*Chlorella variabilis* PTA-12198) cultivated in solar salt pans was used for making B100 biodiesel which was used to run an unmodified diesel vehicle flagged off by late Shri Vilasrao Deshmukh on 30th March' 2012 at CSIR Headquarters, New Delhi.

Keywords: microalgae, *Chlorella variabilis*, biofuel, mass cultivation

Poster No. 5

Mycamoeba gemmipara nov. gen., nov. sp., the first cultured member of the environmental Dermamoebidae clade LKM74 and its unusual life cycle

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Since the first environmental DNA surveys, entire groups of sequences called "environmental clades" did not have any cultured representative. LKM74 is an amoebozoan clade affiliated to Dermamoebidae, whose presence is pervasively reported in soil and freshwater. We obtained an isolate from soil that we assigned to LKM74 by molecular phylogeny, close related to freshwater clones. We described *Mycamoeba gemmipara* based on observations made with light- and transmission electron microscopy. It is an extremely small amoeba with typical lingulate shape. Unlike other Dermamoebidae, it lacked ornamentation on its cell membrane, and condensed chromatin formed characteristic patterns in the nucleus. *M. gemmipara* displayed a unique life cycle: trophozoites formed walled coccoid stages which grew through successive buddings and developed into branched structures holding cysts. These structures, measuring hundreds of micrometres, are built as the exclusive product of osmotrophic feeding. To demonstrate that *M. gemmipara* is a genuine soil inhabitant, we screened its presence in an environmental soil DNA diversity survey performed on an experimental setup where pig cadavers were left to decompose in soils to follow changes in eukaryotic communities. *Mycamoeba gemmipara* was present in all samples, although related reads were uncommon underneath the cadaver.

Keywords: Discosea, eukaryotic diversity, ribosomal genes, yeast, budding

Poster No. 6

The little-known freshwater armophorean ciliate, *Metopus turbo* Dragesco and Dragesco-Kernéis, 1986, originally found in Africa, discovered on the Micronesian island of Guam

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Order Metopida Jankowski, 1980 consists of mainly free-living ciliated protists inhabiting hypoxic environments. Kahl described the lion's share of metopids early in the 20th century and, apart from Anatole Jankowski in the 1960's, there was little further interest in this group. However more recently, Dragesco and Dragesco-Kernéis (1986) described a new species, *Metopus turbo* from a pond in the Republic of Benin (West Africa). *Metopus turbo* was not explicitly described endemic, but Dragesco believed in at least some degree of ciliate endemism in sub-Saharan Africa. Thus, we were surprised to recover a population, apparently conspecific with *M. turbo*, from Guam, the southernmost of the Micronesian Marianas islands. Identification was based on live observation, protargol impregnation, and scanning electron microscopy. The few studies of metopids based on broad geographic sampling suggest a cosmopolitan distribution. The divergence time of armophorean metopids is unknown. The formation of Guam resulted from a collision of the Pacific and Phillipine tectonic plates (56–30 Ma), long after the Gondwanan breakup (about 180–120 Ma). Guam was never part of a continental land mass. Consequently, tectonic vicariance is an unlikely explanation for the disjunct distribution of *M. turbo*, leading to consideration of a dispersive hypothesis. Possibilities include neozoic introduction (due to human activity), aeolian and/or transoceanic dispersal of encysted forms. Transoceanic distribution of plants, animals, and freshwater metazoans has been proposed to explain disjunct distributions. Almost nothing is known about aerotolerance and halotolerance of encysted sapropelic freshwater protists and their possible role in transoceanic dispersal. Future studies of survival of encysted freshwater and terrestrial ciliates in marine conditions might shed light on an additional dispersal mechanism for these organisms. Further, the 18S rRNA gene sequence indicates *M. turbo* is not a close relative of the type species *M. es* and should be transferred to a new genus.

Poster No. 7

First record of gregarine protists (Apicomplexa) from Atacama Desert associated to *Scotobius brevipes* (Coleoptera: Tenebrionidae)

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Gregarine endobionts are known for its appearance in marine, terrestrial and freshwater invertebrates. As trophozoite (feeding stage) they affect the intestines and body cavities of different insects. Their complex morphology and molecular identity makes it difficult to merge them into single clades. We analysed a new gregarine discovered from guts of a tenebrionid darkling beetle *Scotobius brevipes* collected in the Atacama desert, Chile. It was classified to a new genus *Atacamagregarina* next to *Xiphocephalus* on the basis of phylogentic methods. SSU rDNA analysis have yielded that the new species *Atacamagregarina paposa* n. gen., n. sp., *(*Apicomplexa: Eugregarinorida, Stylocephalidae) clusters within the clade of gregarines from other terrestrial invertebrates and forms a cluster with gregarines living in association to North American tenebrionid beetles. Previous assessments of “parasitism” in gregarine–host systems suggest a high degree of host specificity to particular host stages and host species. More information on the biodiversity of gregarines is necessary to resolve phylogenetic position(s) of these species rich group of protists, which still remains poorly understood.

Poster No. 8

Gastrointestinal ciliates (Alveolata, Ciliophora) in Brazilian herbivorous mammals

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In Brazil, the most studies on gastrointestinal ciliates identifies these microeukaryotes at the genus level and there are few detailed surveys about these group in the country. The aim of this study was to document and assess the prevalence, average relative abundance and density of gastrointestinal ciliates in Brazilian herbivorous mammals. Gastrointestinal samples were obtained from four Hostein x Gir cattle (*Bos primigenius taurus* L. x *Bos primigenius indicus*), 22 Morada Nova sheep (*Ovis aries*), eight domestic horses (*Equus caballus*) and one capybara (*Hydrochoerus hydrochaeris*). 96 species and morphotypes of ciliates were identified, belonging to ten families, Allantosomatidae, Isotrichidae, Blepharocorythidae, Buetschliidae, Cycloposthiidae, Ophryoscolecidae, Protocaviellidae, Protohallidae, Pycnotrichidae and Spirodiniidae. Among these ciliates, 35 species were identified for the first time in Brazil and five in the American continent. *Buissonella tapiri* was recorded for the first time in the host species *Equus caballus* and *Holophryozoon bovis*, which was originally described by Jirovec (1933) and not found since, was rediscovered in the present work. Additionally, new morphological and molecular data were obtained for several ciliated species (*Cycloposthium bursa*, *C. caudatum*, *C. compressum*, *C. cristatum*, *C. elongatum*, *C. hydrochoeri*, *C. incurvum*, *C. lenticularis*, *C. minutum*, *Diplodinium anisacanthum*, *D. polygonale*, *Eodinium posterovesiculatum*, *Eremoplastron rostratum*, *Hydrochoerella intestinalis*, *Monoposthium cynodontum*, *Muniziella cunhai*, *Protohallia nana*, *P. uncinata* and *Uropogon urai*). These data increase knowledge about the gastrointestinal ciliates biodiversity and will contribute to a better understanding of the phylogenetic relationships within the subclass Trichostomatia.

Poster No. 9

Two tales of fish parasites *Ichthyophthirius multifiliis* and *Cryptocaryon irritans*

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Ciliated protozoan parasites *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* cause highly lethal white spot diseases in freshwater and marine fish, respectively. Outbreaks are reported globally, and often result in huge economic losses in mariculture/aquaculture and ornamental fish industries. While both parasites exhibit similar life cycles, they belong to two different families. The parasitism is therefore a result of convergent evolution. We have been studying the population structures of both parasites in recent years. Using both mitochondrial and somatic sequences, we are able to resolve isolates of both of parasites into approximately three populations. However, in contrast to *I. multifiliis*, populations of *C. irritans* display unexpectedly high genetic divergences (> 9%), indicating that speciation might have been ongoing. I will discuss the significance of these findings, and how they change our perspectives on studying these two parasites.

Keywords: cox, SSU, Oligohymenophorea, sexual reproduction, syngen

Poster No. 10

Ultrastructural and immunological characteristics of intermediate-type filament in several free-living ciliates

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The ultrastructural characteristics of intermediate-type filament system were observed and compared by the scanning electron microscope in five species of free-living ciliates treated with a patented technology. And the immunology experiments aimed at the intermediate-type filament (IF) of three representative ciliates species were conducted to identify the kinds of these IF and their distribution inside the cell. So that to speculate the biological function of these IF.

The important results are as follows: 1. The three-dimensional characteristics of IF distributed subcortical, around cytopharynx, cytoplasm, nucleus and vesicular structures in ciliates were illuminated at the first time or given a recommendation on submicrostructural level for the five ciliates species. 2. Intermediate-type filaments existed widely in ciliates, but their distribution area and ultrastructure morphology and combination mode with other cell internal structurse are different among different species. As if the closer genetic relationship, the more similar those characteristics in the ciliates. 3. Through immunological experiments, not only above results were confirmed, but also different kinds of IF protein in different species or in different areas of a single cell of ciliates were indentified. There were mainly three kinds of IF protein in these ciliates, keratin, vimentin and lamin protein. 4. A special IF in *Euplotes vannus* around the nucleus were tested by immunofluorescence experiments and confirmed that it does not belong to anyone of above keratin and vimentin or lamin protein. Maybe it is a unique intermediate-type filament protein of ciliates. Its nature and function still need further research.

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Keywords: intermediate-type filament, ultrastructure, immunofluorescence experiment, ciliates

Poster No. 11

Euduboscquella sp. (Dinoflagellata, Syndinea), an intracellular parasite of the ciliate *Helicostomella longa* (Brandt, 1906) Kofoid & Campbell, 1929: Morphology and molecular phylogeny

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Syndinean dinoflagellates that infect tintinnid ciliates cause host mortality which can lead to the decline of blooms and promote species succession. We provide data on parasitism of the tintinnid, *Helicostomella longa*, for weekly samples collect from the southern coast of Korea over a two-year period. Parasite morphology was examined using material and specimens processed by quantitative protargol staining (QPS). Molecular phylogeny of the parasite was inferred using nuclear 18S ribosomal RNA gene sequences. The intracellular parasite develops in the host cytoplasm. At the end of vegetative growth cycle, the mature trophont emerges from the host and undergoes sporogenesis with early separation of the sporocytes. Sporogenesis produces three different types of spores: (1) about 80 dinospores (ca. 8 µm in length), (2) about 450 non-motile spherical spores (ca. 2 µm in diameter), (3) about 320 triangular spores (ca. 7 µm in length). Infected *H. longa* showed 100% mortality rate. Based on the morphological and molecular analyses, the intracellular parasite was identified as a new species of *Euduboscquella* (Dinoflagellata, Syndinea). Further investigations will estimate parasite prevalence in natural host assemblages and examine relationships between infections and environmental conditions.

Keywords: *Euduboscquella*, *Helicostomella longa*, parasitic dinoflagellate

Poster No. 12

The plastid genomes of early-branching rhodophyte algae reveal unprecedented levels of self-splicing intron proliferation

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The new red algal subphylum Proteorhodophytina unites all four classes of mesophilic non-seaweed red algae (Compsopogonophyceae, Stylonematophyceae, Porphyridiophyceae and Rhodellophyceae). Proteorhodophytineans exhibit great diversity in terms of ultrastructure, morphology, and ecology, but their plastid genomes (ptDNA) seem to have some unifying features. One of them is the massive proliferation of group II introns that has occurred during their divergent evolution. Among our six newly sequenced proteorhodophytinean plastid genomes, the most intron-poor ptDNA is that of *Bangiopsis subsimplex* UTEX LB2854 which has 39 predicted introns, while the most intron-rich is that of *Corynoplastis japonica* NIES-2662 with 311 introns. This is more than double the number found in *Euglena gracilis* strain Z, which was the most intron rich plastid genome known prior to this study. Two of the other six genomes also have more introns than *E. gracilis* strain Z. The intron density across the 6 ptDNAs ranges from 0.203 introns per protein-coding gene in *B.* subsimplex to 1.737 introns per protein-coding gene. The second largest ptDNA, that of *Bulboplastis apyrenoidosa* NIES-2742, is notable not only for the role of introns in its expansion but also due to evidence for at least 31 intergenic bacterial insertion sequences whose transposases appear to now be pseudogenized. This marks the first time insertion sequences have been seen in an organellar genome. The sizes and architectures of even the most intron-poor of these newly sequenced red algal plastid genomes contrast strongly with those of the better-studied red algal classes Bangiophyceae and Florideophyceae, which are highly compact and gene-rich, contain few or no introns, and are thought to closely represent the genome of the common ancestor of all plastids. A closer look at red algae reveals a dramatic inflation of plastid genome size, driven in large part by the proliferation of self-splicing introns and bacterial insertion sequences.

Keywords: group II introns, ptDNA, red algae, rhodophyta, transposase

Poster No. 13

Smaller where it´s warmer, bigger where it´s colder: The genome size variation in European populations of *Synura petersenii* (Stramenopiles, Chrysophyceae)

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The amount of nuclear DNA may have substantial impact on cell functioning and its size. A pronounced variation in DNA content was observed in many groups of organisms. While this topic have received considerable attention in higher plants and seaweeds, much less is known for marine microorganisms, and there is almost no knowledge of genome size variation in freshwater protists. We present the first insight into genome sizes in the Chrysophyceae lineage. Using flow cytometry, we analysed DNA content of representatives of *Synura petersenii* populations across Europe. Our pilot data have shown substantial variation in genome sizes among populations, spanning 1.05–1.80 pg of DNA. The source of this diversity is more likely gradual increase/decrease in DNA content than genome doubling (polyploidization). Interestingly, genome size variation seems not to be random but rather follows geographical trends. The genome size of individuals in *S. petersenii* populations increases with latitude in Europe. Given that higher nuclear DNA content usually leads to increase in cell size, our results are consistent with the so called “temperature-size rule” predicting increase in cell size with decreasing mean temperatures. Also, the genome size diversification may indicate independent evolution of lineages within *S. petersenii* and thus cryptic diversity.

Keywords: DNA content, flow cytometry, *Synura petersenii*

Poster No. 14

Multidisciplinary re-description of *Plasmodium* (*Novyella*) *paranucleophilum* in Brazilian wild birds of the Atlantic Forest kept in captivity

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Haemosporidian blood parasites of the *Plasmodium* genus are the causative agents of avian malaria in many parts of the world. Despite the great diversity of Brazilian avifauna, few studies have been conducted to examine the haemosporidians of wild birds found in the Brazilian Atlantic forest, especially those kept in captivity. This study aimed to re-examine and further characterize the South American avian parasite *Plasmodium paranucleophilum* using a multidisciplinary approach. Blood samples were collected from 68 captive birds representing 15 species found in the Atlantic forest of southeastern Brazil. Morphometric and morphological characterization was performed, in addition to PCR and sequencing of the mitochondrial cytochrome *b* gene and subsequent phylogenetic analysis. The overall prevalence of *P. paranucleophilum* infection in the study was 13.23% (n=9), with a mean parasitemia of 0.58%. We observed the highest parasitemia of 3.88% in *Rupornis magnirostris*. In our phylogenetic analysis, *P. paranucleophilum* and *P. nucleophilum* formed distinct, highly supported clades, with a mean genetic divergence of 2.48%. This study provides new morphological and molecular data, expanding our knowledge of the haemosporidians of wild birds in Brazil and highlighting the need for further investigation. The true depth of diversity in Brazilian avian haemosporidians remains largely unknown and, given the enormous variety of vectors and avian species, there may be many more species of these blood parasites yet to be described.

Keywords: avian malaria, Haemosporida, neotropical birds, Atlantic forest

Poster No. 15

Diversity of tintinnids (Ciliophora, Choreotrichida) from the coast of São Paulo, Brazil

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Tintinnids are the most abundant ciliated components of the marine trophic foodwebs, acting as predators of nano- and picoplankton. Most species of this group display loricae polymorphism and are still little known, lacking studies from modern microscopy techniques. From August to December of 2010, a survey of planktonic ciliates from the coast of the state of São Paulo, Brazil, was conducted along the area from 23° 47' 59.5" S, 45° 23' 2.4"W to 23° 50' 4.2"S, 45° 25' 19.9"W; 23° 51' 3.4"S, 45° 26' 35.7"W. As result, we found ca. 90 species, distributed among the following genera: *Tintinnopsis* (ca. 25), *Eutintinnus* (8), *Codonellopsis* (6), *Favella* (6)*, Epiplocylis (4), Dadayiella (5), Dictyocysta* (3), *Tintinnidium* (*3), Stenosemella* (3), *Nolaclusilis* (3), *Undella* (3), *Helicostomella* (2), *Metacylis (*2)*, Ascampbelliella* (2), *Codonella* (2), *Salpingella (2), Coxliella* (1), *Steenstrupiella* (2), *Amphorides* (1), *Amphorellopsis* (1), *Rhabdonella* (1), *Xystonellopsis* (1), *Cymatocylis* (1), *Leprotintinnus*(1), *Protocymatocylis* (1), *Paraundella* (1), *Ormosella* (1). Corroborating the recent literature, we find that loricae polymorphism can be problematic when the taxonomy of tintinnids is solely based on the morphology of the lorica, as exemplified by *Tintinnopsis parva* vs. *T. parvula*, which produce similar loricae, but the former has eigth macronuclear nodules, while the latter has two, as in most congeners. We emphasize the importance of detailed live observations of the proper cells (not only the loricae or fixed cells), but also the extensive use of protargol-impregnation and electron microscopy.

Keywords: ciliates, plankton, lorica, morphology, taxonomy

Poster No. 16

Frontonia vernalis – what the ciliate is?

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The genus *Frontonia* composes of about 40 species. Freshwater *Frontonia* spp. were investigated earlier and at the beginning better than brackishwater ones. Nevertheless, the latter group has been recently studied too. However, several misidentifications took place in both ecological groups. All molecular trees dedicated to the genus *Frontonia* included a sequence indicated in GenBank database as *F. vernalis* (U97110), deposited from UK in 1997 and never published. This sequence refers to freshwater frontoniid populated with *Chlorella*-like symbionts previously studied by some of the co-authors of sequence entry (e.g. Berninget et al., 1986; Finlay et al., 1987). *Frontonia* mentioned in these publications does not fit to the original description of *F. vernalis* (Ehrenberg, 1838) and do not match *F. vernalis* description by Bullington (1939) that, indeed, differs in some features respect to Ehrenberg's description. *F. vernalis sensu* Ehrenberg has not yet a clear taxonomical status. According to Ehrenberg (1838), it should have two contractile vacuoles whereas *Frontonia* sp. corresponding to sequence U97110 has only one (Finlay et al., 1987; Esteban et al., 2010). However, it was never properly described in any publication of UK colleagues. Morphological and molecular investigation made in the present study on two different populations of green *Frontonia* spp. (from Italy and Russia), revealed that they present a set of morphologically similar but distinguishable species differing among themselves and with the one from UK. The Italian population consists of 2 different species and represent a sister clade of *F. paramagna*, which is in turn sister of *F. leucas*. The Russian population of green frontoniids also could be subdividing into two different species, probably identical with Italian representatives. Phylogenetic position of the *Frontonia* spp. from Russia is presently under study. Morphological characters of the frontoniids suitable for these ciliates discrimination are discussed.

Keywords: ciliates, *Frontonia*, discrimination

Poster No. 17

The ruby-crowned tanager *Tachyphonus coronatus* (Passeriformes) as a new host for avian malaria (*Plasmodium* spp.) lineages from Brazilian Atlantic Forest

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Avian malaria is a vector-borne disease caused by *Plasmodium* spp. (Apicomplexa, Haemosporida), which are globally distributed occurring in most bird species. The ruby-crowned tanager *Tachyphonus coronatus* is an endemic thraupid bird from South America. The aim of this study was to evaluate the prevalence and cyt *b* lineages diversity of *Plasmodium* spp. in the ruby-crowned tanager from Brazilian Atlantic Forest. Blood smears from 109 bird specimens were analyzed by light microscopy where *Plasmodium* spp. infections were detected in 64 individuals (58.7% prevalence), in which five distinct gametocytes were identified and had their cytochrome b gene sequenced. Lineage A (TCJB79) occurred in one bird and emerged in a monophyletic clade with *Plasmodium unalis*; Lineage B (TCCA94) also was also found in one bird and grouped in a clade containing *Plasmodium elongatum* morphospecies; Lineage C (TCJB472, TCJB851) occurred in two animals and formed a clade along with *Plasmodium lutzi* morphospecies; Lineage D (TCJB1821) was found infecting one specimens and emerged as an independent monophyletic clade with no correspondence with any haemosporidian morphospecies described so far. Our results demonstrate a high diversity of *Plasmodium* lineages in this same host species and suggest that these lineages have a low host specificity since they were described previously in association with different hosts. This study provides the first record of haemosporidian lineages in *Tachyphonus coronatus* and suggest high diversity of *Plasmodium* lineages in this bird species.

Poster No. 18

Altered forms of Rubisco as an indicator of ROS stress in marine diatoms

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The Rubisco protein represents 2-8% of the total cellular protein in marine diatoms (Wu et al. 2014 *Front. Mar. Sci.*) and its octamer form ($RbcL_8RbcS_8$) presents many targets for protein crosslinking or cleavage products. During previous immunoquantitations of Rubisco we identified the differential presence of RbcL by-products in light-stressed *Thalassiosira* species (Li et al. 2017 *J.Phycol.*). We hypothesized that these Rubisco by-products are caused by reactive oxygen species (ROS) and are correlated to other indicators of ROS stress.

We are particularly interested in understanding ROS detoxification strategies in diatoms based on their cell size. The Black Queen Hypothesis (Morris et al. 2012 *mBio*) posits that in a mixed community of microbes there will exist some beneficiaries with reduced metabolic capabilities that depend on other members for vital cellular functions. Smaller diatoms would be better positioned to take advantage of such community mediated ROS detoxification strategies because of their ability to unload harmful ROS to the environment.

To explore this hypothesis, we exposed centric diatoms *Thalassiosira pseudonana* CCMP1335 (2.5-15 µm) and *Coscinodiscus radiatus* CCMP 312 (30-180 µm) to methyl viologen to induce internal ROS production, or to hydrogen peroxide as an external ROS stress. Cell physiology was monitored using Fast Repetition Rate Fluorometry (FRRF), then protein fragments were assessed using mass spec analysis for confirmation of Rubisco complexes or characteristic degradation products. Cells were also surveyed using widely available lipid peroxidation and superoxide dismutase assays to confirm the use of immunoblotting technique as a proxy for ROS stress in diatoms.

Keywords: Rubisco, reactive oxygen species, marine diatoms

Poster No. 19

The folliculinids of Coobowie Aquatic Reserve

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As part of a taxonomic survey of Australian Folliculinidae this paper presents the results from a year-long study of the Coobowie Aquatic Reserve in South Australia. Folliculinidae comprise 32 genera, and except for a few freshwater species are marine. They secrete and live in chitinous lorica, which their motile larval stage or “swarmer”, secretes.

A new species is described and its wider distribution in South Australia is documented. Light and electron micrographs along with detailed morphometric measurements and DNA sequences are presented.

Keywords: lorica, folliculinid, peristomal wing

Poster No. 20

The testate amoeba *Penardeugenia* is no chlamydophryid, but imbricatean

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Penardeugenia bathybioticus Deflandre 1958, first described under the invalid name *Pamphagus bathybioticus* Penard 1904, is a testate amoeba which gained only very little attention since its discovery. Although it has been very well characterized in its description, nowadays barely any reports can be found, even a Google search yields only in two results. Despite described to bear silica scales, it has been grouped into the scale-lacking Chlamydophryidae, mainly because many as "*Pamphagus*" described amoebae have been transferred to them. We here present one cultured isolate; with light and electron microscopy we make a strong case that this amoeba has to be closely related to *Penardeugenia bathybioticus.* SSU phylogeny reveals that *Penardeugenia* groups within the silica scale-bearing Imbricatea in which it resembles a thaumatomonadid lineage lacking flagella. Removal of *Penardeugenia* from the Chlamydophryidae makes the family substantially more homogeneous; nonetheless the name-giving genus *Chlamydophrys* still lacks molecular data and with that a phylogenetic placement.

Keywords: *Thaumatomonas*, scale evolution, phylogeny, taxonomy, Chlamydophryidae

Poster No. 21

Isolation of diverse flagellates from a hypersaline soda lake

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Hypersaline soda lakes are type of a polyextreme habitat found in several clusters around the world, but there is very little known of their eukaryotic microbial diversity. We applied cultivation, light microscopy and ribosomal small subunit phylogenetics to material from a sample from Lake Manyara, Tanzania (pH 10.5, 16% salinity). Several flagellates were isolated into stable culture, including: 1) a halotolerant trichomonad parabasalid, 2) a *Pharyngomonas* species with an unusual morphotype, 3) an extremely slender colpodellid, which preys on the *Pharyngomonas*, 4) a flagellate that is related to the heterolobosean amoeba *Selenaion*, and 5) a novel deep-branching metamonad. The basic life cycle of the colpodellid was reconstructed using light microscopy. The *Pharyngomonas* species and *Selenaion* relative are most closely related to taxa isolated from non-alkaline hypersaline samples; the other three strains are not specifically related to known halophiles or halotolerant clades. These findings hint at an abundance of yet-undescribed novel lineages in haloalkaline habitats, and invite further protistological examination of soda lakes around the world.

Keywords: extremophiles, haloalkiliphiles

Poster No. 22

The genus *Gomphonema* Ehrenberg from Yunnan Province, China

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The diatom genus *Gomphonema* Ehrenberg is a large and complex genus in terms of its taxonomy, systematics and biogeography. Fourtanier & Kociolek (2011) list over 500 taxa in the genus. Although *Gomphonema* is a large, widely distributed genus, the species in China are not well known. Shi (2004) offered a summary of the genus in China and Skvortzow (1929) presented species from the eastern part of the country. More recently, taxa from China have been described by Fan *et al*. (2004), Li *et al*. (2006, 2010), Liu *et al*. (2013) and You et al. (2005). There are a number of *Gomphonema* Ehrenberg species in China that are endemic to the region, such as *Gomphonema kaznakowi* Mereschkowsky, found in the higher elevations of southern China (Li et al. 2003) etc.

Yunnan Province, located in southwestern China. The unique combination of topographic complexity and favorable moisture conditions in the region supports an enormous richness of biological diversity and high degrees of endemism (Zhang 1999). During the investigation of diatom flora from Yunan in 2014, over 100 samples were collected from south and west of this province. Parameters as pH, temperature and conductivity were measured at the field. The sampling habitat include: river, strean, spring, swamps, ponds, lakes and wet-wall. LM and SEM were using to observe the slides. Totally, 29 taxa were found. Based on the morphological characters, three of these taxa were described as new to science.

This project has a great significance for taxonomical research of Gomphonemaceae in China.

This project supported by National Science Foundation No. 31470308

Keywords: *Gomphonema*, Yunnan Province, China, new species

Poster No. 23

Neobodonids are dominant kinetoplastids in the global ocean plankton

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This phylum Kinetoplastea consists of free-living marine or freshwater bacteriovorous flagellates formerly termed 'bodonids', and parasitic trypanosomatids infecting a broad range of organisms: insects, vertebrates, and plants. The key genera, *Trypanosoma* and *Leishmania*, belong among the best-studied protists. However, our knowledge of diversity and distribution of bodonids is rather limited, despite their potential importance for understanding the evolution of parasitism in kinetoplastids. We have analyzed 18S rDNA barcodes (V9 region) in a large global dataset, combining 124 stations of the Tara Oceans expedition. We found 512 OTUs belonging to the Kinetoplastea, but relative abundance of kinetoplastids was quite low (on average 0.2% of eukaryotic reads per sample, ranging from 0% to 14.8%). Kinetoplastids were more abundant in the mesopelagic (347–852m depth) than in the photic zone (5–185m depth), and in the piconano-plankton (*Neobodo* species complex accounted for 59% of all kinetoplastid reads and 127 OTUs, while unknown Neobodonida accounted for 21% of kinetoplastid reads and 172 OTUs. The third most prominent kinetoplastid group was *Rhynchomonas* (36 OTUs and 18% of reads). Kinetoplastids were dominated by 14 cosmopolitan OTUs (six unknown Neobodonida, five *Neobodo* OTUs, two *Rhynchomonas* OTUs, and one from the Eubodonida clade) representing 94% of total kinetoplastid abundance. Three of those OTUs, belonging to unknown Neobodonida, are putative parasitic species since they were prominent in two very different size fractions (0.8–5 µm and 180–2,000 µm) and can be associated with planktonic appendicularian host species. Rarefaction curves revealed that kinetoplastid diversity was saturated in the whole dataset, as well as in separate depth zones, size fractions, and oceanic provinces. Our results suggest kinetoplastids are rare but ubiquitous component of the global plankton.

Keywords: kinetoplastids, metabarcoding, 18S rRNA, marine plankton

Poster No. 24

Comparative analysis of the diversity of cercozoan taxa from the phyllosphere and rhizosphere of different plant species

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The phyllosphere comprises the aerial parts of plants and is dominated by the leaves. Plant leaves are forming the largest biological surface on Earth with 10^8 km² globally, an area approximately twice as large as the land surface. Bacteria are by far the most dominant colonizers with numbers often exceeding 10^7 cells/cm² leaf surface. However, microbial communities of leaves are taxonomically more diverse and include also filamentous fungi, yeasts, algae and protists. Phyllosphere protists are characterized by diurnal life cycles with short active periods at nighttime when dew accumulates on plant leaves or in the event of rain. Although the occurrence of protist taxa on plant leaves has long been recognized, systematic studies are scarce. Recently it was shown that bacterivore Cercozoa exhibit a high diversity in the plant phyllosphere. With their ability to rapidly excyst, feed and multiply within hours cercomonad Cercozoa are perfectly adapted to the fluctuating environmental conditions in the phyllosphere. We aimed to characterize the diversity of plant-associated cercomonads since it is not clear whether special phyllosphere cercomonads exist or plant-associated cercomonads colonize the phyllosphere and rhizosphere of the same plant. We isolated and identified cercomonads from the phyllosphere and rhizosphere of 66 plants belonging to three functional groups (grasses (*Poa sp.*), legumes (*Trifolium sp.*) and non-leguminous forbs (*Plantago sp.*)) to gain an overview on the diversity of cercomonads on different plant species.

Keywords: Cercozoa, diversity, phyllosphere, rhizosphere

Poster No. 25

Three to eight weeks: *Choreocolax polysiphoniae* development

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Red algal parasites have independently evolved multiple times within the Florideophyceae. Many of these parasites infect a host with which they share a recent common ancestor (adelphoparasites), while others have a more phylogenetically distant relationship with their host (alloparasites). As an organism, red algal parasites are morphologically reduced, lack pigmentation and establish cellular connections with their host. These cellular connections, termed pit connections, are crucial to the success of the infection as they enable the parasite to enter the host cell. The alloparasite *Choreocolax polysiphoniae* develops an internal network of rhizoidal cells that form pit connections with host cells. Eventually, these rhizoids grow towards the surface of the host creating the mature pustule of the parasite. Microscopy has been instrumental in our understanding of the morphological development of these parasites, but molecular tools now enable the exploration of parasitic genetic expression during the infection. Using the MALBAC single cell transcriptomics protocol, amplified mRNA was sequenced from *Choreocolax polysiphoniae* pustules ranging from three to eight weeks in age. Samples were also collected for *in situ* hybridization microscopy in order to tie together morphological development with genetic expression of the parasite. Combining these datasets has produced new insights into parasitic interactions in the red algae.

Keywords: parasite, development, red algae

Poster No. 26

Cytomorphological, ultrastructural and immunocytochemical study of *Acanthamoeba* cysts and new findings in encystment

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Free-living amoebae of the genus *Acanthamoeba* Volkonsky, 1931 are causative agents of serious human diseases: granulomatous amoebic encephalitis (GAE) and *Acanthamoeba* keratitis (AK). The life cycle of acanthamoebae includes two stages: active trophozoite and dormant cyst which due to the cyst wall organization is highly resistant to biocides and represents a serious problem in treatment of *Acanthamoeba* infections. We present unique cytomorphological and ultrastructural features of cysts and encystment in *A. lugdunensis* using a wide spectrum of methods of light, confocal, and electron microscopy. For the first time, microtubular network was detected in encysting cells and the presence of filamentous actin suggests its involvement in late phases of encystment. Cellulose filaments – the main component of endocyst were demonstrated in inter-cystic space, and unexpectedly in the ectocyst thus proving the presence of cellulose in both layers of the cyst wall. New findings on the encystment process include clustering of intramembranous particles (IMP) and their density alterations in cytoplasmic membrane. We propose a hypothesis that in the phase of endocyst formation, IMP clusters represent cellulose microfibril terminal complexes involved in cellulose synthesis which after the cyst wall completion are reduced. Our findings uncover new information elucidating the encystment process in acanthamoebae and contribute to their cell biology understanding.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0123. It was supported with grants VEGA 1/0365/16, KEGA 041UK-4/2015 and SK-CZ-2013-0058. This publication is the result of the project implementation: Comenius University Science Park – 2. phase, ITMS 2014+ 313021D075 supported by the Research & Innovation Operational Programme funded by the European Regional Development Fund. Travel expenses of AV were funded by the project 7AMB14SK008 from the MEYS of the Czech Republic.

Keywords: *Acanthamoeba*, encystment, cyst, cellulose, ultrastructure

Poster No. 27

Cultures from the Edge: a glimpse into the diversity of Arctic phytoplankton

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The Arctic Ocean has been undergoing extreme environmental changes related to ice cover loss, which can drastically change the phytoplanktonic community structure and production. The aim of the French Canadian Green Edge project is to investigate the dynamics of the phytoplankton spring bloom (PSB). The bloom takes place in the ice-edge at the seasonally-covered ice zone and has been estimated to be responsible for more than 50% of annual primary production in the region. In order to bring into culture its major players, we applied several strategies for algal isolation, such as serial dilution, enrichment and flow cytometry cell sorting. From more than 1,000 cultures obtained, we identified 562 isolates by 18S rRNA. The group with most cultured representatives was Bacillariophyta, with 423 strains affiliated to Mediophyceae (267 strains), Bacillariophyceae (155 strains) and Coscinodiscophyceae (one strain). The most frequently isolated genera among diatoms were *Attheya*, *Chaetoceros* and *Fragilariopsis*. With respect to photosynthetic flagellates, Chlorophyta (102 strains) was the most abundant group, with Chlorophyceae, Pyramimonadophyceae and Mamiellophyceae representatives. The latter class was largely dominated by *Micromonas pusilla* (50 strains). A new strain of *Bathycoccus prasinos* was obtained, which correspondence with the "coastal" genotype was validated by sequencing the ITS region of the rRNA operon. Two strains of *Mantoniella* sp. with low similarity levels to known 18S rRNA sequences may represent undescribed taxa. From the 14 isolates affiliated to Cryptophyta, 13 strains belonged to *Rhodomonas* and one to *Falcomonas* genera. Other isolated strains were assigned to Haptophyta (5 strains, mainly *Isochrysis* and *Phaeocystis*) and Ochrophyta (15 strains), including two undescribed Pelagophyceae. This work will help identifying key phytoplankton species involved in the triggering and development of PSB, allow ecological experimentation and provide validation for future metabarcoding studies in the Arctic region.

Keywords: Green Edge Project, Arctic Ocean, Roscoff Culture Collection, polar phytoplankton

Poster No. 28

Observations on the argyrophilic extrusomes of *Epicarchesium granulatum* (Kellicott, 1887) Jankowski, 1985 (Peritrichia, Vorticellidae)

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Epicarchesium granulatum was originally classified as *Carchesium,* but in 1985, Jankowski observed that the pellicular striations of this ciliate were reticulated, and thus, was transferred to *Epicarchesium.* In 1997, Foissner and Leitner redescribed this species from live specimens and after silver impregnation. The specific name of the species "*granulatum*" refers to small spherical projections that are visible in live organisms, of which the nature is herein investigated from light and scanning electron microscopy (SEM) preparations of a Brazilian population from wastewater treatment plants in Rio de Janeiro. Two kinds of "granules" were reported for *E. granulatum*: internal argyrophilic granules, only visible after silver impregnation; and cortical blisters, visible in live specimens. In the present study, we demonstrate that the two kinds of granules are actually different instances of the same structures, herein hypothesized as argyrophilic extrusomes. Our data indicates that the argyrophilic granules correspond to extrusomes still located beneath the cell cortex (perhaps immature extrusomes), whereas the blisters are extrusomes which have docked below the cell pellicle.

Keywords: Ciliophora, ultrastructure, Oligohymenophorea, cortical granules, taxonomy

Poster No. 29

Morphology of *Tintinnopsis everta* (Alveolata, Ciliophora, Spirotricha) suggests homology of posterior and second dorsal kinety

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Molecular and preliminary cladistic analyses strongly indicate that the current lorica-based classification of the tintinnid ciliates is artificial. To fully understand the phylogeny and ecology of tintinnid ciliates (Alveolata, Ciliophora, Spirotricha), knowledge on their cell features is urgently required beyond the lorica characteristics. *Tintinnopsis everta* was discovered in the Kiel Bay by Laackmann in 1908 and lives in the plankton of marine and brackish coastal waters. The species is redescribed from the east coast of the USA based on live observation, protargol-stained material, scanning electron microscopy, and genetic analyses. It falls into the large group of tintinnids with the most complex somatic ciliary pattern (with a right, left, and lateral ciliary field as well as ventral, dorsal and posterior kineties), but demonstrates some unique features: the posterior kinety is left of the left ciliary field (vs. below the left or lateral ciliary fields) and the distance between the ciliary fields and the collar membranelles is extraordinarily wide. Its unique position and dikinetidal structure with cilia associated only with the posterior basal bodies suggest a homology of the posterior kinety with the second (left) dorsal kinety. Congruent with the morphological findings, the species is consistently placed among the tintinnids with the complex ciliary pattern in the gene trees, but its closed relatives could not reliably be determined as the species' placement differs somewhat between the Bayesian Inference, Maximum Likelihood, Maximum Parsimony, and Neighbor Joining analyses and is not well supported.

The study was financially supported by the Austrian Science Fund (Project P 28790).

Poster No. 31

An exploration of diversity of Pyramimonadales and Euglenophyceae in environmental samples

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Euglenophyceae is a group of photosynthetic flagellates that possess a secondary derived plastid of green algal origin. The closest known relative to their plastid is a prasinophyte alga, *Pyramimonas parkeae*. We explored the diversity of Pyramimonadales and Euglenophyceae in environmental samples with the hope to find lineages splitting close to the endosymbiotic event. In the first step, we constructed phylogenetic tree based on 16S rRNA sequences of Euglenophyceae and Chlorophyceae downloaded from GeneBank database and the database of TARA expedition. This tree confirmed the position of Euglenophyceae as a sister lineage to Pyramimonadales and placed marine environmental TARA sequences into Pyramimonadales and basal Eutreptiales (marine euglenids), but also within Euglenales considered so far as strictly freshwater group. In the second step, we have amplified and sequenced small pieces of euglenid chloroplast genomes from our marine environmental samples. For this amplification we have used two primer pairs amplifying chloroplast gene clusters uniquely shared by euglenids and *P. parkeae* (*rps*4-*rps*11 and *trn*C-*rps*2). Phylogenetic trees were constructed from obtained sequences. Both trees revealed the presence of a large number of environmental sequences related to Pyramimonadales and Euglenophyceae. Some of these sequences branched again within freshwater Euglenales, though the low support for the internal nodes did not rule out the possibility that the marine environmental sequences form a sister lineage to Euglenales. We have not detected any new lineage branching close to the point of euglenid plastid origin. Nevertheless, the existence of this unnoticed marine diversity of euglenids suggests a potential for further research in this direction.

Keywords: Pyramimonadales, Euglenophyceae, environmental diversity, phylogeny

Poster No. 32

Description of two new species of marine saprotrophic sphaeroformids in the Mesomycetozoea isolated from the subarctic Bering Sea and draft genome of type strain B5

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The genus *Sphaeroforma* previously encompassed organisms isolated exclusively from animal symbionts in marine systems. The first saprotrophic sphaeroformids (Mesomycetozoea) isolated from pollen grains in the near-shore estuarine environment of the Subarctic Bering Sea are described here. *Sphaeroforma sirkka* and *S. napiecek* are also the first species in the genus possessing endogenous DNA-containing motile propagules and central vacuoles - traits that have previously guided morphological differentiation of sphaeroformids from the genus *Creolimax*. The Mesomycetozoea phylogenetically branch near the animal-fungal divergence and are expected to be important to understanding the origins of multicellularity. Phylogenetic analysis of DNA sequences from the 18S rRNA and the ITS1-5.8S-ITS2 loci place *S. sirkka* and *S. napiecek* within *Sphaeroforma*, extending the number of known species to six within this genus. To explore the genetic diversity within *Sphaeroforma sirkka*, we used Illumina HiSeq reads to produce a draft genome of the type strain B5 with an N50 of 46,896 base pairs.

Keywords: *Sphaeroforma*, Mesomycetozoea, rRNA

Poster No. 33

Molecular phylogeny suggests transfer of the diatom genus *Hemidiscus* into *Actinocyclus* (Bacillariophyta, Coscinodiscales, Coscinodiscophyceae)

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Hemidiscus cuneiformis, type of the family Hemidiscaceae, is a diatom with a distinctive semi-circular shape in valve view. A strain of *H. cuneiformis* was established from the coastal region of the tropical southwestern Atlantic Ocean off Brazil. Cells of this strain showed semi-circular to asymmetrically elliptical valves, indicating morphological plasticity. In the small subunit (SSU) rDNA phylogeny, the type species of *Hemidiscus* branched between two clades of *Actinocyclus*, one of which was a sister group, the other in a basal position. Beyond cell shape, there are no significant morphological differences between *Hemidiscus* and *Actinocyclus*. We propose the transfer of *H. cuneiformis* and *H. kanayanus* into the genus *Actinocyclus*. The family Hemisdiscaceae remains for the genus *Actinocyclus*.

Keywords: Bacillariophyta, molecular phylogeny, morphology, pseudonodolus

Poster No. 34

Proposal of *Monorhizochytrium globosum* gen. nov., comb. nov. (Stramenopiles, Labyrinthulomycetes) for former *Thraustochytrium globosum*

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Thraustochytrium is the type genus of the family Thraustochytriaceae in the class Labyrinthulomycetes. This genus is characterized by zoospore formation, namely, shape of the cell wall of sporangia and presence or absence of a proliferous body. However, there are several issues associated with the taxonomy of this genus, and these include polyphyletic taxa and overlapping of taxonomic features among species. In particular, the first and second species, *T. proliferum* and *T. globosum*, were described based on observations of the morphological features of natural samples in the absence of culture conditions. Before addressing the taxonomic issues associated with this genus, it is important to consider the taxonomic features of each species, i.e., the life history under culture conditions and the phylogenetic position. The objective of the present study was to isolate *T. globosum*, the second described species in the genus *Thraustochytrium*, from the type locality. We successfully isolated strain NBRC 112723, which exhibited characteristic features of *T. globosum*. Under culture conditions, strain NBRC 112723 exhibited taxonomic features observed in other thraustochytrid species. Our molecular phylogeny indicated that this strain isolated from the type locality was located in an unidentified thraustochytrid group; moreover, some strains located in this group exhibited characteristic features of strain NBRC 112723. We clearly distinguished *T. globosum* based on the taxonomic criteria used to classify the *T. proliferum* type species. Therefore, we propose the establishment of a new genus, *Monorhizochytrium*, for the species *T. globosum* in the family Thraustochytriaceae.

Keywords: ectoplasmic nets, life history, *Monorhizochytrium* gen. nov., proliferous body, zoospore formation

Poster No. 35

Morphology, ontogeny and molecular phylogeny of a new heterotrich ciliate *Blepharisma sinicus* sp. nov.

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The morphology, ontogeny and molecular phylogeny of the heterotrich ciliate *Blepharisma sinicus* sp. nov. from Shanghai, China were investigated based on living morphology, infraciliature, and small subunit (SSU) rDNA sequence data. The new species is recognized by the following combination of characters: size about 110–140 µm × 30–40 µm *in vivo*, cell red coloured; peristome extending to middle of body; 48–61 adoral membranelles; 25–31 somatic kineties, including 13–18 postoral kineties; one anterior kinety fragment and one shortened postoral kinety; single ellipsoidal macronucleus; 2–5 globular micronucleus; brackish water habitat. Stomatogenesis usually commences from the shorted postoral kinety, three neighboring kineties are involved subsequently in the anarchic field production. The oral anlage divides longitudinally to form the right paroral and the left adoral primordium. The adoral structures of the proter are maintained except the posterior dikinetids of the paroral which are reorganized *in situ*. The subgeneric classification of *Blepharisma* based on nuclear types are not supported by the ontogenetic nor the SSU rDNA sequence data available. The validity of the family Blepharismidae needs to be reinvestigated especially based on the molecular data of more genera.

Keywords: protozoa, new species, morphogenesis

Poster No. 36

Two new vorticellid species candidates (Ciliophora: Peritrichia: Vorticellidae) from Korea and one proposal to describe colonial peritrichous ciliates using new type morphological character

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Two new vorticellid species candidates were discovered from Korean freshwater environments. Their morphology was investigated by the standard methods. The two new species are *Apocarchesium* n. sp. and *Vorticella* n. sp. and their morphological characteristics are as followings. *Apocarchesium* n. sp. is characterized by the lollipop shaped colony, presence of macrozooids and microzooids, one oval macronucleus with numerous nucleoi, row 2 and 3 fusion of P3, absence of epistomial membrane. *Vorticella* n. sp. is characterized by inverted and elongated bell shaped body, length 55–75 µm, width 22–33 µm, single layered and diameter 25–34 µm peristomial lip, spirally contractile stalk, single epistomial membrane, diagonally truncated shaped cylindroid swammer, deep funneled shaped infundibulum, abstomally shortened row 3 of P3, transverse silverlines 59–107 rows in between peristome and trochal band, 16–31 rows in between trochal band and scopula. In this study, we also discussed about the direction of zooid, which is a relatively overlooked character among the characters required for the morphology of colonial peritrichous ciliates.

