MICROSPORIDIAN INFECTION IN A WILD NEMATODE VARIES DEPENDING ON THE SEX AND LIFE STAGE OF THE HOST

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Microsporidia are obligate endoparasitic organisms that infect animals and a few protists. We discovered and characterized a novel microsporidian parasite, Sporanauta perivermis, that infects the hypodermal, muscle, and reproductive tissues of the free-living marine nematode Odontophora rectangula. In this study, we performed fluorescent in situ hybridization (FISH) to track the infection at different life-stages of the host. We observed two patterns in juvenile O. rectangula nematodes: 1. Pronounced infection of the hypodermal and muscle tissues 2. Infection of the reproductive tissues. Sex cannot be determined in juveniles. However, FISH examination revealed that the patterns observed in juveniles could correspond to the patterns observed in adult males and females. In adult males, S. perivermis causes a pronounced infection of the hypodermal and muscle tissues without affecting the reproductive tissues. On the other hand, infection in adult females is considerably less abundant in the hypodermal and muscle tissues, while the reproductive tissues and eggs (if present) are heavily infected. Our results suggest that sex in juveniles may be predicted based on the infection pattern. We further predict that juveniles destined to be males are those in which the infection affects the hypodermal and muscle tissues only, while juveniles destined to be females are those in which the infection involves the reproductive tissues. Overall, our results suggest that host-parasitic interactions between S. perivermis and O. rectangula vary depending on the sex and life stage of the host, and that S. perivermis is transmitted vertically.
MITOCHONDRIAL GENOME SCAFFOLDS FROM THREE LABYRINTHULOMYCETE DRAFT GENOME ASSEMBLIES

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Labyrinthulomycetes comprise ubiquitous, abundant, and diverse fungus-like marine protists. They are likely to play important roles in the decomposition of particulate organic matter in the oceans, including refractory substrates. They also produce, de novo, large amounts of essential polyunsaturated fatty acids, which may give them an important role in the nutrition of marine metazoans. To gain insight into the physiological ecology of these organisms, we are sequencing and annotating the genomes of *Aplanochytrium* sp. PBS07, *Schizochytrium aggregatum* ATCC 28209, and *Aurantiochytrium limacinum* ATCC MYA-1381. Among the assembled scaffolds we identified one from each organism consistent with mitochondrial genomes (mtDNA), and analyzed these in comparison to the previously reported mitochondrial genome from *Thraustochytrium aureum* (GenBank Accession AF288091). The *Aurantiochytrium* and *Schizochytrium* scaffolds were both similar in size to the gene-rich region of the *Thraustochytrium* mtDNA (34418, 36775, and 31570 bases, respectively). The *Aplanochytrium* mtDNA scaffold was much longer, 55616 bases, largely because of a repeat region containing several genes combined with greater intergenic spacing, not because of greater content of distinct genes. In fact, *Aplanochytrium* has transferred more genes from the mtDNA to the nuclear genome than the thraustochytrids. 21 tRNAs were identified in each of the three thraustochytrids, while 33 were identified in *Aplanochytrium*. Only one of the additional *Aplanochytrium* tRNAs was a type not present in the thraustochytrids, and the other 11 were duplicated in the repeat region. The *Aplanochytrium* scaffold also requires a unique genetic code to produce conserved proteins; in particular, TAG apparently encodes for tyrosine.
ORDER PHYSARALES: THE FIRST REPRESENTATIVE MOLECULAR APPROACH

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The order Physarales constitutes the most diverse and widely distributed group among Myxomycetes, which are protists with a complex life cycle including trophic (plasmodium), reproductive (sporocarps) and dormant stages. These organisms have been extensively studied from a morphological point of view. However, little is known about the phylogenetic relationships among members of this taxon since previous molecular studies that included Physarales were only focused on understanding higher-level relationships. A more detailed molecular study is being performed to build a representative phylogeny of the group, with the aim of unraveling the relationships among different species and genera. Sampling effort was directed to cover morphological and geographic variation, so at least two individuals of each morphospecies were selected from different locations. Several molecular markers (i.e. SSU, LSU and EF1α), are being sequenced using both published and newly designed primer pairs. So far, almost 60 SSU sequences have been obtained, revealing that some genera may not be monophyletic as previously thought. Moreover, available morphological information has been compiled and processed in order to determine the most taxonomically informative characters. The global analysis of molecular results combined with a well-documented morphological data set and ecological information will allow us to propose an updated classification of the order.
LARGE-SCALE PHYLOGENOMIC ANALYSIS REVEALS THE PHYLOGENETIC POSITION OF THE PROBLEMATIC TAXON PROTOCRUZIA AND UNRAVELS THE DEEP PHYLOGENETIC AFFINITIES OF THE CILIATE LINEAGES

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The Ciliophora is one of the most studied protist lineages because of its important ecological role in the microbial loop. While there is an abundance of molecular data for many ciliate groups, it is commonly limited to the 18S ribosomal RNA locus. There is a paucity of data when it comes to availability of protein-coding genes especially for taxa that do not belong to the class Oligohymenophorea. To address this gap, we have sequenced EST libraries for 11 ciliate species. A supermatrix was constructed for phylogenomic analysis based on 158 genes and 42,158 characters and included 16 ciliates, four dinoflagellates, and nine apicomplexans. This is the first multigene-based analysis focusing on the phylum Ciliophora. Our analyses reveal two robust superclades within the Intramacronucleata; one composed of the classes Spirotrichea, Armophorea, and Litostomatea (SAL) and another with Colpodea and Oligohymenophorea. Furthermore, we provide corroborative evidence for removing the ambiguous taxon Protocruzia from the class Spirotrichea and placing it as incertae sedis in the phylum Ciliophora.
CULTIVATION OF A NOVEL BREVIAITE REVEALS CLOSE SYNTHROPHIC ASSOCIATION BETWEEN ANAEROBIC PROTISTS AND DENITRIFYING BACTERIA

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Heterotrophic protists are major consumers of bacteria in marine habitats and frequently found in different types of sediment. Many species live in sediments that are devoid of oxygen but instead exhibit high concentrations of alternative electron acceptors like nitrate, nitrite or nitrous oxide. These electron acceptors are used by many prokaryotic species for anaerobic respiration. Unlike bacteria most protists are not able of anaerobic respiration and thus it is generally believed that the presence of these electron acceptors has no or only little effect on protistan growth.