Keywords: taxonomy, ciliate, peritrich

Poster No. 37

Identification of *Azadinium* species and a new azaspiracid from *Azadinium poporum* in Puget Sound, Washington State, USA

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The identification of a new suite of toxins, called azaspiracids (AZA), as the cause of human illnesses after the consumption of shellfish from the Irish west coast in 1995, resulted in interest in understanding the global distribution of these toxins and of species of the small dinoflagellate genus *Azadinium*, known to produce them. We obtained clonal isolates of four species of *Azadinium*, *A. poporum, A. cuneatum, A. obesum*, and *A. dalianense*, which were isolated from incubated sediment samples collected from Puget Sound, Washington State in 2016. These *Azadinium* species were identified using morphological characteristics confirmed by molecular phylogeny. Whereas AZA could not be detected in any strains of *A. obesum, A. cuneatum* and *A. dalianense*, all four strains of *A. poporum* produced a new azaspiracid toxin, based on LC-MS analysis, named AZA-59. The presence of AZA-59 was confirmed at low levels *in situ* using a solid phase resin deployed at several stations along the coastlines of Puget Sound. Using a combination of molecular methods for species detection and solid phase resin deployment to target shellfish monitoring of toxin at high-risk sites, the risk of azaspiracid shellfish poisoning can be minimized.

Keywords: *Azadinium*, azaspiracid, Puget Sound, Washington State, harmful algal bloom

Poster No. 38

Naegleria fowleri infection induces the NLRP3-inflammasome activation in target cells

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Pathogenic free-living amoebae*, Naegleria fowleri* are widely distributed in soil and water and can cause primary amoebic meningoencephalitis (PAM). During swimming and other recreational water activities, *N. fowleri* trophozoites penetrate the nasal mucosa and invade the olfactory bulbs, resulting in intense inflammatory reactions in the forebrain tissue. To investigate what kinds of inflammasome molecules are expressed in target cells due to *N. fowleri* infection, human macrophage cells (THP-1 cells) were co-cultured with *N. fowleri* trophozoites under the non-contact system. By western blotting and ELISA analysis, the caspase-1 activation and IL-1β production from THP-1 cells and the culture supernatant were observed after co-cultivation. In addition, the increased expression of ASC and NLRP3, which consist of inflammasome complex, was also observed after co-cultivation. To confirm the caspase-1 activation and IL-1β production via NLRP3 inflammasome in THP-1 cells triggered by *N. fowleri* trophozoites, several inhibitors were pretreated on THP-1 cells. The inhibition assay showed that CA-074 (cathepsin B inhibitor), glybenclamide (NLRP-3 molecule inhibitor) and Z-VAD-FMK (caspase-1 inhibitor) reduced the caspase-1 activation and IL-1β production from THP-1 cells. This study suggests that *N. fowleri* infection induces the NLRP3-inflammasome, which activates the caspase-1 and subsequently produces the IL-1β, thus resulting in inflammation against *N. fowleri* infection.

Keywords: *Naegleria fowleri*, PAM, inflammasome, IL-1β, NLRP3

Poster No. 39

Novel phylogenetic lineages of the smallest Vannellida (Amoebozoa, Discosea): morphological and molecular perspectives for the amoebozoan genera

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The comprehensive molecular revision of the order Vannellida (Amoebozoa) performed by A.V. Smirnov and co-authors in 2007 resulted in a re-definition of the genus *Vannella* and establishment of the genus *Ripella* comprising small freshwater vannellids. Since then, another genus of small freshwater vannellids, *Paravannella*, was described in 2014, and an extensive search of the smallest vannellid species in marine and freshwater habitats worldwide led to discovery of a number of new phylogenetic lineages of Vannellida that will be presented in this communication. These are long-branch, highly divergent lineages that group at the base of the Vannellida in SSU rDNA and COI phylogenetic trees. They represent at least four new vannellid genera. Based on the molecular analysis, these genera are grouped into at least three higher-level clades that may correspond to the families. The main problem of this grouping is that currently available morphological data do not allow us to find any traits that might support it. Members of the discovered clades are very poor in the light microscopic characters, and distantly related species often share similar locomotive and floating forms. The same patterns of variation in glycocalyx structure are also shared between distantly related lineages. In particular, a variation between glycocalyx with pentagonal glycostyles and a fuzzy layer without glycostyles occurs among closely related species in many of these lineages. This situation makes a morphological definition of the genera in a classical way hardly possible and urges us to use node-based definitions for these newly found clades. Extensive analysis of the other amoebozoan taxa shows that similar problems may arise when more diversity of small long-branch Amoebozoa is discovered and analyzed. The results obtained by now show seemingly the same problem with the definition of the basal genera of the Dactylopodida (e.g. in *Vexillifera* spp.).

Support: grant 15-04-06239 (RFBR).

Keywords: Amoebozoa, biodiversity, taxonomy, phylogeny, Vannellida

Poster No. 40

Morphological redescription of *Brachonella caduca* (Ciliophora: Armophorea) from Korea

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Brachonella caduca (Kahl, 1937) Jankowski, 1964, collected from the crater lake in Jeju Island, May, 2013 and freshwater in Seoul, August, 2016. We investigated the morphologic, morphometric, molecular characterization, and phylogeny of two strains of *B. caduca.* We also provided the first correlation of morphology, morphometrics, and 18S rRNA gene sequencing for *B. caduca.* Body size about 70–100 x 50–60 um *in vivo*. This species is a helmet-shaped with massive preoral dome and compressed laterally. The posterior end of body is serrated. Macronucleus is globular in mid of body. 15–20 somatic ciliary rows, about 6 preoral dome kineties. Distinctly long caudal cilia lined posterior end. Perizonal ciliary stripe invariably comprised four rows. About 70 adoral zone of membranelles. We submitted 2 new 18S rRNA gene sequences to GenBank.

Keywords: *Brachonella*, 18S rRNA, phylogeny, morphology

Poster No. 41

New marine species of a genus *Pseudoparamoeba* (Amoebozoa, Dactylopodida) from Korea

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The genus *Pseudoparamoeba* Page 1979 in the modern system of Amoebozoa belongs to the family Paramoebidae (Discosea, Flabellinea, Dactylopodida). This genus differs from closely related genera in presence of glycostyles with hexagonal base and dome-shaped apex. In contrast with *Neoparamoeba* and *Paramoeba* amoebae of the genus *Pseudoparamoeba* have no kinetoplastid symbiont — *Perkinsela* amoebae-like organism (or PLO) in their cytoplasm.

Pseudoparamoeba is a relatively rare genus. Since the description of a type species, only one more species was isolated from freshwater pond in Croatia. It was described as *Pseudoparamoeba microlepis* in 2016. This species differs from *P. pagei* in details of a cell coat structure, but these data should be checked. We found another marine species isolated from sample from intertidal marine sediments of Garorim Bay (Korea) designated here as *Pseudoparamoeba* sp. Molecular phylogenetic analysis shown that our isolate probably represents a separate species, the third species of the genus *Pseudoparamoeba*. 18S rDNA sequence of *Pseudoparamoeba* sp. never forms a clade with marine *P. pagei*, but branches separately at the base of *Pseudoparamoeba* clade. This tree topology could mean that *Pseudoparamoeba* is primarily a marine genus, with some species secondary passed to freshwater habitats.

Supported with the Russian Science Foundation 17-14-01391 grant.

Keywords: *Pseudoparamoeba*, 18S rDNA, Paramoebidae

Poster No. 42

Small free-living heterotrophic flagellates (Protista) from Garorim Bay (Yellow sea), Korea

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In order to contribute to an understanding of the geographic distribution of free-living marine heterotrophic flagellates, the diversity of heterotrophic flagellates was investigated at 6 stations in the Garorim bay (Yellow Sea), Korea from April 2015 and November 2016. A total of 65 morphospecies (from 55 genera) of heterotrophic flagellates including 2 new species are reported with uninterpreted records based on light-microscopy. 22 species were from water column and 58 species from bottom sediments. The records consist of three apusomonad, two cercomonad, eight choanoflagellate, three cryptomonads, ten euglenids, two heteroloboseid, seven kinetoplastids, eight stramenopiles, one thaumatomonads and 21 of other or uncertain affinities. Most flagellates described here was previously reported elsewhere and appear to be cosmopolitan. I am unable to assess if the new species are endemic because of the lack of intensive studies elsewhere.

Keywords: heterotrophic flagellates, diversity, cosmopolitan, Garorim Bay

Poster No. 43

Trichomonas vaginalis alpha-actinin 2 modulates host immune responses by inducing tolerogenic dendritic cells via IL-10 production from regulatory T cells

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Trichomonas vaginalis is a pathogen that triggers severe immune responses in hosts. *T. vaginalis* a-actinin 2, Tva-actinin 2, has been used to diagnose trichomoniasis. This study was undertaken to examine the role of Tva-actinin 2 as an antigenic molecule to induce immune response from humans. Western blot analysis using anti-Tva-actinin 2 antibodies indicated its presence in the secreted proteins of *T. vaginalis*. Enzyme-linked immunosorbent assay was employed to measure cytokine production by vaginal epithelial cells, prostate cells, mouse dendritic cells (DCs), or T-cells stimulated with *T. vaginalis* or Tva-actinin 2 protein. Both *T. vaginalis* and rTva-actinin 2 induced cytokine production from epithelial cell lines, including interleukin (IL)-10. Moreover, $CD4^{+}CD25^{-}$ regulatory T-cells (Treg cells) incubated with rTva-actinin 2-treated DCs produced high levels of IL-10. These data indicate that Tva-actinin 2 modulates immune responses via IL-10 production by Treg cell.

Keywords: *Trichomonas vaginalis*, alpha-actinin 2

Poster No. 44

Biodiversity of planktonic ciliates in Southern China Sea

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Planktonic ciliates are important components of marine microplankton. However, the biodiversity and distribution of planktonic ciliates in tropical and subtropical waters of China seas are poorly studied. The biodiversity studies on planktonic ciliates in the coastal and offshore biotopes of southern China sea were carried out in the last decade. In northern coast of the South China Sea, 1) a total of 43 aloricate oligotrich (s.l.) ciliate species belonging to 17 genera of six families were identified; 2) the intertidal zone and mangrove wetland have high oligotrich species richness; 3) fifteen new species and four new genera were reported and their morphologies were studied in detail by living observations and protargol impregnation; 4) the SSU rDNA sequences of 34 species have been analysed, and the molecular phylogeny and systematic relationships of some taxa were discussed based on both molecular and morphological data. In northern offshore area of the South China Sea, 1) the planktonic ciliates community becomes even more species-rich especially concerning loricate tintinnids; 2) horizontally, the distribution of planktonic ciliates was effected by physical processes such as diluted water from estuary and water eddies in the ocean; 3) vertically, planktonic ciliates tend to concentrate in 25-50 m depth with higher abundance than other depths. Our preliminary work indicated there is high planktonic ciliate diversity in the subtropical waters of China seas and should have lots of new findings in the further studies on ciliate biodiversity in this marine area.

This study was supported by the "National Natural Science Foundation of China", project number 31761133001.

Keywords: biodiversity and distribution, aloricate oligotrich (s.l.), loricate tintinnids, mangrove wetland, coastal and offshore

Poster No. 45

Myxobolus pseudowulii n. sp. (Myxozoa, Myxosporea), a new skin parasite of yellow catfish *Tachysurus fulvidraco* (Richardson) and redescription of *Myxobolus voremkhai* (Akhmerov, 1960) Landsberg et Lom, 1991

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In order to examine the myxosporean diversity in yellow catfish *Tachysurus fulvidraco* (Richardson), a survey of this fish species in China was conducted. Light microscopic examination observed a known species, identified as *Myxobolus voremkhai* (Akhmerov, 1960) Landsberg et Lom, 1991 based on morphology, was obtained from gills of yellow catfish and supplement the morphological and molecular data. Besides, a *Myxobolus* species infecting the skin was inconsistent with all of known myxosporean species and formed round, black or milky white with black spots plasmodia in the skin of its fish host. Myxospores are pyriform in frontal view, lemon shaped in lateral view, measuring 14.55 ± 0.67 (12.88-16.16) µm in length, 9.41 ± 0.49 (8.11-10.78) µm in width, 6.99 ± 0.38 (6.12-8.09) µm in thickness. Two ampullaceous polar capsules are slightly unequal in size, with 7.90 ± 0.44 (7.17-9.54) µm in length by 3.50 ± 0.22 (2.98-3.93) µm in width and 7.41 ± 0.32 (6.86-8.04) µm in length by 3.35 ± 0.22 (2.87-3.90) µm in width with seven to nine turns of polar filaments. Plasmodia developed in the stratum spongiosum of skin dermis, desquamating epithelial cells and causing immunological cell infiltration. The ssrRNA gene sequence of the *Myxobolus* species from host skin in different similarity with all available sequences in GenBank. Given the morphological and molecular differences from this species to other *Myxobolus* species, we proposed the name *Myxobolus pseudowulii* n. sp. for this parasite from the skin of yellow catfish. Interestingly, *Henneguya*-like caudal appendages were observed in posterior end of some *Myxobolus pseudowulii* n. sp. spores. Coupled with the species of *Myxobolus* and *Henneguya* clustered together in phylogenetic tree, caudal appendage may be not a valid character to separate *Myxobolus* and *Henneguya* into distinct genera.

Keywords: *Myxobolus*, morphology, histology, ssrRNA, China

Poster No. 46

Live analysis of ciliate abundance and diversity with FlowCAM

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We analysed ciliate species abundance and diversity from oligo-mesotrophic Lakes Mondsee and Irrsee (Salzkammergut area, central Austria) in biweekly samples over three consecutive years; the outlet from L. Irrsee is one of the three main tributaries of L. Mondsee. Since the lakes are connected, we hypothesised that the abundance and diversity of planktonic ciliates would not differ between these two similar lakes. Live samples were measured using a FlowCAM instrument. The FlowCAM (Flow cytometer, Camera And Microscope, Fluid Imaging technology, Yarmouth, USA) is a semi-automatic optical particle counter that measures total number of particles in a sample and records more than 30 morphological and fluorescence parameters. Post-processing analysis tools allow for selecting cells or colonies of a particular size, shape or fluorescence, and to categorise these particles based upon a combination of their properties. We detected approx. 25 ciliate species in the study lakes, including mainly oligotrichs, gymnostomatids, tintinnids, prostomatids, scuticociliates, and several unidentified species. Ciliate abundance ranged from <0.5-37 cells mL^{-1}; average cell number was higher in Mondsee (10.1±0.2 ml^{-1}) than in Irrsee (7.1±0.2 ml^{-1}). Ciliate species number was also higher in Mondsee than in Irrsee. In Mondsee, distinct ciliate peaks were recorded mainly in autumn and, less regularly, during spring and summer. Ciliate peaks in Irrsee were less pronounced and their occurrence did not always parallel those in L. Mondsee. Peaks of small (*Cryptomonas* spp. and *Rhodomonas* spp.) coincided with those of ciliates, suggesting that small flagellated phytoplankton are the favoured food source of the ciliates. Based upon our data, we reject the above hypothesis that connectivity leads to similar ciliate species number and abundance. These findings have important implications for our understanding of freshwater protist dynamics.

Keywords: ciliate, biodiversity, FlowCAM, freshwater

Poster No. 47

Phylogeny of the curious hypotrichous family, Psilotrichidae (Protozoa, Ciliophora, Spirotrichea), with description of one new genus and one new species from Guam

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Hypotrich ciliates, a large group of mainly free-living protists with highly diverse cortical structures, are commonly found in a wide variety of habitats worldwide. Methodological difficulties and insufficient faunistic studies have limited our understanding of their true biodiversity and phylogeny. Psilotrichidae, Bütschli, 1889, a family of curious hypotrichs, is characterized by the long, sparse and undifferentiated cirri, a rigid cortex and oral primordium developing in a deep pouch as in "euplotids". Members of Psilotrichidae have gone through a confused taxonomic history, and the phylogeny of the family is far from conclusive. In order to increase our understanding of the systematics and phylogeny of the family Psilotrichidae, morphological, morphogenetic and molecular studies from a wider range of isolates are needed. In the present study, we give description of two new psilotrichid species, sp. 1 and sp. 2, both of which were collected from Guam. The sp. 1 is characteristed by the *Euplotes*-like body shape, three conspicious ribs on dorsal side, on average a total of 31 cirri in three ventral, one postoral, and one right and one left marginal row, and the numerous flagellates with a red eye-spot. The pyriform/obconical sp. 2, is easily recognized by the table tennis racket-shaped appearance and the virid body due to the numerous flagellates. We also give first record of the 18S rRNA gene for both of the two genera. The two new sequences and the only avaliable psilotrichid sequence, *Urospinula succisa* (Müller, 1786) Esteban et al., 2001, cluster in a highly supported clade (99% ML, 1.00 BI) in the phylogenetic trees, which is consitent the morphologic and morphogenetic features, and reveal the monophyly of the family Psilotrichidae.

Poster No. 48

Biodiversity study on a younger planktonic genus, *Parallelostrombidium* Agatha, 2004 (Protista, Ciliophora)

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Parallelostrombidium Agatha, 2004 is a younger oligotrich genus, and differs from the other genera in that its ventral kinety follows posterior portion of dextrally spiralled girdle kinety, thus its ventral kinety and girdle kinety are with same orientation. To date, up to nine species has been assigned to it, viz. *Parallelostrombidium conicum* Liu et al., 2013, *P. ellipticum* Liu et al., 2015, *P. faurei* (Dragesco, 1960) comb. nov. (basionym: *Strombidium faurei* Dragesco, 1960), *P. grande* (Levander, 1894) comb. nov. (basionym: *Strombidium grande* Levander, 1894), *P. jankowskii* (Xu et al., 2009) comb. nov. (basionym: *Omegastrombidium jankowskii* Xu et al., 2009), *P. obesum* Liu et al., 2015, *P. paralatum* Xu, et al., 2006, *P. rhyticollare* (Corliss & Snyder, 1986) Agatha, 2004 (type; basionym: *Strombidium rhyticollare* Corliss & Snyder, 1986), and *P. siculum* (Montagnes & Taylor, 1994) Agatha, 2004 (basionym: *Strombidium siculum* Montagnes & Taylor, 1994). Among them, seven species were reported once from coastal waters and mangrove wetlands of northern and southern China, respectively, implying this group of microorganism might be highly diverse in terms of species richness in China seas. In 2014, Agatha & Strüder-Kypke proposed a division of *Parallelostrombidium* into two subgenera: *Parallelostrombidium (Parallelostrombidium*) and *Parallelostrombidium (Asymptokinetum*). Species that with ventral kinety entirely parallel to girdle kinety belong to subgenus *Parallelostrombidium*, that only posterior portion of erected ventral kinety parallel to girdle kinety belong to subgenus *Asymptokinetum*. SSUrRNA gene sequences data are available for seven species, all from Chinese researches. Phylogenetic analyses reveal that 7 *Parallelostrombidium* species cluster into two groups in both ML and BI trees, which, however, does not agree with the subgenera proposed by Agatha & Strüder-Kypke, but is coincident with morphologically based classification (dorsal-ventrally flattened vs. cylindrical with posterior end pointed).

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Keywords: Oligotrichia, ciliate, *Parallelostrombidium*, taxonomy, infraciliature

Poster No. 49

Screening for ciliates in 100 Finnish lakes

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In order to compare ciliate diversity and biomass data obtained via the microscopic method with those obtained with NGS (next-generation sequencing; rRNA), samples were taken from 103 lakes in Finland during summer seasons 2015 and 2016. Roughly, the oligotrophic and mesotrophic lakes were equally represented. Samples were fixed with Acid Lugol, Bouin post-fixed and analyzed using Quantative Protargol Stain method.

The assemblage composition revealed three typical scenarios already observed in pristine and acidified lakes, both above and below the timberline.

(i) The lakes dominating by algivorous hunting prostomes (genera *Urotricha*, *Holophrya* and *Prorodon*; in less extent, *Balanion planctonicum*) more eutrophic or acidified lakes from forested areas. Though the presence of picoplanktivorous or omnivorous ciliates was not scarce, they dominated the assemblages only occasionally: e. g. *Halteria* spp. and minute *Rimostrombidium* spp.

(ii) Mixotrophic coarse filter–feeders *Limnostrombidium pelagicum*, *Pelagostrombidium mirabile*, and an unidentified oligotrich (possibly minute subpopulation of the latter ciliate), *Rimostrombidium velox* and *Pelagohalteria viridis* dominated many of the assemblages, resembling those from pristine oligo- to mesotrophic lakes. However, a succession from mixotrophs to algivorous prostomes and minute oligotrichs represented by *Halteria grandinella* might be possible.

(iii) In true oligotrophic lakes, the dominant ciliates were frequently the gymnostomes *Mesodinium* spp. and three species of the genus *Askenasia*, accompanied by mixotrophic ciliates.

Scuticociliates have rarely been recorded. Within mixotrophs, *Vorticella chlorellata*, *Urolep*tus willi, *Euplotes daidaleus*, *Prorodon* / *Pelagothrix* sp., *Coleps* spp. and/or *Askenasia chlorelligera* were also found. In several lakes, plankton *Epistylis* spp. and anabaena (*Dolichospermum*)-colonizing *Pseudohaplocaulus infravacuolatus* were even biomass-dominating. *Gymnostomes* were represented also by minute *Monodinium* sp., *Lagynophrya* sp. (frequently with zoochlorellae) and *Enchelys* spp. In particular, the shallow lakes presented dense tintinnid (*Codonella cratera*, *Tintinnidium* / *Tintinnopsis*) populations. As littoral-related species, *Rimostrombidium lacustris* and *Stentor* sp. were defined.

The study results are waiting for molecular biology confirmation.

Keywords: ciliates, QPS, lakes, oligotrophic, mesotrophic

Poster No. 50

Morphology and molecular taxonomy of ciliates from freshwater bodies in Delhi, India

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Ciliates are common members of freshwater habitats but their biodiversity is not well documented from Delhi, India. In the present investigation, attempt has been made to map ciliate diversity from fresh water bodies of Delhi. Till date, 27 ciliate species belonging to 23 different genera have been identified by *in vivo* studies. Ciliates belonging to classes Spirotrichea (40%) and Oligohymenophorea (30%) are represented in higher proportions in comparison to other species belonging to classes Litostomatea (8%), Karyorelictea (2%) and Prostomatea (2%). This study also aims to specifically throw light on the phylogenetic position of the *Oxytricha granulifera, Gastrostyla* n. sp. and *Architricha indica* isolated from river Yamuna in Delhi, India using morphological and molecular markers. For molecular analysis, SSU rRNA gene was sequenced and phylogenetic tree was constructed. *Oxytricha* and *Gastrostyla* is shown to cluster along with the *Architricha* in a common clade in the phylogenetic tree. The relevance of this observation was further investigated by looking into the morphological and morphometric characteristics of the three genera, which was then further compared with other members of the group. All three are freshwater inhabitants with flexible body and undulating membrane in *Oxytricha* pattern. *O. granulifera* is characterized by the presence of cortical granules, 18 fronto-ventral-transverse cirri pattern; *Gastrostyla* n. sp. is characterized by the absence of cortical granules and presence of a midventral row of cirri; and *Architricha indica* is characterized by the presence of cortical granules and having more than one marginal cirral rows. The differences in their morphological traits combined with the molecular data show that these traits are autapomorphic in origin and therefore, supports the phylogenetic position of this clade.

Keywords: freshwater ciliates, morphology, Oxytrichidae, phylogeny, SSU rRNA gene

Poster No. 51

Nomenclature for the nameless: a proposal for an integrative molecular taxonomy of cryptic diversity exemplified by planktonic foraminifera

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Investigations of biodiversity, biogeography and ecological processes rely on the identification of "species" as biologically significant, natural units of evolution. In this context, morpho-taxonomy only provides an adequate level of resolution if reproductive isolation matches morphological divergence. In many groups of organisms, morphologically defined species often disguise considerable genetic diversity, which may be indicative of the existence of cryptic species. The diversity hidden by morphological species can be disentangled through genetic surveys, which also provide access to data on the ecological distribution of genetically circumscribed units. These units can be identified by unique DNA sequence motifs and allow studies of evolutionary and ecological processes at different levels of divergence. However, the nomenclature of genetically circumscribed units within morphological species is not regulated and lacks stability. This represents a major obstacle to efforts to synthesize and communicate data on genetic diversity for multiple stakeholders. To circumvent this problem, we have designed a stable, reproducible and flexible nomenclature system for genetically circumscribed units, analogous to the principles of a formal nomenclature system. Our system is based on the definition of

unique DNA sequence motifs collocated within an individual, their typification (in analogy with holotypes), utilization of their hierarchical phylogenetic structure to define levels of divergence below that of the morphospecies, and a set of nomenclature rules assuring stability. The resulting molecular operational taxonomic units (MOTUs) remain outside the domain of current nomenclature codes, but are linked to formal morphospecies as regulated by the codes. Subsequently we show how this system can be applied to classify genetically defined units using the SSU rDNA marker in planktonic foraminifera and we highlight its potential use for other groups of organisms where similarly high levels of connectivity between molecular and formal taxonomies can be achieved.

Keywords: cryptic species, planktonic foraminifera, MOTUs, molecular nomenclature, genetic diversity

Poster No. 52

Mitogen-activated protein kinases (MAPKs) as therapeutic targets for the treatment of babesiosis and theileriosis

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Order Piroplasmida (phylum Apicomplexa) comprises the most important pathogenic protozoan parasites including *Theileria* and *Babesia* species cause to the east Coast fever, tropical theileriosis, and babesiosis prominently in vertebrates. Reporting the drug resistance against *Theileria* and *Babesia* species in recent years lead to quest novel drugs and particularly drug target candidates. *Mitogen-activated protein kinases (EC 2.7.11.24)* are enzymes that belong to the class of serine/threonine kinases which are activated by a series of phosphorylation cascades and modulate numerous cellular events including proliferation, differentiation and development. Due to these vital and remarkable biological outcomes, MAPKs are considered as valuable targets for human diseases and protozoan parasites. In this study, we have analysed *mitogen-activated protein kinases of the genus Theileria* and *Babesia* by bioinformatics approaches. To understand structural features, we have modelled MAPK 1 and 2 from *Theileria annulata* and *Babesia bovis*, determined the overall structural stability through 50 ns long molecular dynamics study and docked some known MAPK inhibitors. Although conserved regions for both MAPKs, MAPK2 from parasites possess some motifs which are not found in vertebrate homologs. Molecular docking studies revealed that the known MAPK inhibitors could also affect piroplasmida MAPKs. As a result, efforts on drug development for protozoan parasites *mitogen-activated protein kinases are notable and promised drug targets.*

Keywords: Piroplasmida, mitogen-activated protein kinase, drug development, bioinformatics

Poster No. 53

Analysis of the interaction interface of the possible protein kinase CK2-alpha 1 (CK2A1)-histone deacetylase 1/2 (HDAC1/2) complexes in *Trypanosoma brucei* by molecular dynamics

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Acetylation of the histone tails is one of the most important post-translational modification besides phosphorylation, methylation and ubiquitination. The reverse reaction of the acetylation is the deacetylation of the lysine residues which cause to gain positive charge of histone tails and allows to closed chromatin structure. Deacetylation of the histones are conducted by enzymes called as histone deacetylases (HDACs) in eukaryotes. Targeting of the various classes of histone deacetylases is used as a therapeutic approach for cancers, neurological diseases and additionally parasitic diseases. Phosphorylation of the HDACs proteins by protein kinase CK2 enzyme cause to HDAC activation and regulation. In our previous study, we have showed possible phosphorylated sites of HDAC 1 and 2 by protein kinase CK2 enzyme in *Trypanosoma brucei* by using bioinformatics tools and obtain interacting complexes by molecular modeling and docking approaches. In this study, we have showed that the structural stability of these protein complexes by 40 ns long of molecular dynamics simulations and identified interacting hot spot residues and pockets as well. Inactivation of the histone deacetylation by dissecting binding of the protein CK2 enzyme could be an alternative method for the drug design strategy against to the African trypanosomiasis and as well as for the other kinetoplastid organisms.

Keywords: kinetoplastids, *Trypanosoma brucei*, protein kinase CK2, histone deacetylase, drug design

Poster No. 54

Taxonomy-free molecular diatom index for high-throughput DNA biomonitoring of watercourses

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Current biodiversity assessment and biomonitoring are largely based on morphological identification of selected bioindicator taxa. Recently, several attempts have been made to use eDNA metabarcoding as an alternative tool. However, until now, most of applied metabarcoding studies were based on taxonomic assignment of sequences that refers to morphospecies ecology. Usually, only a small portion of metabarcoding data can be used due to limited reference database and lack of phylogenetic resolution. Here, we investigate the possibility to overcome these limitations by using a taxonomy-free approach that allows computing molecular index directly from eDNA data without any reference to the morphotaxonomy. As a case study, we use the benthic diatoms index, commonly used for monitoring the biological quality of rivers and streams. We analysed 87 epilithic samples from Swiss rivers, which ecological status was established based on microscopic identification of diatom species. We compared the diatom index inferred from eDNA data obtained with or without taxonomic assignment. Our taxonomy-free approach gives promising results by providing a correct assessment in 77% of examined sites. The main advantage of our approach is that almost 95% of OTUs could be used for index calculation, compared to 35% in the case of the taxonomic assignment approach. Its main limitations are the under-sampling and need to calibrate the index on microscopic assessment of diatoms communities. However, once calibrated, the taxonomy-free molecular index can be easily standardized and applied in routine biomonitoring, as a complementary tool allowing fast and cost-effective assessment of the biological quality of watercourses.

Keywords: biomonitoring, biotic index, metabarcoding, high-throughput sequencing, diatoms

Poster No. 55

The protistan megafauna of the deep sea: new observations on xenophyophores (Foraminifera) in the abyssal eastern Pacific Ocean

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Xenophyophores are monothalamous Foraminifera (Rhizaria) that are common on sedimented and hard seafloor substrates in deep-sea environments at water depths between 500 and almost 11,000 m. They construct fragile agglutinated tests that are typically several centimetres in size and sometimes reach dimensions well in excess of 10 cm. Their internal organisation is very distinctive, comprising a branching, multinucleate cell body containing numerous barite crystals and large accumulations of waste material (stercomata) that occupy much of the test interior. Xenophyophores are believed to play a crucial role in deep-sea ecosystems, providing habitat structures for meiofaunal and macrofaunal organisms and enhancing the organic content of sediments surrounding their tests. Analysis of seafloor photographs reveals that these giant foraminifera are a dominant constituent of the abyssal megafauna in the equatorial Pacific Clarion Clipperton Zone (CCZ) (Amon et al., 2016, Sci. Rep. 6. http://dx.doi.org/10.1038/srep30492), a region where commercially important deposits of polymetallic nodules occur on the seafloor. As part of a baseline study of benthic communities we made extensive collections of xenophyophores in two areas (UK-1 and OMS) licensed for exploration by the International Seabed Authority. Based on test morphology, we distinguished 36 morphospecies (34 new to science) among 130 specimens, many of them sessile on nodules. Twenty of these morphospecies yielded 184 DNA sequences, a 14-fold increase in genetic data for xenophyophores that confirms their high diversity in the eastern CCZ. A further 15 morphospecies (11 new to science) were recognised in samples from two other areas (APEI-6 and Russian licence area) within or adjacent to the CCZ. This large number of undescribed species confirms previous evidence that the CCZ is a focal area for xenophyophore diversity. More broadly, it represents an unprecedented increase in the known global diversity of xenophyophores and suggests that many species remain undiscovered in the World's oceans.

Keywords: deep-sea protists, polymetallic nodules, foraminifera, diversity, phylogeny

Poster No. 56

Impact of Piper betle extracts on *Giardia intestinalis* infection in gerbil model

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Giardia intestinalis is an anaerobic flagellated protozoan parasite of mammalian species, including humans and is considered the most common cause of protozoan diarrhoea. It has a worldwide distribution and symptomatic infections occur in both developing and developed countries. There are several compounds known to effectively treat *Giardia* in human patients. The most frequent treatment is the use of nitro-imidazoles such as metronidazole. Unfortunately, they can produce undesirable side-effects and resistance to these treatments has been described in previous studies. For these reasons, the search for new therapeutic alternatives to treat giardiasis has become necessary. *In vivo* models are the most reliable for testing new drugs and therefore better knowledge is needed about the nature of infection in model hosts.

In our study, eighteen gerbils were inoculated orally with viable cysts of *G. intestinalis*. Cyst shedding was monitored daily through floatation method and the number of cysts were counted. After starting cyst shedding, gerbils were treated with *Piper betle* extracted in three different solvents: water, methanol or methanol:tetrahydrofurane. Ten days after the start of treatment, gerbils were sacrificed and dissected. Their duodena were processed for examination using histological sectioning and scanning electron microscopy. Antigiardial activity was evaluated through the course of cyst shedding during the entire experiment. A significant decline in cyst shedding evaluated through linear regression was found in gerbils treated with the aqueous extract. Our results represent important insight into this issue indicating that the aqueous extract of *P. betle* shows giardicidal effects.

This study was financially supported by the UMI – Saving of Pongidae Foundation: project "Parasites and Natural Antiparasitics in the Orang-utan", Czech Academy of Sciences, Grant No. P505/11/1163 and Scholarship program to support creative activity – research collaboration, No. PřF_04_27.10.2006.

Keywords: antigiardial activity, drug of choice, *Giardia intestinalis*, natural antiparasitics, Piper betle

Poster No. 57

Genomic and informatic characterization of membrane coat protein complexes in *Carpediemonas membranifera* and parasitic diplomonads

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Giardia intestinalis, a fresh water-contaminating parasite responsible for the dysenteric illness Giardiasis, and *Spironucleus salmonicida*, a fish pathogen, are well known parasites that belong to the Diplomonad clade within the Excavate Supergroup. These parasites possess unusual modification within their membrane trafficking organelles, including unstacked Golgi bodies, a functional merging of the ER and endosomal compartments and a separate multi-functional endolysosomal organelle, the peripheral vacuole. The molecular machinery underpinning the membrane-transport pathways is clearly reduced in diplomonads, and yet sequence divergence has made robust classification of the specific components into their protein sub-families difficult to obtain. Free-living relatives that are less divergent are a fruitful way to tackle parasite evolution and comparative cell biology. We have used the basal, free-living, diplomonad relative, *Carpediemonas membranifera*, as a model system for studying both *Giardia intestinalis* and *Spironucleus salmonicida*.

C. membranifera possesses a standard set of membrane-trafficking organelles and encodes much less diverged protein sequences than *Giardia*. Therefore we hypothesized that membrane-trafficking coat loss has occurred at the free-living to parasitic transition in diplomonads, with *C. membranifera* possessing a more complete complement of vesicle coats than its relatives. In order to study these systems, we took a bioinformatics approach to analyze newly obtained genomic scale data for the identification coat proteins associated with endosomes and the Golgi. Results revealed a structured pattern of loss in the adaptor proteins complexes (AP). From a reconstructed complement of 5 AP complexes in the metamonad ancestor, we confirm the presence of AP1-4 in *C. membranifera*, AP1-3 in *S. salmonicida* and at least AP1-2 in the various *Giardia* genomes examined. These results have fundamental implications in our understanding of the evolution of membrane trafficking from free-living lineages to parasitic lifestyles with modified endosomes coats within the Diplomonad clade.

Keywords: adaptor protein complex (AP), endomembrane system, diplomonads, parasitism, bioinformatics

Poster No. 58

New freshwater centrohelidian species *Acanthocystis siemensmae* sp.n., *Acanthocystis lyra* sp.n. and *Acanthocystis amura* sp.n. (Haptista, Heliozoa, Centrohelea) from Russia

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Centrohelid heliozoans are ubiquitous protists inhabiting benthos and periphyton in both marine and freshwater ecosystems. Most of them are characterized by mineralized scales having peculiar species-specific shape. *Acanthocystis* Carter, 1863 is one of the most diverse centrohelid genera with about 30 described species at present. The most important diagnostic characters for the species identification within this genus are structure of the plate scales and organization of spine scales tips that can bear teeth varying in shape and quantity.

The aim of this study is a description of new centrohelidian cultures isolated from lakes and rivers of the South Urals (Russia). The following three new species of centrohelids have been described with electron microscopy.

Acanthocystis siemensmae has elliptical or pear-shaped plate scales with a thin marginal rim and spine scales of two types. The long spine scales have shafts slightly branching towards the apex with 14–16 teeth. Extended part of the apex contains four symmetrically oriented longitudinal ridges; each ridge is ended by four teeth of different length. The short spine scales with narrow shafts and expanded cup-shaped apices looks like a maple leaf due to division onto 3–5 ridges of irregular shape. Each ridge has a few teeth of different length.

Acanthocystis lyra has elliptical plate scales with an axial thickening and spine scales of two types. The long spine scales have apices are divided into two pointed, deflected S-shaped branches with 6–8 short teeth on inner edge, outer edge is smooth. The short spine scales broadly bifurcate at their apices. Scales possess marked primary and secondary bifurcations with two short teeth on each apex of the secondary branch. The spine scales have circular basal plates with a clearly expressed marginal rim.

Acanthocystis amura has oval plate scales with an axial thickening and a thin border as well as two types of spine scales. The long spine scales have tips with four small hooks. The short spine scales have four teeth at the distal end.

This study was conducted in the Center of Shared Scientific Equipment "Persistence of microorganisms" of ICIS UB RAS and supported by RFBR (15-29-02749 and 15-29-02518).

Keywords: heliozoa, centrohelids, *Acanthocystis*, new species, electron microscopy

Poster No. 59

Diversity of genus *Chlamydodon*, with morphological and molecular descriptions of six species from China

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Chlamydodon is a group of highly specialized ciliates and can be easily recognized by having characteristic cross-striated band (CSB) that encircles the perimeter of the cell. The species of the genus are algivorous and distributed in brackish water and marine habitat worldwide. In the present work we isolated six species from China, and investigated them with standard methods. Four new species, *C. granuliferus*, *C. oligochaetus*, *C. crassidens*, and *C. similis*, are established. *C. granuliferus* is diagnosed by complete CSB, regularly arranged cortical granules, 22–28 somatic kineties. *C. oligochaetus* can be separated from its congeners by elongate body, and relatively fewer somatic kineties. *C. crassidens* is unique by wide and posteriorly interrupted CSB, and a huge cytostome. *C. similis* is different from other *Chlamydodon* mainly by 38–49 ciliary rows. A poorly-known species, *C. exocellatus* is re-described and the ciliature and a diagnosis are given. A population of *C. triquetrus* is also re-described. Additionally, we provide five SSU rDNA sequences and checked the morphological data of the sequences from NCBI database. Phylogenetic analyses (Maximum Likelihood, ML, and Bayesian Inference, BI) based on SSU rDNA sequences support the establishments of these new species, and confirms the paraphyly of the genus *Chlamydodon* and the monophyly of the family Chlamydodontidae. This work indicates that the morphological and molecular diversities of *Chlamydodon* are much higher than we previously thought, and the number of known and described *Chlamydodon* species is just the tip of an iceberg of a hitherto undiscovered species.

Keywords: ciliates, cyrtophorians, morphology, phylogeny, SSU rDNA

Poster No. 60

Occurrence of gymnamoebas in mixtures of domestic and textile wastewater

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Gymnamoebas are nude amoebae that are characterized by the absence of a protective structure in its vegetative form; they are cosmopolitan and have a wide distribution in the nature, they are mainly in the soil and aquatic environments. Gymnamoebas have been found in different kinds of water, even in domestic wastewater; however, wastewater from the textile industry contains a variety of chemical substances such as dyes, which may inhibit their presence. The objective of the study was to determine the occurrence of gymnamoebas in mixtures of domestic wastewater (DW) and textile wastewater (TW) in different proportions. The mixtures of wastewater were from 100% of DW and 0% of TW up to 30% of DW and 70% of TW. The samples of wastewater mixtures were inoculated in non-nutritive agar with the bacterium *Enterobacter aerogenes* (NNE), the cultures were incubated at 30 °C, and were observed with an inverted microscope. The isolated amoebae were identified morphologically. Ten genera of Gymnamoebas were identified: *Acanthamoeba*, *Mayorella*, *Polychaos*, *Rosculus*, *Saccamoeba*, *Thecamoeba*, *Valhkampfia*, *Vannella*, *Vermamoeba* and *Vexillifera*; of which the most frequent were *Vannella*, *Polychaos* and *Rosculus*, while the genera *Vermamoeba* and *Vexillifera* were the less frequent. In 100% of DW, and in the mixture of 10% DW and 90% TW were found 8 genera of gymnamoebas, but the number of genera decreased drastically to 2 when the proportion of TW increases to 20%; however when the proportion of TW goes of 40 to 70%, the number of genera increased slightly and remained between 4 and 5. The gradual increase in textile wastewater in the mixtures allowed the Gymnamoebas to acclimate to this type of water containing a variety of chemical substances.

Keywords: Gymnamoebas, domestic wastewater, textile wastewater

Poster No. 61

Some tintinnids from the Gulf of California: a focus on the water masses

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The Gulf of California is a marginal sea from the Mexican Pacific Ocean. Is located between the arid territories of the peninsula of Baja California and the Sonora and Sinaloa states. This environment is characterized by a high primary productivity by the coastal upwelling event. Despite his importance and the wide variety of studies developed in this region, the tintinnids, an important component of the microplankton, have not been studied in this environment. The analyzed samples come from the cruises Vaquitas 0217, Exfinife 1609 and Marias 1609 for the north, center and south of the Gulf, respectively. Approximately 35 L of water were collected with Niskin bottles and filtered through a 20 or 34 µm sieve. All the samples were fixed with formol at 4 %. For the species identification was used an optic microscope at 40x. According with the temperature and salinity values of the sampled stations, was collected water from the Gulf of California (GCW) in the north and center of the system and the Tropical Surface Water mass (TSW) in the south. Until now, have been identified twenty-four species of tintinnids. The species number was greater in the GCW than in the TSW mass. Differences in the composition and abundance between the GCW mass were found: a greater species richness and abundance in the center zone. Only seven species were common between the GCW and TSW. The northern part of the GCW did not share any specie with the center part of the same mass and other areas. In the north of GCW small tintinnids were predominant, while, in the center of the GCW and TSW, prevailed tintinnids with a body size greater. The low species number in the north part of GCW may be a consequence of the isolation of this water mass.

Keywords: Gulf of California, tintinnids, water mass, composition, abundance

Poster No. 62

Morphologic and molecular characterization of an ecologically diverse basal lineage of Armophorea, Ciliophora

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Armophorea is a class comprising solely anaerobic ciliates that thrive as endocommensals in various vertebrates and invertebrates and as free-living organisms in various freshwater, brackish, and marine sediments. Although many species of its the three orders, Clevelandellida, Armophorida, and Metopida have been known for long time, the class itself has been established relatively recently, based on the 18S rRNA gene analysis, and thus is called a "riboclass". However, the internal relationships within this class remain poorly understood due to the persisting lack of molecular data and because only a few representatives have been studied in detail using modern morphological methods, such as protargol staining techniques or scanning electron microscopy, while ultrastructural data concerning the cortical structures and hydrogenosomes are sorely lacking for the entire class. *Tropidoatractus acuminatus* Levander, 1894 is a flagship species of Metopida with a conspicuous and unique spiralized rib-like rigid pellicle. Here, we identify the deepest-branching lineage of Metopida, which we will describe as a new family in the future. It comprises two genera, freshwater *Tropidoatractus* and marine *Palmarella*, and six species, including two novel ones. We analyzed the 18S rRNA gene sequence of all six species to reveal their phylogenetic relationships and also studied their morphology and ultrastructure with protargol methods, scanning and transmission electron microscopy.

Keywords: Armophorea, anaerobic ciliates, Metopida, phylogeny, protargol

Poster No. 63

Global comparative analysis of prokaryotic and eukaryotic diversity contributing to oceanic photosynthesis using data from Tara Oceans and Malaspina expeditions

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Motivation: Phototrophic picoeukaryotes or PPEs (organisms measuring ≤ 3µm) contribute to half of the oxygenic photosynthesis activity in open oceans and play key trophic roles for the functioning of these ecosystems. Despite their ecological value, the dynamics and taxonomic composition of these communities are poorly known at global scales, whereas the distribution of cyanobacteria (the only prokaryotes known to be capable of oxygenic photosynthesis) has been widely studied. Nowadays, molecular methods based on high-throughput technology allow us to do new valuable steps towards the comprehension PPEs communities structure. In this study, we analyze 160 samples from surface waters (3m to 5m) collected along *Tara* Oceans and Malaspina circumglobal expeditions with PRC-based amplicons and metagenomic sequencing approaches for characterizing the global relative abundance and richness of PPEs and cyanobacteria.

Results: We identify Chrysophyceae, Dictyochophyceae, Pelagophyceae, Prasinophyceae and Prymnesiophyceae as dominant PPE groups in the photosynthetic communities of global open ocean surface waters. Prymnesiophyceae, Prasinophyceae and Dictyochophyceae emerge as the classes with the highest diversity and abundance of plastidial sequences. When comparing PPEs and cyanobacteria distribution, we observe that cyanobacteria are more widely distributed and abundant. The evolutionary diversification of both communities has been described at class and genus level based on ribosomal DNA sequencing data obtained through two molecular approaches, (PCR-based amplicons and metagenomes), for which we report contradictory results and limitations in the quantification of cyanobacteria and PPE groups.

Keywords: phytoplankton, metagenomics, phylogenetics

Poster No. 64

A novel chytrid species parasitic on the green algae, *Microglena* (Volvocales)

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Chytrids are early diverging linages of true fungi that reproduce with posteriorly uniflagellate zoospores. During asexual reproduction, they form a sac-like structure called the zoosporangium, in which zoospores are produced. In aquatic systems, chytrids parasitic on algae influence the population dynamics of phytoplankton species. Furthermore, chytrids have been shown to play an important role in aquatic food webs. However, the diversity and biology of parasitic chytrids on algae in aquatic systems remain largely unknown due to difficulty of culturing them. Here, we report a novel chytrid culture (KS100) parasitic on *Microglena coccifera*. (Volvocales, Monadinia clade). We determined its taxonomic position based on thallus morphology, zoospore ultrastructure and molecular phylogeny using 18S, 5.8S, 28S rDNA sequences. Host range of this chytrid was determined by the cross-inoculation experiments using nine cultures of volvocalean green algae.

Thallus morphology of KS100 was characterized by 1) spherical or subspherical zoosporangium which becomes slightly angular during zoospore discharge, 2) zoospore discharge through the 2–3, small inoperculate pores, 3) rhizoids branching at the base and extending like fan shape in the host cell. These characters were distinguished from those of any known chytrids. In the molecular phylogeny, KS100 belonged to the order Rhizophydiales and were distinguished from any known families in the order. The zoospore of KS100 possessed the kinetosome-associated structure whose morphology and position were unique among the Rhizophydiales. Based on these results, we concluded that KS100 is an undescribed species, for which a new family and genus should be proposed in the Rhizophydiales. As the results of cross-inoculation experiments, KS100 was revealed to infect only on the three cultures of *Microglena* spp. which are belong to Monadinia clade. No infection was observed in the green algae which belong to other clades in Volvocales such as clades Chlorogonia, Moewusinia, Phacotinia and Reinhardtinia.

Keywords: chytrid, host specificity, *Microglena*, parasite, Rhizophydiales

Poster No. 65

The transcriptome and draft genome of the gut parasite *Blastocystis* sp. isolated from the cockroach *Blatta orientalis*

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Blastocystis is a non-flagellated anaerobic stramenopile widely prevalent in the intestinal tracts of humans and in the digestive tracts of other mammals, birds, reptiles, and insects. *Blastocystis* has been associated with irritable bowel syndrome (IBS) but they have also been detected in the guts of healthy individuals as well. The draft genomes of subtypes (ST) 1 through 9 have been published – all were isolated from humans or monkeys. A previous study on the human ST1 has shown that it acquired genes such as those involved in carbohydrate scavenging, anaerobic amino acid and nitrogen metabolism, oxygen-stress resistance, and host immune evasion through lateral gene transfer. Other peculiarities found in the nuclear genomes of human STs include 3'- polyadenylation of mRNAs to generate termination codons. However, there is a lack of genome information from more basal *Blastocystis* spp. outside of mammals and our project focuses on strains isolated from the oriental cockroach *Blatta orientalis,* which, despite its name, has a cosmopolitan distribution. Unlike its mammalian relatives that thrive at body temperature, cockroach *Blastocystis* grows at room temperature and we expect it to have different adaptations given the different microflora of mammals and cockroaches. Preliminary analysis of the transcriptome suggests that at least 2 strains were present, with some of the sequences being homologous to the rRNA of subtype C4, an isolate from the cockroach *Periplaneta americana*. We have initiated PacBio long-read sequencing and will use this dataset to analyse the cockroach *Blastocystis* genomes and compare them to existing STs genomes to identify common or unique adaptations.

Keywords: *Blastocystis*, cockroach, PacBio, genome

Poster No. 66

Toxoplasma GRA15II-polarized macrophages facilitate adverse pregnancy outcomes of mice

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Toxoplasma gondii (*T. gondii*) is an obligate intracellular parasite which infects a variety of warm-blooded mammals worldwide including humans. Congenital toxoplasmosis is one of the major causes of adverse pregnancy outcomes. It has been known that the virulence and subversion of *Toxoplasma* strains to host immunity are correlated to polymorphic effectors in different genotypes of the parasite. Previous studies indicated that $GRA15_{II}$, one of the molecules secreted by dense granules of *Toxoplasma* tachyzoites, may drive macrophages to M1 polarization *via* phosphorylating of NF-κB p65, and induce high innate immunity against *T. gondii* infection. Here we explored the adverse effect of $ToxoGRA15_{II}$ on abnormal pregnancy by subverting the physiological immune tolerance on maternal-fetus interface. Macrophages presented M1 features following transfection with *gra15II,* showing high production of nitric oxide and inflammatory factors (IL-6, IL-23, IL-1β, IFN-γ, IL-17). Transfusion of the polarized M1 to pregnant mice lead to the increased absorptivity of fetus, stillbirth, and hemorrhage of placenta, with a high level of IL-17 and decreased number of Tregs in splenocytes. We conclude that $ToxoGRA15_{II}$-driven M1 immune response may contribute to the adverse pregnancy outcomes in *Toxoplasma* infection, in addition to direct parasite invasion.

Keywords: *Toxoplasma gondii*, macrophage, GRA15II, adverse pregnancy

Poster No. 67

Morphology, ultrastructure and phylogeny of a new species of *Glissandra* (Protista incertae sedis)

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Glissandra is an understudied genus of free-living marine biflagellates of which taxonomic position remains uncertain. These flagellates are characterized morphologically by possessing a short ventral groove and two long flagella inserted laterally from the groove. Both flagella tightly hold to substrate and only the tip of the anterior flagellum waves when gliding. Two species, *G. innuerende* Patterson and Simpson, 1996 and *G. similis* Lee, 2007, have been described so far. However, neither ultrastructural nor molecular study has been performed due to the absence of the laboratory culture. We recently established a culture of a new species of *Glissandra* from a seaweed sample of the Republic of Palau. In this study, the cultured *Glissandra* cell was subjected to light and electron microscopic observations, as well as a molecular phylogenetic analysis. Light microscopic observation indicated that the flagellate had the characteristics of *Glissandra*, such as a short ventral groove and two long flagella that attach to substrate. However, we noticed two differences between the new *Glissandra* species and the two species previously described. Firstly, the former cell is smaller in size than *G. innuerende* or *G. similis*. Secondly, the two flagella were inserted longitudinally in the new species, not laterally as observed in the two previously described species. In addition, the new species appeared to possess an oval depression, which is apart from the groove, at the ventral side of the cell. The transmission electron microscopic observation revealed that the cell membrane is lined by a thin theca, which is similar to that of apusozoans, and the rim of the oval depression is supported by a microtubular band. An 18S rRNA gene phylogeny recovered no strong affinity between the new species and any known eukaryotes/assemblages (including Apusozoa), suggesting that *Glissandra* represents a previously overlooked branch of the tree of eukaryotes.

Keywords: flagellate, *Glissandra*, Protista incertae sedis, molecular phylogeny, ultrastructure

Poster No. 68

RS165 – a newly isolated protist with possible relationships to *Belonocystis* and *Luffisphaera*

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A mixed culture of a new protist with uncertain affinities was isolated from Red Sea shore near Eilat, Israel (N29.50; E34.92). The cell diameter is 9.20-21.94 (ca. 14.63) µm. The cytoplasm was homogenous and very active. It contained single eccentric nucleus and several large conspicuous presumable food vacuoles. Numerous small granules were present. Contractile vacuoles were not seen. The organism moved by producing very slender filopodia. Electron microscopic study has shown the complex structure of the scales, covering the cell. Their length was 2.96-4.48 (ca. 3.68) µm. Each scale was divided into spike (2.23-3.58 (ca. 2.86) µm) and widened base (0.68-0.95 (ca. 0.82) µm), bearing a skirt-like structure (1.98-3.00 (ca. 2.36) µm). The spike had a hyperboloid shape with six ribs on its wall composed of fine fibrils, forming a lattice network with equilateral triangle-shaped openings. Skirt-like structure was formed with left hand twisted fibrils. Along with big scales this organism had small scales (93.05-186.11 (ca. 119.16) × 49.53-79.37 (ca. 64.35) nm). The spike was connected to the base with an electron-dense ring. EDX analysis has shown the organic nature of the scales. The appearance and especially the scale structure were reminiscent of those in protists known as *Belonocystis* Rainer, 1968, which recently were shown to be the members of Variosea-clade in Amoebozoa, as well as enigmatic organisms *Luffisphaera* Belcher et Swale, 1975 and *Paraluffisphaera* Esteban et al., 2005, probably also related to *Belonocystis* but currently lacking determined position. Those organisms are similar in the presence of organic scales of resembling structure, covering the whole cell surface and the tendency to produce a slender non-branching outgrowths (not known for *Luffisphaera* and *Paraluffisphaera*). Small scales of RS165 resemble those of some amoebae (*Squamamoeba*, *Sapocribrum*) and so called plate-scales of *Paraluffisphaera*.

Study support: RFBR grant 15-04-18101.

Keywords: ultrastructure, Amoebozoa, *Belonocystis*, *Luffisphaera*, scales

Poster No. 69

RS161 – a new morphologically and genetically distinct colonial centrohelid

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Samples containing a new heliozoon were collected from Gulf of Aqaba shore, Israel (N29.50; E34.92) and Indian ocean shore near Terekhol river, India (N15.72; E73.69). Spherical colonies (54.22-77.11 (ca. 65.39) µm) were composed of 7-10 individual cells. Each cell (9.71-15.20 (ca. 11.85) µm) produced axopodia with motile granules, sometimes exceeding the cell diameter up to 11 times. Those originated from the side facing the surface of the colony. No cytoplasmatic bridges between the cells were observed. Each cell was covered with its own layer of siliceous plate-scales, while the whole colony was surrounded with a thick layer of another type of scales. The colonies were capable of rolling movement, floating, but most of them were attached to the substratum. According to EM examination outer scales (1.67-3.99 (ca. 2.72) × 1.43-3.24 (ca. 2.51) × 1.35-3.12 (ca. 2.10) µm) had a pot-like shape with axial rib (0.92-1.96 (ca. 1.31) µm) and numerous conical papillae (32.08-66.10 (ca. 51.11) × 33.01-81.68 (ca. 45.87) nm) on its wall, forming irregular rings around the scale. The axial rib was also ornamented by conical papillae. Plate-scales (2.98-3.80 (ca. 3.45) × 1.28-1.76 (ca. 1.50) µm) had a simple structure and boar only axial thickening (2,00-2.79 (ca. 2.38) µm) and an inflected margin (0.95-2.74 (ca. 1.66) µm) on its outer surface. Described morphology is quite unusual for centrohelids. Previously described colonial centrohelids have more space between cells and no common layer of scales with its own morphology. Pot-like shape of the colony scales is also unique among centrohelids expressing no obvious similarity to any morphotype known so far. Molecular phylogenetic analysis, based on 18S rDNA sequences has shown a deep-branching position of a new heliozoan at the base of the order Acanthocystida and most probably it represents a new centrohelid family.

Study support: RFBR grants 15-04-18101, 16-34-60102.

Keywords: ultrastructure, marine protists, heliozoa, centrohelida, scales

Poster No. 70

Morphology, biology and phylogeny of *Phalansterium arcticum* sp. n. (Amoebozoa, Variosea), isolated from ancient Arctic permafrost

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The genus *Phalansterium* includes solitary and colonial heterotrophic flagellates with a single flagellum; the proximal part of the flagellum is surrounded by a collar-like structure. Many basic aspects of the biology of *Phalansterium* are still not known, including details of the mode of reproduction and feeding. Molecular phylogeny demonstrated that species of the genus *Phalansterium* form a highly supported monophyletic clade within the class Variosea. The relationships of this clade with other groups of Variosea are not yet clear. A new species – *Phalansterium arcticum* sp. nov. was isolated from the Arctic permafrost sample of 8580 years old. This organism normally lives as a sedentary uniflagellated cell enclosed in a thin, flexible mucilaginous sheath, but can form naked swimming cells and amoeboid cells with eruptive pseudopodia accompanied with the formation of short, filopodia-like projections. In a SSU rDNA phylogenetic tree it robustly groups with other species of this genus. For the first time, we describe cell division of *Phalansterium* and add more details on the feeding process in this organism.

Support: grants 17-54-150003 and 15-04-05267 (RFBR).

Keywords: Amoebozoa, permafrost, phylogeny

Poster No. 71

Taxonomic revision of freshwater Foraminifera with the description of new agglutinated species

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Most of foraminifera inhabit marine habitats, but some species of single-chamber monothalamiids have been described from freshwater environments, mainly from Swiss water bodies and over 100 years ago. Recent environmental DNA surveys revealed the presence of four major phylogenetic clades of freshwater foraminifera. However, until now only one of them (clade 2) has been associated to morphologically described species (*Reticulomyxa*). Here, we present morphological and molecular data for the genera representing the three remaining clades. We describe two new agglutinated freshwater genera from China and the Netherlands, *Lacogromia* and *Limnogromia*, which represent clades 3 and 4, respectively. We also report the first rDNA sequences of the genus *Lieberkuehnia*, which placed this genus within clade 1. Our study provides the first morphotaxonomic documentation of molecular clades, showing that the environmental DNA sequences correspond to the agglutinated monothalamous species, morphologically similar to those described 100 years ago.

Keywords: foraminifera, Monothalamea, taxonomy, DNA barcoding

Poster No. 72

Diversity and phylogeny of amoebae of the family Thecamoebidae (Amoebozoa, Discosea)

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Amoebae of the family Thecamoebidae are widely distributed in the environment. These organism are relatively easy to isolate and cultivate, also the frequency of species occurrence is very different. There are rather common species like *Thecamoeba quadrilineata*, *T. striata* and *T. orbis* as well as numerous species known from few findings or never re-isolated since initial description. Many of these species were studied only at the light-microscopic level and require investigation with modern methods, including electron microscopy and molecular studies (this especially concerns the genus *Thecamoeba* and genera of unclear systematic position like *Pseudothecamoeba* and *Thecochaos*). Our studies show that "hotspot" of *Thecamoeba* diversity is terrestrial habitats – soil, grass, dry leaves and surface of trees. During our studies we isolated 33 strains of *Thecamoeba*, including approximately 10 unique species from different geographic locations. Some were identified as known species (*Thecamoeba aesculea*, *T. similis* and *T. quadrilineata*), but there are several strains, which are evidently new for science. The phylogenetic analysis based on 18s rRNA gene revealed two clades inside the genus *Thecamoeba*. This dichotomy is well-supported and probably has a morphological rational as well. The first clade includes amoebae with one large central nucleolus (likes *T. quadrilineata* and *T. aesculea*), while the second clade unifies species possessing many peripheral nucleoli (like *T. similis*). Our data show that species diversity of thecamoebid amoebae remains considerably underexplored.

Supported with Russian Science Foundation (RSF) 17-14-01391 research grant.

Keywords: Amoebozoa, diversity, phylogeny, systematics

Poster No. 73

Biofilms for monitoring presence of Microsporidia in environmental water

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The development of molecular methodologies for targeting pathogens such as the Microsporidia has greatly improved our monitoring capabilities and initiatives. This study analyzed samples collected from five locations in Pensacola, Florida, USA for the presence of Microsporidian pathogens. To circumvent various impediments associated with water collection and filtration, we utilized biofilms as sentinels for detection of Microsporidia. We implemented membrane- dissolution and sample purification in a single confined step followed by real-time PCR to confirm pathogen presence. The results of this study demonstrate that microsporidia are present in environmental water sites in the Florida panhandle and that biofilms may serve as another alternative mode to circumvent filtration methods for their detection.

Keywords: Microsporidia, pathogen, biofilm, monitoring

Poster No. 74

Phylogeny and morphology of five new diplonemid species

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Diplonemids are a sister group of ecologically important and well-studied euglenids and kinetoplastids within the Euglenozoa clade. For a long time, diplonemids were considered as a small and rare group of heterotrophic marine flagellates with only three genera and less than a dozen species described. However, the recent *Tara* Oceans 18S RNA metabarcoding survey on numerous plankton samples across the globe discovered that diplonemids, which total over 45,000 operational taxonomic units, are the most species-rich marine planktonic eukaryotes. Furthermore, they show cosmopolitan distribution with different lineages being abundant and often dominant in most oceanic niches.

Despite their remarkable diversity, altogether there are only four diplonemid species, for which cultures, morphological description and sequence data are available: two representatives of *Diplonema* - *D. papillatum* and *D. ambulator*, one *Rhynchopus* species – *R. euleeides* and the single representative of the *Hemistasia* clade – *H. phaeocysticola*. To the best of our knowledge, no new diplonemid species have been recently described. In order to fill that gap, we attempted to establish axenic cultures by manually picking diplonemid-like cells from samples collected in surface waters on the east coast of Japan. We report the description of four new diplonemid species now available in culture: two new members of the *Rhynchopus* genus and the establishment of two new genera. In addition to this, we create a novel genus to accommodate a protist incorrectly classified as *Diplonema* sp. 2 (ATCC 50224). The description is based on nearly full-size 18S rRNA gene sequences and morphological observations using light and electron microscopy.

Poster No. 75

Phylogenetic diversity and distribution patterns of vannellid amoebae: lineages from the polar regions

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Vannellids (Amoebozoa: Vannellidae) are flattened and fan-shaped free-living amoebae ranging in size from about five to tens of micrometres. They are widespread throughout the world, abundant in both freshwater/soil and marine habitats, and play an important role in communities of microorganisms preying on bacteria. Phylogenetically, they are divided into freshwater and marine clades. In comparison with other major groups of Amoebozoa, vannellids only rarely form cysts.

We analysed our large vannellid dataset including 13 novel strains of Arctic (Svalbard) and Antarctic (James Ross Island) origin. A fragment of cytochrome oxidase I gene was amplified using the Folmer primers and sequenced directly or following a molecular cloning. A translation to amino acids confirmed an RNA-editing in most strains. In total, 240 sequences including those of molecular clones (representing 90 strains of vannellid amoebae) were analysed by Maximum likelihood. Interestingly, the phylogenetic analysis revealed two polar strain lineages consisting of strains from the both polar regions. Of these, one lineage unites strains of the freshwater/soil origin whereas the other one those of the marine origin. Both these lineages have an extreme disjunct, bipolar distribution.

Bipolar distribution is known in various organisms; however, vannellids deserve a special attention. They can be kept in a culture quite easily; therefore they offer an excellent opportunity of studying factors which favour their polar distribution. Our preliminary results suggest that the growth of polar vannellids in the laboratory is not inhibited by a higher (20 °C) temperature.

Poster No. 76

Morphology of *Thuricola kellicottiana* (Stokes, 1887) Kahl, 1935 (Ciliophora: Peritrichia) from a wastewater treatment plant in Brazil

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This study aims to improve the knowledge on the little-known ciliate *Thuricola kellicottiana* based on live observations, protargol-impregnation and scanning electron microscopy. Specimens were isolated from samples collected from the aeration tank of a wastewater treatment plant in Rio de Janeiro, Brazil, in the years of 2016 and 2017, and studied using basic light and electron microscopy ciliatological techniques. The Brazilian population of *T. kellicottiana* is characterized as: one or two zooids per lorica (rarely three). Zooids 249 x 17 µm in vivo on average; body trumpet-shaped with anterior region enlarged and posterior region tapered towards the stalk. Macronucleus ribbon-shaped located along the longitudinal axis of body. One contractile vacuole at dorsal wall of vestibulum. Peristomial collar about 6 µm thick and 27 µm wide; peristomial disc plane and smooth, 21 µm wide, moderately elevated and oblique in relation to the peristomial collar. Infundibulum oblique, extending ca. 19 µm into body. Lorica 186 x 44 µm on average, more or less obconical at posterior third. Aperture and base ca. 37 µm and 16 µm wide, respectivelly; aperture elliptical; shutting valve ca. 36 µm wide, at about 140 µm from the base of lorica. Oral ciliature with outer haplokinety and inner polykinety performing one circle and a half before diving towards infundibulum. Polykinety 1 the longest; polykinety 3 the shortest. All infundibular polykinetids three rowed. Aboral ciliary wreath at about 115 µm from the scopula, composed of monokinetids. *T. kellicottiana* forms pseudo-colonies up to five loricae occupying the same substrate. Loricae were seen attached to activated sludge flocs, organic matter or debris, and even loricae of other protists. Since no available type material is mentioned in the literature, neotypification of *T. kellicottiana* is also proposed.

Keywords: Vaginicolidae, taxonomy, silver staining, scanning electron microscopy, southeast Brazil

Poster No. 77

Molecular and morphological characterization of four trichodinid ectoparasites (Ciliophora: Trichodinidae) from freshwater fishes in China

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Trichodinids are ciliated protozoan parasites or symbionts of marine and freshwater organisms. During a parasitic ciliate survey in China from 2013 to 2015, four *Trichodina* species were isolated from freshwater fishes. *T. paranigra* and *T. reticulata*, were isolated from gills of an exotic fish *Hypseleotris swinhonis* in Tibet. *T. acuta* and *T. hyperparasitis* were isolated from body surface of loach *Misgurnus anguillicaudatus* and gills of yellow catfish *Pelteobagrus fulvidraco* respectively in Hubei. The four species were investigated on morphological identification based on silver-strained specimens, and their small subunit ribosomal RNA gene (SSU rDNA) sequences were sequenced. Morphologically, *T. paranigra* is characterized mainly by the large adhesive disc with obliquely quadrilateral blade and *T. reticulata* is mainly characterized by the 8–12 spherical or elliptical granules in central zone of adhesive disc. *T. acuta* is characterized mainly by the acute sickle-shaped blade with well-developed sharp blade apophysis, the irregular circular granule, and the robust ray. *T. hyperparasitis* is mainly characterized by the broad blade and the inconspicuous round ray apophysis. Phylogenetic analyses revealed that the four *Trichodina* species investigated in the present study were nested within a clade including several freshwater *Trichodina* species. This indicates that the central granule is a useful taxonomic feature, but it may not be an important phylogenetic characteristic. For our knowledge, the present study is the first record of trichodind ectoparasites from an exotic fish in Tibet, which reveals that the invasion of *H. swinhonis* may cause a potential threat to native fishes by carrying or spreading parasitic ciliates.

Keywords: *Trichodina paranigra*, *Trichodina reticulata*, *Trichodina acuta*, *Trichodina hyperparasitis*, phylogenetic analyses

Poster No. 78

Phylogeny, ultrastructure, and mitochondrial genome of a novel discobid nanoflagellate from the Solomon Islands

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Discoba is one of the major eukaryotic assemblages, and comprises four major subgroups, which include Jakobida, Euglenozoa, Heterolobosea and *Tsukubamonas*. Some discobid protists are considered good models for understanding early eukaryotic evolution. For example, the most gene-rich mitochondrial genomes are found among members of Jakobida. Recent environmental studies suggested that our current understanding of the diversity of discobid protists is limited significantly by under-sampling. Here, we report a novel nanoflagellate, strain JB, that was isolated from a lagoon of the Solomon Islands. The flagellate is about 4 µm in length, bears two flagella, and displays a conspicuous ventral feeding groove. The flagellate exists in two morphotypes: a suspension feeder, which bears flagella that are about the cell length, and a swimmer, which has longer flagella. In 18S rRNA gene phylogeny, JB branched just outside of Discoba, although this topology was not supported well. In a tree based on analyses of 151 proteins, JB is sister to the jakobid clade. Together with ultrastructural data and the complete mitochondrial genome, we discuss the significance of JB in the context of better understanding the evolution of Discoba.

Keywords: Discoba, phylogeny, ultrastructure, mitochondrial genome

Poster No. 79

New plastid markers for cryptophycean phylogeny and diversity studies

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Cryptophycean algae are unique eukaryotic algae with nucleomorph, which originated from red algae, i.e. the secondary endosymbiosis. *Rhodomonas* and *Teleaulax* are important components of the coastal ecosystem and some lineages contribute to significant algal blooms from winter to spring at the southwestern region of Korea. Recent studies of cryptophycean systematics have applied complete plastid (pt) and mitochondrial (mt) DNAs. However, no comparative analysis of gene characteristics for molecular marker selection has been done because only a few taxa are available. In this study, we tested nine plastid genes (*atp*A, *atp*B, *chl*I, *clp*C, *dna*K, *rpo*C1, *sec*A, *psa*A, and *tuf*A) with conventional well-known genes such as nuclear 18S rRNA and plastid 16S rRNAs, by using 30 selected taxa (12 cryptophycean lineages) in order to test new markers for cryptophycean phylogeny and diversity researches. We described sequence statistics such as proportions of conserved, variable, and parsimony informative sites of each gene. And compared and discussed the distribution of p-distance and saturation tests for from the inter- and intra-cryptophycean lineages; and bootstrap supports for monophyletic nodes within the cryptophytes.

Keywords: cryptophytes, plastid markers, phylogeny, diversity

Poster No. 80

Establishing a novel drug discovery platform for the identification of anti-microbial compounds against the "brain-eating amoeba"

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Naegleria fowleri ("the brain-eating amoeba") is a unicellular amoeba-flagellate and an opportunistic parasite to humans, which causes Primary Amoebic Menignoencephalitis (PAM). PAM is an acute disease affecting the central nervous system associated with a case fatality rate > 95%. Presently, the anti-fungal drug amphotericin B is considered to be the first choice of treatment against this disease, however, it is frequently associated with severe side effects and might not always be effective. Over the past decade there has been a rise of infections of *Naegleria fowleri* in developing countries, thus, it is crucial to identify and develop new anti-microbial drugs against this fatal pathogen. We have established a novel screening platform and performed an initial drug-screen by employing a collection of drugs and established anti-microbial agents using the non-pathogenic *Naegleria gruberi* as an easy-to-handle model organism for the discovery and investigation of anti-*Naegleria* therapeutics. The viability of the treated cells was monitored using an in house established colorimetric assay. By employing a library of 1443 FDA approved drugs, we have a performed a high throughput screening on *N. gruberi*. Current preliminary data indicate an effective response of certain anti-microbial agents against *Naegleria gruberi*, whereas others contradict already published data. The described screening platform will promote the discovery of anti-*Naegleria* drugs and provide the basis for similar discovery platforms for additional parasitic microbes as well as enabling systematic chemical biology approaches designed to decipher *Naegleria's* biology.

Keywords: *Naegleria gruberi*, *Naegleria fowleri*, brain-eating amoeba, drug screening, therapeutics

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Poster No. 81

DYNAMO project: Characterization of the diversity and function of plankton associated microbiota

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Ever since living organisms arose in the oceans, they have competed for resources and space. However, many planktonic species developed peaceful cooperation involving mutualistic interactions between two partners. Within unicellular eukaryotes, such symbiotic association between heterotrophic hosts and microalgae is widespread and sustain ecologically important taxa, such as the Rhizaria, in the open ocean. While knowledge of bipartite symbiosis has greatly improved, it is only recently that scientists disclosed more intricate relationships involving additional partners such as bacteria. Wherever bacterial communities (i.e. microbiota) have been found in tight association with other organisms (e.g. humans or plants), its critical role for the biology and ecology has been demonstrated. The DYNAMO project explores the diversity of multipartite symbiosis in the plankton and characterize the cellular metabolites they produce. Radiolarians and foraminifers are used as relevant model for marine ecology. An original combination of single-cell sorting and sequencing coupled with microscopy are used to characterize and specifically localize the partners of the association. The essential first step of the project is a description of the plankton associated microbiota, specifically the bacteria always found in association with their host (i.e. core microbiota) *versus* the bacteria occasionally detected. In order to get a more precise perspective on the spatio-temporal diversity of protist associated microbiota, the plan is to collect as many replicates as possible from different sampling sites and over the seasons.

Poster No. 82

Lake warming and seasonal successions of ciliates. A case study from Lake Zurich

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Planktonic ciliates are of great relevance in microbial food webs where they link lower with higher trophic levels. This study in Lake Zurich, Switzerland, aimed to follow the long-term dynamics (2014-2016) of ciliate assemblages in times of climate change by i) assessing limnological parameters and describing plankton dynamics, and ii) characterizing ciliate abundances, alpha-diversity, and similarities of annual assemblages. Water samples for ciliate identification were collected bimonthly at 5 m depth in the open waters from March 2014 to December 2016. A total of 80 samples were quantitatively and qualitatively analysed at high taxonomic resolution using quantitative protargol staining (QPS), microscopic counts and live observations. Alongside, abiotic and biotic parameters were measured.

Abundance of ciliates presented a re-occurring bimodal pattern during the multi-annual investigation. However, this annual pattern did not follow the expected Plankton Ecology Group (PEG) model. Ciliate spring blooms occurred, but abundances were lower than previously described for Lake Zurich. Additionally, clear shifts of ciliate maximal abundances towards summer were observed after clear-water phases. Recurrent extremely warm winters (owing to climate change) resulted in reduced water turnover depths and decreasing transfer of orthophosphate from the deep to the surface. Consequently, bacterio-, phyto- and zooplankton dynamics also deviated from respective expected models, affecting ciliate assemblages and their seasonal successions.

Throughout the study, 46 distinct ciliate morphospecies were identified. Nevertheless, 65% of ciliate standing stocks were due to only five morphospecies, namely *Balanion planctonicum*, *Urotricha* spp., *Halteria/Pelagohalteria* spp. (without endosymbionts), and two representatives of the genus *Rimostrombidium*.

This study showed striking changes within the microbial food web in Lake Zurich. Clear deviations from known occurrence patterns were observed for ciliate morphospecies. However, even in times of lake warming, ciliate seasonal successions followed repetitive patterns from one year to the next.

Keywords: ciliates, Lake Zurich, long-term dynamics, Plankton Ecology Group (PEG) model, global warming

Poster No. 83

Euglenoid movement: an avoidance strategy against algivores?

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Euglenoids have a unique pellicle composed of pellicular strips, which contributes to cell stability but also enables the characteristic euglenoid movement (metaboly). Some euglenoids, for example certain *Euglena* species of the subgroup Serpentes (also known as "*Euglena deses* group"), are capable of extreme deformations of the cell, whereas other representatives are rigid and fast swimmers. During feeding experiments with algivorous vampyrellid amoebae (Vampyrellida, Rhizaria) we observed differential feeding success depending on the euglenoid species. The vampyrellid *Leptophrys vorax* consumed several species of *Euglena*, *Phacus*, *Monomorphina* and *Trachelomonas*, but displayed markedly reduced growth with *Euglena mutabilis* and *Euglena deses*. This inspired to study the feeding strategy of the *Leptophrys vorax* in more detail by time-lapse microscopy and to perform a quantitative feeding experiment with selected euglenoids. Rigid and metabolic euglenoids were offered to *Leptophrys vorax* in the living and dead condition, the latter eliminating any motility. The vampyrellid multiplied successfully with dead cells of all tested euglenoids and with live cells of *Phacus smulkowskianus* and *Euglena deses*. Interestingly, live cells of *Euglena mutabilis* clearly prevented vampyrellid growth. As shown by detailed microscopy, *Leptophrys vorax* attempted to phagocytose *Euglena mutabilis*, but had severe problems to ingest the highly metabolic euglenoids. These data suggest that metaboly in certain phototrophic euglenoids might serve as an effective avoidance strategy against microbial predators.

Poster No. 84

Molecular phylogenetic analyses suggest inconsistencies in systematics of the genus *Eremoplastron* Kofoid & MacLennan

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The validity of genus *Eremoplastron* has been historically questioned due to morphological similarities with *Eudiplodinium* (ciliates in both genera have a single skeletal plate). The present work investigates the validity of genus *Eremoplastron* with 18S-rDNA phylogeny. According to our results, ciliates belonging to genera *Eudiplodinium* and *Eremoplastron* do not constitute a monophyletic group, since *Eudiplodinium maggii* emerged as a sister-group of a clade composed of *Entodinium*, *Epidinium*, *Metadinium* and *Ophryoscolex* and apart of *Eremoplastron* sequences, suggesting that these genera are not synonyms, even though they have a single skeletal plate. *Eremoplastron* ciliates, however, also do not constitute a monophyletic group. *Eremoplastron rostratum* positioned as a sister-group of all ophryoscolecid ciliates and *Eremoplastron neglectum* and *Eremoplastron dilobum* constituted a monophyletic group with *Diploplastron affine*, ophryoscolecid ciliate with two skeletal plates. This study demonstrates that the number of skeletal plates, character widely used in the taxonomy of the family Ophryoscolecidae, is a homoplastic character and highlight the need of reorganization of the taxonomy of the genus *Eremoplastron*.

Poster No. 85

GPSit: A simple and unsupervised method to study the evolutionary positions of nonculturable eukaryotes using single-cell sequencing

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Phylogenetic reconstruction is one powerful method to trace evolutionary history. High throughput genomic data has been upgrading phylogenetic analysis based on several loci to phylogenomics using hundreds of genes. Here, we propose the first timesaving and unsupervised method "Guided Phylogenomic Search in trees" (GPSit), which can be easily utilized by non-experts. GPSit is compatible with genome sequencing and transcriptome sequencing data, as well as with single-cell genome sequencing data that solves the sampling problem of nonculturable eukaryotes. We tested the efficiency and accuracy of GPSit by incorporating available online data and new single-cell sequencing data of three nonculturable marine ciliates (*Anteholosticha monilata*, *Deviata* sp. and *Diophrys scutum*). Compared with previous protocols, our method streamlines the whole workflow with all essential but miscellaneous and exhausting operations integrated. On the premise of precisely reconstructing phylogenetic relationships that is demonstrated by comparing the results with that of previous studies, it can greatly reduce manual operation time from hours to minutes, and thus lower the barrier for non-experts and relieve researchers of the unnecessary burden. Besides, GPSit enables researchers to perform analyses using "supermatrix" and "supertree" methods at the same time, and our result indicates that the former could stably reconstruct "deep" phylogenetic relationships while the latter supports the "shallow" phylogenetic divergence better. Benefited from the high efficiency of GPSit, we evaluated the impact of different levels of missing data on phylogenetic research with both maximum likelihood (ML) and Bayesian inference (BI) methods based on empirical phylogenomic data, and we found that BI is less sensitive to missing data.

Keywords: GPSit, phylogenomics, single-cell sequencing, nonculturable ciliates, missing data

Poster No. 86

Planktomania: 3D technologies to promote education and outreach on plankton

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Plankton is invisible to the naked eye, yet it plays key roles in aquatic ecosystems and in global biogeochemical cycles. It produces 1/2 of the oxygen in the atmosphere, it is at the basis of aquatic food chains and it comprises an amazing diversity of life forms.

Planktomania (http://www.planktomania.org/en) carries general public and kids into the fascinating world of plankton in 3D thanks to unique tools created specifically to facilitate the discovery of the amazing shapes and life histories in this microscopic world. Virtual and augmented reality are promising educational tools enhancing attention and effective learningf

Keywords: plankton, education, virtual reality, augmented reality, outreach

Poster No. 87

The family Urosporidae Léger, 1892: biodiversity, morphological plasticity, and molecular phylogeny, as inferred from SSU rDNA

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The representatives of the family Urosporidae are highly diverse in their morphology at light and electron microscopic level. In addition, they parasitise a wide range of marine and freshwater invertebrates: Annelida, Mollusca, Nemertea, Sipuncula, and Echinodermata. Among species investigated so far on ultrastructural level, there are eugregarines (some *Urospora* spp.) with typical lecudinid-like morphology: the cell body consisting of a single compartment with an attachment apparatus at the apical end, gliding motility, and the presence of longitudinal epicytic folds on the cell surface. Additionally, there are gregarines (*Pterospora* spp. and *Urospora chiridotae*), which are traditionally referred to this family, and possess the completely modified cell-body and cortex organisation. Moreover, some urosporids exhibit the mode of motility that significantly differs from the gliding. Despite these, it is assumed that all urosporid species, *sensu lato*¸ possess at least one common feature in the oocyst morphology: heteropolar, with a tail or tails at one of the end. It was shown that, in SSU rDNA sequences phylogenetic analyses, gregarines from the family Urosporidae grouped altogether with representatives of the family Lecudinidae into the superclade Lecudinoidea (= Urosporoidea). To this superclade, the unusual gregarine *Veloxidium leptosynapta*, isolated from a holothurian and initially belonging to the family Archigregarinidae, affiliated as well. We performed the phylogenetic analyses comprising all lecudinid and urosporid species along with environmental sequences available in GenBank.

The resulting phylogenetic tree as inferred from SSU rDNA sequences confirms that family Urosporidae comprises several clades to which some lecudinid species affiliated. In contrast, some urosporid species are affiliated to the family Lecudinidae. Interestingly, the coelomic gregarine *Urospora chiridotae,* described from blood vessels of the holothuroid *Chiridota laevis,* affiliates to the clade comprising *V. leptosynaptae*. In conclusion, the taxonomy of the families Urosporidae and Lecudinidae should be reviewed.

Financial support provided by GAČR No. P505/12/G112 (ECIP).

Keywords: Urosporidae, morphological diversity, SSU rDNA, phylogenetic analyses, Lecudinoidea

Poster No. 88

First records of epibiont ciliates *Loricophrya bosporica* (Suctoria) and *Cothurnia* sp. (Peritrichia) from methane enriched sediments

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Suctorian ciliate *Loricophrya bosporica* was found at nematode *Metachromadoroides remanei* whereas peritrich *Cothurnia* sp. at nematode *Spirinia* sp. from methane seep of the the Ria Formosa lagoon (Southern Portugal). Consider sediment properties analysis lagoon sediments allow to attribute the hypoxic (periodically anoxic) methane seepage environment. The morphological characterizations of these ciliates with emphasis on poorly studied stylotheca structure in suctorian ciliate and macronucleus morphology in peritrich are presented. *L. bosporica* was described from the Black Sea hydrogen sulfide zone. The re-discovery of the latter ciliate in habitat with deficiency of oxygen and the presence of hydrogen sulfide may be indicative about prevalence of *L. bosporica* to extreme conditions.

Keywords: ciliate, epibiont, methane seep, Ria Formosa lagoon

Poster No. 89

Evaluating information content of molecular phylogenies in tintinnid ciliates (Alveolata, Ciliophora, Choreotrichia)

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Molecular phylogenies based on different rDNA loci are an important tool for inferring relationships. Especially in tintinnid ciliates with their artificial, lorica-based classification, gene sequence analyses provide the most comprehensive insight into genealogies. While taxon diagnoses in ciliates comprise morphological characters, autapomorphies in genetic data, i.e., signature nucleotide positions, are usually not determined. Likewise, the quality of the historical signal contained in the alignments is not examined prior to establishing the phylogenetic trees in tintinnids. Therefore, two different methods were applied for assessing the phylogenetic content of sequence data of choreotrichid ciliates and specifically of the order Tintinnida. They allowed distinguishing historical (molecular synapomorphies) from non-historical signals (molecular homoplasies) in the ribosomal DNA sequences obtained from GenBank. First, a phylogenetic network was computed, visualizing the dataset and characterizing consistent and conflicting information. Secondly, a split spectrum analysis was applied for identifying the number and quality of the nucleotide positions responsible for the statistical support of tree nodes. The results demonstrate the potential as well as the limitations of the presently used molecular markers for resolving the relationships of certain tintinnid groups. They also indicate accidental sequence resemblance which can cause high support values in tree calculations, but are actually the result of long-branch artefacts. The implementation of these methods might also reveal apomorphies in the sequence data usable for characterizing particular tintinnid clusters in the phylogenies.

This study is supported by the Austrian Science Fund (FWF Project I 3268).

Poster No. 90

First transcriptome-based analysis of the Myxogastria (Amoebozoa) further resolves its basal phylogenetic relationships

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Myxogastria, the most species-rich group in Amoebozoa, are amoeboid protists characterized by a complex life cycle that culminates in the formation of spores inside fruiting bodies (sporocarps). Myxogastria are traditionally classified based on both micro and macromorphological characters. However, phenotypic traits of sporocarps can be greatly affected by environmental conditions, making it difficult to find reliable taxonomic criteria.

Recent phylogenetic analyses of 18S rRNA gene sequences have substantially improved our knowledge by revealing the existence of two major clades (i.e., dark-spored and bright-spored). The relationships within these lineages are not well resolved. Moreover, the largest order, Physarales, has been consistently neglected in previous studies and consequently, the circumscription of most genera remains controversial.

Using a phylogenomic approach including 46 newly-generated transcriptomes and a comprehensive taxon sampling we resolve the phylogeny of Physarales, as well as Myxogastria. We include representatives from most families and all five orders traditionally assigned to Myxogastria (Echinosteliales, Physarales, Stemonitales, Trichiales and Liceales), and analysis provides enhanced phylogenetic resolution by using 88 proteins. This dataset represents the largest generated for this group to date.

Our highly resolved phylogeny supports the monophyly of Myxogastria while recovering three fully supported clades, i.e. bright-spored (Lucisporidia), echinostelids, and dark-spored (Fuscisporida). Liceales and Stemonitales appear paraphyletic while the monophyletic Echinostelids are basal to the dark-spored clade. Physarales is a monophyletic group nested within Stemonitales, with Physaraceae recovered as monophyletic but Didymiaceae seems to be paraphyletic. Enigmatic species *Elaeomyxa cerifera* and *Kelleromyxa fimicola* cluster together Stemonitales and Physarales, respectively.

These results form a foundation for future evolutionary analyses of structures across Myxogastria and stress the need for a taxonomic revision of this subclass.

Keywords: Myxogastria, phylogeny, transcriptomics

Poster No. 91

Single cell analysis linking ribosomal (r)DNA and rRNA copy numbers to cell size and growth rate provides insights into molecular protistan ecology

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Ribosomal (r)RNA and rDNA have been golden molecular markers in microbial ecology. However, it remains poorly understood how ribotype copy number (CN)-based characteristics are linked with diversity, abundance and activity of protist populations and communities observed at organismal levels. Here, we applied a sing-cell approach to quantify ribotype CNs in two ciliate species reared at different temperatures. We found that in actively growing cells, the per-cell rDNA and rRNA CNs scaled with cell volume (CV) to 0.44 and 0.58 powers, respectively. The modeled rDNA and rRNA concentrations thus appear to be much higher in smaller than in larger cells. The observed rRNA:rDNA ratio scaled with CV^0.14. The maximum growth rate could be well predicted by a combination of per-cell ribotype CN and temperature. Our empirical data and modeling on single-cell ribotype scaling are in agreement with both the metabolic theory of ecology and the growth rate hypothesis, providing a quantitative framework for linking cellular rDNA and rRNA CNs with body size, growth (activity) and biomass stoichiometry. This study also demonstrates that the expression rate of rRNA genes is constrained by cell size, and favors biomass rather than abundance-based interpretation of quantitative ribotype data in population and community ecology of protists.

Keywords: Body size, copy number variation, growth rate, rRNA:rDNA ratio, warming

Poster No. 92

Effective control of *Poterioochromonas malhamensis* in *Chlorella sorokiniana* culture by maintaining CO_2-mediated low culture pH

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Although predators in microalgal culture can often be protozoa reducing biomass productivity and culture stability, there are few effective approaches to control them. This study investigated the effect of culture pH (i.e., 6.0, 6.5, 7.0 and 7.5) maintained by supply of compressed air bubbles containing various concentrations of CO_2 on death of the flagellate *Poterioochromonas malhamensis* and several other protozoa in the culture of the green microalgae *Chlorella sorokiniana* GT-1. *C. sorokiniana* GT-1 grew well at pH 6.0 and 6.5 and a sustainable biomass concentration of 1.61 g L^{-1} was obtained from the cultures maintained at pH 6.5. The cultures maintained at pH 7.0 and 7.5 collapsed on days 7 and 4 of culture, respectively, as a result of contamination by *P. malhamensis* and to less extent by other protozoa (e.g., ciliates and amoebae). Further experiments revealed that it was the actual dissolved CO_2, not the low pH itself, or reduced dissolved oxygen in the culture medium that prevented the occurrence of *P. malhamensis.* It is speculated that increased CO_2 partial pressure in the culture media may enhance diffusion of CO_2 into the cytoplasm of *P. malhamensis* that lowers the intercellular pH, and thus results in cell death. The method developed in this study can be effective in protozoan control in large-scale *Chlorella* culture in open raceway ponds. It is suggested that a low pH maintained temporarily or constantly by supply of CO_2 may be a promising approach to control *P. malhamensis* and alike in microalgal culture.

This work was partially funded by the State Development & Investment Corporation and China Electronics Engineering Design Institute, China.

Keywords: *Chlorella sorokiniana*, *Poterioochromonas malhamensis*, contamination control, CO_2, pH

Poster No. 93

Seasonal patterns of protist biodiversity in a New England vernal pool

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Vernal pools are temporary freshwater ecosystems primarily located in temperate forests. A number of well-studied species live in these bodies of water, including frogs, salamanders and fairy shrimp; microbial communities in vernal pools are understudied, and past studies focuses largely on bacteria and fungi. Here, we aim to catalogue the protist community over the course of the hydrolytic year as the vernal pool transitions from winter, when the surface of the pool is frozen over, until summer, when the pool is completely dry. Our study centers on the SAR clade (Stramenopila, Alveolata, Rhizaria), capturing many trophic levels within the microbial food web. We use high-throughput sequencing of DNA and RNA PCR amplicons targeting a hypervariable region of small subunit rDNA, to capture the whole (active and quiescent) and active community members. Genetic data are coupled with enumeration data of general morphotypes generated from concentrated Lugol's fixed samples. Together, these data reveal diversity of SAR that show dynamic changes during the transition to desiccation. Both genetic and enumeration data support the idea that microbial communities exhibit a distinct seasonality from winter to spring to summer while alpha diversity counts remain relatively consistent month to month.

Keywords: amplicon, high throughput sequencing, protist, SAR, vernal pool

Poster No. 94

Evolution of ribosomal RNA genes in Euglenozoa

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Eukaryotic ribosomes are composed of a variety of proteins and usually four RNA molecules. Genes coding three of them, 18S, 5.8S and 28S rRNA are maintained in a single operon and transcribed together. They are separated by the internal transcribed spacers (ITS1 and ITS2), which are removed in the post-transcriptional process. The rDNA cistron arrangement is different in the members of Euglenozoa group.