Here we report the cultivation of a novel anaerobic Breviate that is dependent on the presence of nitrate, nitrite or nitrous oxide and lives in a syntrophic association with denitrifying Epsilonproteobacteria. By combining results from metagenomic studies with classical growth experiments we could unravel the phylogenetic placement of the Breviate, its metabolic capabilities as well as the mechanisms behind the syntrophic association. Our study demonstrates that interactions between heterotrophic, free-living protists and bacteria can be very complex and extent beyond the commonly assumed predator-prey relationships.
PROTIST DIVERSITY IN LAGOON ECOSYSTEMS OF THE SOUTH PACIFIC

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Our current understanding of protist diversity in nature remains far from complete. In light of this, we surveyed diversity of protists in poorly studied lagoon waters sampled through the AMNH Explore21 expedition to the Solomon Islands that took place in September 2013. Microbial biomass was collected on polycarbonate membrane filters and remained frozen until processed in the laboratory. Total DNA extracted from these filters was used to construct 18S rDNA amplicon libraries, which were sequenced on the MiSeq platform. In addition, live samples were brought back and used for isolation and culturing work. Together, we have thus far identified about a dozen of novel microbial taxa, including some that might represent new deeply branching lineages.
Oxymonads are a small group of anaerobic flagellates with most known representatives being obligatory symbionts of wood-eating insects. Most oxymonads are unculturable and they are in many ways one of the least understood groups of protists. Until recently, the standard way to obtain data from unculturable organisms was environmental sequencing and metagenomics, which provide information about the diversity and ecology of the environment itself, but very little about the biology of the actual organisms. The emerging technique of single-cell transcriptome sequencing may represent an optimal way to obtain large amounts of sequence data from an organism of interest without the necessity of established culture. In the present study we used a commercially available single-cell transcriptome kit to test the feasibility of such a protocol for use in protistology. Firstly, we tested the protocol on several ciliate species to gain baseline information on the efficiency of the protocol. Secondly, we isolated several single cells of oxymonads *Pyrsonympha*, *Dinenympha* and *Streblomastix*, amplified their mRNA, and sequenced it with MiSeq Illumina technology. Subsequently, we used the obtained data to resolve phylogenetic relationships and analyze basic metabolic processes of these protists. This demonstrates the significance of single-cell transcriptomics for studying hard-to-culture species.
Dating the Rise of Silica Biomineralization in Arcellinids and Euglyphids

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Biomineralization of silica is widespread among eukaryotes. The typical silica biomineralizer use the product as a protective cover, such as in diatom frustules. The two major groups of testate amoebae, arcellinids and euglyphids, are capable of producing their own silica particles in order to construct their shells. These two groups are unrelated phylogenetically, arcellinids are tubulinid amoebozoans and euglyphids are cercozoan rhizarians. Hence, these groups have independently acquired the ability to biomineralize silica. Nebelids, a group within arcellinids, are predators of euglyphids. Nebelids are reportedly capable of using silica scales mineralized by the euglyphids in order to construct their own shells. We present novel evidence for this phenomenon, and coupled with ancestral state reconstructions and a re-analyses of the literature, we have determined that the ability to “steal” euglyphid scales is ancestral in Nebelids, meaning that euglyphids must have been already present when this group arose. Thus, it is possible to determine a minimum age for the rise of euglyphids given a dated phylogenetic reconstruction of nebelids, a group where much more fossil data is available. We present such reconstruction and conclude that both euglyphids and nebelids must have arisen in the early Carboniferous (~370 million years ago).
YOU ARE WHAT YOU EAT: HORIZONTAL GENE TRANSFER IN EUGLENOIDS:

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The history of euglenoids began nearly two billion years ago. These early phagotrophs fed upon cyanobacteria, archaea, and eubacteria. The appearance of red algae and chromalveolates (e.g. diatoms, cryptophyes and dinoflagellates) approximately 1.8-1.2 billion years ago, provided euglenoids with additional food sources. Following the appearance of green algae, euglenoids acquired a chloroplast via a secondary endosymbiotic event with a green algal ancestor. This event involved not only the acquisition of the chloroplast, but massive transfer of thousands of nuclear encoded genes from the symbiont nucleus to the host nucleus. Prior to this chloroplast acquisition, euglenoids may have had nearly a billion years of history and interaction with red algal and chromalveolate lineages allowing for the possibility of other pre-chloroplast endosymbiotic events, or gene transfers to occur. This research shows that non-photosynthetic, housekeeping genes were also transferred to the nucleus from red or chromalveolate lineages. Using BLAST searches against our euglenoid EST database we have detected over 300 putative instances of gene transfer. The data for biotin synthase, glutamate tRNA synthase, prolyl tRNA synthetase, protoporphyrin oxidase, coproporphyrinogen III oxidase, β-ketoacyl-CoA synthase, citrate synthase and others show that genes have been acquired as a result of EGT/HGT and exemplify the findings for many of the 300 genes. This demonstrates that the euglenoid nuclear genome is a mosaic comprised of genes from the ancestral lineage plus genes transferred by EGT/HGT from green, red, and chromalveolate lineages.
Towards multigene analyses of phagotrophic euglenid phylogenetics and biodiversity based on single-cell methods

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The taxon Euglenida contains organisms with a broad range of morphological appearances and various nutritional modes including phagotrophy, osmotrophy, phototrophy and mixotrophy. Phagotrophic euglenids suffer from a low taxon sampling since very few of them are cultured and the whole assemblage is thus understudied. Current taxonomy is based on a variety of morphological features, but as increasingly more molecular data are collected, the misleading nature of most of these morphological characters has become obvious. We have sequenced SSU rRNA genes from single phagotrophic euglenid cells identified with high-quality light microscopy. Resulting phylogenies show that Anisonema and Dinema are admixed, and the polyphyly of the genus Heteronema. But although useful, the SSU rRNA gene in euglenids can be very divergent, and likely contains insufficient reliable signal to robustly reconstruct euglenid phylogeny. To tackle this issue, we modified and adapted existing multiple displacement amplification (MDA) protocols for single cell methods on phagotrophic euglenids, with the aim of obtaining sequences from multiple marker genes per cell using shotgun Illumina sequencing. In our modified MDA we included primers targeting specific genes (e.g. SSU, LSU, Hsp70, Hsp90, etc.) with regular random hexameric primers. We will discuss the applicability of this method on the basis of our preliminary results, any findings based on multi-gene phylogenies, and the future option of single-cell transcriptomics to obtain still greater data depth.
BIOINFORMATIC APPROACH TO THE INVESTIGATION OF ADAPTOR PROTEIN (AP) COMPLEXES IN HAPTOPHYTES

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Emiliania huxleyi is a marine haptophyte with a significant influence on our carbon cycle through the formation of calcium carbonate shells (coccoliths). The process of biomineralization, or shell formation, the details of which still remains unclear, is carried out via the membrane trafficking system (MTS), a system which modulates transport between organelles, as well as endocytic and exocytic processes. Adaptor Protein (AP) complexes are an integral part of this MTS and these ancient proteins mediate transport between plasma membrane, the Golgi, endosomes, and lysosome-related organelles, imparting specificity in cargo selection.

Several key losses of adaptor proteins have been identified in the recently sequenced E. huxleyi strain CCMP1516. In order to elucidate the mechanism of biomineralization in an organism with a reduced MTS, the modulation of the AP complexes of the naked haptophyte, Isochrysis, and calcifying haptophytes, Gephyrocapsa and several E. huxleyi strains, were examined using comparative genomics, phylogenetic analysis, and transcriptomic analysis.

We report haptophyte-specific losses of two AP complexes, AP-3 and AP-5, and calcifying haptophyte-specific duplications in AP-complexes that represent a major difference between biomineralizing and non-biomineralizing haptophytes. In addition, there is evidence of novel AP-related proteins in all haptophytes examined. These include TCUP; which is a subunit of TSET, a new heterohexameric trafficking complex related to AP complexes. Furthermore, we identified proteins that convergently resemble Golgi-localizing, gamma-ear containing, ARF binding (GGA) proteins previously found in humans and yeast, with role in sorting between trans-Golgi network (TGN) and endosomes.
COMPARING GENE EXPRESSION OF *OCHROMONAS*, A MIXOTROPHIC CHRYSOPHYTE, UNDER DIFFERENT RESOURCE AVAILABILITY

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Mixotrophic protists are a diverse and ecologically important group of organisms whose role in the ecosystem can be affected by resource availability. Investigations of how resource availability affects the physiology and gene expression of these organisms provide understanding of their functional roles in the ecosystem. Species of *Ochromonas* are mixotrophic chrysophytes (phagotrophic phytoflagellates) that are found ubiquitously in many aquatic environments and are potentially major consumers of bacteria. We studied how the availability of light and bacteria affected the growth and gene expression of a freshwater *Ochromonas* species (strain BG1). Cultures of *Ochromonas* were fed with heat-killed bacteria and grown in the presence or absence of light, and then cells were harvested for RNA during the exponential growth phase. RNA was also harvested from cells reaching stationary phase in the light, 4 – 5 days after heat-killed bacteria were depleted. Growth of *Ochromonas* was similar whether or not light was available, indicating *Ochromonas* is a predominantly heterotrophic mixotroph. Gene expression profiles differed significantly between cells in stationary phase and exponential phase in the light. Gene expression profiles of cells grown with or without light in the presence of bacterial prey in exponential phase were similar. This investigation will allow us to identify genes whose expression are highly reactive to changes in resource availability and may aid in the understanding of the physiology of this organism and its role in the environment.
Despite the ecological and economic importance of protists, broad surveys of protistan diversity in environmental and human samples have rarely been conducted. This is largely due to the lack of universal high-throughput sequencing methods for the comparison of microbial eukaryotic communities. A promising protocol intended for use on the Illumina platform and published by the Earth Microbiome Project, targets the V9 region of the 18S rRNA marker gene and includes a ‘vertebrate blocking primer’ designed to reduce the amplification of vertebrate DNA. Contamination with, for example, human DNA can be problematic in studies of microbial eukaryotes. We conducted a pilot study to determine 1) whether this protocol can detect a variety of protists, 2) establish the utility of the vertebrate blocking primer and 3) determine if the protocol is suitable for use with environmental samples. First we tested the utility of the primers by Sanger sequencing amplicons from 17 species of protist obtained from ATCC, including Cryptosporidium parvum, Giardia intestinalis, Toxoplasma gondii and several species of trichomonad. We then optimized the library protocol for 2x100 Illumina MiSeq sequencing using artificial mixtures of the DNA used for Sanger sequencing plus environmental samples from the NYC subway and sewage systems. The results demonstrate that the V9 region can detect and identify a variety of protist and vertebrate DNA that may be present in environmental samples, and that the blocking primer reduces the amount of host DNA amplified, thus providing an effective way to detect microbial eukaryote genetic material in environmental samples.
THE CALVIN-BENSON CYCLES COMES FULL CIRCLE IN EUGLENOIDS

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Euglenoids are an ancient eukaryotic lineage that may have existed as early as two billion years ago. In contrast, a mere 64 years ago, Melvin Calvin and Andrew A. Benson performed experiments on *Euglena gracilis* and elucidated the series of reactions by which carbon is fixed and reduced during photosynthesis. However, the history of this pathway (Calvin-Benson Cycle) in euglenoids was more complex than Calvin and Benson could have imagined. The chloroplast present today in photosynthetic euglenoids arose from a secondary endosymbiosis between a phagotrophic euglenoid and a green alga. A long period of evolutionary time existed before this secondary endosymbiotic event took place which provided opportunity for other endosymbiotic events or gene transfers to occur prior to the establishment of the green chloroplast. This research revealed the evolutionary history of the major enzymes of the Calvin-Benson cycle throughout the photosynthetic euglenoid lineage and showed that the majority of genes for Calvin-Benson cycle enzymes shared an ancestry with red algae and chromalveolates - which suggested that they were likely horizontally transferred to the nucleus prior to the acquisition of the green chloroplast. The history of each enzyme will be summarized, but the phylogeny of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sedoheptulose-bisphosphatase (SBPase) and triosephosphate isomerase (TPI), will be presented to illustrate how the phylogeny of each enzyme was determined.
GENE TRANSFER AND GENOME EVOLUTION IN CRYPTOPHYTE AND CHLORARACHNIOPHYTE ALGAE

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The process of secondary endosymbiosis, in which a eukaryotic endosymbiont establishes itself inside a eukaryotic host, has played a major role in the spread of plastids (chloroplasts) across the tree of eukaryotes. The cryptophytes and chlorarachniophytes harbor plastids derived from red and green algal endosymbionts, respectively, and are unusual amongst secondary plastid-bearing algae in that the nucleus of the endosymbiont persists in a miniaturized form termed the nucleomorph. The Archibald Laboratory uses genomic tools to study the evolution of nucleomorph-bearing algae. We recently led an international effort to sequence the nuclear genomes of the cryptophyte Guillardia theta and the chlorarachniophyte Bigelowiella natans. Here we present preliminary results of an Illumina-based effort to sequence the nuclear, nucleomorph, mitochondrial, and plastid genomes of two additional species: the cryptophyte Chroomonas mesostigmatica CCMP1168 and the chlorarachniophyte Gymnochlora stellata CCMP2057. In addition to comparing the gene content of these genomes to their counterparts in G. theta and B. natans, we are particularly interested in the frequency and mode(s) of organelle-to-nucleus DNA transfer. We wish to test the ‘transfer window hypothesis’ of Barbrook, Howe and Purton, which posits a relationship between the number of organelles per cell and the frequency of DNA transfer. The significance of bona fide organelle-derived DNAs in the C. mesostigmatica and G. stellata nuclear genomes will be discussed.
EXPERIMENTAL TRANSCRIPTOMICS OF THREE LABYRINTHULOMYCETES SPECIES: INSIGHTS ON CARBON DEGRADATION AND VITAMIN METABOLISM