Euglenozoa (Excavata) consists of three major phyla: Euglenida (auto- or heterotrophs), Kinetoplastea (free-living predators or parasites) and Diplonemea (marine predators). The structure of rDNA cistron was examined in several parasitic kinetoplastids and revealed that their operons contain more than three typical rRNA genes. It is caused by the presence of additional ITS sequences in DNA fragment corresponding to 28S rDNA. However, the most complex rDNA structure is observed in *Euglena gracilis* (Euglenida). Its operon consists of 15 rRNA genes separated by 14 ITS sequences. Furthermore, rRNA genes of *E. gracilis* are located on extrachromosomal circular particles. Fragmented 28S rRNA seems to be a common feature in Euglenozoa. However, many problems require further studies: the mechanism of removing additional spacers from pre-rRNA, the mechanism of acquisition of new spacers or the potential role of those additional elements in genes. In our work, we try to trace the evolution of rDNA within Euglenozoa, focusing particularly on the distribution of ITS sequences. We analyzed rDNA sequences from several species of kinetoplastids, diplonemids and euglenids. Based on previously published data we tried to predict the arrangement of rRNA genes in species for which no such analysis was conducted and identify events of acquisition of new spacers in different phylogenetic lineages.

Keywords: Euglenozoa, rRNA genes, evolution, phylogeny

Poster No. 95

Giant formation and surviving strategy in *Blepharisma*

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Ciliate *Blepharisma* usually prey bacteria, but it is known that cannibalism occurs under starved conditions, forming giant cells. In the previous studies, nuclear morphology and cell size of giant cells have been reported, but factors that affect giant cell formation (GF) and the mechanism and the biological significance of GF are not well understood. Here, we studied (1) morphology of the giant cell formed by cannibalism, (2) environmental factors affecting GF, and (3) advantages of GF by cannibalism. We found that GF was affected by cell density and variation of cell size during stationary phase in *Blepharisma*. Although GF occurs by other than cannibalism, cannibalism is the least risky way, and GF is advantageous for long-term survival and uptake of the prey.

Keywords: ciliate, *Blepharisma*, cannibalism

Poster No. 96

Grazing of three protozoa on *Aureococcus anophagefferens*

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The algal species *Aureococcus anophagefferens* is responsible for brown tides which recurred in estuary areas in the east China during the period 2009–2014, and tropical interactions between marine protozoa and this species remain poorly understood. In this study, grazing by three marine protozoa including *Oxyrrhis marina*, *Uronema marinum*, and *Euplotes vannus* on a Chinese strain of *A. anophagefferens* was investigated, offered either mono-algal or mixed-algal diets consisting of *A. anophagefferens*. A typical Michaelis-Menten pattern generally existed between ingestion rate and food level on mono-algal diets. *E. vannus* had the highest maximum ingestion rate (I_{max}) of all and I_{max} of *O. marina*, *U. marinum*, and *E. vannus* were 45%, 70%, and 41% lower feeding on exponential phase cells of *A. anophagefferens* on *Isochrysis galbana.* Maximum growth rates were sequentially 0.63, 0.48, and 0.57 d^{-1}, lack of consistent trend compared with *I. galbana* as diet. Offered mixtures of exponential phase cells of *A. anophagefferens* and *I. galbana* with different proportions, three protoza selected for *A. anophagefferens* cells. When stationary phase cells of *A. anophagefferens* were used, *O. marina* and *U. marinum* avoided *A. anophagefferens* while *E. vannus* tended to select cells with dominant cells, exhibiting opportunistic feeding. Finally, quantification of extracellular polymeric substances in *A. anophagefferens* cells revealed that transparent expolymer particles (EPS) content was 17.8±0.4 pg Xeuiv.cell^{-1} in stationary phase cells, significantly higher than that in exponential phase cells (14.5±0.4 pg Xeuiv.cell^{-1}). Our study suggested that *O. marina* and *U. marinum* may act as a control during initial bloom proliferation while *E. vannus* may exert stronger grazing pressure throughout a bloom. Grazing pressure of some protozoa such as *O. marina* may weaken at the bloom climax due to production of a large amount of EPS by *A. anophagefferens* cells.

Keywords: brown tide, protozoa, grazing selectivity, transparent expolymer particles

Poster No. 97

An evolutionary evidence of an heme iodoperoxidase in *Tisochrysis lutea*

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Tisochrysis lutea is a small haptophyte used in aquaculture as a feedstock for shellfish and shrimps. Recently, an uptake mechanism of iodide, very similar to that proposed for iodine-accumulating marine algae Laminaria, has been found in *T. lutea*. Furthermore, the iodine-containing thyroid hormone thyroxine has been identified in *T. lutea* as well as a gene that encodes a putative peroxidase involved in the oxidation of iodide and iodide metabolism, the so-called "*perox*" gene. This supports the idea that an haloperoxidase could be involved in the oxidation of iodide in *T. lutea* and this *perox* gene is the best explanation to date. However, there is still a surprising lack of knowledge about the evolution of the haloperoxidases family in microalgae. In this study, we conducted a phylogenomic analysis of the *perox* gene to approximate its evolutionary history and to unravel more details about its origin and its posible haloperoxidase function in *T. lutea.* We only found 14 homologous sequences present in only 4 species; two ciliates (*Oxytricha trifallax* and *Stylonychia lemnae)*, one haptophyte *T. lutea* and the amoebozoa *Acanthamoeba castellanii* (Ac). The absence of this *perox* gene in microalgae contrasts with the 11 microalgae species which have peroxidase activities or organoiodine compounds. Furthermore, our Maximum Likelihood phylogeny suggests that Ac could be a vehicle for transmission of gene *perox* to ciliates and haptophyte by HGT. Despite being scarcely distributed, we found that the *perox* gene is under strong purifying selection and the function for *perox* homologous could be included in "peroxidase-cyclooxygenase family". Our results indicate that *perox* could explain the presence of iodotyronine in *T. lutea*; to put it in other words, *perox* seems to be an heme iodoperoxidase acquired by HGT with a putative primitive function linked to an ancestral antioxidant inorganic compound like iodide.

Keywords: peroxidase, evolutionary history

Poster No. 98

Tropical ciliates of North America, novel flagships and new species

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Large freshwater tropical ciliates are unicellular eukaryotic organisms that play an essential role in microbial loops and food webs. The biodiversity and biogeography of many freshwater ciliates is an understudied area within microbial ecology. Some species once thought to be endemic to a restricted geographical area have been shown to have wider distributions when sampling efforts are increased. Many distinct tropical ciliate species were originally documented in Africa, and had been unseen elsewhere. These ciliates are mostly known only from drawings, with little ecological information and no photographic records existing. Intensive sampling was conducted in numerous freshwater areas of Florida (USA) during the 2016/17 season. We record novel observations of several tropical flagship ciliates discovered as thriving locally. Our findings here include: the first record outside of Africa for some large, flagship ciliate species; their first records for the Americas; the first photomicrographs for several species such as *Frontonia vesiculosa*; and the first report of the ecology for these species. Our discovery of new species in genera such as *Sonderia* and *Woodruffides* are also reported here, highlighting the largely unknown world of tropical microbial diversity. Some ciliate hosts have locally been found to contain an interesting ecto and potentially endo symbiotic bacterial consortia. These ciliates may form a relationship involving all three domains of life, with EM images revealing a symbiosis between an anaerobic eukaryote as host to dense layers of prokaryotic life. Such investigations are important for understanding global biodiversity, tropical systems ecology, and the actual dispersal of microorganisms.

Keywords: biogeography, ciliate, ecology, flagships

Poster No. 99

Long-amplicon environmental sequencing of eukaryotes using PacBio

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The small subunit (SSU) RNA gene is a widely-used marker for environmental sequencing to assess eukaryotic biodiversity, and discover new species in various environmental samples. This approach typically employs next generation sequencing technologies such as Illumina, which generates millions of reads through massive parallelisation. One major limitation of this approach is sequence length, which has resulted in an unprecedented wealth of data but for only short (≤500bp) regions of the SSU gene. Consequently, the phylogenetic resolution of current data is generally poor. This problem may be overcome by the advent of the high-throughput, long-read sequencing platform PacBio. While PacBio sequencing has successfully been used to amplify the complete SSU in prokaryotes for metabarcoding, a similar approach has not yet been tested for eukaryotes. To address this, we obtained three soil samples and used long-range PCR to amplify a 4000-4500 bp region of the rRNA operon, including 18S, ITS1, 5.8S, ITS2, and 28S. Amplicons were sequenced on three SMRT cells on the new Sequel system, generating a total of ~ 200,000 long reads. Here, we will present our pipeline for cleaning and analysing eukaryotic ribosomal data generated with this method and show its potential to assess eukaryotic diversity using the stronger phylogenetic signal of longer reads. Most notably, this approach allows to reliably infer taxonomic affiliation using phylogenetic trees rather than simpler homology binning, which represents a key advantage to discover novel lineages. It also significantly develops databases of ribosomal genes other than the SSU, increasing taxon sampling and phylogenetic coverage of ITS, 5.8S, and 28S regions.

Keywords: PacBio sequencing, environmental samples, rRNA operon, biodiversity, soil protists

Poster No. 100

Response of under-ice phytoplankton populations to light exposure during the Arctic spring bloom

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Marine phytoplankton are responsible for approximately 50% of global primary production and are at the base of marine food webs. In the Arctic Ocean, one important factor controlling phytoplankton productivity is sunlight, as sea-ice and snow cover limits light penetration in the water column. Over the last three decades, the extent of the seasonal sea-ice and snow cover has decreased in response to climate change, resulting in changes in the dynamics of light penetration. These changes may cause important fluctuations of the spring and summer phytoplankton blooms dynamics with unknown effects on Arctic marine food webs and carbon cycle. In order to better understand the effects of light on Arctic nano- and pico-phytoplankton growth, a series of six incubation experiments were performed between May and July 2016 during an ice camp campaign (www.greenedge-project.info) in Baffin Island (67°28.78 N, 63°47,37 W). Sea-ice coverage at the ice camp was about 90% until mid-June and decreased rapidly down to approximately 10% at the end of July. The experiments were performed with surface sea-water collected through a permanent hole in the sea-ice and incubated in the laboratory for 7 to 8 days at 4°C in the dark (control) and in the light at 100 µE m^{-2} s^{-1}. Phytoplankton growth was analyzed by flow cytometry. We observed a rapid increase in pico- and nano-phytoplankton cell concentration, up to 15-fold with light for the two experiments performed before the onset of the spring phytoplankton bloom. The four other experiments performed later in the season showed a clear decrease in cell concentration demonstrating that light was clearly not limiting any more. We are currently determining how the structure of community changed during these incubations by 18S rRNA metabarcoding.

Poster No. 101

Discrepancy between morphological and molecular traits for species discrimination in the tintinnid ciliates *Parafavella* spp.

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Parafavella spp. were collected during the cruise of the Korean research ice-breaker Araon 2012 in the North Pacific Ocean and from the coastal water off Korea. The tintinnids are usually identified based on the morphology of loricae rather than infraciliatures. However, *Parafavella* which is one of the genera in tintinnids has a high variation on lorica morphology in size and shape that implies an existence of synonym or convergence on the lorica morphology. To clearly delineate the inter-specific boundary of *Parafavella* species, we analyzed the lorica morphology with their genetic traits. Based on the lorica, five species were identified as follows: *Parafavella denticulata* (1 indv.), *P. gigantea* (3 indvs.), *P. hemifusus* (1 indv.), *P. parumdentata* (3 indvs.), and *P. subrotundata* (2 indvs.). With respect to the molecular trait, nuclear (SSU–LSU D1/2, ca. 2,400 bp) and mitochondiral (CO1, ca. 480 bp) genes were newly sequenced and analyzed. The nuclear gene sequences of all five morphospecies were completely identical each other while the CO1 sequences split into three clades (*P. parumdentata* clade 1, *P. parumdentata* clade 2, *P. denticulata–P. gigantea–P. hemifusus–P. subrotundata* clade) with the cutoff of 3% genetic dissimilarity. Based on the results, we assume that the five morphospecies could be defined as two species (*P. parumdentata* vs. *P. denticulata–P. gigantea–P. hemifusus–P. subrotundata*), and the clades 1, 2 of *P. parumdentata* could have 1) sibling species or 2) hybrid or 3) pseudogene. Further study on the infraciliatures and the genetic variations are necessary to clarify their species boundary.

Poster No. 102

Molecular phylogeny of the amoeba genus *Deuteramoeba* (Amoebozoa, Tubulinea)

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An amoeba genus *Deuteramoeba* belongs to the family Amoebidae, order Euamoebida, within the class Tubulinea. This is one of the "core" amoebae genera, representing typical "proteus-like" organisms, most frequently associated with "an amoeba" for a general auditorium. In the same time, knowledge on the phylogeny and systematics of amoebae of the family Amoebidae remains at the surprisingly low level. Except for the genera *Amoeba* and *Chaos*, other members of the family are very poorly studied and known from few findings (or were never seen since initial description). No molecular data are available for the genera other than two above mentioned. We have obtained 18S rRNA gene sequences from *Deuteramoeba algonquinensis* CCAP 1530/5 strain and a new *D. mycophaga* isolates and found that this genus is a neighbour of *Amoeba* + *Chaos* clade in the phylogenetic tree. This corresponds to the relationships deduced from morphology.

Supported with RFBR 16-04-01454 research grant.

Keywords: Amoebozoa, phylogeny, systematics

Poster No. 103

Duplex real-time PCR method for simultaneous detection of *Acanthamoeba* spp. and *Naegleria fowleri* in water

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Acanthamoeba spp. and *Naegleria fowleri* are free-living amoebas (FLA) widely distributed in natural environments and are associated with some severe diseases in humans. In our previous study, we set up a duplex real-time PCR method to detect FLA in water. In this study, we investigated two kinds of FLA, *Acanthamoeba* spp. and *Naegleria fowleri*, in raw water samples. A total of 12 samples of raw water were collected from three major streams in Daejeon. For each stream, two different samples were collected in twice. The raw water samples were filtered and concentrated by centrifugation, and the final pellet was inoculated on non-nutrient agar plates and incubated for 6 days at 30°C. Then, the total genomic DNA was extracted and analyzed using the duplex real-time PCR method. Ten out of the twelve samples (83%) contained *Acanthamoeba* spp. and three samples (25%) were positive for *N. fowleri*. Although the number of analyzed smples (n=12) were not much, our results showed that *N. fowleri* is not as common as *Acanthamoeba* spp. To identify the distribution of FLA in raw water in more detail and to secure the public water safety in Korea, further surveys should be conducted.

Keywords: *Acanthamoeba* spp., *Naegleria fowleri*, free-living amoeba, duplex real-time PCR

Poster No. 104

Evolutionary dynamics and lineage-specific gene loss in the plastid genomes of cryptophyte algae

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Cryptophytes are an ecologically important group of largely photosynthetic unicellular eukaryotes. This lineage is of great interest to evolutionary biologists because their plastids are of red algal secondary endosymbiotic origin and the host cell retains four different genomes (host nuclear, mitochondrial, plastid, and red algal nucleomorph). Here, we report a comparative analysis of plastid genomes from six representative cryptophyte genera. Four newly sequenced cryptomonad plastid genomes of *Chroomonas mesostigmatica*, *Ch. placoidea, Cryptomonas curvata*, and *Storeatula* sp. CCMP1868 share a number of features including synteny and gene content with the previously sequenced genomes of *Rhodomonas salina, Teleaulax amphioxeia*, and *Guillardia theta*. Our analyses of these plastid genomes reveal examples of lineage-specific gene loss and numerous intron insertions. In particular, the *chl*B/*chl*L/*chl*N genes, which encode light-independent (dark active) protochlorophyllide oxidoreductase (LIPOR) proteins, show recent gene loss and pseudogenization across the cryptomonad lineages. Comparison of plastid and nuclear genome based phylogenies suggest the existence of a single red algal secondary endosymbiosis in the ancestor of chlorophyll-*c* containing algae. This event was putatively followed by additional rounds of eukaryotic endosymbioses that spread the red lineage plastid to diverse groups such as haptophytes and stramenopiles.

Keywords: plastid genome, cryptophyte, horizontal gene transfer

Poster No. 105

Dynamics of planktonic diatom *Chaetoceros tenuissimus* and its infectious viruses for five years in Hiroshima Bay, Japan

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Diatom dynamics are affected by diverse environmental factors, such as water temperature, salinity, light, nutrients, and water movement. Diatom viruses are considered to be important in diatom dynamics in nature. However, diatom and virus relationships have not been widely examined and thus are not well understood. In this study, we conducted field research to understand the relationship between the planktonic diatom *Chaetoceros tenuissimus* Meunier (Bacillariophyta, Centrales) and its viruses, single-stranded DNA virus (ssDNAV) and single-stranded RNA virus (ssRNAV), in Hiroshima Bay, Japan, from April 2010 to March 2015. Diatom cell number and viral abundance were determined by real-time PCR methods and the most probable number method, respectively. We also identified the dominant viral species. Blooms of *C. tenuissimus* were observed from May to September in every year, with the maximum reaching as high as ~10^4 cells mL^{-1}. Increases viral counts were observed during the host bloom periods, indicating that ecological relationships were present in those seasons. Most viral isolates during early summer were identified as ssRNAV, after which ssDNAVs were predominant during midsummer and autumn. These results suggest that seasonal temperature and salinity changes owing to rainfall affect viral infection and/or proliferation, resulting in increased viral abundance and succession of dominant viruses. Ecological environments might affect diatom population dynamics directly and indirectly through virus infections.

Keywords: algae, diatom, virus, ecology, population dynamics

Poster No. 106

Features of the peritrichous ciliates (Ciliophora, Peritrichia) spread in the river Uzh

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Peritrichous ciliates (Peritrichia Stein, 1859) take part in transform processes of organic substances in the freshwater reservoir biocenoses, in sustaining biological balance in the reservoirs and they are indicators of water reservoir sanitary hygienic state. The aim of the research – is to study species composition, the structure of domination of peritrichous ciliates, determine the water quality of the river Uzh for the dominant peritrichia species.

The peritrichia species composition in the river Uzh (the town Korosten') are researched. 18 peritrichia species are identified: *Epistylis chrysemydis* Bishop et Jahn, 1941, *E. plicatilis* Ehrenberg, 1831*, Campanella umbellaria* (Linnaeus,1758)*, Opercularia nutans* (Ehrenberg, 1838)*, Vorticella campanula* Ehrenberg, 1831*, V. convallaria* (Linnaeus,1758)*, V. microstoma* Ehrenberg,1830*, V. striata* Dujardin, 1841*, V. submicrostoma* Ghosh, 1922*, V. alba* Fromentel,1874*, V. banatica* Lepsi, 1935*, V. mayeri* Fauré-Fremiet,1920*, Carchesium batorligetiense* Stiller, 1935*, C. polypinum* (Linnaeus, 1758)*, Zoothamnium kentii* Grenfell,1884*, Z. parasiticum* Stein, 1859*, Vaginicola crystallina* (Ehrenberg, 1830) та *Platycola decumbens* (Ehrenberg, 1830).

In the results of the peritrichia species richness analysis in the river Uzh found increasing number of species (8-9) in the period from May to October, when conditions were the most favorable for their development. The genus *Vorticella* Linnaeus, 1767 is identified throughout the study period.

The peritrichia population density varied from 2,15 to 4,36 sp/sm^2 by seasons. Peritrichia of the *Epistylis* Ehrenberg, 1830 and *Vorticella* genuses were dominating in spring, summer and autumn. Population density of the *Epistylis* was 2,27, 2,49, 1,11 sp/sm^2 and *Vorticella* –1,93, 1,45, 0,93 sp/sm^2 in accordance. Only *Vorticella* species are founded in the winter (1,85 sp/sm^2).

In the results of the peritrichia domination structure analysis established 7 "basic" species: *Epistylis chrysemydis, E. plicatilis, Vorticella campanula, V. alba, V. striata, V. mayeri* and *V. convallaria.* They are indicators of the mezosaprobic zone.

The research results can be used for monitoring freshwater ecosystems in urban areas.

Keywords: peritrichous ciliates, Peritrichia, species richness, population density, "basic" species

Poster No. 107

Inter-annual differences in phytoplankton spring bloom community structure in a high Arctic fjord (Adventfjorden, Svalbard)

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We investigated the size and trophic structure of the annual planktonic protist community structure in the ice-free Adventfjorden in relation to environmental factors. Our high-resolution (weekly to monthly) study was conducted in a period of high inter-annual variability in Atlantic water advection to the fjord (January 2012 – October 2013). The study showed clear inter-annual differences in the spring community structure, most likely the response to various hydrographical conditions. In both years, the spring blooms, manifested by a sudden increase in the total protist abundance and biomass, took place in the second half of April, i.e., about a month earlier compared to the ice-covered West Spitsbergen fjords. Bacillariophyceae dominated the spring bloom when local water masses prevailed, while *Phaeocystis pouchetii* dominated the spring bloom when Atlantic water prevailed. In addition, the study showed that intrusions of Atlantic waters can strongly modify the quantitative composition of local communities in Adventfjorden, as evidenced by, *inter alia*, an order of magnitude greater protist abundance in the spring of 2012, corresponding with a substantially higher phototropic protist biomass (chlorophyll *a*) in surface waters adjacent to Isfjorden. In the light of the progressive increase of Atlantic water inflow to the Arctic, the shift from Bacillariophyceae-dominated to *P. pouchetii*-dominated spring bloom may become more frequent and can negatively affect the energy transfer efficiency in the marine food web.

This study was funded by the National Science Centre, Poland within the Let's Sea Project (2015/17/N/NZ8/01642).

Keywords: planktonic protists, seasonality, Adventfjorden, West Spitsbergen

Poster No. 108

Soil ciliated protist communities from agroecosystems and natural sites of Region Marche (Italy)

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In the framework of the *BioPrint Pilot Project* and for the first time in Italy, we have investigated the biodiversity and the community structure of soil ciliates from agroecosystems and natural sites of Marche Region. The aims of the study were: i) to evaluate the capacity of ciliates to discriminate between different types of land uses; ii) farming management practices; and iii) to assess relationships among ciliate community and abiotic parameters. Soil samples were collected twice from 10 sites (5 natural sites: FORest (virgin soils); and 5 agricultural fields: 3 ORGanic (minimum tillage) and 2 CONventional (sod seeding). Ciliate communities were studied by means of qualitative (*non-flooded Petri dish*) and quantitative methods. Soil chemical-physical (texture, CEC NPK, OM, C/N, soil moisture, temperature) parameters were also measured. Qualitative ciliate analysis allowed us to identify a total of 59 species representing 29 genera and 12 orders (plus 10 species new to science). ORG sites were the richest in species followed by CON and FOR. Multivariate analysis showed statistically significant differences between natural sites (FORest) and agricultural sites, as well as between the ORGanic and CONventional management farming systems. CCA analysis showed correlations between the distribution of species with environmental parameters indicating the importance of these parameters in shaping the ciliate communities in the different type sites. Altogether, these results showed the bioindicative potential of ciliate communities in discriminating between natural sites (FORests) and agroecosystems, as well as their capacity to discriminate, at least preliminary, between different soil management systems (ORG *vs* CON).

Keywords: soil ciliates, agro-ecosystems, soil health, organic farming, soil management practices

Poster No. 109

Cytotoxicity of single and bimetallic mixtures of heavy metals and antioxidant defenses in the soil ciliated protist *Rigidohymena tetracirrata*

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Ciliated protists are ubiquitous eukaryotic microorganisms which are well adapted for life in soil ecosystems. Ciliates exert a key role in soil microbial food webs by mediating the fluxes of nutrients and energy between different trophic levels. Further, they promptly sense and react to various soil perturbations and toxicants. All together, this made them valuable bioindicators of soil health. In this study, we analysed the toxicity (LC_{20} and LC_{50} at 24hrs) of single metal (Cd, Cu, and Zn) and bimetallic mixtures (Cd + Zn, Cd + Cu, and Cu + Zn) and, antioxidant responses in the soil ciliate *Rigidohymena tetracirrata*. The LC_{20} values for Cd, Cu, and Zn were 0.53, 0.22 and 23.0 mg l^{-1}, respectively; LC_{50} values were 1.16, 0.37 and 32.7 mg l^{-1}, respectively. The order of decreasing toxicity was Zn >> Cd > Cu. Analysis of the bimetallic mixture treatments using the Concentration Addition (CA) model, unveiled that the Cd + Zn mixtures were able to prevalently generate antagonistic effects as compared to the other mixtures (Cd + Cu, and Cu + Zn). Antioxidant activities were measured in (intra- and extra-) cellular extracts using different *in vitro* tests. The total phenolic contents (TPC) were significantly higher in extra- Cu LC_{20} ($p \leq 0.01$) and intra-cellular Cd LC_{20} extracts ($p \leq 0.001$). The intra- Zn LC_{50} and extra- cellular Cd LC_{50} extracts showed significantly higher α,α-diphenyl-β-picrylhydrazyl (DPPH) scavenging activities ($p \leq 0.05$). The extra- Cd LC_{50} and intra-cellular Cu LC_{50} extracts showed significantly higher hydroxyl radical scavenging (HRSA) activities (Cd LC_{50} $p \leq 0.001$; Cu LC_{50} $p \leq 0.001$). Overall, *R. tetracirrata* seem to have a good potential to be used as test organism in ecotoxicological analysis of soil contaminated with heavy metals.

Keywords: soil ciliates, heavy metals, cytotoxicity, bimetallic mixtures, antioxidant responses

Poster No. 110

Species composition and abundance of tintinnids (Ciliophora, Protista) at an anchored station in Garorim Bay (Yellow Sea), Korea

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Species composition and abundance of tintinnids were investigated at an anchored station in the Garorim Bay (Yellow Sea), Korea during spring tide and neap tide in August 2016. A total of 20 species (9 genera) of tintinnids were found (spring tide: 15 species from 6 genera, neap tide: 19 species from 9 genera): *Amphorellopsis acuta, Ascampbelliella urceolata, Codonellopsis ostenfeldi, Codonellopsis* sp.*, Eutintinnus lususundae, Favella ehrenbergii, Helicostomella longa, Stenosemella pacifica, Stenosemella parvicollis, Tintinnopsis baltica, Tintinnopsis beroidea, Tintinnopsis campanula, Tintinnopsis cylindrical, Tintinnopsis dadayi, Tintinnopsis nordqvisti, Tintinnopsis parvula, Tintinnopsis radix, Tintinnopsis tocantinensis, Tintinnopsis uruguayensis, Leprotintinnus* sp. The abundances of tintinnids during the spring tide and neap tide varied 255-1,175 (avg. 722) cells/l and 2,110-3,635 (avg. 2,738) cells/l, respectively. On a spring tide, the abundance had a strong positive relationship with tidal heights, but had a weak relationship on a neap tide. Also the abundance had a positive relationship with chl-a concentrations. The dominant species were *Amphorellopsis acuta* and *Tintinnopsis tocantinensis. Amphorellopsis acuta* is known to be an oceanic species. This species occupied about 25% of the total abundance during the spring tide and about 84% during the neap tide, while *Tintinnopsis tocantinensis* occupied 46% and 6%, respectively. This study suggests that the water mixing process may affect the species composition and the temporal distribution pattern of tintinnids during the study, and *A. acuta* may be imported from outer bay by the Yellow Sea Warm Current, which is a branch of the Kuroshio Current.

Keywords: Tintinnid, Ciliophora, Garorim Bay, *Tintinnopsis tocantinensis*, *Amphorellopsis acuta*

Poster No. 111

Detection of mixotrophic behaviour within aquatic photosynthetic picoeukaryotes

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There is increasing evidence that many aquatic organisms are not strictly heterotrophic or autotrophic but rather mixotrophic. Mixotrophy is an intermediate nutritional strategy, merging autotrophy and heterotrophy to acquire organic carbon and/or other elements. It has been demonstrated that mixotrophic algae, in particular those <5 µm in size, can be major grazers of bacterioplankton in oligotrophic marine systems, being regularly responsible for 50% of the total bacterivory. These findings fundamentally change our view of open ocean food web functioning. However the estimation of the functional importance of mixotrophs in natural ecosystems is made delicate, on one hand, because of the difficulty identifying these organisms among the photosynthetic eukaryotes and, on the other hand, because their relative abundance is very low compared with the other elements of the planktonic community (picocyanobacteria, bacteria, ciliates and heterotrophic flagellates). Moreover, little is known on the specific taxonomic classes capable of mixotrophy as well as the environmental conditions favoring this behavior especially in freshwater lake ecosystems. We are therefore developing different approaches to detect mixotrophic picoeukaryotes in lakes. We performed short term grazing experiments coupled with TSA-FISH (tyramide signal amplification - fluorescent *in situ* hybridization) on natural populations. Haptophytes, chlorophytes, cryptophytes and chrysophytes which are the main components of the photosynthetic populations have been targeted by specific probes. Moreover, we used flow cytometry sorting coupled with Lysotracker staining to target mixotrophic populations.

Poster No. 112

Systematic studies on ciliates (Alveolata, Ciliophora): progress and achievements based on molecular information

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Due to the complex morphological and convergent morphogenetic characters, the systematics of ciliates has long been ambiguous for several groups. Since 1990, the research group in the Laboratory of Protozoology, Ocean University of China, in collaboration with a number of other research groups worldwide, has carried out a series of integrative investigations on ciliate systematics, based mainly on phylogenetic analyses of gene sequence data along with considerations of morphological and morphogenetic characters. To date, genomic DNA has been extracted from more than 1700 ciliate species/strains, and several marker genes have been sequenced. Phylogenetic analyses have been performed for about two thirds of ciliate orders, based on gene sequence data along with considerations of morphological and morphogenetic characters. Here, we summarize the main findings of our phylogenetic studies for five main ciliate groups: hypotrichs, euplotids, scuticociliates, oligotrichs and peritrichs. 1) Phylogenetic classifications of about 50 species of hypotrichs have been resolved, although the monophylies of three orders remain unconfirmed; 2) the subclass Euplotia and orders Euplotida and Discocephalida are all monophyletic, and all seven families are well supported monophyletic assemblages; 3) *Lynnella* represents an order-level taxon that is separated from the subclasses Oligotrichia and Choreotrichia, both of which are strongly supported monophylies and are sister to each other; 4) Separation of the peritrich families Zoothamniidae and Vorticellidae is supported and *Zoothamnium* exhibits a higher genetic diversity than suggested by the gross morphology of its species; 5) The scuticociliate order Philasterida is monophyletic with the separation of loxocephalids from Philasterida being supported, and the thigmotrichids should be regarded as a suborder within Pleuronematida; 6) In an overview of high taxon-rank classification of the ciliates, 14 classes were recovered including one new class, Protocruziea, and Mesodiniea is basal to the well-supported subphyla Intramacronucleata and Postciliodesmatophora.

Keywords: ciliate, molecular systematics, phylogeny, multi-gene

Poster No. 113

The sanguicolous apostome *Metacollinia luciensis* (Colliniidae, Apostomatia) is not closely related to other sanguicolous apostomes

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The family Colliniidae includes apostomes that are adapted to the haemocoel/blood of various invertebrates, particularly crustaceans. Our research on colliniids parasitizing the haemocoel of euphasiids and planktonic amphipods has lead to recognition of the family Pseudcolliniidae to include the new genera *Pseudocollinia* and *Fusiforma*, which are morphologically separated from "true" colliniids (Chantangsi et al. 2013. Protist 164:793). However, we have as yet no genetic data on “true” colliniids.

To explore further the phylogeny of sanguicolous apostomes, *Metacollinia luciensis*, identified at Roscoff by de Puytorac & Grain (1975. Protistologica 11:61) as *Collinia orchestiae*, was recollected in August 2015 at Roscoff from the same amphipod host *Orchestia gammarella*. Five amphipods had infestations of *M. luciensis.* The apostomes were transferred by finely drawn Pasteur pipettes to 0.22-µm filtered sea water, then to microfuge tubes, pelleted, and preserved in >80% ethanol.

Ciliates were Protargol stained and DNA was extracted. The small subunit rRNA (SSUrRNA) and cytochrome c oxidase subunit I (*cox*1) genes were amplified. Morphologically these isolates were identical to those described by de Puytorac & Grain (op. cit.). We therefore conclude that they are conspecific with the 1975 populations.

Molecular phylogenetic analyses of SSUrRNA genes unambiguously grouped *M. luciensis* with other apostomes with robust bootstrap support, but separated it distinctly from the pseudocolliniid clade. While there are only *cox*1 sequences for a subset of these apostomes, *M. luciensis* was also distant from the pseudocolliniids and separated from them by *Hyalophysa* spp. These results confirm the distinctness of the families Colliniidae and Pseudocolliniidae. We are now aiming to find the sanguicolous colliniids *Collinia circulans* and *Paracollinia branchiarum*, which infest the freshwater amphipod *Asellus aquaticus* and the freshwater gammarid *Gammarus pulex* respectively, to test the monophyly of the family Colliniidae.

Supported by Natural Sciences and Engineering Research Council of Canada.

Keywords: apostome, *Collinia*, phylogeny, small subunit rRNA, ciliate

Poster No. 114

Assessing how temperature affects growth rate: a mechanistic approach using the model ciliate *Tetrahymena*

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Ambient temperature is possibly the most important abiotic factor affecting the specific growth rate of free-living protists. Providing robust, predictive models for the response of growth rate to temperature is, therefore, important for studies ranging from environmental issues to industrial applications. Numerous studies have investigated this phenomenon, and several have provided predictive functions. Generally, the underlying mechanism associated with such functions assumes they are driven by the thermodynamics of a single enzyme, which may be inhibited at low and high temperatures. Here we explore the veracity of this assumption, by fitting a range of plausible mechanistic functions to thermal growth responses. Specifically, to test the appropriateness of models we have collected data on the thermal responses of several species of the model ciliate *Tetrahymena*, which collectively cover a wide range of temperature. Our data include not only the influence of temperature on growth rate but also on cell size, allowing us to assess "production" (growth rate x cell size). We then explore several models, including those associated with single and multiple enzyme and non-enzyme driven mechanisms, and determine which best fits the data. To do so, we apply Akaike Information Criterion (AICc). Ultimately, our goal using this model system, is to establish a general, mechanistic function for predicting protistan responses to temperature.

Keywords: temperature, growth rate, model

Poster No. 115

Two-decade changes of the ciliate assemblage in the temperate Slapy reservoir (Czech Republic)

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Twenty three-year (1994–2016) data on the ciliate assemblage in the Slapy reservoir (Vltava River, Czech Republic) were analysed to provide a detailed case study of the combined effects of concurrent climate change and eutrophication. Comparing to the other reservoirs, Slapy exhibits longer spring overturn, quick onset of stratification and the stable epilimnion, which water residence time is significantly longer compared to the reservoir retention time (~30 days) producing reduced nutrient availability during the entire vegetation season.

Ciliates were identified and the biomass evaluated via using Quantitative Protargol Stain in the regular three-week samples, Acid Lugol-fixed and Bouin postfixed. Due to a high variation in the species composition, the data were first analysed using an ecological – feeding behaviour attempt, followed by species/genus analysis. The fine filter-feeders (bacterivorous and omnivorous picoplankton feeders) frequently dominated the assemblages during the first decade of the study. However, the maximum ciliate peaks reflected mostly biomasses of the other groups: mixotrophic coarse filter-feeders (mainly oligotrichs and choreotrichs), algivorous ciliates, tintinnids, or even *Stentor* sp.

During the last decade of study, several trends are observed. Within the fine filter-feeders, *Halteria* spp., *Pelagohalteria viridis* and minute *Rimostrombidium* spp. were the most important, followed by peritrichs, which dominate the group only occasionally during last 5 years. The mixotrophic coarse filter-feeders are becoming more important; *Pelagostrombidium* spp. dominate over *Limnostrombidium* spp. while *Rimostrombidium velox* appears only occasionally. Within algivorous ciliates, prostomes *Urotricha* spp. and *Balanion planctonicum* were alternating the dominant position but now, much larger *Histiobalantium* sp. is replacing them more frequently. *Askenasia*, *Mesodinium* and *Lagynophrya* have been typical raptorial (possible flagellate hunters) genera in the reservoir since the start of the study.

Untill now, significant biological interactions were not statistically proven; changes in the limnological regime of the reservoir seemingly control the ciliate assemblage much more than previously supposed.

Keywords: ciliates, feeding behaviour, biomass, long-term, water reservoir

Poster No. 116

Delimitation of functional traits and intra-specific variability of test morphology in freshwater Arcellinida: the 'ECOTRAIT' Project

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Arcellinida (testate lobose amoebae), a group of freshwater benthic protists, are sensitive indicators of water quality change. Their ability to construct robust tests (e.g. shells) that preserve well in lake sediments makes Arcellinida a valuable proxy in palaeolimnological studies. Genetic analyses have demonstrated that test morphology is not always a reliable indicator of genotype, underlining the need to better understand the extent to which Arcellinida are able to develop ecophenotypical responses to environmental pressures.

Recent studies of peatland testate amoebae confirmed that functional traits - interspecific morphological features that vary with environmental change - can be used to reconstruct hydrological change independently of species data, thus circumventing potential taxonomic issues. Minimal traits-based work has been undertaken with lake Arcellinida, which typically consist of larger tests and show a greater range of test variability. We describe the 'ECOTRAIT' project, an EU funded project which aims to examine the character and causes of functional trait variability in modern and palaeolimnological settings, develop novel biometric approaches to aid in trait delimitation and to apply genetic sequencing techniques to quantify to what extent variations in test morphology are a result of genotype versus phenotypic plasticity.

We present preliminary results two lake sediment cores: Bodham Rail Pit, Norfolk, a shallow eutrophic pond characterised by episodes of *Lemna* development; and Loch Leven, Scotland, a large shallow lake characterised by a historical water level drop and increasing eutrophication. We use geometric morphometrics to visualise and quantify morphological variability in Arcellinida tests. Our results demonstrate that test size and relative aperture width co-vary with proxy-inferred environmental changes, underlining the potential applicability of functional trait-based approaches. The presence of a large degree of intra-specific variability in test morphology in both cores potentially represents an important and overlooked response to environment change.

Keywords: Arcellinida, plasticity, morphology, ecophenotype, functional traits

Poster No. 117

Soil ciliate (Protozoa; Ciliophora) diversity in and around Delhi, India and its ecological implications

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Soil is a mixture of minerals, water, air, organic matter and countless organisms inhabiting and utilizing this elaborate resource. The soil community consists of bacteria, fungi, protozoa and nematodes. Larger fauna increase the rate and amount of mineralization in soil and add nutrients for the plant growth. Protozoa are bacterial grazers and enhance nutrient recycling, increases the amount of nutrients and making them more available to plants. Soil ciliates are integral part of the soil community but the information on them is still rudimentary particularly from India. Biological components of the soil can vary from one site to another depending upon the organic and inorganic make-up of the soil. In the present study, the ciliate diversity was investigated from different habitats in and around Delhi Region, India, i.e. from human inhabitant land (Acharya Narendra Dev College campus, IP Park, Govindpuri petrol pump), agricultural land (Mahendergarh, Baghpat, Bhiwani), sewage treatment plant (Rithala) and dump yards (Karnal, Okhla and Ghazipur). The differences in the composition of the soil samples were compared by physico-chemical analysis of soil samples. Samples were analysed for pH, nitrogen content, organic carbon content using standard procedures. Ciliate abundance was correlated with physico-chemical properties. In total, ciliates belonging to 5 classes, 7 orders, 15 genera and 20 species were found with maximum number of ciliates belonging to class Spirotrichea. Maximum ciliate diversity was found in ANDC and Rithala sewage treatment plant whereas ciliate diversity was least in Mahendargarh and Bhiwani. *Spathidium* sp. was specifically present in the Govindpuri petrol pump site. *Colpoda* were present in the entire sites examined but most abundant in sewage site.

Keywords: ciliates, diversity, physio-chemical, soil, Spirotrichea

Poster No. 118

The use of urea and glycine as C and N substrates by dinoflagellates

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Urea and free amino acids are generally the most available dissolved organic compounds in coastal waters. Many phototrophic dinoflagellates can use them as nitrogen source, often in preference to inorganic nitrate. It is less clear whether these microorganisms also consume carbon originating from organic compounds. Using stable isotope tracers and isotope ratio mass spectrometry, we investigated concurrent uptake of nitrate and urea or glycine by cultures of dinoflagellates *Prorocentrum minimum* and inspected both organic nitrogen and carbon use by these organisms. In our experiments, urea and glycine uptakes significantly exceeded the concurrent uptake of nitrate. Both urea and glycine were predominantly used as nitrogen sources. Urea-C and glycine-C consumption was 12–20 times lower than the theoretically predicted (according to the structure of urea and glycine molecules) and was less than 1-2 % of the inorganic carbon uptake. Using bioinformatical approach, we identified putative proteins involved in urea and glycine metabolism in the transcriptome of *P. minimum* from MMETSP database. The observed uncoupling of organic nitrogen and carbon utilization can be explained by metabolic processing of urea and glycine in a cell. We argue that such uncoupling may decrease the net efficiency of inorganic carbon uptake fueling the process of photosynthesis, since feeding on urea and glycine as N sources leads to inorganic carbon release.

Funded by the Russian Science Foundation, project 16-14-10116.

Keywords: dinoflagellates, ecophysiology, nutrition, *Prorocentrum minimum*

Poster No. 119

Cryptic species diversity in *Paramecium* (Ciliophora): more search, more find

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Paramecium (Ciliophora) is one of the most studied genus among ciliates, and it is divided into five subgenera. The subgenus *Cypriostomum* comprises four morphological species (*P. calkinsi*, *P. nephridiatum*, *P. polycarium*, and *P. woodruffi*), which are rather difficult to identify and remain poorly investigated.

We analyzed 57 strains belonging to this group of species, which were collected from different regions all over Eurasia. All strains were grouped according to morphological features (cell morphometry, type, number and location of micronuclei, type and number of pores of contractile vacuoles), and their phylogenetic relations were inferred from 18S rDNA and COI gene sequences.

Two species (*P. nephridiatum* and *P. polycarium*) were easily recognizable by morphological criteria, while it was not possible to discriminate between *P. calkinsi* and *P. woodruffi*. However, we managed to determine several morphological groups fitting existing descriptions of those species. Furthermore, these groups were also confirmed by molecular phylogeny. Thus, the status of *P. woodruffi* is debatable and *P. calkinsi* should be redescribed.

According to our morphological observations, we found two peculiar groups of strains: one was characterized by a peculiar novel for *Paramecium* type of micronucleus. In the other group general characteristics of *P. calkinsi* were combined with the micronucleus typical for *P. polycarium*. Dendrogram shows that these strains constitute also separate phylogenetic groups, and we suppose that they may represent two novel species of *Paramecium*.

Supported by RFBR 16-04-01195. Sequencing was performed in the RRC "Molecular and Cell Technologies", St Petersburg State University.

Keywords: cryptic species, *Paramecium* biodiversity, new morphospecies, molecular analysis, *Cypriostomum*

Poster No. 120

Environmental drivers to annual variation of protozoan communities in a sub-tropic urban wetland ecosystem, southern China

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With ease of collection, short life cycles, and rapid response to environmental changes, increasing attention is being focused on protozoa as favorable indicators for bioassessment. To reveal the environmental drivers to annual variation of protozoan communities in a sub-tropic urban wetland, their species composition, abundance, diversity, and their correlations with abiotic factors were studied in Xixi wetland, Hangzhou, China. A total of 89 protozoan species comprising 34 ciliates, 13 flagellates, and 42 rhizopods, 7 of these were dominant species. The protozoan abundance range from 3×10^4 ind. l^{-1} to 19.65×10^4 ind. l^{-1}, ciliates (69.3%) were the most primary contributors in terms of relative abundance. The cluster analysis discriminated the annual protozoan communities into three stages of spring, summer-autumn and winter at a 30% similarity level with a significant difference. Multivariate correlation analysis showed that temporal variation in protozoan communities was significantly related to the changes of environmental variables, especially water temperature, dissolved oxygen, chemical oxygen demand (COD) and nutrients. All three diversity (species richness, diversity and evenness) indices were significantly correlated with the COD and nutrients. The results demonstrated that the annual variation in protozoan abundance represented a clear seasonal shift in response to environmental changes and thus may be used as a potential indicator for assessing water quality in a sub-tropic urban wetland ecosystem.

Keywords: annual variation, protozoan communities, urban wetland, water quality

Poster No. 121

What can ciliates tell us about looking good, getting drunk, and vaping?

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To ensure human and environmental safety, developed nations now mandate the evaluation of many substances. To this end, standard acute toxicity tests exist, using animal models. Animal testing, however, is both expensive and morally questionable. Ciliates are single celled whole-organisms that exhibit animal-like traits in behaviour, physiology, and biochemistry and share a substantial amount of their genome with vertebrates. Thus, ciliates can act as a good replacement for animal models, especially as: 1) clonal cultures are simple to establish and maintain; 2) axenic cultures can be established; 3) stock cultures can be purchased from a range of sources or isolated locally; 4) culturing is inexpensive, requiring virtually no space and little effort; and 5) doubling times are short, and thus tests are rapid (~48 h) and can provide multiple replicates. We provide three examples of tests that we have conducted using ciliates, and in doing so argue that further work in this research area is both timely and profitable. Specifically, we will present work that assesses the toxic effect of mascara, whiskeys, and e-cigarettes.