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Labyrinthulomycetes is a ubiquitous, diverse, and abundant group of predominantly marine Stramenopiles that live mainly as saprobes, obtaining their nutrition from non-living particulate organic matter of algal, higher plant, or animal origin. Thus, even though labyrinthulomycetes are not fungi, they function as fungi in an ecological sense. Despite the important role these organisms may play in the cycling of marine carbon and other elements, they still remain under-studied, and most of their metabolic peculiarities, such as the potential capability for decomposing a wide range of biopolymers, including crude oil and tarballs, remain unknown. The genomes of three species (Aurantiochytrium limacinum ATCC MYA-1381, Schizochytrium aggregatum ATCC-28209 and Aplanochytrium kerguelense PBS07) have been sequenced at DOE-JGI, evidencing genome sizes ranging from 35 to 60Mbp. Each of these species has also been grown in four different culture media (790By+, horse serum, Artemia detritus, and Spartina detritus) and their RNA extracted and sequenced as a part of the Marine Microbial Eukaryote Transcriptome Sequencing initiative of the Moore Foundation.

Having access to both genomes and a set of different transcriptomes represents an excellent opportunity to gain insight into the hydrolytic and metabolic repertoire of each species. We have mapped RNAseq Illumina reads to corresponding genomes and this showed patterns of expression that were common to each species but, more interestingly, there were differences in expression associated to different culture media. The analysis of these transcriptional differences allows investigating genes involved in organic carbon degradation and vitamin metabolism.

Submitted for a POSTER
COMPLEX EVOLUTION OF PLASTID GAPDHs IN THE DINOFLAGELLATE SPECIES WITH GREEN ALGA-DERIVED PLASTIDS.

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Typical photosynthetic dinoflagellates possess red alga-derived plastids. However, some minor groups replaced their original plastids by the plastids of other eukaryotic algae enslaved as endosymbionts. The nuclear genomes of these dinoflagellates encode the genes with the apparent phylogenetic affinity to the endosymbiont, suggesting that plastid replacement triggered endosymbiotic gene transfer.

In this study, we investigated the origins of plastid glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) in two novel dinoflagellate species with green alga-derived (Chl-a+b) plastids, strains TRD and MRD. Considering the plastid origins in strains TRD and MRD, we anticipated the ‘green algal’ version of plastid GAPDH genes in both two strains. Unexpectedly, both strains were found to possess haptophyte-type GAPDHs for their green alga-derived plastids. Intriguingly the haptophyte-type plastid GAPDH is also known in Lepidodinium chlorophorum, which bears a Chl-a+b plastid (Takishita et al. 2008) but is distantly related to strain TRD or MRD in the dinoflagellate phylogeny. Altogether, multiple lateral gene transfers are necessary to explain the distribution of haptophyte-type plastid GAPDH gene in the three dinoflagellates with green alga-derived plastids.
RNA-SEQ ANALYSIS ON RANDOMLY-FRAGMENTED LINEAR MITOCHONDRIAL GENOME OF AN OYSTER PATHOGEN *PERKINSUS MARINUS*

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Mitochondrial genome is essential for oxidative phosphorylation and thus conserved throughout eukaryotes living in aerobic environment. However, those of protists heavily vary in their size, topology, and gene content; sometimes how to encode proteins is modified. *Perkinsus marinus* is a notorious unicellular parasite infecting farmed oyster population. We have recently sequenced full-length mitochondrial *cox1* gene of this organism, which requires frequent and thus efficient frameshift during translation. To obtain a whole picture of this peculiar phenomenon, we are now going to determine genetic information supporting the frequent frameshift. Previously, we have sequenced the mitochondrial genome. The genomic contigs extended up to 67 kbp in total, but series of experiments showed that they physically consists of randomly-fragmented linear DNA molecules with varying length of 1 to 10 kbp. It exhibited highly AT-rich composition, and encoded only three proteins, *cox1*, *cox3*, and *cob*; large majority of the 67-kbp information seems non-coding. In the present study, we have performed RNA-seq to see what part of the genome is transcribed, and how are the ribosomal RNAs encoded. Since the ribosomal RNAs were estimated to be fragmented as in apicomplexan parasites, small RNA fraction was prepared form the whole cell extract, and subjected to RNA-seq using Ion PGM. Out of 4.5 million reads, ca. 10% was mapped to mitochondrial genome, chiefly on the non-coding region. Analysis is still on-going, and we will present the results up to the minute.
GENOMIC INSIGHTS INTO PARAMOeba INVADENS AND ITS Kinetoplastid Endosymbiont

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Endosymbiosis – the process in which one cell evolves inside another – has been a driving force in evolution. However, endosymbiotic events involving the uptake and integration of a heterotrophic, eukaryotic cell by another eukaryote are extremely rare. An interesting example can be observed in the parasitic amoeba Paramoeba, which harbors a kinetoplastid endosymbiont (PLO, Perkinsela-like organism) in a stable (obligate) endosymbiotic relationship. Very little is known about the reasons for, and nature of, the interactions between these two organisms. To enlighten the endosymbiotic relationship of Paramoeba invadens, the causative agent of recurrent mass mortality of sea urchins, and its kinetoplastid endosymbiont, we are sequencing the host and endosymbiont nuclear genomes using Illumina technology. Here we present a preliminary analysis of these genomic data, including comparison with the recently sequenced genome of Paramoeba pemaquidensis (formerly Neoparamoeba). Our focus is on the extent of genomic and metabolic interaction between host and endosymbiont. To this end, we are exploring the secreted proteins encoded in both genomes and are using phylogenomics to search for endosymbiotic gene transfers. Our long-term goal is to understand the extent to which the PLO is integrated with its host, and the mechanisms that led to the establishment of this unusual endosymbiosis. We also hope to gain insight into the nature of the pathogenic capabilities of Paramoeba species, which infect a wide variety of marine animals.
Investigations into the eukaryotic diversity at hydrothermal vent ecosystems have been limited in the scope of sampling compared to prokaryotic diversity studies. Sampling effort at any particular site has been limited to a few samples of sediment or heavily diluted hydrothermal fluids with little regional sampling coverage. Recent studies of prokaryotic 16S rDNA diversity at both mid-ocean ridge and volcanic arc seamounts have demonstrated distinct patterns in community composition linked to geographical isolation. Significant differences have been shown in prokaryotic populations along the Mariana Arc both between seamounts and individual vents on the same seamount without significant links to chemical parameters. Preliminary results are presented of a unique regional scale survey of the eukaryotic 18S rDNA diversity in diffuse hydrothermal fluids at seven seamounts along the Mariana Arc. Results show a trend similar to that of Mariana prokaryotes of little overlap between protistan assemblages at different seamounts. The majority of sequences grouped among the alveolates and stramenopiles; however, diversity within these groups was high. Particular focus is placed on NW Rota-1 which is a dynamic, actively eruptive volcano and which contained the highest proportion of protists in the 18S sequence libraries and the highest diversity. The continuity of hydrothermalism along the Mariana Arc may provide a natural laboratory in which to identify levels of endemicity of various eukaryotic species as well as investigate community responses to changing conditions in dynamic settings. Further research is proposed to assess the nature of the 18S rDNA and to address questions regarding its sources.
MITOCHONDRIAL GENOME OF *PALPITOMONAS BILIX*: UNIQUE GENOME STRUCTURE AND ANCESTRAL CHARACTERISTICS IN GENE CONTENT

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*Palpitomonas bilix* branched at the basal position of a clade of cryptophyes, goniomonads, and kathabrepharids in a recent phylogenomic analysis. In this study, we determined the mitochondrial (mt) genome of *P. bilix* by using a next generation DNA sequencing technique. The *P. bilix* mt genome was assembled as a single linear molecule with approximately 75 kbp in length. The mt genome appeared to comprise a single copy region (~15 kbp) and nearly identical inverted repeats (~30 kbp) flanking at both 5’ and 3’ ends of the single copy region. Intriguingly, the overall structure of *P. bilix* mt genome strikingly resembles that of a colponemids-like organism recently reported by Janouškovec et al. (2013), although the two organisms belongs to two distantly related groups in the tree of eukaryotes (Cryptista and Alveolata). We identified 40 protein-coding genes, three ribosomal RNA genes, and 20 tRNA genes in the *P. bilix* mt genome. Comparisons of mt gene content between *P. bilix* and diverse eukaryotes illuminated the ancestral characteristics of the mt genome determined in this study. Significantly, some of the protein-coding genes found in the *P. bilix* mt genome have not been found in any mt genomes determined to date, except those of jakobids.
SNARE EVOLUTION AND THE CELL BIOLOGY OF APICOMPLEXAN PARASITES

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Genome sequence information is now available for diverse microbial species, and coupled with advances in phylogenetic analysis, has helped to elucidate many aspects of eukaryotic evolution. Focusing on experimentally tractable species in the phylum \textit{Apicomplexa}, we combined evolutionary and genomic approaches with cell and molecular biological manipulation to test phylogenetic hypotheses, and define key molecular players in the evolution of these important human and veterinary pathogens. Apicomplexans harbor a distinctive endomembrane system characterized by the presence of numerous organelles essential for the survival and replication of these parasites. The biogenesis and organization of these organelles is likely to be regulated by SNAREs (Soluble N-ethylmaleimide-Sensitive Factor Adaptor Protein Receptors), a family of protein that constitutes the central component driving membrane interaction in all eukaryotic cells. We have identified 25 \textit{Toxoplasma} and 24 \textit{Plasmodium} SNAREs that group into conventional R/Qabc-SNARE subclasses. Phylogenetic reconstruction, comparison with well-characterized human and fungal SNAREs, and subcellular localization permits identification of clear orthologs for most SNAREs involved in the early secretory pathway, but late SNAREs are either absent or highly diverged. Unusual apicomplexan SNAREs include several localizing to the parasite plasma membrane, rhoptries, or uncharacterized cytoplasmic vesicles. This study provides a map of SNAREs likely to be involved in distinctive parasite functions.
PARASITES IN PARADISE: COMPARATIVE GENOMICS OF NEPHROMYCES AND CARDIOSPORIDIA

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Apicomplexans are highly successful and often devastating parasites, infecting every major metazoan lineage. Until recently it was believed the apicomplexan phylum was entirely composed of obligate parasites, but species in the genus Nephromyces appear to be tunicate symbionts, inhabiting all of the members of the Molgulidae family. The closest relative of Nephromyces, Cardiosporidia, is a tunicate blood pathogen. This provides an unusual opportunity to study the transition from a parasitic to commensal lifestyle. We have performed preliminary sequencing of the Nephromyces and Cardiosporidia genomes to determine the metabolic pathways that enabled Nephromyces to become a symbiont. Our sequence data support the systematic placement of the Nephromyces and Cardiosporidium clade among the XXXX Apicomplexa. Additionally, sequence data reveal multiple different Nephromyces species inhabiting a single tunicate host, highlighting the need for further sampling. Our data support an earlier claim that Nephromyces harbor bacterial endosymbionts, further complicating the metabolic picture. The metabolic capabilities of both Nephromyces and its bacterial endosymbionts will be discussed, along with their relationship with the tunicate host.
Euglena gracilis is a secondary green alga related to trypanosomes that derives from a secondary endosymbiosis between a phagotrophic ancestor and a prasinophycean green alga. Our general objective is to study the metabolic interactions established between the secondary plastid and the mitochondrion after the endosymbiotic event and to determine the phylogenetic origin of the genes encoding the proteins involved in the energetic pathways. As a first step, we analysed the subunit composition of the mitochondrial respiratory chain, both in silico and by targeted proteomics, to assess the extent of its similitude with the respiratory chain of Trypanosomatidae. We have shown that Euglena shares many additional subunits with trypanosomes, which suggests that these subunits are not especially associated to a parasitic lifestyle. As a second step, we sequenced the total transcriptome of Euglena and determined the phylogenetic origin of each predicted transcript using a database of about 1000 complete proteomes representing the diversity of life. These analyses confirmed that Euglena recruited its genes from a very diverse set of sources. As a third step, we performed a high-throughput analysis of the mitochondrial proteome of Euglena. Our MS/MS experiments, taking advantage of the availability of our transcriptome, mostly recovered mitochondrial proteins, which indicates that our mitochondrial extracts were quite pure. The identified proteins encompassed about 15 different mitochondrial pathways. We are now in the process of comparing the expression levels of both the transcripts and the corresponding proteins across a range of culture conditions selected to differently stimulate the mitochondrion and the plastid.
SHOULD *Paramecium sonneborni* BE A MEMBER OF THE *Paramecium aurelia* SPECIES COMPLEX? INSIGHTS FROM MOLECULAR, GENETIC, AND CYTOLOGICAL STUDIES