Poster No. 122

Planktonic foraminifera-derived environmental DNA extracted from abyssal sediments preserves patterns of plankton macroecology

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Deep-sea sediments constitute a unique archive of ocean change, fueled by a permanent rain of mineral and organic remains from the surface ocean. Until now, paleo-ecological analyses of this archive have been mostly based on information from taxa leaving fossils. In theory, environmental DNA (eDNA) in the sediment has the potential to provide information on non-fossilized taxa, allowing more comprehensive interpretations of the fossil record. Yet, the process controlling the transport and deposition of eDNA onto the sediment and the extent to which it preserves the features of past oceanic biota remains unknown. Planktonic foraminifera are the ideal taxa to allow an assessment of the eDNA signal modification during deposition because their fossils are well preserved in the sediment and their morphological taxonomy is documented by DNA barcodes. Specifically, we re-analyze foraminiferal-specific metabarcodes from 31 deep-sea sediment samples, which were shown to contain a small fraction of sequences from planktonic foraminifera. We confirm that the largest portion of the metabarcode originates from benthic bottom-dwelling foraminifera, representing the in-situ community, but a small portion (< 10%) of the metabarcodes can be unambiguously assigned to planktonic taxa. These organisms live exclusively in the surface ocean and the recovered barcodes thus represent an allochthonous component deposited with the rain of organic remains from the surface ocean. We show that planktonic foraminifera DNA is preserved in a range of marine sediment types, the composition of the recovered eDNA metabarcode is replicable and that both the similarity structure and the diversity pattern are preserved. If these observations apply to the rest of the pelagic community, it would pave the way for surveys of seafloor sedimentary eDNA covering the entire spectrum of pelagic biodiversity and its interaction with the climatic history of the oceans.

Keywords: planktonic foraminifera, eDNA, metabarcoding, DNA preservation

Poster No. 123

Phytomyxids – diverse, abundant parasites of plants and algae at sea and on land

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Phytomyxids (Rhizaria, Endomyxa) are largely elusive group of microbial pathogens of a wide range of hosts and only a couple of species are well studied (e.g. clubroot of brassicas, powdery scab of potato). So far it is estimated, that there are 30–40 species of phytomyxids – with DNA-data available for 10 species only. Using a targeted approach, we could identify more than 120 new lineages within all major groups of the Phytomyxea, suggesting that the number of species is considerably higher. A large novel clade basal to *Spongospora nasturtii* was identified in samples form fresh water habitats predominantly inhabited by aquatic plants, mosses and liverworts. This clade is highly diverse and contains at least 7 new clades with more than 30 new 18S-rDNA lineages. However, the majority of the newly found lineages is marine, with at least 14 new clades and 61 18S-rDNA lineages. This indicates that the previously described 9 marine species largely underestimate phytomyxid diversity in the sea. Two new species parasitic on brown algae could be found. One is a new species of *Maullinia* which is parasitic on the bull kelp *Durvillea antarctica*, while the second new species is a parasite of filamentous brown algae, which morphologically fits the description of Karlings *Phagomyxa algarum*. The latter belongs to a very diverse clade accounting for approximately half of the above mentioned marine diversity and lineages belonging to this clade were found in samples with worldwide origin. Understanding these new species and identification of the yet undescribed diversity within phytomyxids will not only increase our understanding about the biodiversity of the group, but will also impact on a number of important research areas such as seagrass ecosystems and algal aquaculture.

Poster No. 124

The toxic effects of nano-TiO2 on the ultrastructure of *Euplotes eurystomus* (Ciliophora, Hypotrichida)

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In order to explore the influence of nano-TiO_2 on the ultrastructure of ciliate *Euplotes eurystomus*, this study used transmission electron microscope to detect and compare the changes of cellular ultrastructure after treating with two sizes of nano-TiO_2 (5nm, 25nm). The results demonstrated that the nano-TiO_2 can perforate the nuclear membrane and mitochondrial membrane. It can also cause a series of changes on cellular ultrastructure such as partial subpellicular microtubule layer deficiency, cytoplasm vacuolation, nucleolus reduction, chromatin gathering and condensation, mitochondrial cristae breakage and disappearance, mitochondria disintegration and so on. Besides, we found the 5nm nano-TiO_2 has made a more serious damage on the cellular structure than 25nm nano-TiO_2.

This work was supported by grant (No. 31672249; 31572223) from The National Natural Science Foundation of China.

Keywords: nano-TiO2, *Euplotes eurystomus*, ultrastructure

Poster No. 125

Morphology, morphogenesis, and molecular phylogeny of a soil ciliate, *Gonostomum kuehnelti* Foissner, 1987 (Ciliophora, Hypotrichia), from Northwest China

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The morphology, morphogenesis of a soil hypotrichous ciliate *Gonostomum kuehnelti* Foissner, 1987, isolated from Northwest China, was investigated based on live observations and protargol staining. Photomicrographs and detailed morphological description of living cells, cortical granules and the number of dorsal kineties were provided in this study. Our form resembles the original population in terms of their live characters and ciliary patterns. Morphogenesis of *G. kuehnelti* corresponds well with that of the type population. The main events during binary fission are as follows: (1) parental adoral zone of membranelles retained completely; (2) the six streaks of the undulating membrane and cirral anlagen are segmented in a 1: 2: 2: 2: 4: 4 pattern from left to right, and form three frontal, two frontoventral, one buccal, two frontoterminal, three postoral ventral, two pretransverse ventral, and transverse cirri, respectively; (3) marginal rows develop intrakinetally; and (4) dorsal kineties originate by intrakinetal proliferation in the parental structures. In addition, the SSU rDNA sequence of *G. kuehnelti* was first provided in this work. Phylogenetic analyses based on SSU rDNA sequences showed that the genus *Gonostomum* is nonmonophyletic. *G. kuehnelti* is closely related to *G. strenuum* and three congeners.

This work was supported by the National Natural Science Foundation of China (project numbers: 41361055, 31522051, 31430077, 31401963), the International Research Group Program (IRG14-22), Deanship of Scientific Research at King Saud University for funding this work through Research Group (RGP-242) and the Young Teachers' Promotion Program at Northwest Normal University (NWNU-LKQN-16-11). Many thanks are given to Professor Weibo Song, OUC and to Mr. Yongqiang Yang and Mr. Guanhong Wan for collecting the samples.

Keywords: gonostomatids, hypotrichs, ontogeny, SSU rDNA, taxonomy

Poster No. 126

Influence of light on locomotion of *Amoeba proteus*

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Sunlight is one of the most important factors to live for almost all species. In addition to energy sources, the sunlight gives organisms information about environment, so that they decide their behaviors and survival strategies to adapt to drastically changing environment. These responses to light have been classified into three behaviors, phototaxis, photophobic responses and photokinesis. The phototaxis is defined by the movement forward or backward to the light source. Photophobic responses are observed upon a sudden increase or decrease of light intensity. Dependence of the moving speed on the constant photo-irradiation is called photokinesis. These three reactions are crucial in behavior decision of living creatures including non-photosynthetic organisms. A large free-living amoeba, *Amoeba proteus*, has been used as a model system of amoeboid locomotion for a long time. Though several reports about phototaxis and photophobic responses of *A. proteus* have been published, photokinesis has still been unclear. Then we investigated the behavior of *A. proteus* on the constant photo-irradiation.

Without light stimulation *A. proteus* exhibited two modes of movement, which were termed active and static modes. When blue light was illuminated on the cells, the cells with the static mode changed their motions into the active mode. It is consistent with the fact that movement of the static mode had not been reported in normal conditions because of using white light as observation light in ordinary past-experiments as far as we know. Then we tried to describe quantitatively differences between the active and static movements using statistical methods. As a result, the active mode of *A. proteus* was described as the similar characters to the amoeboid locomotion of other types of cells. On the other hand, oscillations of trajectories were represented in the static mode. This study contributes to progresses of the ethology of *A. proteus*.

Keywords: amoeboid movement, *Amoeba proteus*, photokinesis

Poster No. 127

Morphology and phylogenetic analyses of three novel *Naegleria* isolated from freshwaters on Jeju Island, Korea, during the winter period

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The genus *Naegleria* is one of the best known heterolobosean groups, and is the causative agent of primary amoebic meningoencephalitis. This group is rarely studied in temperate regions during winter. Here, three novel *Naegleria* were isolated from freshwaters on Jeju Island, Korea, during winter. Two isolates were amoeboflagellates, and one isolate was an amoeba. All amoebae had eruptive pseudopodia, and the layer of refractile granules around a large nucleus. They formed a cyst with ~2 pores in the cyst stage. The amoeboflagellate form had two flagella and no division in the flagellate stage, and no cytostome. These features are very similar to typical *Naegleria*. Furthermore, our isolates were able to grow at > 30 ^{o}C, suggesting that they had different thermophilicity from *Naegleria* in polar regions. Based on the 18S rRNA gene and the ITS1-5.8S rRNA gene-ITS2 sequences, the phylogenetic analyses consistently revealed that the isolates are members of the *Naegleria* group. However, the isolates differ from other species in both phylogenetic trees. Thus, *Naegleria* in cold habitats appeared to have a high degree of novelty, but their thermophilicity may be dependent on locality.

Keywords: cold habitats, freshwater, Heterolobosea, molecular phylogeny, *Naegleria*

Poster No. 128

Plastid & mitochondrial genomes of *Minerva aenigmata*, an early diverged species of Bangiales (Rhodophyta)

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Minerva aenigmata is a species in the Bangiales that has unbranched, erect thalli, initially forming uniseriate filaments that become multiseriate at maturity. This simple filamentous structure is morphologically indistinguishable from other filamentous Bangiales taxa. *M. aenigmata* is known as the earliest diverged species of Bangiales that is distributed in the upper intertidal zone along the New Zealand coast. Currently, one *Bangia*, two *Porphyra*, and six *Pyropia* plastid genomes are available, however, these taxa all belong to the stem group of the Bangiales. To better understand organelle genome evolution, here we sequenced and annotated the complete plastid and mitochondrial genomes of the early diverged *M. aenigmata*. The plastid genome length is 189.5 kbp with 32.3% GC content. The circular plastid genome contains 247 genes including 203 protein-coding genes, 6 rRNAs and 38 tRNAs. The mitochondrial genome is 29.7 kbp in size with 30.8% of GC that encoded 21 protein-coding genes. Comparative analyses of organelle genomes show highly conserved genome structures including genome sizes, gene contents, gene orders and intron regions both in plastid and mitochondrial genomes. In this presentation, we will discuss possible evolutionary scenarios for the high conservation of organelle genomes of the Bangiophyceae.

Keywords: Rhodophyta, organelle genome, Bangiophyceae, *Minerva aenigmata*

Poster No. 129

Pelagibaca bermudensis promotes growth and lipid productivity of *Tetraselmis striata* in broad range of stressors in addition to the release of HHQ

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Pelagibaca bermudensis and potential biofuel strain *Tetraselmis striata* co-cultivated under varying pH, salinity, temperature and light conditions, and it compared with the *Tetraselmis striata* cultivated under axenic conditions. In all of the experimental sets, biomass and lipid productivities of the co-cultivated conditions were higher than the axenic conditions. The cell abundance were several magnitudes higher under optimal condition in presence of *P. bermudensis*. An independent investigation of the effect of crude extracts containing metabolite and nutrients released by the *P. bermudensis* on the microalgal cells exhibited the elevated biomass and higher growth. However, it leads to slight decrement of lipid content but gave clues for the enhanced survival of microalgal cells in presence of the *P. bermudensis*, which could release metabolites in broad spectrum of environmental stressors. This co-cultivation strategy can be used for the amelioration of *T. striata* biomass production in the saline effluents/ seawater based media with broad pH range and with varying temperature/light conditions. We also studied the bacterial growth dynamics in presence of this microalga and results showed unique growth trends in each case. The bacteria were found to release quorum sensing precursor HHQ in most of the varying environmental conditions which could not hamper the growth promoting effect to the microalgae and could lead to harmonious typical growth curve for the survival. Although, most of other quorum sensing molecules were found to be absent. *P. bermudensis* could not grow in the absence of *T. striata* in microalgal growth media (O3).

Keywords: co-cultivation, stressors, biomass, lipid, quorum sensing precursor

Poster No. 130

New records of heterolobose amoeba *Willaertia* sp. in water bodies of Ukraine

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Heterolobosean amoebae are a group of amoeboid protists which according to current system of eukaryotes belongs to the super-group Excavata Cavalier-Smith, 2002 (Adl et al. 2005), and includes 140 described species (Pánek et al. 2012). Heterolobosea are found in a broad spectrum of habitats. The representatives of this class are distributed in marine and freshwater ecosystems, and soils. The class includes some strictly marine or freshwater species. There are free-living and parasitic forms. Regardless of ecological and morphological diversity of known heterolobosean amoebae, most of them are still poorly studied or not described. Studying amoebian fauna of Ukrainian water bodies of different kinds we continuously register both new species and new habitats of these protists (Patsyuk 2014, 2015).

Genus *Willaertia* De Jonckheere, Dive, Pussard et Vickerman, 1984 – unicellular vahlkampfiids, with amoeboid stage and cysts with a lot of pores, uninuclear. The flagellates are with four anterior flagella, 19–20 µm in length without cytostome (Lee et al. 2000). Amoebae of this genus are of the eruptive morphotype. We have found only one amoeba of the genus *Willaertia*, but the data on its morphology is insufficient to identification to species level. Thus the found amoeba is identified as *Willaertia* sp.

Willaertia sp. move with wave-like eruptive (intermittent) crescent projections of the frontal part of the cell. The uroid is convex, with very thin threads, divided from the main part of the cell. The cell is elongated. The amoeba length is 100–120 µm, width 30–40 µm, L/B ratio is 3–4.5. The nucleus is singular, located in the centre of cytoplasm, 12–22 µm in diameter. Cyst formation and flagellate stage were not observed in culture. Habitat locations: we found the species in Teteriv river, in Zhytomyr city. New habitats of the species are Horyn' river (Ternopil and Rivne regions of Ukraine).

Keywords: heterolobose amoebae, morphotype, Ukrainian water bodies

Poster No. 131

Influence of different nitrogen sources on morphological and physiological parameters of dinoflagellates *Prorocentrum minimum* growing in nitrogen-limited continuous cultures

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Dinoflagellates are able to assimilate diverse dissolved organic and inorganic compounds as nitrogen substrates, which gives them a competitive advantage in eutrophied habitats. Our aim was to examine the impact of various nitrogen sources on morphological and physiological parameters of the *Prorocentrum minimum* cells. *P. minimum* cultures were grown in chemostats under nitrogen-limiting conditions with the addition of equal amounts of nitrate, ammonium, urea or glycine as nitrogen sources. After reaching a steady-state, we collected samples to analyze cell shape and size, nitrogen and bicarbonate uptake, natural fluorescence, and RNA synthesis rate in *P. minimum* using diverse techniques, such as fluorescent microscopy, stable and radioactive isotope tracers, liquid scintillation counting, and isotope ratio mass spectrometry. The analysis of cell size and natural fluorescence of cellular photosynthetic pigments revealed no significant differences between cultures grown on different N sources, but demonstrated size heterogeneity among distinct cells within the same culture. The analysis of the nitrogen and carbon uptake rates, as well as the rate of H^3-uridine incorporation, showed that physiological activity differed in cells growing on various N sources. For example, a very high rate of RNA synthesis was observed in urea-grown cultures as compared to the cultures growing on other nutrients. The knowledge about physiological and morphological response of dinoflagellates to their N diet is very important in the light of ongoing eutrophication of coastal regions.

Funded in part by RFBR, project 15-29-02706.

Keywords: dinoflagellate, nitrogen metabolism, chemostats

Poster No. 132

Improving the taxonomic sampling of excavates to solve the root of the eukaryotic tree

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If most of the eukaryotic superphyla are commonly accepted as monophyletic, the relationships between them remain mostly unresolved, particularly the root of the eukaryotic tree, defining the first bifurcation among these superphyla. Nowadays, two main hypotheses are debated, the Unikont/Bikont hypothesis (UB), which places the root between the Unikonts, including Metazoa, Fungi, the amoebozoans and some protist lineages, and the bikonts, i.e. all the other lineages, including the Archaeplastida and a large diversity of unicellular eukaryotes. The second hypothesis, Neozoan/Excavate (NE), proposes the root to be between the excavates, a very diverse group of protists, and all the other eukaryotes. Some excavates possess unusual cellular and genomic features absent in other eukaryotes, like a large and bacterial-like mitochondrial genome (jakobids) or a unique complex flagellar apparatus found in many excavates. Each of these hypotheses has major implications for the nature of the last eukaryotic common ancestor (LECA), and for the early evolution of eukaryotes. In the case of the UB hypothesis, LECA would have been a complex organism, most of the modern eukaryotic features already having been acquired before LECA. In the NE hypothesis, the specific features of excavates could be ancestrally present in LECA, when most of the evolution leading to the common features of the other groups would have been acquired after LECA, and the excavates would have a unique evolutionary history. To solve this fundamental evolutionary question, we need to improve the phylogeny of eukaryotes, in particular a broader taxonomic range of genome sequencing of excavates, which is currently dominated by fast-evolving parasite genomes. Here we present a phylogeny of excavates including *Diplonema papillatum* and a preliminary analysis of the first genome of this newly sequenced free-living excavate. Our initial results demonstrate the extreme genetic diversity of the excavates within the free-living as well as parasitic lineages.

Keywords: eukaryotes, phylogeny, eukaryotic root, *Diplonema papillatum*, excavates

Poster No. 133

Comprehensive assessment of protistan diversity in deeply continental saline water bodies with metagenomic and cultural approaches

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Despite saline lakes are widespread in arid and subarid areas of Eurasia, their microbial diversity has not been investigated yet appropriately in contrast to freshwater, marine and ocean sites. Only few metagenomic studies have investigated eukaryotic communities in such water bodies, whereas more articles describe known and new taxa of heterotrophic protists there. Thus understudying of eukaryotic communities in the saline waters is clear well. Modern methods of high-throughput metagenomic sequencing, based on detection of the 18S gene fragment, give the opportunity to study protistan biodiversity thoroughly and to compare it in different water bodies. The aim of this investigation was to use and compare two methods such as 18S metagenomic sequencing and cultural method, for characterization of structure and biodiversity of protistian communities in saline deeply continental water bodies of the South Urals (Russia) with salinity more than 6,5%. Water and sediment samples from the saline lakes were examined with high-throughput sequencing of the 18S gene, V4 region, in MiSeq (Illumina). At the same time enrichment cultures from some samples were examined under microscopy. After comparison of the data obtained with different approaches for the same samples, drawbacks of every method were found. Another conclusion was concerned to a global comparison of protistan communities from saline waters of different continents with proper recognition of common and specific eukaryotic taxa for saline waters.

This study was conducted in the Center of Shared Scientific Equipment «Persistence of microorganisms» of ICIS UB RAS and supported by RFBR (17-04-02079, 17-04-00135, 15-29-02749, 15-29-02518).

Keywords: protistan diversity, saline lakes, NGS, metagenomic sequencing, cultivation of protists

Poster No. 134

Associations between choanoflagellates and bacteria in the marine environment

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Microbial associations are widespread in marine environments but poorly characterized. To investigate associations between bacteria and heterotrophic protists in the eastern North Pacific Ocean, we identified active predatory protists using food vacuole staining and isolated them individually by high-throughput Fluorescence Activated Cell Sorting (FACS). Based on 18S rRNA gene amplicon sequencing, 249 of 464 sorted unicellular eukaryotes were uncultured choanoflagellates belonging to 13 different OTUs (97% identity threshold) – coming from at least 7 different genera. Paired 16S rRNA gene screening showed that 32% of the choanoflagellate cells had physically associated bacteria. Comparison of the relative abundance of these bacteria in size-fractionated environmental samples allowed us to distinguish putative prey that were enriched in the free-living fraction (primarily Alpha- and Gammaproteobacteria) from others that were enriched in the >3 µm sample, suggesting a particle-associated lifestyle (mostly Flavobacteria, Verrucomicrobia, and Planctomycetes). Two divergent proteobacterial taxa were also found to be associated to a specific choanoflagellate taxon. These two were rare in the free-living fraction and other water column samples, but their relative abundance in amplicon data was correlated to the choanoflagellate. Our results suggest a specific interaction (possibly a symbiosis) between the choanoflagellate and the divergent bacteria, and the targeted metagenomes of these cells are currently being analyzed. Overall, our data suggest that choanoflagellates represented 15-40% of unicellular microbial predators at the time of sampling. Thus, we are also characterizing distributions and associations in other datasets to investigate the prevalence of specific choanoflagellate taxa and associated prokaryotes in other marine regions.

Keywords: choanoflagellates, Fluorescence Activated Cell Sorting, interactions, Bacteria

Poster No. 135

Molecular phylogenies challenge generic classification of spathidiid ciliates

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Reconstruction of evolutionary history of the ciliate order Spathidiida has proven elusive. There are weakly statistically supported deeper nodes in 18S rRNA gene phylogenies on one hand, while several statistically strongly supported clusters containing morphologically dissimilar taxa that usually lack any common apomorphies on the other hand. To clarify whether silent aspects of the spathidiid phylogeny are results of methodological problems of tree-building algorithms, we examined informativness of the macronuclear rRNA locus using an increased taxon and marker sampling as well as a complex statistical approach. Likelihood mapping revealed that the macronuclear rRNA locus has enough phylogenetic information to infer spathidiid relationships. However, some noise and conflicts hamper inference of deeper branching events, as documented by short parallelograms in the star-like central part of the split graphs and by low numbers of phylogenetically informative nucleotide homologies supporting deeper nodes of phylogenetic trees. Based on the diversification analyses and the c-statistic, we assembled a body of evidence that the spathidiid phylogeny retains the signature of one or several rapid radiations in the Palaeozoic and a subsequent gradual extinction that has started in the Mesozoic. A combination of these two phenomena along with polyphyly of three large spathidiid genera (*Spathidium*, *Epispathidium* and *Arcuospathidium*) are speculated to be the main reasons for fuzzy phylogenetic picture within the order Spathidiida. Because natural classification of spathidiids cannot be provided at the present state of knowledge, we suggest to keep the existing morphology-based generic classification, but stress that the large spathidiid genera are artificial collective groups.

Keywords: 18S rRNA gene, ITS region, *Litostomatea*, oral ciliary pattern, Spathidiida

Poster No. 136

Active microbial eukaryotes in the Movile Cave chemosynthetic ecosystem

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Located near the coast of the Black Sea on the Dobrogea Plateau in Mangalia, Romania, the Movile Cave is a unique underground cave ecosystem. This karstic cave is thought to have been sealed off from the outside world for several million years. Since its discovery in 1986, several studies have been carried out to study troglodytic metazoa but also the diversity of bacterial and archaeal communities responsible for carbon fixation in this chemosynthetic ecosystem. However, the presence of microbial eukaryotes has never been investigated. Here, we present our first results about the diversity of microbial eukaryotes in the oxygen-depleted waters and floating loose biofilms of the Movile Cave using a metabarcoding approach. We amplified the V4 and V5 regions of the 18S rRNA marker gene and applied high-throughput techniques (paired-end Illumina MiSeq). High quality assembled reads were clustered in operational taxonomic units and assigned to eukaryotic taxa using an in-house pipeline. Our results show a relatively large protist diversity dominated by ciliates, cercozoans and stramenopiles. In addition, we have generated metatranscriptomic data in order to identify active lineages and metabolic pathways that are active in this ecosystem.

Keywords: ecology, protist diversity, metabarcoding, chemosynthetic ecosystem, metatranscriptomic

Poster No. 137

Peatland micro-eukaryotic biodiversity in changing climate – a field experiment

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Climate change affects the diversity of living organisms, including micro-eukaryotes inhabiting peatlands. Although covering only 3 % of the land area on Earth, peatlands store ca. one third of the global terrestrial carbon stock. This function is controlled by the abiotic conditions of peatland soils: waterlogged conditions leading to low oxygen concentration, which makes them vulnerable to climate change effects. Shifts in plant and soil communities in response to climate change threaten the C-sequestration of peatlands as well as their characteristic biotic communities. The aim of our study was to assess the influence of warming and reduction of precipitation on the structure and diversity of micro-eukaryotic communities as determined by high-throughput sequencing of the V4 hypervariable region of the 18S small-subunit ribosomal RNA. In the active warming field experiment in a poor fen in W Poland infrared radiators were used to simulate climate warming, while automated retractable curtains were used to reduce the amount of precipitation. We hypothesised that: 1. a high taxonomic and functional diversity of micro-eukaryotes would be present, including many unknown and characteristic taxa/clades; 2. warming and reduction of precipitation would affect micro-eukaryotic diversity as well as community structure, those changes being correlated to altered ecosystem functioning (modification of carbon flux); 3. reduction of precipitation and warming would cause a decrease in abundance and diversity of mixotrophs; 4. Reduction of precipitation and warming would decrease the total diversity of micro-eukaryotes and especially the abundance and diversity of taxa characteristic for stable hydrologically *Sphagnum* mires.

Poster No. 138

Ciliate and algae species from Texcoco lake, a Mexican saline basin, with notes on their geographic distribution

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The lacustrine area of Texcoco is located northeast of Mexico City (Mexico), it is characterized by its high dissolved salt content. Free living ciliates and planktonic algae were surveyed during five months in Texcoco Lake, Mexico. For ciliate species, water samples were obtained with some roots and mud, and for algae, water samples were sieved through a mesh of 10 µm. We recorded 11 free-living species of ciliates and three species of algae. Ciliate species are included in five subclasses: Cyrtophoria, Haptoria, Hypotrichia, Peniculia and Scuticociliatia, and algae are included in Cyanoprokaryota and Heterokontophyta divisions. Ciliate species geographic distributions include 37 countries, and the algae species have been recorded in 47 countries. The distributional data could be explained on the basis of suitable environmental conditions through the world, including the Texcoco lake basin.

Acknowledgments. To Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (DGAPA-PAPIIT-UNAM) for the support to the project IN229811.

Keywords: ciliates, algae, Mexico, Texcoco

Poster No. 139

Epistylis plicatilis Ehrenberg, 1831 (Ciliophora: Peritrichia) recorded on a new host and contribution on its worldwide geographic distribution

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Several species of *Epistylis* occur as free-living, but numerous species are usually epibionts of algae, aquatic plants, mollusks, and crustaceans, and also on inorganic substrates. The goal of this study is to provide some cytological characteristics of *E. plicatilis* Ehrenberg, 1831 attached to a freshwater gastropod, with its global geographic distribution. *Epistylis plicatilis* was identified as an epibiont on the shell surface of the gastropod *Physa* sp. collected from an urban lake in Mexico City. This species was previously recorded in *Pomacea figulina* Spix, 1827 from Brazil. We present some notes about its cytological characteristics. Considering all available data, we conclude that *Epistylis plicatilis* has not been recorded in Australian and Ethiopic regions.

Acknowledgments. To Biól. M. Reyes-Santos (Facultad de Ciencias, UNAM) by her help with technical procedures. To P. B. L. Flores-López (Facultad de Ciencias, UNAM) by gastropod sample collection.

Keywords: *Epistylis*, *Pomacea*, epibiont, Mexico

Poster No. 140

Distributional patterns of some free-living ciliate (Alveolata: Ciliophora) species

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We analyzed the geographic distributional patterns of 29 ciliate species from six freshwater localities in Mexico (Ocotal Lake, Tiacaque Spring, Analco Lake, Azucena Reservoir Pond, Montebello Lake, and Tzsicao Lake) by using panbiogeographical methods. Ciliate species are included in 11 classes, 22 families and 22 genera. For the 29 species, we obtained all available data to construct their individualized tracks. Three generalized tracks were obtained based on the distribution of only 20 ciliate species: a) Eurasian-American, b) American-Afroeurasian and c) American-Afroeurasian-Australian tracks. The Atlantic Ocean is considered as the main baseline. Ciliate species studied have a wide Gondwanic and Laurasic distributional history. We identified nine nodes (representing composite areas and rich in species of Ciliophora), four of them disjunct between the Old and the New World. We conclude that free-living ciliates follow the moderately endemicity model. The historical biogeographic approach used to analyze their distributional patterns are good tools to identify their biogeographical history.

Acknowledgments: To Posgrado en Ciencias Biológicas and CONACyT for the grant to D. Méndez-Sánchez for master studies. From Facultad de Ciencias, UNAM, to Biól. M. Reyes-Santos for her micrographic technical assistance, to M. en C. O. Alcántara for his help with distribution data assemblage, and to Dr. P. González-Ávila for her assistance with biogeographical analysis.

Keywords: Ciliates, panbiogeography, track analysis, Mexico

Poster No. 141

Does water level fluctuation in floodplains influence beta diversity patterns of planktonic ciliates?

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Temporal variations in environmental heterogeneity and in levels of connectivity among habitats may dictate the magnitude of species sorting and dispersal processes, respectively, causing variation in species composition. Thus, one can anticipate that spatial beta diversity varies strongly through time. However, the temporal dynamics of beta diversity are often overlooked in community ecology. Here we test the hypothesis that ciliate β-diversity is lower during high water periods than during low water periods, due to the homogenizing effects of floods. We use data on ciliate communities of four South American floodplains (Paraná, Pantanal, Araguaia and Amazon). Our results indicate an effect of water level fluctuation on ciliate β-diversity and environmental heterogeneity between sampling periods for the Amazon and Paraná floodplains, but not the Araguaia and Pantanal floodplains. We also found positive relationships between uniqueness in biological space and uniqueness in environmental space, during both low and high water periods. Thus, although we found a homogenizing effect of floods in two of the four Neotropical floodplains, the lack of differences in ciliate β-diversity in the other two floodplains indicates that this homogenizing effect may not be a general rule for planktonic ciliates. Our results suggest that environmental filtering importantly influences the community assembly of ciliates in Neotropical floodplains, adding evidence to the idea that environmental processes are key factors affecting microbial community assembly.

Poster No. 142

Strombidium hongkongense n. sp. feeds on progametes of *Noctiluca scintillans*

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Strombidium hongkongense is a new marine oligotrich ciliate, isolated from a bloom of *Noctiluca scuntillans* near Port Shelter, Hong Kong. It is different from its congeners by the combination of the following characters: cell usually heart-shaped, cell size mostly 20-35 × 20-30 µm in vivo; deep buccal cavity extending obliquely to about 1/2 of cell length; prominent apical protrusion; the adoral zone of membranelles divided into 17-19 collar membranelles and four buccal membranelles; one ball-shaped macronucleus; the girdle kinety forming a closed loop which obliquely surrounds the body; ventral kinety absent. We documented for the first time *S. hongkongense* that feeds on *N. scuntillans*'s progametes undergoing stages 5 to 9 of nuclear division. It frequently swam on or around gametogenic and some vegetative *N. scuntillans* cells. *S. hongkongense* associated with gametogenic cells had significantly lower swimming speed and changed direction more frequently than those associated with vegetative cells, which overall increased their time spent around the food patches (progametes). This trophic interaction constitutes an up-side-down predator-prey link, in which ciliates within the typical size range of *N. scuntillans* prey, become the predators. Based on the phylogenetic tree (maximum-likelihood), there are 14 environmental clones similar to *S. hongkongense* found in other coastal waters, where *N. scuntillans* presence or blooms have been reported. This novel predator-prey relationship could therefore be common in other *Noctiluca* habitats. Additional studies are needed to assess the magnitude of its impacts on *Noctiluca* population dynamics and plankton bloom succession.

Keywords: *Strombidium hongkongense*, feeding behavior, swimming pattern, *Noctiluca*, progametes

Poster No. 143

Morphology and multi genes-based integrative phylogenies of *Spirostomum* ciliates (Ciliophora, Heterotrichea, Spirostomidae) give us insights on their evolution

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Spirostomum is a genus of free-living ciliate protists, belonging to the family Spirostomidae among 10 families within class Heterotrichea. The species of *Spirostomum* are found in both salt and fresh water. All of them are elongated, flexible and highly contractile. Although they are unicellular, members of some species can grow as long as 4 mm. Morphological and molecular delimitation of *Spirostomum* species is currently under debate. In this study, we present hypotheses of the evolution and species delimitation within the genus *Spirostomum* based on multi-genes (18S-rRNA, ITS1-5.8S-ITS2 region, 28S-rRNA, alpha-tubulin and mt COI) and morphological data analyses. Phylogenetic analyses included Bayesian inference, Maximum likelihood, Maximum parsimony methods, and Phylogenetic network analyses were performed. Additionally, we estimated a species tree for *Spirostomum* spp. using the coalescence-based method. The results show that: (1) single gene trees are inconsistent and the concatenation of all data improved the resolution of deep nodes; (2) the coalescent-based species tree is consistent with phylogenies based on the 18S-rRNA gene and dividing the *Spirostomum* species into two major lineages; (3) high degree of genetic variability at population level by mitochondrial CO1 gene but not the nuclear gene marker indicates, the possible presence of several cryptic species within the genus *Spirostomum*.

Keywords: *Spirostomum*, species, multi-genes, morpholgy, phylogeny

Poster No. 144

Born in America: A molecular phylogeography of *Hyalosphenia papilio* (Amoebozoa; Arcellinida)

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Assessment of the spatial distribution and diversity of free-living protists remains an open and unresolved question among the scientific community. Recent studies show that some groups of microbial eukaryotes present limited dispersion, sometimes even narrow endemism and contradict the paradigm of cosmopolitan distribution illustrated by the tenet "everything is everywhere, but, the environment selects". However, most studies aimed at demonstrating or contradicting this paradigm, and very few have evidenced phylogeographic processes driven by allopatric speciation. Here, we present a survey of the molecular diversity within a morphospecies of testate amoeba, *Hyalosphenia papilio* through a large part of the Holarctic realm. These organisms are infeodated to raised bogs and are distributed all over the Northern hemisphere. Previous studies show that this group hosts a wide diversity of cryptic species, some of which had potentially a narrow distribution. We barcoded single cells using the mitochondrial COI (partial cytochrome oxidase subunit 1 sequences) as the reference gene. Our dataset was based on sequences obtained through data mining in GenBank plus our own data and included 61 sites and a total of 418 sequences, resulting on 13 different lineages (i.e. "molecular species"). From these, nine lineages showed narrow restricted geographical distributions while four others were well distributed all across the Holarctic realm. Eurasian peatlands hosted only panholarctic lineages and no narrow endemics. This evidence, in addition to our reconstruction of ancestral distributions based on a phylogenetic tree, suggests that *Hyalosphenia papilio* originated probably in the West coast of North America where it survived the glaciation in existing refuges. We could estimate that the Palaearctic realm has been colonized four times, independently.

Keywords: dispersal, Hyalospheniidae, peatland, phylogeography, *Sphagnum*

Poster No. 145

High genetic diversity of amoebae belonging to the genus *Mayorella* (Amoebozoa, Discosea, Dermamoebida) in natural habitats

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Amoebae of the genus *Mayorella* are widespread in marine and freshwater habitats; some species are also known from soil. These amoebae possess the low number of morphological characters allowing species differentiation, so the number of described species remains limited. These organisms are hard to maintain in culture, the most of them are polyphagous and carnivorous; they require a variety of food objects, including other protists, to live and multiply well. Thus they are difficult objects for molecular studies; only two sequences of named *Mayorella* strains were available until recently. For the present study we have isolated 8 strains of *Mayorella* from different locations worldwide, documented them with light-microscopy and sequenced 18S rRNA and Cox I genes. The resulting trees show that *Mayorella* is a robust clade of Dermamoebida; inclusion of so many sequences improved the support for this branch of Amoeboozoa in SSU trees. The vast majority of our strains probably are new for science; this evidences that the diversity of mayorellas in natural habitats is high and the genus is rather species-rich, comprising no less that 11 species. Our results show that sequence data are necessary for reliable identification of *Mayorella* species.

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Keywords: Amoebozoa, phylogeny, diversity

Poster No. 146

Benthic foraminifera from the Northeastern Gulf of Mexico shelf and slope

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Benthic foraminifera are widely distributed protozoa used as ecological indicators in both current and palaeo oceanography. Knowledge of the ecology and distribution of these organisms in various regions of the Gulf of Mexico is limited. The BP Deepwater Horizon oil well failure in the northern Gulf of Mexico highlighted the need to better understand the distribution and abundance of these organisms relative to environmental factors and ecosystem perturbations such as the oil spill. Sediment samples were collected using a Shipek grab along transects on the northwest Florida GOM shelf. Clone libraries were developed from PCR amplified 18S rDNA genes for sequence analysis. Analysis of random clones from libraries were used as a proxy for community structure (presence and relative abundance) to be evaluated for foraminiferan spatial and temporal dynamics on the Northwest Florida Shelf in the NE GOM. Additional continental slope samples were obtained by multicore and treated in similar fashion. This analysis revealed a limited species richness with a majority of sequences aligning to known organisms listed in the NCBI database. A few taxa were shown to dominate both the coastal communities and deep water sites. These include *Glabratellina sp., Trochammina hadai,* and *Trochammina sp.,* and *Textularia sagittula* and *Bathysiphon argenteus* as well as members of genera *Astrammina, Bolivina, Cibicides and Cibicidoides*. Other species were more restricted in their distributions, and the data indicate a degree of spatial specificity with respect to depth and sediment type.

Keywords: Foraminifera, marine, benthic, Gulf of Mexico, oil spill

Poster No. 147

Single cell genomics of uncultured Marine Alveolates (MALVs) shows paraphyly of basal dinoflagellates

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Marine Alveolates (MALVs) are diverse and widespread early-branching dinoflagellates, but most knowledge of the group comes from a few cultured species that are generally not abundant in natural samples, or from diversity analyses of PCR-based environmental SSU rRNA gene sequences. To more broadly examine MALV genomes, we generated single-cell genome surveys from seven individually isolated cells. Genes expected of heterotrophic eukaryotes were found, with interesting exceptions like presence of proteorhodopsin and vacuolar H^+-pyrophosphatase. Phylogenetic analysis of concatenated SSU and LSU rRNA gene sequences provided strong support for the paraphyly of MALV lineages. Our findings indicate that multiple independent origins of several characteristics early in dinoflagellate evolution, such as a parasitic life style, underlie the environmental diversity of MALVs, and suggest they have more varied trophic modes than thought to date.

Poster No. 148

Morphological protist diversity in the marine oxygen minimum zone of northern Chile

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Oxygen minimum zones (OMZ) harbor diverse eukaryotic microbial assemblages, some of them potentially restricted to these zones as revealed by molecular surveys applied across oxygen gradients. However, most of these organisms remain undescribed to date. Here we present the morphological diversity of protists along a depth oxygen gradient in the OMZ off Northern Chile from four sampling stations between November and December 2015. Morphological analyses were performed under epifuorescence with the help of the fluorochoromes iodurum propidium and calcofluor that allowed the visualization of nuclei and cellulose/chitin cell walls, respectively. Morphotypes were differenced based on their size and the general shape of the cells, presence/absence of a cell wall evidenced by calcofluor as well as characteristics related to the nucleus (size, shape and position in the cell). With exception of only one unidentified dinoflagellate morphotype that was detected in all depths, distinct composition patterns were detected along the oxygen gradient. As expected, oxic depths (10-50 m) were dominated by chain-forming diatoms (e.g., *Thalassionema*, *Thalassiosira*, *Chaetoceros*) and large dinoflagellates (mainly *Protoperidium* and *Ceratium*). Some dinoflagellates seemed to be infected by parasites resembling the final infection stage of the genus *Amoebophrya* (Syndiniales). Suboxic and anoxic depths (100-400 m; OD < 20 µM) were dominated by small naked dinoflagellates and *Thraustochytrid*-lyke organisms. These results are consistent with molecular surveys performed for OMZs and point to differential functional diversity in these systems.

Keywords: eukaryotes, anoxic zones, plankton ecology

Poster No. 149

Does the bottleneck effect shaped the current population structure of *Paramecium biaurelia* (*P. aurelia* species complex, Ciliophora)?

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The information on the spatial variability of free-living and freshwater microbial eukaryotes inhabiting remote ecosystems are relatively rare, because in many cases the determination of boundaries between species might be difficult due to the lack of its uniform definition, scarce data on natural populations as well as existence of cryptic diversity.

Species (or cryptic species) identification in microbial eukaryotes often requires at least morphological and molecular approach (Boenigk et al., 2012), and if possible mating reaction tests which allow to be sure that, for example, distant populations are in fact one species. Therefore in our opinion that *P. biaurelia* (one of the fifteen cryptic species of *P. aurelia* complex) collected worldwide from 89 sampling points during 62 years with application of three presented above approaches seems to be an appropriate model for testing protistan biogeography hypotheses.

Despite the large distance, the most of studied populations of *P. biaurelia* do not differ from each other (rDNA fragment), or differ slightly (*COI* mtDNA fragment). The obtained results may suggest that in the past the predecessors of the present *P. biaurelia* population probably went through a bottleneck, and its current distribution is the result of a recent dispersal by natural or anthropogenic factors. Another explanation for the low level of genetic diversity despite the huge distances between the collecting sites may be a slow rate of mutation of the studied DNA fragments revealed in some species of the *P. aurelia* complex. In contrast, the other member of the complex, characterized wide range of occurrence – *P. primaurelia* revealed a much larger number of nucleotide substitutions between particular *COI* haplotypes. The presence of low genetic variability of two loci and occurrence of very similar or identical haplotypes around the world may support the ubiquity model in spatial distribution of *P. biaurelia.*

Keywords: free-living microbial eukaryotes, spatial variability, *Paramecium aurelia* species complex, haplotype varation, ubiquity model in spatial distribution

Poster No. 150

Whether "sampling the neighborhood" supports the EiE hypothesis in the case of the *Paramecium aurelia* species complex? Insights from the local and seasonal COI haplotype variability of natural populations

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Despite that ciliates from the *Paramecium* genus are one of the most characteristic microorganisms of aquatic ecosystems, already known in the seventeenth century thanks to Leeuwenhoek observations, their biogeography and distribution (similarly as well as the majority of the other protists) remain poorly understood in comparison to animals or plants. Currently two models, with different points of view describe observed distribution patterns of microbial eukaryotes - "ubiquity model" (UM) (Finlay et al., 2006) and "the moderate endemicity model" (MEM) (Foissner et al., 2008). Due to the fact that the information on the spatial and seasonal genetic variability of microbial eukaryotes inhabiting natural ecosystems are relatively rare, the purpose of the current project was to test whether a deeper sampling of local water reservoirs reveal new *COI* haplotypes in comparison to haplotypes obtained from distant as well as close *P. aurelia* populations.

During initial studies (seasons 2015-2016) we sampled 28 locations (the Southern Poland, mainly in Kraków), 49 water reservoirs, and 177 sapling points. Some reservoirs were sampled 2-6 times, which consequently led to 374 sampling points examined - in 15.77% of them *P. aurelia* was detected. In the studied area, based on genetic crosses (the biological species concept) and *COI* mtDNA analysis (the phylogenetic species concept) we found 8 out of 15 known *P. aurelia* cryptic species and identified 18 *COI* haplotypes. Only two haplotypes (from *P. sexaurelia*) were new, the remain 16 was shared with the other, often distant *P. aurelia* populations. What's interesting in the case *P. biaurelia* the majority of the global *COI* variability was detected within Kraków area. Therefore it seems that our initial findings tend to support "Everything is Everywhere" hypothesis.

Keywords: "Everything is Everywhere" hypothesis, *Paramecium aurelia* species complex, COI haplotype variation, deep sampling of natural populations, the Southern Poland

Poster No. 151

Multigene phylogeny of deep branching stramenopiles

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Stramenopiles are one of the important eukaryotic assemblages including very diverse species such as photosynthetic unicellular algae and seaweeds, fungus-like osmotrophic organisms and many phagotrophic free-living flagellates. Although photosynthetic stramenopiles have been well studied and their phylogenetic relationships are mostly clear, the phylogenetic relationships among deep branching heterotrophic stramenopiles are not well resolved. This is due to lack of adequate genomic/transcriptomic data for representatives of each deep branching lineage, which prevented the robust reconstruction of early diversification events of stramenopiles. In this study, we performed multigene phylogeny to resolve the phylogenetic relationship among deep branching stramenopiles. We sequenced two transcriptomes of deep branching stramenopiles; one is a newly established culture of unidentified Marine Stramenopiles (MAST-6)**,** and the other is *Platysulcus tardus*, a heterotrophic flagellate with an uncertain phylogenetic position in stramenopiles. We prepared a phylogenomic alignment of 120 genes and 76 taxa, including these two new transcriptomes. In this 120-gene phylogeny, ***P. tardus*** was found to be basal to other stramenopiles. Also, MAST-6 was sister to MAST-4 clade. The resolved relationships of deep branching stramenopiles provide some insights about the characters that were possibly present in the last common ancestor of stramenopiles and their evolutionary patterns in stramenopiles.

Poster No. 152

Effects of temperature and salinity on virus-mediated diatom cell death

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Diatoms (Bacillariophyta) are unicellular, photosynthetic, eukaryotic algae found worldwide in many aquatic environments. They account for a large proportion of marine biomass. Several studies have demonstrated that viral infection is an important factor affecting diatom dynamics in aquatic environments. To understand the relationships between host diatoms and their infectious viruses, the effects of various environmental factors on host-virus interactions must be characterized. Among diverse environmental factors, water temperature and salinity are considered important for regulating phytoplankton growths, as are light and nutrient levels. To examine the effects of water temperature and salinity on host-virus interactions, we used two strains of the marine planktonic diatom *Chaetoceros tenuissimus* and four viruses with different host specificities. The time necessary for a virus to lyse half the diatoms within a culture (CR_{50} = days required for chlorophyll a fluorescence intensity of host cells to decrease by >50%) was significantly affected by changes in both water temperature and salinity. In several host-virus combinations, the environmental suitability for host growth and the CR_{50} of the viruses were significantly correlated. Environmental factors may be important factors in determining the dominant virus species in a diatom bloom.