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Recently recorded European strains of *Paramecium sonneborni* from Cyprus (*P. aurelia* complex), the species known before from only one stand (Northern America, Texas, USA), were recognized based on the results of strain crosses, cytological slides, and molecular analyses of one ribosomal (ITS1-5.8S-ITS2-5’LSU), four mitochondrial (i.e. *CytB* gene) and three nuclear loci (i.e. GSPATG00000363001). Characteristic features of *P. sonneborni* as type of micronuclei, new macronuclear anlagen, and phylogenetic trees based on the studied fragments revealed that *P. sonneborni* seems closer to *P. jenningsi* and *P. schewiakoffi* than to the other members of the *P. aurelia* complex. The results allows us to consider exclusion of *P. sonneborni* from the *P. aurelia* spp. complex and propose introduction of the new group of species *P. sonneborni, P. jenningsi,* and *P. schewiakoffi.*
ENGAGING IN EVOLUTIONARY EVALUATION OF ENDOBIONTS: TOWARD A MULTIGENE PHYLOGENY OF THE PROTIST TRICHOMYCETE ORDERS ECCRINALES AND AMOEBIDIALES

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The Mesomycetozoea is a relatively understudied class of unicellular symbionts that molecular phylogenies have placed at the divergence of animals and fungi. Subsumed in this class are the cosmopolitan orders Eccrinales and Amoebidiales, which are considered obligate commensal endobionts of various arthropods, including marine, freshwater and terrestrial hosts. Once thought to be members of the fungal class Trichomycetes due to their thallus-like growth form and ecological similarity, molecular evidence has necessitated reclassification. However, evolutionary relationships within and between them are still unclear as the number of taxa sampled and/or the amount of gene data gathered have been factors limiting resolution. Preliminary trees using 18 and 28S rDNA indicate a non-monophyletic Amoebidiales, which with the Eccrinales, are sister to Ichthyophonus (a fish parasite). Unexpectedly, Paramoebidium (Amoebidiales), aquatic insect associates, show some host sorting and possible cryptic speciation. We also update our ongoing efforts to establish a protein-coding gene for an enlarged data set.
MAPPING THE DIVERSITY OF METOPIDS AND REVEALING NEW MARINE ANAEROBIC CILIATES

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Anoxic sediments host a wide variety of ciliates. Anaerobiosis has independently arisen in several lineages of ciliates; so far, anaerobes have been found in at least six lineages of ciliates. To deepen our knowledge about their diversity, we have cultivated more than 50 strains from fresh water, brackish, and marine anoxic sediments worldwide. We determined their SSU rDNA sequences, performed protargol staining techniques, and studied light-microscopic morphology. In addition, we used transmission electron microscopy to assess the ultrastructure of some of the strains. Several novel clades of metopids, the free-living anaerobic ciliates of the class Armophorea, were identified. The distribution of one clade is limited to South America, whereas at least some of others are cosmopolites, found in different continents from habitats of various altitudes, from tropical marsh soils to high mountain lakes. Importantly, two new deep lineages of marine anaerobic ciliates were discovered. According to the SSU rDNA analysis, the first one is related to SAL group (Spirotrichea, Armophorea, Litostomatea) with Cariacotrichea, but forms a separate lineage, possibly a novel class. The second one clusters within Prostomatea, but is not specifically related to the anaerobic Plagiopylidae. We conclude that marine anoxic sediments harbor a high diversity of undescribed anaerobic ciliates.
ARCHIGREGARINES OF THE ENGLISH CHANNEL REVISITED

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Gregarines represent an important transition step from free-living predatory (colpodellids s.l.) and/or photosynthetic (Chromera and Vitrella) apicomplexan lineages to obligate intracellular parasites of metazoans such as coccidians and haemosporidians. (Un)fortunately, the damage these invertebrate parasites cause doesn’t match their theoretical importance. Thus, they belong to the most under-sampled and least-described protists. Among the gregarines, archigregarines represent one of the traditionally recognized groups with a unique mixture of ancestral (myzocytosis) and derived (lack of apicoplast, presence of subpellicular microtubules) features. Here we are revisiting the archigregarine diversity of the genus Selenidium (including the type species Selenidium pendula) from polychaetes of the English Channel. Out of the 14 different polychaete species examined, seven were found positive for the presence of archigregarines (Nerine cirratulus, Sabella pavonina, Sabellaria alveolata, Amphitrite gracilis A. johnstoni, Cirriformia tentaculata, and Cirratulus cirratus). The phylogenetic analysis of SSU rDNA revealed again the paraphyly of archigregarines. The five different archigregarines, formed a clade with other Selenidium representatives of, mainly tube-forming polychaetes. Based on morphology and host-profile these were identified as S. pendula, S. hollandei, S. sabellariae, S. sabellae and a yet to be identified or newly described Selenidium spp. For the first time, we expand the description of these species with DNA data and for some of them even with light and electron microscopy.
ABSCISIC ACID BIOSYNTHETIC PATHWAYS HAVE BEEN LOST IN A NON-PHOTOSYNTHETIC PLASTID OF PERKINSUS MARINUS, A PROTOZOAN PATASITE OF AN OYSTER

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The plastids are photosynthetic organelles that are spread over distinct eukaryotic lineages. On the other hand, some plastid-harboring organisms have lost photosynthetic ability during their evolution, and they mostly retain the non-photosynthetic plastids. Apicomplexan parasites (e.g. Plasmodium falciparum, Toxoplasma gondii etc.) also have non-photosynthetic plastids, termed the apicoplast, which conserves essential function(s) for parasite growth. However, the function remains controversial. In 2011, Yeh and DeRisi showed that isopentenyl pyrophosphate (IPP) biosynthesis is the only essential function in P. falciparum. And Nagamune et al showed that abscisic acid (ABA), a universal plant hormone biosynthesized from IPP, is produced and controls cell cycle regulation in T. gondii. Therefore we hypothesized that ABA has critical functions in cell cycle regulation even if the plastids lose photosynthetic ability. To elucidate this, we investigated whether ABA plays an important role in another non-photosynthetic plastid-harboring parasite Perkinsus marinus that is early diverged from Dinoflagellata, close to Apicomplexa. An ABA biosynthetic inhibitor fluridone blocked cell growth of P. marinus, but ABA supplement did not influence the parasite growth and rescue the parasite from the inhibition. In a liquid chromatography-mass spectrometry analysis, ABA was not detected in a parasite cell extract. These results suggest that ABA biosynthesis and ABA regulatory mechanisms have been lost in P. marinus and justify further studies to understand evolutionary relationships between cell cycle regulation and ABA in non-photosynthetic plastid-harboring organisms.
IDENTIFICATION OF MATING TYPE DETERMINING GENES IN SELECTED SPECIES OF THE *PARAMECIUM AURELIA* COMPLEX

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In each species of the *Paramecium aurelia* complex, two mating types are distinguished: O (Odd) and E (Even). The O and E types are homologous in all species, but they are determined by one of three different systems: maternal inheritance (synclonal), random determination (caryonidal), or Mendelian determination. Previous work in *P. tetraurelia* identified three genes that are required for expression of mating type E (*mtA, mtB, and mtC*). *mtA* encodes an E-specific transmembrane protein involved in agglutination with O cells, while *mtB* and *mtC* appear to encode transcription factors required for *mtA* transcription. In *P. tetraurelia*, mating type O is determined during macronuclear development by the excision of the *mtA* promoter as an IES; the rearrangement is regulated by the scnRNA pathway, explaining the maternal inheritance of mating types.

We have started a survey of selected species from each of the 3 systems to test whether mating type E is characterized by expression of *mtA* orthologs in all species, and to determine whether this always requires *mtB* and *mtC* orthologs. The final aim is to identify the mating-type determination mechanism in each species and to determine how is it possible to evolve from a maternal inheritance system to a random determination one, or the reverse, or to a Mendelian one.

The results obtained so far are consistent with the idea that *mtA* expression is always associated with type E, but the mechanisms of mating-type determination appear to vary widely among *P. aurelia* species.
Members of the genus *Paramoeba* (Amoebozoa) are best known as parasites that cause disease in a variety of marine animals including fish, sea urchins and lobster. These amoebae possess a distinctive perinuclear body historically known as the parasome. The parasome is in fact a eukaryotic endosymbiont belonging to the Kinetoplastida and has been given the name *Perkinsela* sp. The nature of the endosymbiotic relationship between *Paramoeba* and *Perkinsela* is poorly understood. We are using genomic, molecular, and phylogenetic approaches in an attempt to better understand the evolution of *Paramoeba* and the *Perkinsela* sp. living inside it. Here we present molecular sequence data and light microscopic observations from novel strains isolated from the Big Island of Hawaii. The impact of these findings on the origin and co-evolution of *Paramoeba* and *Perkinsela* sp. will be discussed.
TWO NEW SPECIES OF THECAMOEBA THAT DISPLAY UNIQUE TRANSIENT “DOUGHNUT-SHAPED” MORPHOLOGIES

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Two amoeboid organisms were isolated from the soil between the annex and lecture hall of Harned Hall on the Mississippi State University Campus. The two organisms are large, highly wrinkled amoebae with a single nucleus that contains nucleolus consisting of several parietal lobes. Amoebae also exhibit a unique “doughnut-shape” that as far as we know is not described in any primary literature. The isolated organisms share some similar morphological features with species in the family Thecamoebidae, in particular the genus Thecamoeba. Photographic comparison of the two isolated strains with previously described species of Thecamoebidae revealed morphological differences that show the two isolated species do not superficially belong to any previously described species in the family Thecamoebidae. Genetic comparison of the small subunit ribosomal DNA of the two isolated organisms with all of the available genetic data from Thecamoebidae confirms that the two isolated organisms are new species in the genus Thecamoeba and the phylogenetic relationship between the two new species and previously described species is examined. Further investigation is needed to examine the unique doughnut form using scanning electron microscopy and the structure of the glycoprotein cell coat (glycocalyx), an indicative character of Thecamoebidae amoebae, using transmission electron microscopy.
GENETIC AND EPIGENETIC CONTROL OF DNA DELETION IN PARAMECIUM: A GENOMIC PERSPECTIVE

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Since mating type inheritance studies in the late 1930s in Paramecium, ciliates have served as models for what has become known as epigenetic inheritance. We have now substantially advanced these studies by genome-wide analyses of the interplay between epigenetic and genetic control of DNA deletion. In order to transform a copy of Paramecium tetraurelia's micronuclear genome into a new macronuclear genome during sexual development it is necessary to target and precisely delete \( \approx 45,000 \) unique, Internal Eliminated Sequences (IESs). Two classes of small RNAs (scnRNAs and iesRNAs) are employed in IES targeting, and a PiggyBac-derived transposase, PiggyMac, is the putative IES excisase. scnRNAs relay epigenetic information from the parental macronucleus to the developing macronucleus, while iesRNAs are produced and used in the developing macronucleus.

Why only some IES deletion is epigenetically controlled has been enigmatic. By studying the effects of silencing of three genes: \textit{PGM} (DNA excision), \textit{DCL2/3} (scnRNA production), and \textit{DCL5} (iesRNA production) on a genome-wide scale, we identify key properties associated with IES deletion. Based on these analyses, we propose that, depending on the exact combination of their lengths and end bases, some IESs are less efficiently recognized or excised and have a greater requirement for targeting by scnRNAs and iesRNAs. We suggest that the variation in IES retention following silencing of \textit{DCL2/3} is not primarily due to scnRNA density but rather the genetic properties of IESs. Our studies suggest that the underlying genetic properties of developmentally deleted DNA sequences modulate the degree to which they are epigenetically controlled.
Preaxostyla (Excavata: Metamonada) is one of the least studied eukaryotic lineages. All members of Preaxostyla are anaerobic and are divided into free-living, morphologically relatively uniform genus *Trimastix* (3 species), and endobiotic, morphologically diverse oxymonads (more than 100 species living mostly in termites). In order to examine the diversity of free-living Preaxostyla more deeply, we isolated and cultured 35 freshwater and two marine strains morphologically consistent with *Trimastix*, and determined their SSU rDNA sequences. Results of phylogenetic analyses showed that the strains are extensively diversified. Two marine strains form either a clade or two paraphyletic basal lineages of the whole Preaxostyla. The freshwater strains constitute several lineages that form a robust clade with oxymonads. Although the precise phylogenetic position of the oxymonads is not completely resolved, it seems that they are closely related to a novel free-living clade represented by four strains.

The strains were examined also by means of light microscopy. Morphology of both marine and several freshwater strains roughly corresponds to *Trimastix marina* suggesting that this species is polyphyletic. Most freshwater strains are similar to *T. pyriformis*, but are phylogenetically far too diverse to represent a single species. Three strains forming a clade within the *T. pyriformis* complex possess tiny cells in comparison with the other strains.