Keywords: diatom, virus, water temperature, salinity

Poster No. 153

Diversity of benthic ciliates from sandy beaches in Southern Brazil

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Sandy beaches represent 2/3 of littoral zones and complex factors as winds, waves and sediment characterize this environment. Ciliophora is one of the most diversified unicellular eukaryotic phylum and, in marine sediments, there are records of 40 species/cm³. Despite its importance, the lack of knowledge about interstitial community regarding ecological e taxonomic aspects is still a problem. In the present work, we characterized the assemblage of interstitial ciliates from sandy beaches located in the north coastal area of Rio Grande do Sul state, southern Brazil. A total of 14 sites were sampled in the swash zone of seven beaches. In each sampling point, eight subsamples of sand were taken with a PVC tube of 10cm in length and 3cm in diameter. Until now, six samplings campaigns were carried out: two during winter, two during spring and two during summer. For extraction of the organisms from the sediment, the Uhlig's modified method was used and organisms were observed in vivo using an optic microscope. A total of 49 species/morphotypes were identified among 37 genera and ten classes. Classes Spirotrichea (31%) and Karyorelictea (21%) accounted for 42% of the ciliate species richness in the studied environment. *Trachelonema oligostriata* and *Tracheloraphis oligocineta* (Karyorelictea) were the most frequent species, found in 88% of the samples, followed by *Pleuronema* cf. *smalli* (Oligohymenophorea, Scuticociliatia), and *Euplotes aspheniscus* (Spirotrichea, Hypotrichia) found in 62% and 57% of the samples, respectively. These results corroborate that groups as Spirotrichea and Karyorelictea play an important role in the diversity of sandy beaches. Likewise the high frequency of genus like *Tracheloraphis* agrees with the idea of a close relationship between the group, the interstitial habit, and the marine environment.

Keywords: ciliates, interstitial community, sandy beaches, ecology, Karyorelictea

Poster No. 154

Effects of urban development on the composition of marine ciliate assemblage

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Urban development of coastal areas helps to increase recreational activities, but also causes morphological modification of sandy beaches and pollution. In marine environments, Cilliophora is one of the most abundant unicellular eukaryotic phylum, but, the knowledge about its richness, and ecological relationships in the interstitial marine habitat is still incipient, especially in Brazil. The aim of the present study was to identify relationships between urbanization rates and composition of the interstitial ciliate assemblage in sandy beaches in Southern Brazil. A total of 14 sampling sites distributed along the swash zone of seven beaches with different degrees of urbanization (high, intermediary, low and almost null) were sampled during winter, spring and summer. In each point, eight sediment samples, as well as samples to analyze chlorophyll *a* and organic matter, were taken. Uhlig's modified method was used to extract organisms from the sediment. Ciliates were observed in vivo using an optic microscope and were identified based on specialized literature. Chlorophyl *a*, and organic matter were analyzed using standard protocols. A total of 49 species/morphotypes were identified belonging to 10 classes in the Phylum Cilliophora. Highest species richness (42 species) was found in Arroio do Sal beach, characterized by intermediary degree of urbanization. Torres and Tramandaí beaches presented a species richness of 36 and a high degree of urbanization. The site without urbanization (Praia das Cabras) presented the lowest ciliate richness: 25 species. The highest values of chlorophyll *a* (0.009µgV-1), and organic matter (16.80%) were found in Santa Rita de Cassia beach, and in Arroio do Sal beach, respectively. Results of clusters analyses showed different rates of similarity among the beaches, suggesting some regulation of assemblage composition by the environment. Impacts related to urbanization may affect ecological relationships among species, helping to shape the ciliate assemblage composition.

Keywords: ciliates, sandy beaches, urban development, ecology, Karyorelictea

Poster No. 155

Environmental change, temporal heterogeneity and fragmented habitats: modeling eutrophication in a metacommunity microcosm

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Community ecology has long focused on processes that regulate patterns of species distribution and abundance. Facing an era of anthropogenic global change and biodiversity loss, strategies of conservation biology are increasingly relevant. Our research deals with temporal and spatial aspects of two of the biodiversity threats, namely fragmentation and eutrophication. According to literature, pulsed nutrient addition increases phytoplankton diversity by allowing higher number of coexisting species. Furthermore, it is known the benefit of intermediate connectivity in metacommunity landscape in increasing primary producers diversity. However, it is yet unclear if those two factors are additive in increasing diversity and if the grazers responses are related to their prey. With the goal of understanding how eutrophication impacts biodiversity in a metacommunity landscape, we hypothesized that pulsed rather than continuous nutrient addition will increase diversity and these effects will be even greater in a metacommunity landscape. We experimentally manipulated different ways to load nutrients to an aquatic model system. The eutrophication dissimilarity was created by adding nutrients either continuously with a peristaltic pump, or pulsed, pipetting once a week. In addition, two different topologies (isolated patch vs. metacommunity) were also compared. The experimental community was composed by phytoplankton, as primary producers, and microzooplankton, as grazers. The data were analyzed with two-way repeated-measures ANOVA, with local and regional species Shannon diversity being estimated. Grazers were strongly affected by the different topologies, with higher diversity and more stable community dynamics in metacommunities under pulsed nutrient addition. However, in isolated communities, grazer diversity was higher with continuous rather than pulsed nutrient addition. Resource use efficiency was also higher in metacommunities with pulsed nutrient addition. These results revels that pulsing nutrients can increase or maintain the biodiversity of primary producers and grazers in comparison with continuously nutrient load, with the proviso of metacommunity landscape condition for grazers.

Keywords: metacommunity, protists, human impacts, eutrophication, fragmentation

Poster No. 156

Mostly the "usual suspects" but a few novelties as well: Results from an extensive cultivation effort of heterotrophic protists from the Baltic Sea

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The study of cultured strains has a long tradition in protistological research and has greatly contributed to establishing the morphology, taxonomy and ecology of many protist species. However, cultivation-independent techniques, based on 18S rRNA gene sequences, have demonstrated that natural protistan assemblages mainly consist of hitherto uncultured protist lineages. This mismatch impedes the linkage of environmental diversity data with the biological features of cultured strains. Thus, novel taxa need to be obtained in culture to close this knowledge gap. In this study, traditional cultivation techniques were applied to samples from coastal surface waters and from deep oxygen-depleted waters of the Baltic Sea. Based on 18S rRNA gene sequencing, 128 monoclonal cultures of heterotrophic protists were identified. The majority of the isolated strains were affiliated with already cultured and described taxa, mainly chrysophytes and bodonids. This was likely due to “culturing bias” but also to the eutrophic nature of the Baltic Sea. Nonetheless, ~11% of the isolates in our culture collection showed highly divergent 18S rRNA gene sequences compared to those of known organisms and thus may represent novel taxa, either at the species or the genus level. Novelties comprised taxa within a wide variety of phylogenetic groups such as chrysophytes, cercozoans, choanoflagellates, goniomonads, and bodonids. Moreover, we also obtained evidence that some of the isolated taxa are ecologically relevant, under certain conditions, in the Baltic Sea.

Keywords: traditional cultivation, culturing bias, unamended seawater incubation, flagellates, novel taxa

Poster No. 157

Taxonomic re-descriptions of *Kiitoksia ystava* Vørs, 1992 (Rhizaria) and *Ministeria vibrans* Tong, 1997 (Filastrea) based on ultrastructure and molecular characterization

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Many protist species are described solely based on light microscopy. Ultrastructural and/or phylogenetic characterizations are often missing, especially among small taxa with infrequent occurrence. Here, we provide re-descriptions of two nano-sized heterotrophic protist species that were isolated from the Baltic Sea and established as stable cultures. Both were investigated by light and electron microscopy as well as gene sequence analysis (18S rDNA).

One taxon is the tiny flagellate species *Kiitoksia ystava* described in 1992 by Vørs. Although this species was repeatedly detected in marine environments, its phylogenetic position and ultrastructure was, as yet, unknown. Our study revealed that this species possesses a highly reduced second flagellum and tubular mitochondrial cristae. Phylogenetic tree reconstructions placed *K. ystava* as a new branch within Thecofilosea (Rhizaria).

The other taxon, *Ministeria vibrans* a tiny amoeboid species that was described in 1997 by Tong. *M. vibrans* is well-characterized by molecular phylogeny but its ultrastructure was never investigated in detail before. The genus *Ministeria* belongs to Filasterea (Opisthokonta). Our electronmicroscopical study revealed axopodes and additional filopodes but no flagellum. Highly reduced kinetosomes were observed only seldom and mitochondria show flat cristae. Vesicles of uncertain function show a lamellar structure inside.

This work (A. P. Mylnikov) was partially supported by the grants of Russian Foundation of Fundamental Research (17-04-00565 A and 17-04-00899 A).

Keywords: *Kiitoksia ystava*, *Ministeria vibrans*, Baltic Sea, re-description, cultivation

Poster No. 158

Feeding and grazing impact by the mixotrophic ciliate *Mesodinium rubrum* on natural populations of marine heterotrophic bacteria in the coastal waters of Korea

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We explored feeding by the mixotrophic ciliate *Mesodinium rubrum*, heterotrophic nanoflagellates (HNFs), and small ciliates (*M. rubrum* red tides occurred. We also measured ingestion rates of *M. rubrum* on cultured heterotrophic bacteria as a function of bacterial concentration using fluorescently labeled bacteria (FLB) in the laboratory. The ingestion rates of *M. rubrum* on natural populations of heterotrophic bacteria (2.3–16.8 bacteria grazer^{-1} h^{-1}) were comparable to or lower than those of co-occurring HNFs (10.7–41.7 bacteria grazer^{-1} h^{-1}), but much lower than those of co-occurring small ciliates (76.0–462.2 bacteria grazer^{-1} h^{-1}). However, the maximum grazing coefficient of *M. rubrum* (0.245 d^{-1}) on natural populations of heterotrophic bacteria was much higher than that of small ciliates (0.089 d^{-1}), and slightly higher than that of HNFs (0.204 d^{-1}). With increasing bacteria concentrations, ingestion rates of *M. rubrum* on cultured heterotrophic bacteria continuously increased, but became saturated at higher prey concentrations over 1–5´ 10^6 cells mL^{-1}. The maximum ingestion rate of *M. rubrum* on cultured heterotrophic bacteria were 34.4 bacteria grazer^{-1} h^{-1}. Based on the present study, it is suggested that *M. rubrum* may be an important grazer of heterotrophic bacteria and sometimes have considerable grazing impact on natural populations of heterotrophic bacteria.

Keywords: ingestion, bacterivory, protist, red tide

Poster No. 159

Single cell transcriptomics of a facultative anaerobic ciliated protist *Metopus yantaiensis* (Ciliophora, Armophorea)

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A broad swath of uncultivable eukaryotic microbes is inaccessible to genome-wide comparative methods. One promising approach is single cell transcriptome (SCT), whereby transcriptome data are produced by amplifing total cDNA from an individual in nature. Here we tested the efficacy of SCT to generate a draft transcriptome assembly from a single sample of a facultative anaerobic marine ciliate *Metopus yantaiensis* (Ciliophora, Armophorea). Using *de novo* gene prediction, we identified 14125 protein-encoding genes in the *Metopus* transcriptome. This genetic inventory was sufficient to search for app. 300 orthologous genes in ciliates. We preliminary inspected the mitochondria related genes, which may contribute to the anaerobic habituation. With a comparison to the congeneric ciliate *Nyctotherus ovalis*, 73 out of 85 proteins and RNAs likely involved in the mitochondrial metabolism were found in *Metopus*. Among these, two divergent hydrogenase-homologous genes were examined and applied to predict its evolutionary origin for Armophorea. Similar to the symbiotic *Nyctotherus*, both hydrogenases of free-living *Metopus* were revealed polyphyletic and with LGT (Lateral Gene Transfer) origin, possibly facilitating the adaption of *Metopus* to both aerobic and anaerobic habitats. The second type hydrogenase uniquely found in *Metopus*, separated from the one presented in both *Metopus* and *Nyctotherus*, suggesting a free-living origin of the Armophorea hydrogenase.

Keywords: single cell, anaerobic, ciliate, transcriptome, hydrogenase

Poster No. 160

Long-term effects of effluents from wastewater treatment plant on upstream and downstream planktonic protozoa communities in river ecosystem

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Planktonic protozoa communities are highly involved in microbial trophic dynamics and often play major ecological roles in self-purification in urban river ecosystems. There is an advance require in our understanding of the biology of these systems. However, their community structures and dynamics in urban river remain underexplored, especially after the long-term influence of sewage influent release from wastewater treatment plants (WWTPs). Our objective here is to assess the relative importance of dispersal and local environmental factors in shaping protozoa communities and phylogenetic diversity from upstream to downstream on fine-scale with 20 sites samples in 5 rivers of Hai River system. The analyses of ca. 517, 339 Illumina Miseq PE300 sequencing reads of 18S gene revealed high complexity of protozooplankton communities with 430 OTUs (operational taxonomic units). Ciliated protozoa are one of the major groups with 62.8 percent of planktonic protozoa, and in which members of class Litostomatea, Oligohymenophorea and Spirotrichea are dominated. These molecular data correlated with 14 intrinsic environmental factors is used for multivariate statistical analysis. Distinct differences in richness and community composition at the OTU, genus and class level were observed between the upstream and downstream areas, and same patterns were also reflected in environmental factors. Correlation analysis of biotic and abiotic factors revealed that local environmental variables influenced by sewage influent are responsible to community structure dynamics. The origins and significance of these differences are discussed and our work demonstrates that local environmental variables caused by anthropogenic activities have a consistent and significant contribution to the natural protozooplankton assemblages, irrespective of dispersal capabilities.

Keywords: eukaryotic microbes, high throughput sequencing, wastewater treatment plants, ciliates, nutrients

Poster No. 161

Belonocystis is a member of Amoebozoa: an example of dramatic flagella simplification?

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The genus *Belonocystis* Rainer has had an uncertain position within Eukaryota for a long time. We obtained transcriptome data from *Belonocystis marina* and the multiflagellated amoebozoan *Multicilia marina,* and conducted a phylogenomic analysis that identifies *Belonocystis* and *Multicilia* as sister groups branching within the Amoebozoa, class Variosea. This result contradicts previous suggestions of *Belonocystis* belonging to Rhizaria because of its fine outgrowths, and shows the presence of another unusual morphological type within Amoebozoa. Poor taxon sampling of Variosea complicates reconstruction of its evolutionary history, but some preliminary hypotheses can be proposed. *Belonocystis* and *Multicilia* share a spherical cell shape with radiating fine outgrowths and flagella respectively. Both crawl along the substratum, have a complex glycocalyx, and form multinuclear stages. The most interesting question concerns the nature of the *Belonocystis* outgrowths. Study of their ultrastructure has shown that they lack axonemes, suggesting that the outgrowths are pseudopodia. On the other hand, they superficially resemble flagella of *Multicilia* in their position on the cell surface and their mode of movement. One possible scenario is a direct transformation of ancestral *Multicilia*-type flagella (which are already reduced, having singlets instead of doublets in the axoneme) to *Belonocystis* outgrowths through dramatic cytoskeleton reduction; if so, this would represent the first case of an evolutionary transition between flagella and pseudopodia, the two basic motility organelles of eukaryotic cells. The nature of the cystoskeleton present in the *Belonocystis* outgrowths is not yet determined, but transcripts of flagella-specific dyneins were detected in our transcriptome data, which is consistent with a possible persistence of the tubulin-dynein system, in spite of the axoneme loss. More detailed studies of the outgrowths structure and diversity of this newly found Variosea clade are necessary to better understand these evolutionary events.

Study support: RFBR grant 15-04-18101.

Keywords: amoeboid organisms, phylogenomics, ultrastructure, dynein, evolution

POSTER SESSION C (162–242)

Poster No. 162

Genetic transformation of thraustochytrid strains (Labyrinthulea) by *Agrobacterium tumefaciens*

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The thraustochytrids (Labyrinthulea = Labyrinthulomycetes) have been known as the producer of useful organic matters such as poly-unsaturated fatty acids, squalene and carotenoids. The genome information of *Schizochytrium aggregatum, Aplanochytrium kerguelense* and *Aurantiochytrium limacinum* has been reported. The methods of genetic transformation of thraustochytrids have already been established by electroporation or particle bombardment. Cheng *et al.* [Microbiological Research 167 (2012): 179–186] developed a transformation approach for *Aurantiochytrium* using the *Agrobacterium tumefaciens* (=*Rhizobium radiobacter*) binary vector system. In their method, previously the protoplasts were prepared by using digestive enzymes of cell wall, cellulase and snailase. In this study, we made an attempt at developing a simpler protocol and applying the method of genetic transformation by *Agrobacterium* to the phylogenetically diverged thraustochytrids, *Parietichytrium* sp., *S. aggregatum, A. kerguelense* and *A. limacinum*. After co-cultivation of each strains with *A. tumefaciens* harboring pBI101 plasmid containing the neomycin-resistance gene as the selectable marker and GFP gene for confirmation of the gene expression. The transformants of *A. limacinum* were successfully obtained without the procedure of converting to protoplasts and the fluorescence of GPF were clearly observed. However, the transformants of *Parietichytrium* sp., *S. aggregatum,* and *A. kerguelense* have not been obtained yet. It is possible that comparatively thicker cell walls of these species than that of *Aurantiochytrium* prevent the attack by *A. tumefaciens.*

Keywords: *Aurantiochytrium*, *Parietichytrium*, stramenopiles, GFP, transgenic

Poster No. 163

The genetic mechanisms of morphogenesis of single cell organisms

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Protists compile a significant amount of life on earth, and there is a great diversity in protist morphology and evolutionary origin. But what are the genetic mechanisms behind this diversity, and what are the molecular mechanisms involved in the morphogenesis of single cell organisms? Here we present first results from an initiative to dissect the gene expression pattern and localization of mRNA and regulatory RNA in single cells. By cutting *Acetabularia acetabulum* (class Ulvophyceae), into pieces, and utilize quantitative transcriptomic approaches, we aim at obtaining a greater understanding of the transcripts involved in creating its characteristic umbrella shaped morphology.

Poster No. 164

Evolution of gene regulation in nature's smallest nuclear genomes

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Cryptophytes are unicellular algae derived from secondary endosymbiosis, i.e., the uptake of a photosynthetic eukaryote by a heterotrophic cell. Having retained the nucleus of the ingested red alga, cryptophytes are structurally and genetically complex. Each cell contains two cytoplasmic compartments, four genomes, and a plastid contained within four organellar membranes. The genome of the relict red algal nucleus (the 'nucleomorph') is highly reduced: that of *Guillardia theta* is a mere 551 kb and contains 487 genes. How control over gene expression is accomplished in such ultra-compact genomes is not known. To explore the process of gene regulation in the nucleomorph, we studied small regulatory RNAs (sRNAs) in *G. theta* by deep sequencing. We identified thousands of 20-40 nt sRNAs mapping to the nucleomorph genome of *G. theta*. Analysis of RNA secondary structure and conserved sequence motifs identified genes encoding U1, U2 and U4 spliceosomal RNAs, shedding light on the nucleomorph mRNA splicing process. To identify novel regulatory sRNAs, reads were mapped individually to the *G. theta* nuclear, nucleomorph, plastid and mitochondrial genomes. This revealed host nuclear-encoded tRNAs to be a major source of sRNA production. These tRNA-derived fragments (tRFs) derived mainly from the 3' half of mature tRNA and displayed specific isodecoder preferences. In addition, distinct populations of tRFs originating from organelle-encoded tRNAs were identified. Ongoing analyses aim to examine potential differences in length, cleavage position and read abundances between organelle- and nucleus-encoded tRFs. We are also searching the *G. theta* genomes for candidate targets of tRF-guided gene regulation. Finally, to probe the sRNA repertoire in an algal lineage that has independently undergone secondary endosymbiosis, we are sequencing sRNAs from the chlorarachniophyte *Bigelowiella natans*. Comparative analyses in *G. theta* and *B. natans* will reveal to what extent their independently evolved nucleomorph genomes have converged upon similar sRNA-guided gene regulatory pathways.

Keywords: complex plastid, nucleomorph, small RNA, splicing, tRNA-derived fragment

Poster No. 165

Actin cytoskeleton of dinoflagellate *Prorocentrum minimum*: a new look at the organization and functioning

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The actin cytoskeleton (spatial organization, protein form, functions) demands more precisely studying in the cells of dinoflagellates from different taxonomic groups despite of the presence of some works (Roberts and Roberts 1991; Roberts et al., 1992; Soyer-Gobillard et al. 1996; Villanueva et al., 2014). After examination of organization of microtubular cytoskeleton we investigated distribution of different forms and some aspects of functioning of actin in the cells of dinoflagellate *Prorocentrum minimum*. The filamentous actin was stained by TRITC-phalloidin, unpolymerized actin – fluorescent deoxyribonuclease I. The microtubular cytoskeleton was examined with using of transmission electron microscopy and immunofluorescent labeling with anti-α-tubulin monoclonal mouse antibodies. The fixation procedure with using of methanol was applied that provided the better dye penetration and the absence of interfering autofluorescence and allowed us to use fluorescent staining. According to our results that correspond with literature data (Schnepf et al., 1990) cortical microtubular cytoskeleton is absent in *P. minimum* cells. F-actin is mainly distributed in the cortical region of the cytoplasm and in the central area adjacent to the nucleus. Labeled monomeric actin is determined in the cytoplasm and nucleus, with very intensively stained nucleolus; in some cells the most intense signal was observed in the nucleus on the whole. The chromosomes are surrounded by the actin meshwork. The supposed participation of actin in the process of ecdysis was tested by treatment with actin-depolymerizing agent latrunculin A with subsequent high-speed centrifugation inducing cell covering shedding. Decreasing of ecdysis level in the treated sample allows us to assume participation of F-actin in the amphiesma change in *P. minimum* cells.

Funded by the Russian Science Foundation, project 16-14-10116.

Keywords: actin, cytoskeleton, dinoflagellate, *Prorocentrum minimum*

Poster No. 166

Cryptic sex in Euglenozoa? – Detection of genes involved in meiosis

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The phylum Euglenozoa comprises euglenids, kinetoplastids (bodonids and trypanosomatids), diplonemids and symbiontids. While no persuasive evidence for meiosis, sex or any mechanism of genetic exchange exists for euglenids, diplonemids and symbiontids, various lines of evidence for meiosis including the presence of meiosis-spefic genes exist in trypanosomatids. The aim of this study was to find the possible homologs of meiosis-specific and meiosis-related genes in various euglenozoans using several bioinformatic methods. We also conducted phylogenenetic analysis of each studied gene. We were able to identify many meiotic genes in various euglenozoan species suggesting cryptic sex in these species.

Keywords: Euglenozoa, meiosis, meiotic genes

Poster No. 167

Mitochondrial genome of *Vannella croatica* (Amoebozoa, Discosea, Vannellida)

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The Amoebozoa clade combines no less than 2,500 species. Of them, transcriptomic data are available for about 80 species, while mitochondrial genomes - just for 9 species. Those are *Acanthamoeba castellanii* (41,6 kbp), *Acanthamoeba polyphaga* (39,2 kbp), *Dictyostelium discoideum* (55.6 kbp), *Dictyostelium citrinum* (57.8 kbp), *Dictyostelium fasciculatum* (54.56 kbp), *Physarum polycephalum* (62.9 kbp), *Balamuthia mandrillaris* (41,7 kbp), *Vermamoeba vermiformis* (51,6kbp) and *Paramoeba pemaquidensis* (48,5 kbp). Hence the mitochondrial genomes are not yet sequenced even for all major groups of Amoebozoa and the available amount of data does not allow any evolutionary analysis. A complete mitochondrial genome sequence of amoeba *Vannella croatica* (Amoebozoa, Discosea, Vannellida) was obtained using pulse-field gel electrophoretic isolation of the circular mitochondrial DNA, followed by the next-generation sequencing. The mitochondrial DNA of this species has the length of 28,933 bp and contains 13 protein-coding genes, 2 ribosomal RNAs and 16 transfer RNAs. *V. croatica* mitochondrial genome is relatively short compared to other amoebozoan mitochondrial genomes, but is rather gene-rich and contains significant number of open reading frames. Its genome has two duplicated methionine and lysine tRNA genes, which functional significance is not clear. Gene regions of the mitochondrial genome in *V. croatica* contain numerous TAA and a few TGA stop codons within the gene sequence. As suggested by Zlatogursky et al. in amoebae mitochondrial genome TGA termination codon is probably translated as tryptophan. As for TAA codon, we suggest that in *Vannella* mitochondrial genome it may also be a coding one or this site can be further modified during post-translational RNA editing processes. The order of genes in the mitochondrial genome of *V. croatica* also significantly differs from that in other sequenced mitochondrial genomes of Amoebozoa.

Supported with RSF 14-14-00474 (concept and NGS) and RBRF 16-34-60111 grant (bioinformatics).

Keywords: Amoebozoa, Vannellidae, mitochondrial genome, gene order, mitochondrial DNA

Poster No. 168

Gene regulatory regions in *Monocercomonoides*

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Regulation of gene expression is a key ability of every single cell in its development, differentiation and homeostasis. On the other hand, rather sparse amount of information is available for protists and our understanding of regulation of gene expression in eukaryotes is limited to a few model organisms. Our research is aimed at oxymonads, poorly studied group of anaerobic protists, which inhabit digestive tract of some animals. We take advantage of the first complete genome sequence of an oxymonad (*Monocercomonoides* sp. PA203) and characterize structure of promoter regions, 5' untranslated regions and annotate basal transcription and translation initiation factors. Our results are compared to the closest studied relatives – *Trichomonas vaginalis* and *Giardia intestinalis*.

We have identified several conserved motifs in promoter regions of *Monocercomonoides*, including TATA box and TATA-like motif. These motifs potentially play a role in the transcription regulation. 5' untranslated regions are relatively short (typically 20–30 nucleotides) and GC content in these regions is low compared to model organisms. In selected genes, the quality of the automatic prediction of UTR was verified by RACE. We have annotated sets of basic transcription (23 proteins) and translation initiation factors (30 proteins) in *Monocercomonoides* genome. These sets are similar to sets annotated in the genome of *Trichomonas vaginalis* and larger than sets in the genome of *Giardia intestinalis*.

Keywords: gene regulatory region, oxymonads, transcription factor, translation factor, 5' UTR

Poster No. 169

Developing *Corallochytrium limacisporum*, an enigmatic unicellular opisthokont, as a new model organism

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Understanding how evolution has been remodeling more simple organisms into more complex forms of life, is key to understanding the diversity of lifestyles that we see nowadays.

Different and complementary approaches to unravel the course of evolution are necessary to tackle this big question. Genome data is, undoubtedly, the first approach to unravel that diversity and biology, but it is only with the development of molecular tools to experimentally test different hypotheses and obtain functional answers, that we can fully understand this transition.

The eukaryotic clade known as Opisthokonta is an ideal group to address many biological and evolutionary questions, since it comprises two of the most complex eukaryotic lineages, our own animal clade, and Fungi. One of the earliest branching lineages among opisthokonts is the Corallochytrea, with only one taxon described so far: the free-living marine *Corallochytrium limacisporum*, isolated from coral reefs. *C. limacisporum* was first described as a fungus, but molecular phylogenies situated it within the clade that comprises animals and their closest unicellular relatives. Thus, this species is key to understanding animal origins.

We have here developed for the first time essential molecular tools in *C. limacisporum*. Protocols for robust transient and stable transfection have been already established allowing now further steps into genome editing in this organism and therefore allowing us to address more specific biological questions, which will be discussed.

Keywords: molecular tools, *Corallochytrium limacisporum*, stable transfection

Poster No. 170

The encystment-related genes of *Pseudourostyla cristata* and its regulation mechanism were analyzed by comparative transcriptomics

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Many species of protozoa ciliates encyst when they encounter adverse situation. Ciliates encystment may be attributed to the differential expression of genes. Here, this study systematically compared signaling pathways and genes related encystment of *Pseudourostyla cristata* using transcriptomics. Through comparatively analyzed expression genes in resting cysts and vegetative cell of *Pseudourostyla cristata* by RNA – Seq, we obtained 41,965,669 total unigenes and 2,582 differential unigenes. Compared with the vegetative cell, the cyst cell had 316 up-regulation unigenes and 2266 down-regulation unigenes. GO annotation showed 36,934,220 unigenes and 2,519 differential unigenes had GO terms. Among 2,519 differential unigenes, the resting cyst had 430 up-regulation terms and 2,317 down-regulation tems compared to the vegetative cell. These different terns mainly involved cell wall synthesis, intracellular material transportation and the ribosome organelles, etc. KEGG analysis showed that these genes were mapped to 284 differential pathways, compared with the vegetative cell, the resting cyst had 73 heighten pathways and 279 weaken pathways. We find out many important differential genes in these pathways, such as calcium transport signaling pathway existed 24 differentially expressed genes, of which 23 genes were down-regulated and 1 gene was up-regulated in the resting cyst compared with vegetative cell. There were 18 differentially expressed genes in protein degradation depended ubiquitin signaling pathway, and compared with the vegetative cell, the resting cyst had 16 genes expression down-regulated and 2 genes expression up-regulated. Most differentially expressed genes in these two signaling pathways were down-regulation, so the signal effects of these two pathways were weakened. These data indicating that the resting cyst and the vegetative cell had significantly different gene expression profiles, which provided important information for revealing the mechanisms of the encystmen and resisting adversity.

This work was supported by the National Natural Science Foundation of China (31672247).

Keywords: *Pseudourostyla cristata*, encystment, transcriptomics, differentially expressed gene, signaling pathway

Poster No. 171

Morphology, morphogenesis and molecular phylogeny of a soil ciliate, *Pseudouroleptus caudatus caudatus* Hemberger, 1985 (Ciliophora, Hypotricha), from Lhalu Wetland, Tibet

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Pseudouroleptus caudatus caudatus Hemberger, 1985, a soil ciliate isolated from Tibet, was studied in vivo and after protargol impregnation. The Tibetan population is mainly characterized by: elongate body with narrowly rounded anterior end and tapered posterior end; length of buccal area relative to body length ca. 20–25%; cortical granules colourless, round, densely distributed throughout sub-pellicular layer of cell; one parabuccal cirrus; post-peristomial cirrus lacking in 75% of specimens analyzed; left and right ventral rows commence at same level; four dorsal kineties; 3–6 inconspicuous caudal cirri; two macronuclear nodules; 2–7 micronuclei; contractile vacuole located at about 33% of body length near left margin. Morphogenesis is characterized by: (1) parental adoral zone of membranelles retained completely; (2) anterior segments of streaks VI and IV and the whole of streak V form the anterior, middle, posterior segments of the mixed row, respectively; (3) right ventral row originates de novo in both daughter cells; (4) marginal rows develop intrakinetally; (5) dorsal kinety anlage 3 develops de novo in the proter and intrakinetally in the opisthe; and (6) the two macronuclear nodules fuse into a single mass which then divides. Molecular phylogenies corroborate the morphological identification and support the close relationship between *Pseudouroleptus* and *Strongylidium*.

This work was supported by the National Natural Science Foundation of China (project numbers: 31372148 to C. Shao and H. Ma, 31172041 to C. Shao and Z. Yi) and the China Scholarship Council, which funded an extended visit by the principal author to North Carolina Central University, USA. We are grateful to the associate editor Helmut Berger for helpful critical suggestions and comments on the manuscript.

Keywords: Hypotricha, morphogenesis, phylogeny, *Pseudouroleptus caudatus caudatus*, taxonomy

Poster No. 172

Molecular mechanisms of endosymbiosis between *P. bursaria* and *Chlorella* spp.

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Endosymbiosis is one of the major forces driving the evolution of eukaryotic cells, which has occurred multiple times in different lineages during the evolutionary history. However, the initial process of how the endosymbiosis is formed and established in the host cell is still unclear. Here, we use the ciliate *Paramecium bursaria* and the algae *Chlorella* spp. to study the early stage of endosymbiosis. The *P. bursaria* can establish endosymbiosis with various *Chlorella* species. Previous studies have shown that there are some key cytosolic events that establish endosymbiosis. However, the molecular mechanisms or genes responsible for initiating and establishing the relationship between *P. bursaria* and *Chlorella* spp. remain largely unknown. We performed infection experiments of two *P. bursaria* aposymbiotic strains with different *Chlorella* species and found that these two *P. bursaria* strains exhibited different abilities of establishing stable endosymbiosis. Currently we are comparing the genomes of these two strains in order to identify possible genes contributing this difference. In addition, we are interested in the evolutionary trajectories of how chlorella establishes stable endosymbiosis with *P. bursaria*. We have observed that different strains of *Chlorella vulgaris* had different infectivities toward the same aposymbiotic *P. bursaria* strain. By experimentally evolving the chlorella strain with low infectivity to enhance its infectivity, it allows us to dissect the mutations that contribute to chlorella infectivity. Our studies would provide more insights on the molecular mechanisms of incipient stages of endosymbiosis.

Keywords: endosymbiosis, comparative genomics, molecular biology

Poster No. 173

Ancient divergence, and recent diversification of peridinin plastid genomes

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The plastid genomes of peridinin-containing dinoflagellates are highly unusual, retaining only twelve protein-coding genes. Previous studies of peridinin plastid genes have found evidence for unusual evolutionary features, including novel insertions and open reading frames, use of alternative translation initiation codons, and the loss of otherwise conserved residues. However, placing these events in an evolutionary context has been limited by the amount of sequences available, with effectively complete genomes only available for three peridinin dinoflagellate species. We have identified dinoflagellate plastid mRNAs from transcriptome data from NCBI and MMETSP, vastly increasing the number of sequences available. We have used this data to document the evolutionary changes that have occurred in peridinin plastids. The origin of dinoflagellate plastids was accompanied by extremely divergent evolution, specifically underpinned by a change in the selective factors acting on early dinoflagellates. These divergent sequence features have continued to accumulate in individual dinoflagellate lineages. We show that this divergent evolution has been biased throughout dinoflagellate history to photosystem I genes, and the stromal faces of peridinin plastid proteins, suggesting possible evolutionary drivers for the extraordinary events seen in this plastid lineage.

Keywords: dN/dS, chloroplast genome, minicircle, algae, RNA editing

Poster No. 174

The hybrid nature of the *Bigelowiella natans* photosynthetic antenna system: evolution, function and regulation

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Bigelowiella natans is a mixotrophic flagellate and member of the chlorarachniophytes (Rhizaria), whose plastid is derived from a green algal endosymbiont. With the completion of the *B. natans* nuclear genome we are able to begin the analysis of the structure, function and evolution of the photosynthetic apparatus. *B. natans* has undergone substantial changes in photosystem structure during the evolution of the plastid from a green alga. While Photosystem II (PSII) composition is well conserved, Photosystem I (PSI) composition has undergone a dramatic reduction in accessory protein subunits. Coinciding with these changes, there was a loss of green algal LHCI orthologs. There are also a collection of LHCX-like proteins, which are commonly associated with stramenopiles and other eukaryotes with red algal-derived plastids, along with two other unique classes of LHCs— LHCY and LHCZ— whose function remains cryptic. To understand the regulation of the LHC gene family as an initial probe of function, we conducted an RNA-seq experiment under a short-term, high-light (HL) and low-light stress. Several LHCII transcripts were down regulated under HL and up-regulated following a shift to very-low (VL) light, as is common in antenna specializing in light harvesting. Many of the other LHCII and LHCY genes had a small, but significant increase in HL and most were only moderately affected under VL light. The LHCX and LHCZ genes, however, had a strong up-regulation under HL-stress and most declined under VL-light, suggesting that they primarily have a role in photoprotection, as suggested for the LHCX family in stramenopiles. The *B. natans* light-harvesting antenna system appears to be a hybrid between green algae, from which the plastid evolved, and the stramenopiles, where many of the photoprotective functions may have been acquired.

Poster No. 175

Reassessment of the evolution of light harvesting complex superfamily during plastid diversification using non-alignment approaches

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Light-harvesting complex (LHC) and light-harvesting complex-like (LIL) proteins in photosynthetic eukaryotes make up a diverse protein family that is thought to have evolved from small membrane proteins acquired from cyanobacterial endosymbionts during plastid evolution. All members of the LHC/LIL protein family contain a recognizable chlorophyll-binding domain and are likely targeted to the thylakoid membrane inside the plastid. The LHC/LIL protein family is structurally diverse, having one to four predicted transmembrane helices with one or two well-conserved chlorophyll-binding domains. Studying the evolution of this family as it relates to the diversification of light harvesting and photoprotection during plastid evolution is challenging because of high structural diversity and the small size of the proteins. Initial clustering of the family members, as a prelude to multiple sequence alignments, is often based on the number of chlorophyll-binding domains and transmembrane helices. This assumes that all LIL proteins with such structural similarities are directly related through gene duplication or speciation. This assumption may be violated in protein families with multiple functional or structural domains where internal duplications or domain shuffling might have introduced non-homologous regions. To address these issues, we took two approaches to reduce the effect of researcher selection bias. First, we used all-vs-all BLAST queries and protein similarity networks to identify true homologs, and as such which should go in a multiple sequence alignment-based phylogeny. Second, we used the MEME suit for motif discovery and subsequent phylogenetic analysis of the protein family based on short motif presence-absence data without using multiple sequence alignments. We will discuss the potential of using these non-alignment approaches for analyzing diverse protein families, with a focus on the LHC/LIL family for studying the evolution and diversification of photosynthesis and plastids.

Poster No. 176

ADHE enzymes in *Entamoeba*: Molecular & biochemical characterization

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Amoeboid protists taxonomic studies have transitioned from single morphological traits (pseudopodia), to single gene (SSU rRNA) phylogenies. Single-gene analyses of metabolic traits (e.g. alcohol dehydrogenase ADHE) contribute to conflictive phylogenetic depictions, due to horizontal acquisition (horizontal gene transfer, HGT) of genes from prokaryotes and/or unicellular eukaryotes. The intestinal pathogen *Entamoeba histolytica* lacks mitochondria and derives energy from the fermentation of glucose to ethanol. The last two steps of this pathway are catalyzed by *E. histolytica* alcohol dehydrogenase 2 (EhADH2), which belongs to the ADHE family. ADHE bifunctional enzymes have separate N-terminal aldehyde dehydrogenase (ALDH) and C-terminal alcohol dehydrogenase (ADH) domains. All *Entamoeba* ADHE protein sequences (*E. dispar, E. terrapinae*, *E. invadens*-IP-1*, E. invadens*-VK-1:NS, *E. moshkovskii*, and *E. histolytica)* branch together next to a cohesive cluster of low G+C Gram positive and g-proteobacteria. We characterize *E. invadens* IP-1 and VK-1:NS and *E. dispar* ADHE enzymes and compare them to EhADH2. The result shows a similar binding mechanism of acetyl-CoA to the ALDH domain among all three enzymes suggesting a similar evolutionary origin. However, because all four enzymes show different binding affinities for acetaldehyde to the ADH domain, selective pressures within specific host environments and genetic variability might have influenced the adaptations of ADHE homologs to diverse ecological niches (i.e. genetic adaptation to anoxic conditions in the vertebrate/invertebrate gut). The ADHE enzymes are potential targets to better manage amebiasis, due to their essential role in *Entamoeba* survival. We demonstrate that synthetic pyrazolines, natural extracts and commercial compounds can be investigated as inhibitors of both, enzymatic activities and trophozoite growth.

Keywords: alcohol dehydrogenase ADH, aldehyde dehydrogenase ALDH, horizontal gene transfer HGT, growth inhibition, amoebiasis

Poster No. 177

Leishmania species identification by High Resolution Melting (HRM) analysis based on hsp70 nucleotides sequence

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Leishmaniases are considered endemic in 98 countries of five continents. The disease affects 12 to 14 million people worldwide and about 350 million people are at risk of infection. In Brazil, to date, eight *Leishmania* species are involved in human infections, with extensive distribution in the country. The wide clinical spectrum of the disease is the main factor that makes difficult an accurate diagnosis and the correct identification of the *Leishmania* species involved is very important to allow the design of treatment strategies and generate important data for epidemiological and ecological studies. Here, we used High Resolution Melting methodology (HRM) as a practical and robust tool for identifying Brazilian *Leishmania* species, using *hsp70* coding sequence as target. Dissociation curves of 3 different amplicons of *hsp70* based real-time PCR products accurately produced distinct melting profiles, that enable the identification of the reference strains (*L.) infantum chagasi*, *L. (L.) amazonensis*, *L. (L.) mexicana*, *L. (V.) lainsoni*, *L. (V.) braziliensis, L. (V.) guyanensis*, *L. (V.) naiffi* and *L. (V.) shawi*. DNA from *Trypanosoma cruzi*, *T. brucei*, *Crithidia fasciculata* and non infected BALB/c mice were also tested and produced distinct HRM profiles. Less than one parasite can be detected per reaction. To validate the diagnostic potential of the strategy, DNA from naturally infected sandflies, experimentally infected mice and human biopsies were also tested, and the results confirmed the identification obtained by other techniques. *Hsp70*-HRM analysis is a low cost, easy to apply, reliable, potentially automated procedure that is an interesting alternative for the detection, quantification and identification of *Leishmania* species in biological and clinical samples.

Keywords: *Leishmania* identification, polymerase chain reaction, DNA melting profile, nucleotide diversity

Poster No. 178

Otto Bütschli and the Russian protozoological school

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Johann Adam Otto Bütschli (1848–1920) – professor of zoology and paleontology at Heidelberg University, the world-renowned German zoologist-protozoologist and the eminent cytologist made an epoch in investigation of unicellular organisms, first of all in Ciliophora studies. Bütschli was the first to recognize and to demonstrate that conjugation of ciliates was not a reproductive process, but a sexual reorganization similar to fertilization. Protozoa's studies culminated by him in a three-volume monograph (1880–1889), a critical review of the whole field that included much original works made by Bütschli himself. He was the first to identify and order sequentially the stages of nuclear division (mitosis) in several types of animal cells. Bütschli clearly illustrated dividinig cells and noted what he called rodlets (chromosomes) that made up the «nuclear plate». He drew many conclusions from his studies, some of which were well ahead of his time. Prof. Bütschli was not only a great scientist, but excellent teacher. He has grown up several generations of pupils and followers. Among of them were such well-known German biologists as Friedrich Blochmann, August Schuberg, Richard Goldschmidt, Robert Lauterborn and Clara Hamburger. More than 40 Russian students and scientists studied and worked at his institute and in many respects roots of protozoology and zoology of invertebrate in Russia at the beginning of the XX century were connected with the Heidelberg's soil (Fokin, 2013). Wladimir T. Schewiakoff (1859–1930), professor of zoology in St. Petersburg and Irkutsk universities and in Imperial Women Pedagogical Institute as well as vice-minister of the Ministry of Public Education of Russia studied in Heidelberg in 1885–1889. In many respects he became the leader of the national protozoology. Prof. Schewiakoff made a basement of the protozoological school in Russia, then it was developed by his best student Prof. Valentine A. Dogiel (1882–1955).

Keywords: protozoological school, Otto Bütschli, Wladimir T. Schewiakoff, Valentine A. Dogiel

Poster No. 179

Separation and sorting of microbial cells using microfluidic size-based particle separation technology

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The decrease in the cost of massive parallel sequencing is transforming the microbial sciences. Sample preparation is now one of the key limiting factors. Hydrodynamic microfluidic platforms have been proven to be useful and versatile for precisely sorting cells based on their physicochemical properties. We have applied microfluidic size-based particle separation technology with applications in cell separation and enrichment. In comparison with the flow cytometry platform for high-throughput cell sorting, we show that the advancements in microfluidics not only enable cell sorting in a simple unit but also facilitate operation on fragile cell samples with real-time sequencing capabilities. Separation and sorting of micron-sized particles or cells has great potential in diagnostics and biological analyses, food processing and environmental assessment.

Keywords: microfluidic, flow cytometry, cell sorting, real-time sequencing

Poster No. 180

Evolution of aggregative multicellularity in the sorocarpic amoeba *Acrasis kona* (Heterolobosea, Excavata)

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Aggregative multicellularity (also termed sorocarpic multicellularity) has evolved independently many times among both eukaryotes and prokaryotes. When starved or exposed to environmental stress, the cells can cooperate to form fruiting body (sorocarp) consisting of resilient cysts or spores. Among eukaryotes, this type of quasi-multicellular behavior is well studied in dictyostelids (e.g. Dictyostelium discoideum). *Acrasis* species are common soil microbes frequently encountered in the surveys for the "sorocarpic amoebae", and they were long considered the primitive sister taxon to dictyostelids. However, Acrasids belong to one of the earliest major branches of eukaryotes, the excavates, for which there is very limited molecular data and a high potential for the discovery of genetic novelty. We have completed sequencing and annotation of the *A. kona* nuclear genome. We are particularly interested in gene evolution and regulation associated with the development of aggregation. This will be done by comparing genomic data from aggregating versus non-aggregating amoebas. RNAseq analysis will be conducted from the main stages of the *A. kona* life cycle (vegetative growth, starvation, aggregation and excystment). With the extensive experimental literature on dictyostelids and other species, those will serve as guide and comparison in our study of analogous processes in Acrasis, revealing how similar mechanisms have evolved independently and if there are common features they might share.

Keywords: multicellularity, aggregation, comparative genomics, acrasids, dictyostelids

Poster No. 181

Insights into the genome of *Metchnikovella incurvata* (Metchnikovellidae), an early branching member of the parasitic Microsporidia

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Metchnikovellidae is a lineage of highly specialized hyperparasites, which infect and reproduce inside gregarines (Apicomplexa) inhabiting marine invertebrates. They have been known for decades, but their phylogenetic affiliation has been under constant discussion. Confirming some early predictions, the recent analysis of the first almost complete genome for a metchnikovellid, *Amphiamblys* sp., placed them as deeply branching Microsporidia, a lineage of extremely reduced parasites forming the phylogenetic clade of Opisthosporidia together with aphelids (Aphelida) and rozellids (Rozellosporidia = Cryptomycota) in Holomycota. We have obtained the partial genome of the metchnikovellid *Metchnikovella incurvata* through single-cell genomics techniques, by isolating, whole genome amplifying, and sequencing DNA from a single infected gregarine parasitizing a polychaete of the Kandalaksha gulf in the White sea. After decontaminationg of bacterial and host sequences, we assembled and annotated the genome. We have carried out phylogenomic analysis using a multigene dataset, which included *Amphiamblys sp*. Our results confirm that metchnikovellids are the earliest known branch of Microsporidia. However, although *Amphiamblys sp.* and *M. incurvata* form a unique basal group within Microsporidia, they are significantly divergent from each other. The comparative genomic analysis of these two metchnikovellids (the only two obtained to date) with those of more diverged Microsporidia and sister groups provide information about how genome and functional reduction occurred during the evolution of this lineage of highly reduced parasites.

Keywords: phylogenomics, single-cell genomics, Microsporidia

Poster No. 182

Insights into an extensively fragmented eukaryotic genome: de novo genome sequencing of the multi-macronuclear ciliate *Uroleptopsis citrina*

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Genome evolution in ciliated protists has to be interpreted in light of their unusual genome architecture (the presence of a somatic macronucleus and a germline micronucleus in each cell). More interestingly, large-scale genome rearrangements, including fragmentation, DNA elimination, and DNA amplification, occur during macronuclear development, which results in macronucleus genomes fragmented to different extents. However, compared to the high species diversity of ciliates, genomic research has been limited to only a few species due to DNA contamination and obstacles in cultivation. Here we introduce an effective and efficient method combing telomere-primer PCR amplification and high throughput sequencing, which can reduce DNA contaminations, amplify the fragmented genome, and obtain the genome data efficiently. Based on this method, we report the genome of a multi-macronuclear ciliate, *Uroleptopsis citrina*. 1) The telomeric sequence in *U. citrina* are confirmed to be (C4A4)2C4 by directly blunt-end cloning. 2) Genomic analysis of the resulting chromosomes shows a "one-gene one-chromosome" pattern, with a small number of two and three gene chromosomes. 3) Chromosomal analysis shows the existence of an obvious asymmetrical GC skew and high AT bias in the subtelomeric regions of sense-strand, with the detection of an 11bp high AT motif region in the 3' subtelomeric region, which may be related to DNA replication. 4) By focusing on the subtelomeric region, an obvious 40 nt strand-nonsensitive oscillation was found, which may be related to the physical structure of the double helix and responsible for addition of telomere, and initiation of transcription and replication. 5) Compared to *Oxytricha trifallax* and *Stylonychia lemnae*, which each cell has two macronuclei, the multi-macronuclear *U. citrina* is composed of shorter chromosomes. 6) Genome comparison among *Uroleptopsis citrina*, *Oxytricha trifallax*, *Stylonychia lemnae*, and *Tetrahymena thermophila* reveal a core gene pool, which serve as vital factors in the unicellular life of ciliates and are stable at the protein level during evolution. This work provides valuable reference for genomic research and furthers understanding of the dynamic nature of eukaryotic genomes.

Keywords: *Uroleptopsis citrina*, fragmented genome, ciliate, genomic amplification

Poster No. 183

Enzymatic and chemical mapping of nucleosome distribution in purified micro- and macronuclei of the ciliated model organism, *Tetrahymena thermophila*

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Genomic distribution of the nucleosome, the basic unit of chromatin, contains important epigenetic information. To map nucleosome distribution in structurally and functionally differentiated micronucleus (MIC) and macronucleus (MAC) of the ciliate *Tetrahymena thermophila*, we have purified MIC and MAC and performed micrococcal nuclease (MNase) digestion as well as hydroxyl radical cleavage. Different factors that may affect MNase digestion were examined, to optimize mono-nucleosome production. Mono-nucleosome purity was further improved by ultracentrifugation in a sucrose gradient. As MNase concentration increased, nucleosomal DNA sizes in MIC and MAC converged on 147 bp, as expected for the nucleosome core particle. Both MNase digestion and hydroxyl radical cleavage consistently showed a nucleosome repeat length of ~200 bp in MAC of *Tetrahymena*, supporting ~50 bp of linker DNA. Our work has systematically tested methods currently available for mapping nucleosome distribution in *Tetrahymena*, and provided a solid foundation for future epigenetic studies in this ciliated model organism.

Keywords: nucleosome, micrococcal nuclease digestion, hydroxyl radical cleavage, *Tetrahymena*, macronuclei

Poster No. 184

Genetically engineering cyanobacteria into chloroplasts

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Chloroplasts produce the vast majority of oxygen and organic carbon in the biosphere. The ancestry of these photosynthetic organelles has been traced back to a free-living cyanobacterium, which was engulfed and subsequently "domesticated" by a single eukaryotic cell. This ancient endosymbiotic event—called the "primary endosymbiosis"—was a turning point in the history of life, for it allowed eukaryotes to harness photosynthesis, and eventually gave rise to plants and algae. How this symbiosis began and ultimately became permanent is a matter of much speculation, having occured so remotely in evolutionary time (~1 BYA).

Owing to advances in synthetic biology, another approach to understanding the process of endosymbiotic integration is possible. By re-tailoring a cyanobacterium with genes that predispose it to endosymbiosis, the onset of a primary plastid acquisition can be recapitulated. Recently, a strain of the cyanobacterium *Synechococcus elongatus* was engineered to express proteins that allowed it to enter into the eukaryotic cytoplasm, where it continued to grow, divide, and photosynthesize. While the cyanobiont showed no evident harm to human macrophages or embryonic zebrafish, it was not heritable. Therefore, transgenic cyanobionts should be inoculated into protists, which are can transfer endosymbionts over several generations with each cell division.

We present progress in engineering a new primary plastid. a model system for the field of chloroplast evolution, which has amassed a number of hypotheses but currently lacks experimental tools to test them. This work will also provide bioinspiration for a major undertaking in applied biology, which is the bioengineering of synthetic organelles. In principle, synthetic organelles would allow for the installation of bioengineered compartments into natural hosts, as a vehicle for self-contained genetic circuits and biochemistry.

Poster No. 185

Paramecium bursaria sibling species or species complex? Molecular analysis of mitochondrial and nuclear fragments of genome

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Paramecium bursaria (Ehrenberg 1931, Focke 1836) is an archetypical outbreeder, which presumably means that the effective population size is large. The extreme outbreeding strategy may be the reason that the strains of the same syngen (reproductively isolated groups) can be geographically distant but genetically close. The geographic distribution of *P. bursaria* syngens as the other three morphological species i.e. *P. caudatum, P. multimicronucleatum* and *P. aurelia (P. primaurelia, P. biaurelia, P. tetraurelia)* may be regarded as cosmopolitan.

The existence of syngens is the result of the speciation process. The main question is whether it is a complex species structure within *P. bursaria*?

We analysed 70 strains of *P. bursaria* from distant geographical locations. Syngen identification was performed by mating reaction of a studied strain with standard strains representing all the mating types of each syngen. To analyze the degree of speciation within *P. bursaria*, we examined the sequences of nucleotides and constructed phylograms using neighbor-joining and maximum-likelihood methods.

It was shown that *P. bursaria* strains of the same syngens cluster together in all inferred molecular phylogenies. The genetic diversity among the studied *P. bursaria* strains based on rDNA sequences was rather low. The *COI* divergence of *P. bursaria* was also definitely lower than that observed in the *P. aurelia* complex.

The distribution of *P. bursaria* seems to be moderately endemic. The outbreeding strategy, characteristic of *P. bursaria*, has a significant role in reaching new locations, but the genetic conservation of strains belonging to the same syngen makes them geographically isolated from each other. The complex of species serves as a good model to check importance of speciation mechanism – divergence of genomes and reproductive isolation.

Keywords: *Paramecium bursaria*, syngens, molecular analysis, rDNA, COI mtDNA

Poster No. 186

Is there any relationship between *Paramecium bursaria* syngen and the species of endosymbiotic algae? Molecular analysis of a nuclear and plastid genome

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Paramecium bursaria (Ehrenberg 1831) is a ciliate species living in a symbiotic relationship with green algae. The studies objective was to identify endosymbionts of *P. bursaria* and to answer the question whether particular geographical locations of *P. bursaria* strains are related to the specified symbiont species. We analysed 43 symbionts strains of *P. bursaria* based on molecular features. Three DNA fragments were analysed: two of a nuclear genome (a fragment of ITS1-5.8S rDNA-ITS2 region and a fragment of a gene encoding large subunit ribosomal DNA (LSU rDNA) and the fragment of plastid genome containing 3'*rpl36*-5'*infA* genes. The analysis of two ribosomal sequences showed the existence of 28 haplotypes for the ITS1-5.8S rDNA-ITS2 (haplotype diversity Hd = 0.977), 28 haplotypes for the LSU rDNA (haplotype diversity Hd = 0.905) and 36 haplotypes were identified for 3'*rpl36*-5'*infA* genes (Hd = 0.984). The endosymbionts were identified as *Chlorella vulgaris*, *Chlorella variabilis*, *Chlorella sorokiniana* and *Micractinium reisseri*. We rejected the hypotheses concerning: (i) the correlation between *P. bursaria* syngen and the symbiont species; (ii) the relationship between species of a symbiont and geographical distribution; and (iii) the occurrence of the geographical division of symbionts into an American and European group.

Keywords: *Paramecium bursaria*, endosymbiotic algae, molecular analysis

Poster No. 187

New entodiniomorphid ciliates, *Buetschlia minuta* n. sp., *B. cirrata* n. sp., *Charonina elephanti* n. sp., from Asian elephants of Turkey

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Three new entodiniomorphid species, *Buetschlia minuta* n. sp., *Buetschlia cirrata* n. sp., and *Charonina elephanti* n. sp., were described from the hindgut of Asian elephants (*Elephas maximus*) from Gaziantep, Turkey. *B. minuta* n. sp. has an ovoid body shape with a truncated anterior end and a rounded posterior end, an adoral ciliary zone surrounding the cytostome, somatic ciliary rows in the anterior two thirds of the body, an ovoid macronucleus without a constant position, and a concretion vacuole in the anterior one third of the body. *B. cirrata* n. sp. has an ovoid body shape with the anterior end truncated and the posterior end rounded, an adoral ciliary zone surrounding the cytostome, unevenly distributed somatic cilia, an ovoid macronucleus without a constant position, and a concretion vacuole in the anterior one third of the body. *C. elephanti* n. sp. has an ovoid body shape with both ends rounded, an ovoid macronucleus without a constant position, two buccal ciliary zones, an adoral ciliary zone, a vestibular ciliary zone, three somatic ciliary zones, a dorsal ciliary zone, two posterior ciliary zones, dorsal and ventral, and a vestibulum with a Y-shaped infraciliature.

Keywords: Entodiniomorphid ciliate, *Buetschlia minuta* n. sp., *Buetschlia cirrata* n. sp., *Charonina elephanti* n. sp., Asian elephant

Poster No. 188

Rumen ciliate fauna of domestic sheep (*Ovis aries*) in İzmir, Turkey and scanning electron microscopic observations

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Species composition and distribution of ciliates were investigated in the rumen contents of 15 domestic sheep (*Ovis aries*) living in İzmir, Turkey. Twenty-three species and 11 morphotypes belonging to nine genera were identified. The density of ciliates was 118.3 ± 63.5 × 10^4 cells ml^{-1}, and the mean number of ciliate species per host was 13.5 ± 5.3. *Entodinium simulans* was the most abundant species, with a prevalence of 100%. Two morphotypes of *Diplodinium flabellum* were identified, such as *D. flabellum* m. aspinatum and *D. flabellum* m. monospinatum, and *E. simulans* m. dubardi was found for the first time in domestic sheep in Turkey. The scanning electron microscopic observations of *Entodinium longinucleatum*, *E. bursa*, *E. rectangulatum* m. rectangulatum, *E. semahatae*, *E. exiguum*, *E. minimum*, *E. simulans* m. caudatum, *E. dilobum*, *Diplodinium flabellum* m. aspinatum, *D. dentatum*, *Isotricha prostoma*, *Metadinium affine*, *Enoploplastrom triloricatum*, *Dasytricha ruminantium*, *Ophryoscolex caudatus* m. tricoronatus, *Epidinium ecaudatum* m. parvicaudatum, and *Polyplastron multivesiculatum* were studied.

Keywords: ciliate, rumen, SEM, sheep, Turkey

Poster No. 189

Ciliated protozoan fauna in the forestomach of Dromedary camels

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Species composition and distribution of ciliated protozoa obtained from the forestomach of 20 Dromedary camels living in Zawiya, Libya were examined. Nineteen species and 10 morphotypes belonging to eight genera were identified. The mean number of ciliates was 54.2 ± 32.9 × 10^4 cells ml^{-1} in the forestomach contents, and the mean number of ciliate species per host was 6.5 ± 2.9. *Entodinium* and *Epidinium* were the main genera, as these ciliates were found consistently at higher proportions than those of the other genera. In contrast, *Ophryoscolex* and *Polyplastron* were only observed at low frequencies. *Diplodinium rangiferi*, *Entodinium ellipsoideum*, *E. simulans*, and *Polyplastron multivesiculatum* were new endosymbionts recorded from camels.

Keywords: ciliate, dromedary camel, forestomach, Libya, Protoza

Poster No. 190

Molecular mechanisms during food acquisition and gliding locomotion in viridiraptorid amoeboflagellates – a transcriptomic study of *Orciraptor agilis* (Rhizaria)

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The Viridiraptoridae (Rhizaria) are a family of phagotrophic freshwater flagellates, which feed on the protoplasts of green algae. *Orciraptor* degrades the cellulosic cell wall of algae in a well-defined, ring-like pattern, displaces the resulting cell wall disc and subsequently extracts the algal plastid with pseudopodia. Preliminary experiments revealed that *Orciraptor* contains cellulases and pectinases, and that the feeding event involves massive changes in cytoskeletal architecture. Between feeding events, *Orciraptor* displays a flagella-based gliding motility, a still poorly studied locomotory process. Therefore, I intend to explore 1) cell wall degrading enzymes, 2) actin-binding proteins, and 3) motor proteins interacting with microtubules in the viridiraptorid *Orciraptor agilis*. This will be done with a comparative transcriptomic approach and a selection of biochemical methods. My aim is to shed light on the underlying molecular machinery of the above mentioned processes with a focus on carbohydrate-active enzymes involved in cell wall lysis. With this project I expect to provide experimental evidence for enzymatic cell wall degradation in unicellular 'protoplast feeders' with genetic and biochemical methods, to characterise candidate enzymes, and to explore the repertoire of structural and force-generating cytoskeletal proteins involved in feeding and locomotion of viridiraptorid amoeboflagellates.

Poster No. 191

Study on *Pelagovasicola*-like ciliate with sequestered diatom chloroplasts

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Kleptoplasty is the ability of heterotrophic organisms to preserve algal chloroplast through algal predation and partial digestion. In many organisms, it is known that such sequestered chloroplasts maintain their function over a few days to several months. We herein introduce a *Pelagovasicola*-like ciliate (Didinidae, Litostomatea) collected from Lake Biwa, the largest and oldest lake in Japan. This ciliate, which is nearly spherical and about 100 µm in diameter, is filled with hundreds of 2–3 µm ocher-colored granules that impart their color to the whole cell. These granules were indistinct by light microscopy, but examination by TEM showed that they are chloroplasts. Many of the chloroplasts seemed to be at different phases of digestion in food vacuoles (from intact-looking to half-digested), while some chloroplasts did not appear to be inside food vacuoles. These 'naked' chloroplasts were surrounded with three-layered outer membranes. Based on their color and ultrastructure deemed as sequestered diatom chloroplasts, we attempted PCR with diatom *rbc*L-targeted primers from isolated single ciliate cell, which yielded a sequence (662 nt) identical to the centric diatom *Discostella nipponica* (Stephanodiscaceae). Although the diatom nucleus has not been found in TEM observation, diatom nSSU rDNA-targeted PCR from dozens of ciliate cells fruited a 721 nt sequence. This sequence was one-nucleotide different from the diatom symbiont of the dinoflagellate *Peridiniopsis penardii* as well as *D. nipponica*. This result masks from which organism this ciliate sequesters the chloroplasts. In ciliates, kleptoplasty phenomena have been reported in 15 species, but this ciliate would be the first case that retains diatom chloroplasts.

Keywords: kleptoplasty, ciliate, diatom chloroplast

Poster No. 192

Genome and transcriptome draft of the heterotrophic euglenoid *Rhabdomonas costata*

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Euglenoids represent a group of protists with diverse modes of feeding including phagotrophy, osmotrophy, phototrophy and mixotrophy. Until today, there is available neither complete nor partial nuclear genome sequence of any euglenoid. We are trying to partially fill the gap in the sampling of eukaryotic genomes by presenting this genomic and transcriptomic drafts of primary osmotroph Rhabdomonas costata. To avoid bacterial contamination, Rhabdomonas cells were prior to gDNA isolation purified by FACS sorting in combination with laser microdissection and the extracted DNA was subjected to whole genome amplification. Rhabdomonas genomic assembly is too fragmented to be used for genes prediction, nevertheless the comparison of transcriptomic and genomic data allowed us to estimate features of its introns. The set of 39,585 putative Rhabdomonas proteins was predicted from the decontaminated transcriptome. Only 26,052 predicted proteins have any homologue in NCBI and were annotated by KEGG; 16% of these bear recognizable splice leader sequence at their 5'terminus. Annotation of the mitochondrial backbone metabolism provides the first data on Rhabdomonas mitochondrion, which is consistent with our knowledge on the mitochondrion of Euglena gracilis.

Keywords: *Rhabdomonas costata*, intron, splice leader, mitochondrial metabolism

Poster No. 193

Insights into *Anaeramoeba* metabolism and symbiosis via genomics and proteomics

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Anaeramoeba is a recently established lineage of marine anaerobic amoebae and amoeboflagellates with phylogenetic ties to Metamonada. These amoebae have been found to harbour unknown prokaryotic endosymbionts that are intimately associated with the mitochondrial related organelles (MROs) of the amoebae. At the moment, the basis of this endosymbiosis remains unknown. We will seek to determine the complete genome sequence of three *Anaeramoeba* isolates (BMAN, SCHOONER and BUSSELTON) from two different species (*Anaeramoeba ignava* and *Anaeramoeba flamelloides*) and their respective associated endosymbionts via state-of-the art nanopore sequencing. Large-scale culturing of the amoebae has been established and work is underway to establish the taxonomic affiliation and identity of the endosymbionts using 16S single-cell PCR and fluorescence in situ hybridization. These genomes will contribute to increased knowledge about this recently discovered lineage of eukaryotes and enable the use of proteomic techniques to study the dynamics and metabolic profile of the endosymbiosis in detail.

Keywords: genomics, proteomics, *Anaeramoeba*, Metamonada, symbiosis

Poster No. 194

The role of intra macronuclear microtubules in chromatin segregation during formation of Large Extrusion Bodies in *Tetrahymena thermophila* and their programmed nuclear death

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Large extrusion bodies (EBs) were induced in *Tetrahymena thermophila* by treatment of cells with aphidiocolin (APH), followed by shifting them to a drug free medium. The APH induced over replication of macronuclear DNA and reversible cell division arrest (Kaczanowski and Kiersnowska, 2011 Protist 162:616-636). After shifting cells from APH to the drug free medium, a central granule of chromatin (prospective EBs), surrounded by microtubules, developed in macronucleus, prior to the first post treatment division. Subsequently, the chromatin of the central granule appeared in the middle region of the dividing macronucleus during its elongation and it was extruded to the cytoplasm thereafter as the EB. The remaining chromatin segregated to the opposite polar ends of the macronucleus, and was separated from the chromatin by intra macronuclear microtubules, which appeared in the middle region of macronucleus. Next the EBs underwent apoptotic-like degradation and autophagy, similar to that which appear in the Programmed Nuclear Death (PND) of old macronuclei in *Tetrahymena* conjugant cells. It was shown with the TUNEL reaction and within vivo acridine orange staining respectively. Our results led us to two overlapping hypotheses: (i) microtubules play an active role in segregation of minichromosomes to the polar regions of dividing macronucleus, (ii) over replication of DNA with the APH induced appearance of defective DNA copies, which were destined for the EBs and did not segregate to the opposite ends of the macronucleus.

Keywords: extrusion bodies, chromatin segregation, *Tetrahymena*

Poster No. 195

The chlorophyll catabolism in a phycophagic cercozoan *Paracercomonas* sp. strain KMO002: exploring a biochemical/molecular biological approach

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Phycophagic protists ingest cells of microalgae that contain enormous amounts of chlorophylls under illuminated environments, none the less for the significant phototoxicity of these pigments. Recent studies have revealed that many lineages of protists are capable of detoxification catabolism of chlorophylls and produces 13^2,17^3-cyclopheophorbide enols (CPE) as its results. Despite accumulated evidences of CPE production in diverse lineages across supergroups of eukarya, little study has been successfully addressed to identify enzymes and/or genes responsible for on this metabolic process. We isolated a strain of phagotrophic amoeboflagellate (strain KMO002) by micropipetting from a freshwater sample collected in a small reservoir in Japan, and co-cultured with cyanobacterium *Synechococcus elongates* PCC 7942. The molecular phylogenetic analysis of 18S rDNA placed KMO002 in a clade of Cercomonadida, Cercozoa, that exclusively consists of genus *Paracercomonas*. KMO-002 was observed under microscope to actively prey on cells of PCC 7942 as well as other unicellular cyanobacteria and demonstrated to produce CPEs. Interestingly, when it was fed on *Acaryochloris* sp. that dominantly produces chlorophyll *d* instead of chlorophyll *a*, a species of CPE derived in chlorophyll *d*. Because such chlorophyll *d*-producing cyanobacteria are unlikely to be a natural prey in the environment, the results indicate a relatively loose substrate specificity of the enxyme(s) responsible for the CPE catabolism in the *Paracercomonas*. Here, we will also discuss on our transcriptomic analyses on this two-membered culture.

Keywords: Cercozoa, chlorophyll catabolism, cyclopheophorbide enols, phycophagy, two-membered culture

Poster No. 196

Protists in ancient Arctic permafrost: classic and metagenomic approaches

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Permafrost is a unique subsurface complex environment where microorganisms retain viability over a long period of time, from thousands to millions of years. Recently, several new protist species were described, recovered from cysts frozen in permafrost layers. The biggest diversity yielded the Holocene strata of the floodplain deposits of the Gydan Peninsula and the buried soils of the late Pleistocene ice complex on the Kolyma Lowland. In total, we have isolated about 40 strains of viable amoeboid protists. Most of these isolates presumably represent new species to science, several of which have already been described to date.

As a proxy to the overall diversity of protist paleocommunities, we studied six metagenomes from Eurasian Arctic Late-Pleistocene permafrost sediments formed in different conditions. Eukaryotic DNA comprised some 1% of the total, which may be caused by insufficient extraction. In total, members of the supergroups Amoebozoa, Archaeplastida, Alveolata, Stramenopiles, Rhizaria, Excavata, as well as some groups incertae sedis were noted. Autotrophic protists occupied from 3 to 18% of total protist abundance; their proportion was higher in metagenomes from aquatic sediments than in terrestrial ones. Fungi were more abundant in terrestrial samples than in aquatic ones; their diversity was the highest in the sample from freshwater lake and the lowest in the sample of marine origin. Cercozoa (Rhizaria) genes were abundant in all samples. A metagenome of the sample from marine sediments contained a big number of diatom genes. The lowest taxonomic diversity was observed for the sandy marine sediments, the highest – for the freshwater sediments. Community structure revealed in metagenomes corresponded to the conditions of sediment formation.

Support: grants 17-54-150003 and 15-04-05267 (RFBR).

Keywords: Amoebozoa, paleocommunities, permafrost, Arctic, metagenomics

Poster No. 197

Phosphorylation of serine 148 in *Giardia lamblia* end-binding 1 protein is important for cell division

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Giardia lamblia is a unicellular organism, showing a polarity with two nuclei and cytoskeletal structures. Accurate positioning of these organelles is essential for division of *G. lamblia*, which is poorly understood. *G. lamblia* end-binding 1 (GlEB1) protein and *G. lamblia* aurora kinase (GlAK) have been shown to modulate microtubule (MT) distribution during cytokinesis. A direct association between GlEB1 and GlAK was demonstrated. Like GlEB1, GlAK was also found at nuclear envelopes and median bodies of *G. lamblia*. In vitro kinase assays using *Giardia* lysates immunoprecipitated with anti-GlAK antibodies or recombinant GlAK suggested that GlEB1 is a substrate of GlAK. Site-directed mutagenesis indicated that threonine-205 in GlAK was auto-phosphorylated and that GlAK phosphorylated serine (Ser)-148 in GlEB1. Ectopic expression of a mutant GlEB1 (with conversion of Ser-148 into alanine of GlEB1) resulted in an increased number of *Giardia* cells with division defects. Treatment of *G. lamblia* with an AK inhibitor triggered cytokinesis defects, and ectopic expression of a phospho-mimetic mutant GlEB1 (with conversion of Ser-148 into aspartate) rescued the defects in *Giardia* cell division caused by the AK inhibitor. These results suggested that phosphorylation of GlEB1 played a role in cytokinesis in *G. lamblia*.

Poster No. 198

Adaptation responses of individuals to environmental changes in the ciliate *Euplotes crassus*

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Although the response unit of living organisms to environmental changes is at the individual level, most experiments on the adaptation responses of ciliates have been conducted in batches, comprising multiple-individuals, due to their microscopic size. However, here, we confirmed that individuals undergo different division cycles in monocultures of *Euplotes crassus*. They also exhibited transcript variations of 4.63-fold in SSU and of 22.78-fold in Hsp70. Additionally, in salt-stressed *E. crassus* individuals, SSU transcripts of individuals varied by 6.92-fold at 27 psu, 8.69-fold at 32 psu, and 2.51-fold at 37 psu. However, the maximum difference in Hsp70 was only 4.23-fold under all conditions. These results suggest there may be different biological rhythms even in siblings derived from the same parent. It can also be inferred that various environmental factors have different effects on different *E. crassus* individuals. Therefore, to elucidate relationships between organism adaptations and environmental changes, studies at the individual level should be conducted with multi-individual approaches.

Keywords: adaptation, ciliates, environmental change, gene expression, individual difference

Poster No. 199

Mitochondrial genomes of *Euplotes crasuss* and *E. cristatus*: revision of mitochondrial-encoded genes in the genus *Euplotes*

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Euplotes ciliates are common inhabitants of sand interstices of the intertidal zone. They cannot be easily separated on the basis of any morphological characteristic, and there are conflicting views regarding their taxonomy and species status. Mitogenomes for two *Euplotes* have been registered in GenBank. In this study, mitogenomes of undetermined taxa, *E. crassus* and *E. minuta*, was determined, compared and mitochondrial-encoded genes were revised. As results, *Euplotes* mitogenomes were nearly identical in length and gene content. *Euplotes* members strongly support monophyletic clades on the phylogenetic trees constructed with mitochondrial protein-coding genes. The split segments of two rRNA genes was confirmed by the conserved secondary structure of rRNAs. Based on prediction of transmembrane helices and secondary structure, putative split genes were added. In addition, the 18-bp palindromic repeat unit on the central repeat region showed the range of 24 to 56 copies, and the max- and min-copy number were observed for *E. crassus* and *E. minuta*, respectively. These results will enhance our understanding of mitogenomes in ciliates.

Keywords: Ciliates, *Euplotes*, mitogenome

Poster No. 200

Kleptoplasty in foraminifera during the polar night

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Although multichambered foraminifera have never been reported to have own pigments, their cytoplasm often possesses colour. There are several possible sources of such coloration: foraminiferans inhabiting tropical seas tend to form symbiosis with photosynthetic microalgae, the latter giving pigmentation to the host cytoplasm. Foraminiferans from temperate and arctic waters are not known to harbor photosymbionts, however, many species are brightly coloured either due to the digested photopygments of prey algae or to kleptoplasty. Kleptoplasty – a process of sequestering of chloroplasts – has been described in several foraminiferal genera, kleptoplasts being derived from diatoms only. It is debatable for how long a sequestered chloroplast can last in the host cytoplasm since many of its proteins are coded by the algal nuclear genome, destroyed by the foraminifera.

In January 2015 and 2016 we collected living foraminifera in several fjords of Svalbard. All major species (*Nonionellina labradorica, Islandiella helenae, Cassidulina reniforme, Elphidium excavatum, Elphidium bartletti*) possessed species-specific coloration. Epifluorescent microscopy showed the presence of chlorophyll bodies within the cytoplasm of several species. Further investigation with the use of light and transmission electron microscopy revealed abundant kleptoplasts in the cytoplasm of studied specimens. All the observed kleptoplasts were intact, none showed signs of degradation or digestion. During the polar night the only source of kleptoplasts could be the diatom resting spores, nevertheless, we did not find any digestive vacuoles in the foraminiferal cytoplasm or other evidence of active feeding. Therefore, observed plastids should have been retained in host for a long time since the lit period of the year. The function of these organelles in the foraminiferal cell is yet to be determined.

Keywords: benthic foraminifera, polar night, kleptoplasty, cell biology

Poster No. 201

Endosymbionts of *Pelomyxa palustris* and other Archamoebae

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Pelomyxa palustris is a giant anaerobic amoeba, apparently lacking any energy-producing organelles but hosting three different prokaryotic endosymbionts. For a long time the identity of these symbionts has been debated, since they were characterized only by morphological methods. Using DNA extracted from *P. palustris* cysts, we have obtained three SSU rRNA gene sequences, affiliated to three different prokaryotic genera: *Methanosaeta* (methanogenic archaeon), *Syntrophorhabdus* (syntrophic bacterium) and *Rhodococcus* (aerobic chemoorganotrophic actinobacterium). The endosymbionts' identification and available information about metabolic activities of microbes of the corresponding groups allowed us to hypothesize about their possible roles. We suggested that the aerobic bacterium protects the host and other partners from the toxic action of oxygen, while the two other prokaryotes form a syntrophic pair, which helps *Pelomyxa* to get rid of its metabolic waste transforming it to methane. We speculate that the tripartite consortium consisting of *P. palustris* and its endosymbionts resembles to some extent those found in sewage treatment plants. The study of symbiotic consortia in other Archamoebae (9 *Pelomyxa* spp. and *Mastigella nitens*) revealed that they may be either tripartite as in *P. palustris,* or bipartite. None of those species has the same composition as *P. palustris,* although the majority of them also has an actinobacterium and a methanogen. We speculate on the possible differences in metabolism of these amoebae determining their distinct prokaryotic consortia.

Poster No. 202

Developing the ichthyosporean *Creolimax fragrantissima* as an experimentally tractable organism to address evolutionary and cell biological questions

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The origin of multicellular animals from their unicellular ancestor was a pivotal step in the evolution of life. Recent studies on unicellular relatives of animals have revealed that their genome contains genes crucial for metazoan development and multicellularity, including those involved in cell–cell communication, cell proliferation and cell differentiation. What remains unclear is the role of those genes in the unicellular relatives of animals, and how they were co-opted at the onset of Metazoa. To address those questions, we need to perform functional studies on those taxa.

We will here present our work towards development of *Creolimax fragrantissima*, as an experimentally tractable system, to investigate both, the origin of animal multicellularity, as well as its developmental mode through a syncitial stage. *C. fragrantissima* is an ideal organism to be experimentally tractable because of 1) its specific life cycle, which differs from the developmental mode of other close unicellular relatives of animals (such as choanoflagellates and filastereans), 2) its well-annotated genome, 3) preliminary working transgenesis tool, and 4) it is easy to culture.

We have further optimized the tranfection protocol in order to perform functional studies and set up some basis for genome-editing technologies. Moreover, we are currently establishing selection strategies to achieve stable transfection. Progress and the potential implications of our research will be presented and further discussed.

Keywords: origin of multicellularity, unicellular relatives of animals, *Creolimax fragrantissima*, transgenesis tool

Poster No. 203

Genome study of single cellular red alga *Rhodella*

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Red algae (Rhodophyta) played an important role in the evolution of photosynthetic organisms as plastid donor(s) in chlorophyll-c containing algae including cryptophytes, haptophytes, stramenopiles, and alveolates. Most red algae are important components in marine ecosystems contributing as primary producers. Despite of this importance, only several red algal genomes have been reported among more than 7,000 species. From the red algal group of the Rhodellophyceae, there is no fully sequenced genome to date.

To fill the gap of our understanding on red algal genome evolution, here we analyzed a new genome of the red alga *Rhodella*. Using the long-read single molecule sequencing (PacBio) platform, we completed whole-genome sequencing of this species. We discuss the interesting story of red algal genome evolution.

Keywords: red algae, genome, evolution

Poster No. 204

Cells in need of iron. Influence of iron availability on the metabolism of *Naegleria gruberi*

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Iron is a biogenic element essential for all organisms on the Earth. Iron is present in many important enzymes affecting their metabolic activity and becoming a limiting factor for pathogenicity of microorganisms. On the other hand, the presence of excessive amount of iron within the cell leads to Fenton reaction, which is responsible for oxidative stress and cell death.

We decided to study the effect of iron availability on the model free-living heterotrophic amoeba *Naegleria gruberi*, which is close relative to *Naegleria fowleri*, an opportunistic pathogen that causes fatal meningoencephalitis in humans, a disease currently without efficient treatment.

We studied the influence of iron excess and iron depletion on the metabolism of *N. gruberi* by proteomic analysis, metabolomic analysis and spectrophotometric activity measurement of selected metabolic enzymes. Currently the most significant changes between iron excess and depletion were observed in the hemerythrin – a protein with unknown function and enzymes alcohol dehydrogenase and Fe-hydrogenase, suggesting a huge impact on the energy metabolism.

Keywords: iron, metabolism, *Naegleria*

Poster No. 205

From sequencing to genome comparison: an easy to handle automatic pipeline

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An automated workflow of whole genome sequence data enables easy and customizable genome comparisons.

The presented pipeline is designed for genome comparison of different species of Chrysophyceae showing varying nutritional modes. In the course of evolution, Chrysophyceae frequently reduced their plastids and accordingly changed their nutritional mode. Hence, the genome comparison reveals details in dependence of nutrition: essential and optional genes, gene density and arrangement, GC content and genome size. However, it can be easily adapted to other species.

The workflow automates the processing of sequencing data. It applies the Snakemake workflow engine, which automates the use of executable tools and scripts. Usually the work stages including quality control, de novo assembly, gene prediction, gene annotation and gene comparison require time for configuration and interpretation of the intermediate data. The workflow covers these tasks in one step and provides the user with an easy to configure, automated pipeline. The key features are generation of hybrid assemblies from PacBio and Illumina sequencing data, optional filtering of prokaryotic sequences (from non-axcenic cultures) and advanced gene prediction from available RNA sequencing data.

Five strains from the culture collection at the University of Duisburg-Essen were whole genome sequenced using the technologies of Illumina Hiseq XTen and Pacbio RSII. The automated Snakemake workflow was set up to analyse the sequence data. Initially, SPAdes was used to assemble the sequenced reads. Additionaly, in non-axenic cultures the software MaxBin2.0 separated the contigs in eukaryotic and prokaryotic sequences depending on GC content and abundance. If available RNA-Seq data aided the gene prediction process of the programs Tophat2, Augustus, Genemark and Braker. The predicted genes were searched with Diamond against the KEGG database. Finally, gene matches of each species were compared among the different nutritional modes.

This workflow will enable the community to easily perform basic genomic analysis.

Keywords: Chrysophyceae, hybrid assembly, genome comparison, automation, non-axenic cultures

Poster No. 206

Can protist ciliates act as a natural reservoir for bacteria potentially pathogenic for Metazoa? Trans-infection experiments of *Rickettsiales* endosymbionts from the ciliates *Euplotes* and *Paramecium* to the planarian *Dugesia*

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Most of the microorganisms of genera responsible for vector-borne diseases (VBD) has hematophagous arthropods as vector/reservoir. Recently, many new species of such microorganisms were found in various terrestrial and aquatic eukaryotic hosts: numerous new bacterial species belonging/related to the genus *Rickettsia* (*Alphaproteobacteria, Rickettsiales;* with obligate intracellular lifestyle) were discovered in protist ciliates. Although their pathogenicity for either humans or animals is still under study, these bacteria could actually act as etiological agents of possible VBD in aquatic environment. Indeed, an increasing number of massive fish death was recorded in intensive aquaculture facilities during the lasts years due to epidemics caused by *Rickettsia*-like bacteria with unknown natural vector. As ciliates could vector pathogenic organisms possibly responsible for zoonosis, this study was meant to verify the transmission of the *Rickettsiales* endosymbionts hosted by two species of ciliates to a metazoan model, the planarian *Dugesia japonica*. The ciliates were *Euplotes woodruffi* and *Paramecium multimicronucleatum*; the first hosts in the cytoplasm two different *Rickettsiales* endosymbionts, "*Candidatus (Ca)* Megaira polyxenophila" and "*Ca.* Bandiella woodruffii", and the betaproteobacterium *Polynucleobacter necessarius*; the second hosts in the macronucleus a subspecies of the *Rickettsiales* endosymbiont "*Ca.* Trichorickettsia mobilis". Ciliate monoclonal mass cultures were set up for trans-infection experiments via *os*: 1. Washed, concentrated ciliates were homogenized, pelleted, and added to regular food for planarians. 2. Antibiotic-treated planarians were fed on ciliate-enriched food or regular food (control), washed, and let digest for 1, 2, 3, and 7 days. 3. A comparative multidisciplinary investigation through DNA extraction-PCR procedure, and TEM observation was performed. DNA of endosymbionts was recovered up to 7 days after feeding in treated planarians, and we could identified bacteria at TEM in treated planarians' intestine also outside digestive vacuoles, indicating that they somehow are able to avoid animal digestion.

Financial support: PRA 2016, University of Pisa.

Keywords: *Rickettsia*-like organisms, Planaria, trans-infection experiments, ciliates, comparative multidisciplinary investigation

Poster No. 207

PUF proteins in *Giardia intestinalis*

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Regulation of gene expression is a complex process in which DNA and RNA binding proteins play an important role. *Giardia intestinalis,* an anaerobic protozoan parasite, contains highly compact genome with extremely short untranslated regions (UTRs). The regulation of gene expression during giardia cell- and life-cycle has been poorly studied so far. Thus, we have decided to characterize one important factor of the regulation machinery. The 3´UTRs of mRNA mediate the stability as well as the localization of the transcripts. PUF proteins represent family of 3´UTR-binding proteins, which control the function of the target transcripts by their repression, activation or sequestration. These eukaryotic proteins are evolutionarily conserved from protists to metazoans. Each of them contains highly conserved C-terminal domain, which specific binds to 3´UTR of mRNAs. We have identified five PUF proteins in *G. intestinalis* genome. We have confirmed the expression of all genes and localized their products. All five PUFs are naturally expressed in giardia trophozoites and localized in the cytoplasm. Two of these proteins were selected for initial molecular analyses of RNA-protein and protein-protein interactions in *G. intestinalis* using chemical cross-linking followed by affinity purification of biotinylated PUFs. The general aim is the characterization of PUFs in giardia biology with regard to the parasite cell- and life-cycle.

Poster No. 208

Characterization of a phospholipase C-like protein (TbPI-PLC2) from *Trypanosoma brucei*

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Trypanosoma brucei is the causative agent of African Trypanosomiasis, a deadly disease affecting humans and cattle. There are very few drugs to treat the disease and evidence of mounting resistance raises the need for new drug development. The inositol 1,4,5 triphosphate/diacylglycerol (IP_3/DAG) pathway regulates important processes in many organisms. *T. brucei* has an active IP_3 receptor, localized to the acidocalcisome, that is essential for infection in mice. In previous work (King-Keller et al., *Eukaryot. Cell* 14:486-494, 2015) we characterized a phosphoinositide phospholipase C (TbPI-PLC1, Tb927.11.5970) from *T. brucei* that contains a domain organization characteristic of PI-PLCs, such as X and Y catalytic domains, an EF-hand calcium-binding motif, and a C2 domain, but lacks a pleckstrin homology (PH) domain. In addition, TbPI-PLC1 contains an *N*-terminal myristoylation consensus sequence only found in trypanosomatid PI-PLCs. Here we report the presence of a second PI-PLC-like protein (TbPI-PLC2, Tb927.6.2090) that is very similar to TbPI-PLC1 but lacks the Y catalytic domain and the C2 domain and possesses instead a PDZ domain. Recombinant TbPI-PLC2 hydrolyzes neither phosphatidylinositol (PI) nor phosphatidylinositol 4,5-bisphosphate (PIP_2), and does not modulate TbPI-PLC1 activity. However, knockdown of *TbPI-PLC2* expression alone or together with downregulation of *TbPI-PLC1* expression by RNAi resulted in growth inhibition. This is in contrast with the lack of effect of downregulation of expression of *TbPI-PLC1* alone. TbPI-PLC2 has a plasma membrane and intracellular localization and it might be involved in IP_3 binding as has been reported for the phospholipase C-related catalytically inactive protein 1 (PRIP-1) of mammalian cells (Uji et al., *Life Sci* 72:443-453, 2002). The PDZ domain could be involved in this binding and this is being investigated.

Poster No. 209

Changes in fatty acid composition and docosahexaenoic acid (DHA) content of the heterotrophic dinoflagellate *Oxyrrhis marina* fed on different prey species

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The heterotrophic dinoflagellate *Oxyrrhis marina* is known to produce high levels of docosahexaenoic acid (DHA) when fed on diverse algal prey. Large-scale culturing of algal prey species is not easy and requires a large amount of budget, and thus more easily cultivable and low-cost prey is required. Dried yeast was selected as a strong candidate in our preliminary tests. We explored the fatty acid composition and DHA production of *O. marina* fed on dried yeast and compared these results to those of *O. marina* fed on two algal prey species: the phototrophic dinoflagellate *Amphidinium carterae* and chlorophyte *Chlorella* sp. powder. *O. marina* fed on yeast, which does not contain DHA, produced the same high level of DHA as those fed on DHA-containing *A. carterae.* This indicates that *O. marina* is likely to produce DHA by itself regardless of prey items. Furthermore, the DHA content (and portion of total fatty acid methyl esters) of *O. marina* satiated with dried yeast, 52.40 pg per cell (and 25.9%), was considerably greater than *O. marina* fed on *A. carterae* (26.91 pg per cell; 15.7%) or powder of *Chlorella* sp. (21.24 pg per cell; 16.7%). The cost of dried yeast (approximately 10 US dollars for 1 kg yeast) was much lower than that of obtaining the algal prey (approximately 160 US dollars for 1 kg *A. carterae*). Therefore, compared to conventional algal prey, dried yeast is a more easily obtainable and lower-cost prey for use in the production of DHA by *O. marina*.

Keywords: *Oxyrrhis marina*, fatty acid, DHA, algal prey, dried yeast

Poster No. 210

Characterization of the newly discovered pervasive mitosomal protein in *Giardia intestinalis*

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The unicellular eukaryotic organism *Giardia intestinalis* is worldwide expanded anaerobic parasitic protist. Giardiasis, the disease caused by *Giardia*, is one of the most common parasitic disease that causes troubles not only to humans but also to animals. This organism is also interesting for its many unique cellular features. One of them is the presence of mitosomes, the special organelles derived from mitochondria. Similarly to mitochondria, mitosome is limited by two membranes, which accommodate translocases transporting proteins from the cytosol. However, mitosome does not have its own genome and as far as we know, there is only one pathway of the iron-sulphur cluster biosynthesis in this organelle. Using the *in vivo* enzymatic tagging technique, a couple of unique mitosomal proteins were identified, including a GL50803_16424. This method used advantages of biotin ligase enzyme (BirA) for crosslinking of proteins and for subsequent purification. The protein GL50803_16424 attracted our attention by interacting with components of all mitosomal subcompartments - the mitosomal outer membrane, the inner membrane and the mitosomal matrix. In addition, the expression of HA-tagged GL50803_16424 resulted in the formation of structures near the mitosomes never seen before in *Giardia*. Our preliminary data from mass spectrometry shows that the protein is connected in some way with mitosomal membrane and its overexpression induces the accumulation of membrane proteins. Bioinformatic approaches revealed that the GL50803_16424 has domain similar to the myelodysplasia-myeloid leukemia factor 1-interacting protein. The function of GL50803_16424 is still unknown, but our hypothesis is that this unique protein could be a part of mitosomal translocases complexes.

Keywords: *Giardia intestinalis*, mitosome

Poster No. 211

Development of CRISPR/Cas9 in the unicellular holozoan *Capsaspora owczarzaki*

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The emergence of animal multicellularity is considered one of the major evolutionary transitions. Recent phylogenomic analyses have revealed that studying it from the perspective of their unicellular relatives is key to fully understand how this transition happened at a genetic level. For this reason, development of genetic tools for these organisms is becoming essential. Here we show our most recent progress in the development of a genome editing protocol based on the CRISPR/Cas9 technology for *Capsaspora owczarzaki*, a close unicellular relative to metazoa that has a well described life cycle. So far, a methodological pipeline for CRISPR delivery and genotyping has been set up and properly implemented, and different variants of the delivering vectors have been rehearsed. Despite the experimental complexity of current CRISPR analyses and other feasibility hindrances, our results evidence that this organism exhibits a lot of potential for genetic tractability, making functional assays closer than before.

Keywords: CRISPR, genome-editing, multicellularity, model organism, genetic tractability

Poster No. 212

Genetic diversity and a novel genotype in populations of the honeybee pathogen *Nosema ceranae* from Thailand

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Reduction of honeybee populations is an issue for honey production and pollination worldwide. It has been suggested that the microsporidian pathogen *Nosema ceranae* plays a role in this decline. Identification of *N. ceranae* in the Western honeybee (*Apis mellifera*) has been proposed to be the result of a host transfer from its original host the Asiatic honeybee (*A. cerana*). Studies of genetic variation in *N. ceranae* targeting *A. mellifera* hives distant from the pathogen's native range have found high levels of diversity. We investigated the genetic diversity in *N. ceranae* populations in *A. cerana* and *A. mellifera* from Thailand to understand how diversity varies among hosts and within this pathogen's native range. Small ribosomal RNA subunit (*SSU*) sequences obtained from pathogens isolated from both hosts indicate high genetic diversity uncorrelated with host species. Similar to previous studies, we found that nucleotide diversity (pi) is 0.006 on average. A high-fidelity DNA polymerase (PfuUltra II Fusion HS) was used to test for presence of artefactual diversity in our samples. We found that its use lowers detection of diversity by up to 4 times and eliminates evidence of recombination. We also report a new genotype from northern Thailand that is intermediate between *N. ceranae* and its European sister species *N. apis* and could be a representative of a new cryptic species. Overall, our data suggests that genetic diversity in *N. ceranae* populations from Thailand does not vary among hosts and that its detection is highly affected by polymerase fidelity. Finally, the discovery of a distinct *Nosema* genotype indicates that previously unknown taxonomic diversity exists in this important lineage of arthropod parasites. Future whole-genome analyses will shed further light on the amount of parasite genetic diversity that exists in beehives from Thailand.

Keywords: Microsporidia, *Nosema*, honeybee, genetic diversity, novel genotype

Poster No. 213

The autofluorescence of lipophilic organelles in the eustigmatophyte *Vischeria* sp.

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The research interest in the eustigmatophyte algae rapidly grew in the last decade because of their great biotechnological potential, moreover more and more questions have recently emerged for the basic research, too. Eustigmatophyte algae are evolutionary very old group of Stramenopiles, which were defined as a separate class on account of the presence of very specific lipophilic organelles. These include the extraplastidial stigma in zoospores and the reddish globule commonly occurring in vegetative cells, which has been well documented by light and electron microscopy in last fifty years. However, the composition, biogenesis and physiological functions of these organelles are not known yet. The cells contain also lipid bodies consisting of polyunsaturated fatty acids (PUFA) contributing up to 50 % of the biomass dry weight, which is particularly valuable for biotechnologies. Surprisingly, the lipophilic organelles (e.g. lipid bodies and reddish globule) are autofluorescent in eustigmatophytes, but the actual origin and more detailed properties of their autofluorescence have not been well described till now. In our study we want to contribute to the revelation of the fluorecent nature of the lipophilic organelles by means of fluorimetry, confocal Raman microscopy and proteomic analysis of the fraction of lipophilic organelles, mainly the lipid bodies and reddish globule in our model species *Vischeria* sp. BOF79.

This work was supported by the project NPUI No. LO1417.

Keywords: Eustigmatophyceae, lipid body, reddish globule

Poster No. 214

Whole genome studies of two non-model species of euglenids: *Euglena longa* and *E. hiemalis*

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Euglenids are a diverse group belonging to the Euglenozoa and comprises of primary heterotrophs (eg. *Rhabdomonas*), phototrophs (eg. *E. gracilis, E. hiemalis*) and secondary heterotrophs (eg. *E. longa*). Although, euglenids phylogeny has been studied extensively, the sequences of whole nuclear genomes have not yet been introduced. While, the studies of this group's genetic composition shall particularly bring new insights into the studies of atypical introns, such as nonconventional and intermediate ones.

This project regards the *E. longa* and *E. hiemalis de novo* genomes sequencing, assembly and annotation. We also plan to obtain the *E. hiemalis* and *E. longa* transcriptomes, followed by the analysis and comparison to its genomes. Obtained data would be a source of information about the novel genetic regions and their regulation mechanisms. We aim to seek for general patterns of introns distribution, their origin and types.

Total DNA and RNA will be isolated from *E. longa* (SAG 1204-17a) and *E. hiemalis* (CCAP 1224/35) cultures. The RNA sequencing pair-end library with the polyA selection will be constructed, whereas for DNA - PacBio and Illumina pairend and mate-pair libraries will be obtained. Trinity will be used for transcriptome, whereas SPAdes, SOAP, AbySS and MIRA for genome assembly, respectively. Further steps will include *ab initio* gene prediction using Augustus, supported by transcriptomic data mapping and functional annotation with BLASTP and HMMER3.

Recently, we have obtained the preliminary *E. longa* genomic data. The first SPAdes assembly resulted in 212517 contigs. The total length of the assembly is 22.55 Mb with N50 equal to 1058 bp. Roughly 64% of raw reads was mapped to the obtained assembly using the bowtie2. We did not detect signals of contamination. In the closest future, we would like to concentrate on obtaining the preliminary MiSeq data for *E. hiemalis* genome and transcriptome.

Keywords: euglenids, next-generation sequencing, genome assembly, transcriptome assembly, atypical introns

Poster No. 215

Inducible protein stabilization system in *Leishmania mexicana*

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Leishmania are protozoan parasites causative agents of leishmaniasis. They alternate between two developmental stages. The flagellated extracellular promastigotes multiply in the gut of sandfly vectors, while the intracellular non-flagellated amastigotes reside inside the macrophages' phagolysosomes of vertebrates. Targeted regulation of protein levels is an important tool to investigate the role of proteins essential for cell function and development. In recent years, methods based on the *Escherichia coli* dihydrofolate reductase (ecDHFR) have been established and used in various cell types. ecDHFR destabilizes the fused protein of interest and causes its degradation by proteasomes, unless it is stabilized by a specific ligand Trimethoprim or Trimethoprim-lactate. This system has been successfully established in *Trypanosoma cruzi*, however in *Leishmania mexicana*, it turned out to be not functional – the fused protein was not degraded. We therefore decided to make other mutated versions of ecDHFR which fortunately provided almost complete degradation in non-induced state and protein stabilization after the induction by TMP/TMP-lac. With this system, we are able to define function of various proteins, potential virulent factors and, what more, establish a system in which an inducible stabilization of a toxic protein could lead to damage and/or cell death of *L. mexicana*. Such system could be then potentially used as a vaccination.

Keywords: *Leishmania mexicana*, protein stabilization, ecDHFR, TMP

Poster No. 216

Ion dependence of ecdysis in dinoflagellates

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Dinoflagellates possess a very complex cell covering, amphiesma, consisting of the plasma membrane and amphiesmal vesicles underlying it. In armored species, amphiesmal vesicles contain cellulose thecal plates. Amphiesma is a dynamic structure that undergoes rearrangements during the life cycle of a dinoflagellate. The most dramatic changes occur in the process of ecdysis which represents shedding of the upper layers of a cell covering (old plasma membrane, outer membrane of amphiesmal vesicles, and thecal plates). Ecdysis is an important stage of such processes as cyst formation and awakening, as well as adaptations of dinoflagellates to stress conditions. Nowadays, physiological and molecular mechanisms of ecdysis in dinoflagellates are not clear. We investigated the role of Na^+, K^+, and Ca^{2+} in the stress-induced ecdysis of the armored dinoflagellate *Prorocentrum minimum*. We performed the experimental ion substitutions in the external medium and revealed that their presence is essential for ecdysis. Calcium ions had the most pronounced effect on the level of ecdysis in *P. minimum* culture. We also used blockers of ion channels and pumps as well as actin depolymerization agents in order to shed light on the molecular bases of revealed ion effects.

Funded by the Russian Science Foundation, project 16-14-10116.

Keywords: dinoflagellates, ecdysis, ion channels, actin, cell physiology

Poster No. 217

Study of the contractile vacuole using *Dictyostelium discoideum* as a model

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The organelles of the membrane-trafficking system are responsible for the movement of material within eukaryotic cell, as well as exchange with the extracellular environment.

In many eukaryotic cells, specialized secretory organelles allow for release of crucial compounds into the extracellular space. In human cells these organelles include the recycling endosome, multi-vesicular body and lysosome related organelles (LROs), including the secretory granules. Non-mammalian organisms also possess an array of secretory organelles. One of the least understood is the contractile vacuole (CV).

The CV is an osmoregulatory organelle found in a wide variety of amoeba, algae, flagellates, and ciliates. It is composed of a central vacuole with associated set of membranous tubules that collect ions and water molecules feeding into the central compartment. The CV undergoes cyclical swelling and collapse, fusing with the plasma membrane and expelling Ca2+ and water

In order to examine the evolution and function of the CV, we have performed microscopic and transcriptomic analyses using two strains of *Dictyostelium discoideum* (wild type DH1, and mutant strain clmA-) grown in axenic culture, and under inducing and non-inducing conditions during one hour (hypotonic and isotonic conditions).

After confirming induction by observed differences the diameter and contraction rate of the CV, RNAseq data was collected. Preliminary analysis confirms the differential regulation of known membrane-trafficking markers associated with CV function, and should give further insight into the membrane-trafficking machinery involved in CV action in *Dictyostelium*.

These results will be compared with ongoing equivalent experiments in ciliates and other freshwater organisms possessing CVs in order to assess whether CVs across eukaryotes are homologous and to gain insight into the mechanistic underpinning of CV function.

Keywords: contractile vacuole, *Dictyostelium discoideum*, RNAseq, membrane-trafficking machinery

Poster No. 218

The filasterean *Capsaspora owczarzaki* as an experimentally tractable system to understand the origin of animal multicellularity

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The transition to animal multicellularity from a single-celled ancestor is one of the most transcendent events in the history of life. Recent genome data from the closest unicellular relatives to animals have shown that changes in regulatory programs involving cell signaling, cell adhesion, cell communication and transcriptional regulation were probably crucial for the emergence of metazoans. Thus, functional studies of key genes involved in these programs essential for multicellular functions in a unicellular context can give us insights into the molecular mechanisms that drove this transition.

However, traditional model systems cannot address this question. Thus, we need to develop genetic tools among the closest unicellular relatives of animals, which are the only ones with the potential to answer how regulatory programs were co-opted at the onset of Metazoa.

To this end, we have developed the filasterean *Capsaspora owczarzaki,* one of the closest unicellular relatives of animals, as an experimentally tractable system. We have optimized a classical transfection protocol with plasmid DNA, which results in a reasonable efficiency for further functional assays such as overexpression and localization experiments. In this regard, we have created a platform of multiple expression vectors tagging several cellular locations. In parallel, we are evaluating several selection strategies to achieve stable transfection. Preliminary results and implications of this study will be presented and discussed.

Keywords: origin of animal multicellularity, genetic tools, transfection, Filasterea

Poster No. 219

Biodiversity of symbiotic associations between the euryhaline paramecia and prokaryotes

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Symbiotic associations between ciliates and intracellular bacteria frequently occur in brackish waters. A population of paramecia forming bipartite and tripartite symbiotic systems with bacteria was isolated from a pool in the intertidal zone on Sredniy Island (the White Sea, Chupa Inlet). According to morphological data, the population consisted of *P. nephridiatum* and *P. calkinsi*. An intranuclear (most likely, *Holospora curvata* in *P. calkinsi*) and four different new or poorly studied cytoplasmic endosymbionts in various combinations were registered in the strains obtained. The infected strains were studied with FISH, CLSM, AFM and TEM. FISH with the group specific oligonucleotide probes demonstrated that three examined cytoplasmic bacteria belonged to *Alpha-proteobacteria*. One of the cytoplasmic endosymbionts of *P. calkinsi* was positive with the *Holospora*-specific probe and, thus, should belong to *Holospora* genus, so far comprising only intranuclear endosymbionts. However, no invasion tip characteristic for *Holospora* was observed in electron micrographs. Several *P. nephridiatum* strains harbored endosymbionts with a peculiar refractile spheric granule in the center of the cell. By its fine structure (numerous flagellae, individual parasitophorous vacuole and an electron dense spheric inclusion), this endosymbiont resembles *Pseudolyticum minutus,* which has been described only morphologically. This endosymbiont belongs to *Ca*. Midichloriaceae family, as shown by FISH with the Midichloriaceae-specific probe. The third type of cytoplasmic endosymbionts, quickly lost in the laboratory culture, were vibrios, located in individual vesicles. The most frequent cytoplasmic endosymbiont was motile flagellated *Trichorickettsia,* which occurred in both *Paramecium* species, often together with other bacteria. Molecular characterization of the endosymbionts is under way. Bipartite and tripartite symbiotic systems would serve good models to study interactions of the partners; long term observations of laboratory cultures and monitoring nature population could help to clarify the issue of balanced competition of bacterial partners in double infections in ciliates.

Supported by RFBR grant 15-04-06410.

Keywords: symbiosis, *Paramecium*, double infection, *Ca.* Midichloriaceae, *Trichorickettsia*

Poster No. 220

Uncovering the diversity of antimicrobial proteins produced by ciliates

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The arisen of antibiotic multi-resistant bacteria have dramatically increased in recent years and have became a major challenge of public health. Because of that, many efforts have been made toward the discover and development of novel bio-active compounds to serve as an alternative in human therapeutics. Among them, antimicrobial peptides (AMPs), an ancient and diverse class of molecules that plays a critical role in the innate immune system, have received considerable attention in the last few years due to its eficace as natural antibiotic, stability and low level of acquired resistance development by targeted microorganisms. AMPs have been successfully isolated from across all tree of life but have never been found in ciliated protists. In this work, we used bioinformatic tools to identify and characterize putative AMPs from 18 publicly available ciliate genome sequences. First, the genomes were screened by local alignment against patterns derived from well defined AMP families (profile-HMM and regular expressions [REGEX]) to identify potential candidates within these genomes. The antimicrobial activity of these candidates was *then* predicted based on sequence homology and structural properties using machine learning methods and three-dimensional structure modelling, resulting in the identification of ~400 putative AMPs in which includes: Alpha-helical, Beta-strand and mixed Alpha- and Beta-structure peptides from both disulphide and disulphide-free classes of AMPs. Our data highlight the biotechnological importance that ciliates may have as natural sources of AMPs considering also, their enormous diversity and ecological ubiquity (increased probability to find novel AMPs), and that many of them could be in vitro cultured at large industrial scales (suitable for pharmacological applications). Moreover, our data suggests that ciliates may have the assistance of a diversity of AMPs against competitors, predators and pathogens in their natural environment.

Keywords: ciliates, anitimicrobial peptides, bioinformatics, biotechnology, molecular modelling

Poster No. 221

Identification of functional diatom symbiont diversity in a benthic foraminifera with an extremely heat-tolerant symbiosis using a combined approach of algae culturing and genetic fingerprinting

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Symbiont-bearing benthic foraminifera are calcareous marine protists, which play an important role in global carbonate production. Their ecological success depends of the efficiency of their algal symbiosis. Individual foraminifera host multiple algal symbiont types and understanding the specificity of the symbiosis is essential for determining the resilience of the protist to global change stress. We analyzed the symbiont community composition of 40 benthic foraminifera *Pararotalia calcariformata,* a species with an unusually heat-tolerant diatom symbiosis. We carried out a parallel characterization of the symbiont community using algae culturing and genetic fingerprinting. We collected it from four locations along the Israeli Mediterranean coast, inside and outside of a thermally polluted site. The culturing approach unveiled 16 diatom taxa, the most commonly found was the diatom *Minutocellus polymorphus.* The genetic fingerprinting approach identified in most cases a single species belonging to *M. polymorphus*. This species has never been observed in a symbiotic association before. We conclude that *P. calcariformata* hosts a consortium of multiple diatom symbionts, but the symbiosis system to be dominated by *M. polymorphus*. This finding is consistent with the concept of symbiont shuffling whereby the host can enhance its adaptive potential by maintaining (or being able to acquire) a broader spectrum of symbionts.

Keywords: symbiosis, climate change, diatoms, symbiont shuffling

Poster No. 222

PhyloMagnet – Searching for organelle-related lineages in metagenome data

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The diversity of life is still largely unexplored, but becomes more and more accessible through advances in NGS. Most metagenomic studies focus on complex microbial communities, from the human gut to extreme environments in the polar regions or the seafloor. Metagenome datasets can be used to extract metagenome-assembled genomes (MAGs). These can be subjected to phylogenomic placement and serve as genomic representatives of understudied and so far unexplored groups. Extracting all possible MAGs from existing metagenome data is currently not feasible because of practical limitations both in terms of computational capacities and storage space. Therefore, a targeted selection needs to be made in order to reduce the number of samples that will be subjected to further investigation and extraction of MAGs.

To effectively search through large assemblies of metagenomic data such as those deposited in the SRA archive of the NCBI, we are developing a pipeline called PhyloMagnet. Our goal is to circumvent the need for complete metagenome assembly of these samples by focusing on the "gene centric assembly" of a set of carefully chosen phylogenetic markers. Using state-of-the-art bioinformatics tools and approaches, we aim for extremely fast processing of raw sequence data and a phylogenetically informed selection of candidate metagenomic samples.

Here, we will present the PhyloMagnet workflow together with its application to mining publicly available metagenome datasets for bacterial lineages closely related to both mitochondrial and chloroplastic organelles.

Keywords: metagenomics, mitochondria, chloroplasts, bioinformatics, phylogenetics

Poster No. 223

Diatom plastid fate following ingestion by Foraminifera (Rhizaria) in an intertidal mudflat of the French Atlantic coast

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The Bourgneuf Bay is a large intertidal mudflat situated on the French Atlantic coast south of the Loire estuary. This mudflat shelters several species of benthic foraminifers. Three hard shelled species with different feeding strategies dominate this environment: *Ammonia aomoriensis*, *Haynesina germanica* and *Elphidium oceanense*. After TEM and photophysiological studies, *A. aomoriensis* and *E. oceanense* had a large majority of degraded kleptoplasts with low or no photosynthetic efficiency; whereas *H. germanica* retains healthy diatom chloroplasts and uses them for photosynthesis.

This process, known as kleptoplasty, still needs some clarifications to characterize photophysiological and feeding strategies between species, short and long term retention capacity and to link those differences to foraminiferal morphological and molecular characteristics.

In the present study, we investigate the identity of diatoms and chloroplasts ingested by *Ammonia aomoriensis*, *Haynesina germanica* and *Elphidium* oceanense through DNA extraction, cloning and Sanger sequencing of individual foraminifers.

The main objective is to check if foraminifers target specific diatoms, we also compare our results with clones obtained from the “wild and free” microphytobenthos of the same location. The identification of diatoms and chloroplasts is based on the amplification through two primer sets, one targeting diatom nuclear rDNA SSU (18S) and the other targeting chloroplast rDNA SSU (16S). Preliminary results confirm a difference between foraminiferal species for 16S rDNA data. The three species also seem to ingest different diatom species based on 18S rDNA data. These data suggest different feeding strategies possibly linked to diatom size and foraminiferal morphological characteristics.

Keywords: foraminifera, diatoms, kleptoplastidy, DNA barcoding

Poster No. 224

Defined surface traits of microparticles and food bacteria influence ingestion and digestion of *Tetrahymena pyriformis*

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Since the uptake of bacteria by means of phagocytosis followed by escaping from food vacuoles seems to be the common way of symbionts to infect their hosts, it is of interest to know which traits influence ingestion as well as digestion of protists. Studies with pathogenic microorganisms as well as re-infection studies revealed that surface traits are often involved in these processes. To investigate the effects of biochemically and biophysically defined surface traits, synthetical microparticles (approximately 5 µm in diameter) based on bovine serum albumin embedded in a poly-methacrylate matrix were fed to *Tetrahymena pyriformis* and their fate was investigated by fluorescence microscopy. By means of a carbodiimde any substance carrying an amino or carboxyl group can be covalently bound to the particles and, in consequence, alter their surface. Whereas amino acids decrease the number of ingested particles, additional peptides or enzymes increase the number of microparticles per individual. Also, some of these substances alter the digestive process of *T. pyriformis*.

For further investigation on the effects on digestion and finally the frequency of bacterial escapes from the food vacuoles, the chemical modification process was adapted to alter the surface traits of a red fluorescing transformant strain of *Escherichia coli*, resulting in proper ligand binding as well as a sufficient number of viable bacteria after coupling. By fluorescence and transmission electron microscopy the fate of the bacteria was followed with respect to incomplete digestion and single bacterial cells residing in the cytoplasm of *T. pyriformis*. The results suggest that bacteria with either increased surface hydrophobicity or alkalinity are more capable of evading digestion on the one hand, on the other hand these bacteria show higher chances to escape from the ciliate's food vacuoles, indicating that biophysical surface traits strongly influence these processes.

Keywords: bacterial surfaces, digestion, endosymbiosis, food vacuoles, *Tetrahymena*

Poster No. 225

Extensive flagellar remodeling during the complex life cycle of *Paratrypanosoma*, a divergent trypanosomatid

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Paratrypanosoma confusum is a kinetoplastid flagellate that constitutes the most basal branch of the highly diverse obligatory parasitic trypanosomatids. While it has a characteristic promastigote stage, *Paratrypanosoma* also forms a unique haptomonad stage that progressively depolymerizes its single flagellum. It becomes firmly attached to the surface via a large bulge on the base of the flagellum, which is then remodeled into a thin protease-resistant amoeboid attachment pad. Both stages multiply by binary division, and the progeny of a sessilemastigote either remains sessile or grows a flagellum and resumes motility. The *Paratrypanosoma* genome has been streamlined and reduced as in other trypanosomatids. Its life stages coexist in culture and are endowed with very different transcriptomic profiles, reflecting striking differences in their biology. The absence of social motility and some unique morphological and biochemical features indicate that the monoxenous *Paratrypanosoma* retains a few ancestral traits that precipitated evolutionary success of trypanosomatids but tended to be lost or suppressed in all derived extant lineages.

Keywords: *Paratrypanosoma*, flagellum remodeling, trypanosomatids, haptomonads

Poster No. 226

Evaluating approaches for genetic transformation of dinoflagellates

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Dinoflagellates are a large and diverse group of protists present in all aquatic habitats; they are crucial components of global ecosystems involved in the carbon cycle and food webs. Collectively, they occupy multiples trophic levels being capable of photoautotrophy, heterotrophy or both. Some key phenomena such as harmful algal blooms, coral symbioses and bioluminescence also involve dinoflagellates. Their study is severely hampered by the lack of tools and protocols for genetic transformation, a problem that, until very recently, has been addressed only sporadically. Here we describe ongoing efforts to manipulate genetically *Oxyrrhis marina*, an unarmoured, non-photosynthetic dinoflagellate species broadly utilized to address many aspects of protist biology. Due to their many unusual and divergent molecular features, transforming dinoflagellates poses important challenges, including the difficulty to foresee a suitable approach based on what works in other organisms. So far we concentrate on two approaches: DNA-based introduction of transgenes aimed at integration at specific, highly expressed loci; and introduction of artificial dinoflagellate mRNA encoding a reporter. On the other side, we evaluated the efficiency of various methods to introduce DNA into *O. marina* cells, including electroporation and chemical transformation with calcium phosphate and polyethylenimine. The latter two resulted much better than electroporation in achieving high efficiency and low mortality. Our method to use mRNA for heterologous expression relies on in vitro transcription of an artificial gene that includes a 22-base spliced leader present in all natural dinoflagellate transcripts and is thought to be required for efficient translation. AT this point, our preliminary results are suggestive that heterologous expression of proteins is possible, although more work is needed to achieve stable transformation.

Keywords: dinoflagellates, transformation, expression, transfection

Poster No. 227

Unveiling the CIA components of *Trichomonas vaginalis*

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Iron-sulfur (FeS) cluster assembly pathways are extensively studied in model organisms, e.g. *Saccharomyces cerevisiae*, *Homo sapiens*, and more recently in *Trypanosoma brucei*. However, their character is not fully elucidated in anaerobic protists such as *Trichomonas vaginalis*. This human pathogen possesses anaerobic form of mitochondria, the hydrogenosome, in which some component of FeS cluster assembly machinery (ISC) has been identified, whereas the cytosolic CIA pathway has not been studied so far. Investigating the CIA pathway, we found homologues for most of key proteins that are involved, namely Nbp35, Cfd1, Nar1, Cia1 and Cia2. The CIA components that are apparently absent include Tah18, Dre2, MMS19, Grx3/4.

We expressed identified proteins with HA-tag and localized them by cell fractionation and immunofluorescence microscopy in *T. vaginalis*. The results showed the location of the proteins exclusively in the cytosol. Further, we co-immunoprecipitated two Cfd1 paralogues, TvCfd1A and TvCfd1B to search for their interacting partners. These experiments revealed that these two paralogues interact with each other, however we did not observed interaction of any paralogue of Cfd1 with Nbp35, which is known in *S. cerevisiae*. We suggest that not only hydrogenosomal but also cytosolic FeS cluster assembly pathway of this parasite differs significantly from typical models.

Keywords: *Trichomonas vaginalis*, FeS cluster, CIA pathway, co-immunoprecipitation

Poster No. 228

Secretion of β-amylases by *Trichomonas vaginalis*

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Glucose is an essential nutrient for human parasite *Trichomonas vaginalis* to generate ATP via anaerobic fermentation in the cytosol and extended glycolytic pathways in hydrogenosomes. In vaginal fluids, the main source of glucose is likely free glycogen derived from vaginal epithelial cells. To be utilized by *T. vaginalis*, glycogen and glucose-containing polymers needs to be extracellularly digested to monomeric glucose that is transported into the cells. Glycogen hydrolysis is catalyzed by various enzymes of which exo-acting β-amylases hydrolyzes α-1,4-linkages of glycogen from the non-reducing end liberating β-maltose. Next α-glucosidase activity of *T. vaginalis* can hydrolyze maltose to glucose. To get more insight into β-amylases distribution, we search for β-amylase coding genes across eukaryotic supergroups. In addition to *T. vaginalis*, in which we identified 4 genes for β-amylase (BA1-4), we found orthologous genes in related bovine pathogen *Tritrichomonas foetus*, and in *Nagleria gruberi.* β-amylases are also common in land plants, and Amoebozoa group, whereas animals and fungi seem to be devoid of β-amylases. Next we were interested whether *T. vaginalis* β-amalyses are secreted by classical or non-classical secretory pathway. The co-expression of ER-localized biotine ligase (BirA) and acceptor peptide tagged BA1-4 revealed that BA1-3 pass via classical secretory pathway and they are release to the cell environment. This process is inhibited by brefeldin A. In ER, BA-1 appeared to be heavily glycosylated with Asn-linked GlcNAc2Man5. Interestingly, BA4 is trapped in ER and is not secreted. Incubation of *T. vaginalis* under various environmental conditions revealed that presence of glycogen and iron regulate β-amylase gene expression. Our data indicate that β-amylases are novel important members of *T. vaginalis* secretome.

Keywords: *Trichomonas vaginalis*, β-amylases, secretome, glycogen

Poster No. 229

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

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The α-helical C-tail anchored proteins represent a heterogenous group of membrane proteins with a large functional N-terminal domain exposed to the cytosol and a short membrane insertion at their C-terminus. They are components of the outer membrane of organelle such as mitochondria and their relatives, namely hydrogenosomes and mitosomes, as well as they serve as membrane proteins of the endoplasmatic reticulum (ER) or plasma membrane. In general, targeting signals consist of short transmembrane domain (TMD) and positively charged flanking regions. Proteomic analysis of *Trichomonas* hydrogenosomes revealed the presence of twelve C-tail anchored proteins. First, we confirmed the topology of six representative C-tail anchored proteins in the outer hydrogenosomal membrane by protein protection assay. Further we investigated character of targeting signals, which are responsible for delivery of C-tail anchored proteins into the hydrogenosome or ER. Initially we replaced C-terminal domain of protein disulfide isomerase (PDI), which is present in outer membrane of ER, with TMD and charged C-terminus of the hydrogenosomal protein TVAG_272350. Expression of this chimeric protein in trichomonads resulted in its delivery to the hydrogenosomal membrane. When PDI was expressed only with C-terminus from the hydrogenosomal protein with native TMD, the chimeric protein was targeted into ER. We also investigated effect of mutations of the positively charged C-terminal domain. The C-tail anchored protein was targeted to the hydrogenosome and endplasmic reticulum when the C-terminal domain was deleted or the positively charged amino acid lysin was replaced by serin. This phenotype enables to visualize contact sites and a complex network of endoplasmic reticulum and organelles by super resolution immunofluorescence microscopy. These data suggest that structure of TMD and its flanking regions is critical for specific delivery of C-tail anchored proteins into hydrogenosomes and ER.

Keywords: C-tail anchored protein, hydrogenosome, endoplasmic reticulum, protein targeting, super resolution microscopy

Poster No. 230

The role of phenotypic assortment and sex in *Tetrahymena* adaptation and evolution

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Understanding the mechanisms that generate genetic variation, and thus contribute to the process of adaptation, is a major goal of evolutionary biology. Tetrahymena thermophila is a ciliate with an unusual genetic feature, called phenotypic assortment, which may allow for an increase in the amount of genetic variation following sex, thereby increasing its evolvability. To test this hypothesis, I compared the rate of adaptation in T. thermophila populations that were allowed to undergo phenotypic assortment to those that were not. These populations were maintained at two different temperatures and fitness was measured every 25-50 generations for over 1000 generations. Under some environmental conditions, the populations that underwent phenotypic assortment adapted more quickly than those that did not. This suggests that the additional genetic variation generated by phenotypic assortment can increase the rate of adaptation under certain conditions.

Poster No. 231

Transcriptional dynamics during the unicellular to multicellular transition of the sorocarpic amoeba *Fonticula alba* (Nucletmycea: Opisthokonta)

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Sorocarpic amoebae are eukaryotic microbes who live and feed as single amoeboid cells before facultatively aggregating and differentiating to form multicellular structures (known as fruiting bodies) made up of dead and/or dormant cells. Amoebae that exhibit a sorocarpic life cycle are found in every major eukaryotic supergroup with the exception of the Archaeplastida. *Fonticula alba* is a sorocapic amoeba that occupies a particularly interesting position on the tree of life as the only multicellular member of the protistan clade that is sister to the Fungi + Opisthosporidia. Thus, the evolutionary position of *F. alba* suggests that multicellularity evolved at least two times independently on the Nucletmycea branch of Opisthokonta. We aim to investigate the molecular mechanisms that facilitate multicellularity in *F. alba* and compare them to those in the well documented systems in Fungi and in Holozoa (i.e., animals + their closest protistan relatives). To examine the potentially rich commonalities, differences, and/or co-options in these multicellular systems, we deeply sequenced transcriptomes from five biological replicates of aggregating (actively fruiting) and non-aggregating cultures of *F. alba* for expression profiling in these discrete life stages. Armed with our deeply sequenced, publically available genome along with these novel developmental data, our results show of *F. alba*'s 6,457 predicted genes ~300 are significantly differentially expressed during fruiting body development. From these potentially developmentally important transcripts we can now reveal the molecular mechanisms that make *F. alba* able to create emergent fruiting structure with other cells, which is unique among the opisthokonts.

Poster No. 232

Cellular and molecular mechanisms to assess heavy metal toxicity in the freshwater ciliate, *Euplotes aediculatus* from Delhi, India

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Heavy metals are biologically important though increase in the threshold concentration of heavy metal in the environment may interfere with the metabolic activity of organisms and induce reactive oxygen species (ROS) causing DNA damage which can eventually lead to cell death. Increase in anthropogenic activities have contributed to rise in the concentration of heavy metals in the terrestrial and aquatic ecosystems which is becoming a major concern these days. Thus, it becomes necessary to assess the environmental heavy metal toxicity. Ciliates are considered to be the most suitable organisms as they are easily cultured in the laboratory and show quick response towards stress as they do not have cell wall. In this present study, single-cell freshwater eukaryote, *Euplotes aediculatus*, is selected as model organism to evaluate the effect of heavy metal toxicity. Identification of the species was done by silver-network staining, Feulgen staining and by sequencing SSU rRNA. The cells of *E. aediculatus* were treated with cadmium and copper. It was observed that growth rate exhibited inverse relationship with heavy metal concentration. Mortality rate and various doses of heavy metals (control, LC_{30}, LC_{50}, LC_{70}) were determined. LC_{50} values of ciliate were 2 mg/L and 0.2 mg/L for Cd and Cu respectively, indicating copper to be more toxic. In treated cells, ROS generation was studied by specific fluorophore (dihydroethidium). Since, antioxidant enzymes are involved in detoxification of ROS, activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined and were found to increase significantly in the presence of Cd and Cu. Also, GPx was sequenced and the expression of GPx was determined by real-time quantitative PCR. Cd and Cu significantly increased the expression of GPx, suggesting that these genes may be involved in defense mechanisms against oxidative stress and thus, can be used to evaluate cytotoxic effect of heavy metals.

Keywords: freshwater ciliate, glutathione peroxidase, heavy metal, reactive oxygen species, superoxide dismutase

Poster No. 233

Cep164 genes in the transition zone of the mature basal body

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Cep164 proteins are essential components of the transitional fibres (or distal appendages) of basal bodies and centrioles. The transition zone docks the mature basal body to the plasma membrane and without it the basal body cannot nucleate a functional flagellum. Cep164 is one of the most conserved genes found in the basal bodies across the evolutionary spectrum and Trypanosomatid flagellates possess three rather diverse orthologues. Using immunofluorescence microscopy and endogenous tagging approaches in *Trypanosoma brucei* model all three proteins, both C and N terminally tagged versions, localize to the distal end of the mature basal body in a ring around the barrel of the basal body. Colocalization experiment shows that although the signal greatly overlaps within the transition fibres region there are fine differences between the individual proteins. Two of the Cep164 proteins are found in all mature basal bodies, but the third one is absent from the newly maturing basal body during the cell cycle and the signal appears only after abscission. After RNAi ablation of the individual proteins the morphology of procyclic *T. brucei* cell was altered, but the growth of the culture was not affected.

Poster No. 234

The ciliate *Euplotes petzi* is the natural reservoir of the bacterium *Francisella* in the Antarctic region

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Euplotes petzi is a strict psychrophilic ciliate isolated from coastal waters of Terra Nova Bay (Antarctica). In analyzing the results from a draft genome sequencing of total *E. petzi* DNA preparations, we found that more than 3% of the assembled contigs had a bacterial origin. All the bacterial DNA sequences, including one containing rRNA genes, overlapped with DNA sequences of the genus *Francisella,* which comprises a large number of species classified as facultative intracellular γ-proteobacteria potential noxious to their hosts. Based on these findings, we undertook the isolation, characterization and genome determination of the *Francisella*-like bacteria – tentatively named *F. adeliensis* sp. nov. – hosted in the cytoplasm of *E. petzi*. Genome sequence comparisons provide evidence that *F. adeliensis* forms a new clade in the *Francisella* phylogenomic tree. In addition to being well separated from all the recognised pathogenic *Francisella* species, this clade is distinct also from *F. endociliophora,* symbiont in the congeneric species *E. raikovi*, as well as from the *Allofrancisella* species that are collectively known as 'environmental francisellas'. The inclusion of *F. adeliensis* in the *Francisella* phylogenomic tree does not support the separation of this genus between 'intracellular' and 'environmental' forms.

Keywords: ciliate endosymbiont, *Francisella*, *Euplotes*

Poster No. 235

Microbe microbiomes: a new single-cell approach to characterize associations between ciliates and prokaryotes

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Symbioses between ciliates and bacteria are ubiquitous, and frequently very important for the physiology and evolution of both partners. Nevertheless, the number of deeply investigated systems is quite low, mainly limited to a few genera of well-known and easy-to-cultivate hosts. Additionally, due to the lack of appropriate methods for the study of such associations in the natural environment, information on their ecological relevance is scarce. The aim of the present work is to develop and test a new approach for the identification of single ciliate cells and their associated prokaryotic microbiomes without the need for host cultivation. This protocol includes the characterization of the prokaryotic community associated to a single protist host cell by SSU rRNA gene sequencing using the Illumina MiSeq platform. The method was preliminary tested on ciliates grown and maintained in laboratory standard conditions and then on freshly-collected specimens from their natural environment. Samples were collected from freshwater and marine coastal ponds. A total of 31 different ciliate morphotypes were isolated and 61 % of these were successfully processed. The success rate was not influenced by the size of the host, nor by the kind of original environment (freshwater Vs marine). This new approach could be a precious tool for the detection of yet-unknown microbial associations and for investigations on their distribution and ecology.

Keywords: symbiosis, ciliate, bacteria, single-cell

Poster No. 236

Dynamics of encystation of intestinal parasite *Giardia intestinalis*

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G. intestinalis is a human intestinal parasite, which causes diarrhea and difficulties with absorption of lipids. The parasite leaves the body in the form of cysts, which are infectious to other hosts. The encystation is triggered by higher pH and higher concentration of bile in the lower parts of the gastrointestinal tract. This process can also be induced in vitro under laboratory conditions. While the encystation has been studied for a long time, the molecular details and the dynamics of the overall process remain to be understood.

In the proteomic approach, we try to identify proteins associated with the encystation specific vesicles (ESVs), and which are expressed during encystation and which enable the formation of the cyst wall. To accomplish this, the dominant cyst wall marker - cyst wall protein 1 (CWP1) - was fused to the biotin acceptor peptide (BAP) sequence and expressed in the presence of biotin ligase. Usage of this construct enables high affinity purification of CWP1 and its interaction partners.

In addition, we also study the encystation by visualizing the formation of the ESVs and the cyst wall in live. For this purpose, we prepared CWP1 fused to two different fluorescent tags, the Halo-tag and the novel yellow fluorescence-activating and absorption-shifting tag (Y-Fast). These fluorescent tags can be used for long-term live imaging of *G. intestinalis* under anaerobic conditions, which are necessary for the parasite cultivation.

Using these methods we want to obtain more detailed understanding of the encystation of human protist parasite *G. intestinalis.*

Poster No. 237

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

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The special class of membrane proteins, so called tail-anchored (TA) proteins, carry a single C-terminal transmembrane domain that anchors them to organellar membranes. TA proteins mediate interactions among membrane bounded compartments by their N-terminal domains during various processes such as vesicular transport, regulation of apoptosis or protein translocation. In some eukaryotes, the specific pathway controls precise post-translational insertion of tail-anchored proteins into the endoplasmic reticulum membrane – Guided Entry of Tail-anchored proteins (GET) pathway.

Our bioinformatics analyses revealed the absence of most of the GET proteins in majority of the eukaryotic lineages except opisthokonts. However, one of the components of GET pathway (Get3) is conserved in all eukaryotic groups, excavates included. Get3 serves as a ATP-dependent shuttle under nonstress conditions and as an ATP-independent chaperone when its oxidized. We are using *Giardia intestinalis* in order to characterize its GET machinery. We have shown that giardia Get3 is a cytoplasmic protein with affinity to the endoplasmic reticulum. Using chemical cross-linking followed by affinity purification of biotinylated Get3, the specific set of interacting proteins has been identified. We determined one of the isolated proteins as another member of GET pathway – homolog of transmembrane receptor Get2.

We are interested in the characterization of functional GET pathway including the investigation of chaperone function of Get3 in *G. intestinalis*. In addition to giardia-specific information, our general aim is to define the evolution of GET pathway in eukaryotes.

Poster No. 238

Analysis of nonconventional introns in genomes of marine diplonemids

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Diplonemids are a group of flagellates within the Euglenozoa phylum. Marine diplonemids are abundant in the ocean waters, but they have been poorly investigated. Recently, some light has been shed on their genetics as 10 diplonemid cells belonging to 10 different, previously undocumented species were isolated from waters around the coasts of California (USA). Single-cell sequencing and subsequent analysis of their genomes revealed that their genes contain nonconventional introns. Additionally, it has been discovered that one of the introns in the *tubA* gene (in one of those diplonemids' cells) contains an open reading frame coding a protein with reverse transcriptase domain, which may be responsible for the spread of the nonconventional introns throughout the genomes.

Nonconventional introns are found not only in diplonemids, they have been first revealed in their close relatives – euglenids. They are characterized by a lack of the GT/C-AG borders and the presence of stable RNA secondary structure, bringing together the ends of intron and adjacent exons. The most conserved feature of such intron structure is the pairing of +4 ,5, 6 nucleotides at the 5' end and -8, 7, 6 nucleotides at the 3' end of its sequence. Nonconventional introns also seem to be incorporated at the new positions more often than they are lost. Their mechanism of excision is still unknown.

In this study, we perform an in-depth analysis of genomic data of these marine diplonemids. Our aim is to characterize the nonconventional introns and compare them to those found in euglenids. So far, we have analyzed the distribution of introns in seven genes, examined the secondary structure of introns and searched for open reading frames within these introns. In the future, we will focus on the studies of other genes, especially in order to seek for the occurrence of potentially new ORFs.

Keywords: diplonemids, nonconventional introns

Poster No. 239

De novo genome assembly of a new mantamonad strain from a long-read dataset

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Mantamonads represent a deeply-branching 'orphan' lineage of heterotrophic eukaryotes, distantly related to opisthokonts. We sequenced the genome of the undescribed mantamonad strain SRT-306 on the PacBio platform. The protist culture included mixed food bacteria. As a result, bacterial reads were also sequenced. To identify and remove bacterial contaminants from our dataset, we considered BLASTN and BLASTX analyses of partitioned reads and predicted peptides, annotation of spliceosomal introns using PASA [Program to Assemble Spliced Alignments], GC content, and sequence coverage. The cleaned mantamonad assembly included 178 contigs, one of which represents the complete sequence of its mitochondrial genome. The final genome size was 28 Mbp, with an N50 contig size of 305 kbp and a GC content of 59%. We assessed the completeness of the genome using a BUSCO [Benchmarking Universal Single-Copy Orthologs] analysis. This identified 274 out of 303 total (90.4%) conserved orthologs in the all-eukaryote data set, which is consistent with full coverage in some fully-sequenced eukaryote genomes. We also present results from ongoing phylogenetic and metabolic pathway analyses.

Poster No. 240

Mitochondrial genome of bacillariophycean diatoms reveals loss, gain, and re-loss of genes

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Diatoms play a role as primary producers in the marine ecosystem and are involved in energy, carbon, nitrogen, and silicate exchanges between the water column and sediments. In the Bacillariophyceae, Bacillariales and Naviculales are representative orders that are ubiquitous and rich in species, ca. 5,000 and 1,100 spp, respectively. However, systematics and evolutionary studies have largely relied on morphological and ultrastructural characteristic and a handful nuclear and plastid genes (i.e., LSU and SSU rRNA, and *rbc*L). In present study, we determined five new mitochondrial genomes (mtDNA) from Bacillariales and compiled with all available dataset (total 25 mtDNA) to establish a robust phylogeny. The dataset encompassed 33 protein coding genes (7,968 amino acids) of 13 species from two bacillariophycean orders. The best phylogeny reveals a monophyly of Bacillariales and Naviculales with maximum support values and basal position of Fragilariales within the class. Based on the best phylogeny, Naviculales showed the loss, gain, and re-loss of genes, such as atp8, rps7, and rps11 in mtDNA. We demonstrated the implication of mtDNA data to understand evolutionary history of diatoms.

Keywords: diatoms, phylogeny, mitochondrial genome, evolution

Poster No. 241

A heterolobosean strain SRT213 and the putative function of its mitochondrion-related organelle

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An amoeboflagellate, strain SRT213, was isolated from mangrove sediments sampled in the Republic of Palau in 2011, and has been maintained in the laboratory under the micro-aerobic condition with prey bacteria. A preliminary electron microscopic observation identified no typical mitochondrion, but double membrane-bound organelles that resembled superficially to mitochondrion-related organelles (MROs) found in diverse anaerobic/micro-aerophilic eukaryotes. Our small subunit ribosomal DNA phylogeny recovered a strong affinity between SRT213 and a heterolobosean *Creneis carolina* (Panek et al, 2014), suggesting that this amoeboflagellate is a new member of Heterolobosea. Prior to this study, MRO function was investigated only in two heterolobosean species, *Psalteriomonas lanterna* (de Graaf et al., 2009) and *Sawyeria marylandensis* (Barbera et al., 2010). In this study, we generated the transcriptomic data from SRT213 to deepen our understanding of the function of MROs in heteroloboseans. Homology searches against the SRT213 data successfully identified the transcripts encoding enzymes involved in anaerobic ATP generation and hydrogen production, suggesting that the MRO in this species belongs to "class 3/4." In addition, we identified the transcripts for putative MRO proteins that were not detected in the data from the two previously studied heteroloboseans, such as subunits of succinate dehydrogenase (complex II), those of F_oF_1 ATP synthase (complex V), and the enzymes comprising the TCA cycle. In this presentation, we will discuss the differences and commonalities in MRO function between SRT213 and other anaerobic/microaerophilic eukaryotes.

Keywords: Heterolobosea, mitochondrion-related organelles, anaerobic ATP generation, electron transport chain

Poster No. 242

Molecular study of *Blastocystis* isolates from residents and their domestic animals among 10 families in a small Indonesian community with poor hygiene

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An intestinal unicellular eukaryote, *Blastocystis* sp. has been considered as zoonotic parasite based on the molecular epidemiological data regarding small subunit rRNA gene (SSU rDNA) among humans and animals. To evaluate the detailed zoonotic lifecycle of *Blastocystis* organisms in endemic area, we examined *Blastocystis* isolates from residents and their domestic animals among 10 families with poor hygiene in Sumba Island, Indonesia where fecal cross-contaminations among humans and their animals were likely common. To clarify whether the same haplotype is engaged to both human and animal colonizations, the sequences of variable region of the SSU rDNA from the both sources were compared by a cloning method. A total of 48 and 43 of human and animal fecal DNAs were directly extracted respectively, and examined by a set of subtype-specific PCRs. Based on the band intensity profile of the PCR amplicons, 25 out of the 48 human and 28 out of the 43 animal fecal samples were judged as true positive for *Blastocystis*, respectively. Unexpectedly, the most STs detected from the residents and their animals were not matched, while only ST1 was identified in some residents and goats. In the variable region analysis of SSU rDNA, all five haplotypes isolated from the ST1-amplicons from goats were completely matched to those obtained from the residents. Although *Blastocystis* parasite has been believed to be zoonotic parasite, the results of this study indicates that the most STs of *Blastocystis* are maintained as host-specific manner, and the zoonotic transmission seems to be accidentally taking place in the lifecycle. It should be noticed that the direct DNA extraction from fecal samples, which have been widely applied for the molecular epidemiology of this parasite, could detect the mechanical passage of the cysts, thus the determination method for the true colonization of *Blastocystis* sp. might be required.

Keywords: *Blastocystis*, transmission, epidemiology, zoonosis