Our data convincingly show that the phylogenetic diversity of free-living Preaxostyla is more extensive that of the endobiotic oxymonads, despite of the relatively uniform morphology. The example of free-living Preaxostyla indicates that the diversity of free-living anaerobic protists is still underestimated.
IRON-SULFUR ASSEMBLY IN OXYMONADS AND TRIMASTIX

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Oxymonads are anaerobic or microaerophilic protists living in guts of insects and vertebrates. Today they are considered to be the last large group of eukaryotes without confirmed mitochondrion-like organelle. In their closest free-living relative, Trimastix, were found mitochondrion-like organelles resembling hydrogenosomes. In the transcriptomic and genomic projects of Trimastix and oxymonad Monocercomonoides we were not able to identify any transcripts/genes for enzymes of mitochondrial ISC iron-sulfur cluster assembly machinery which is ubiquitous in eukaryotic organisms. Instead we found subunits of SUF (sulphur mobilisation) machinery which is in eukaryotes known only from plastids and from two unrelated anaerobes Blastocystis hominis and Pygsuia biforma. In the Monocercomonoides genome project we identified four subunits of SUF machinery – SufB, SufC, SufS and SufU. All these genes are localised in clearly eukaryotic scaffolds, SufC and fused gene for SufSU contain classical spliceosomal introns. The genes contain all essential domains needed for their function. In the Trimastix transcriptome we found subunits B, C, S, D, and U, but some of them may originate from bacterial contamination. Phylogenetic analyses of concatenated Suf B, C, and S of Monocercomonoides and Trimastix showed that they are sister related, which also indicate their eukaryotic origin. This was also proven by fluorescent in situ hybridization of subunits of SUF system in Monocercomonoides nuclei. Localisations of these enzymes in the cell are still unknown. Substitution of mitochondrial ISC pathway by SUF might serve as a preadaptation for the complete loss of mitochondria in oxymonads.
MEASURING RECLAMATION SUCCESS IN SOILS OF THE ATHABASCA OILSANDS USING PROTISTS AS A BIOLOGICAL INDEX

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Soil plays a central role in the functioning of all terrestrial ecosystems, and as such, soil quality is a key determinant of land reclamation success. The Athabasca oil sands mining area in Alberta, Canada, is a disturbance of over 600 km² of what was originally a mix of black spruce, white spruce and aspen forest. Following surface mining, reclamation involves salvaged surface soils and near-surface geological materials being placed as a new soil cover on reconstructed landscapes. This project characterized the biodiversity of soil mesofaunal populations on natural and reclaimed Athabasca oil sands sites. We focused on soil protists, as they are responsible for much of the nutrient fluxes through the soil food web and have crucial downstream impact on animal and plant biodiversity. Using stratified random sampling, replicated DNA extractions and PCR amplification of the V4 region of SSU rRNA, and Illumina NGS amplicon libraries, we found that the protist communities differ between samples, with diversity being dominated by metazoa and fungi in undisturbed forest, and organic and mineral soil being very similar; while diversity is much more evenly distributed across animals, fungi, plants, chrysophytes, cercozoa, ciliates and dinoflagellates in reclaimed sites, but with differences in diversity and relative abundances between age of reclamation as well as between organic and mineral soil. This study demonstrates for the first time that the protist fauna in reclaimed sites is different from that of undisturbed sites, which has implications for the establishment of undisturbed-equivalent food webs and land capability in reclaimed sites.
PTOLEMEBA N. GEN., A NOVEL GENUS OF HARTMANNELLID AMOEBAE (TUBULINEA, AMOEBOZOA); WITH AN EMPHASIS ON THE TAXONOMY OF SACCAMOeba

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Hartmannellid amoebae are an unnatural assemblage of amoeboid organisms that are morphologically difficult to discern from one another. In molecular phylogenetic trees of the nuclear encoded small subunit rDNA, they occupy at least five lineages within Tubulinea, a well-supported clade in Amoebozoa. The polyphyletic nature of the organisms has led to many taxonomic problems in regards to paraphyletic genera. Recent taxonomic revisions have alleviated some of these problems. However, the genus Saccamoeba is paraphyletic in molecular reconstructions and is still in need of revision as it currently occupies two distinct lineages. Here we report a new clade on the tree of Tubulinea, which we infer represents a novel genus that we name Ptolemeba n. gen. This genus subsumes a clade of hartmannellid amoebae that were previously considered in the genus Saccamoeba, but whose mitochondrial morphology is distinct from Saccamoeba. In accordance with previous research, we formalize the clade as distinct from Saccamoeba. Transmission electron microscopy of our isolates illustrate that both molecularly discrete species can be further differentiated by their unique mitochondrial cristal morphology.
Evolutionary history of MINOS: Evolutionary insight into the evolution of mitochondria-related organelles and the origins of cristae

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In fungi and metazoa, the mitochondrial inner membrane organizing system (MINOS) is a multi-subunit protein complex required for proper cristae formation. MINOS is also thought to tether the mitochondrial inner and outer membranes at cristae junctions. Using comparative genomics we sought to determine if MINOS could account for cristae biogenesis in diverse eukaryotes. We hypothesized that, if MINOS is critical for cristae biogenesis, then MINOS should be present in all organisms containing canonical mitochondrial cristae, but would be absent in organisms that contain degenerate mitochondria-related organelles (MROs) that lack cristae like hydrogenosomes or mitosomes. Here, we demonstrate that MINOS core components are present in all major eukaryote clades indicating a likely presence in the last eukaryote common ancestor. Furthermore, complete absence of MINOS components correlates with the presence of degenerate MROs suggesting that a general mechanism of cristae formation might be shared by diverse eukaryotes. In addition to its ancient origins, we show that some MINOS components are lineage-specific novelties unique to opisthokonts. Finally, our results suggest an α-proteobacterial origin of MINOS.
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 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, there is no effective drug against this disease. 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 using 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 in house established colorimetric assays. 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, will provide the basis for similar discovery platforms for additional amoebae, and will enable systematic chemical biology approaches designed to decipher Naegleria biology.
In the sunlit surface ocean, heterotrophic protists graze on phytoplankton and together with viruses control bacterial numbers. The largest biome in the world by volume, however, is an environment that is extremely limited by the input of organic nutrients from the surface water either through sinking particles, deep convection of dissolved organic matter, and some chemoautotrophic production. In recent years, we enumerated and to some extent were able to sort protists of bathypelagic and abyssopelagic environments into broad taxonomic groups. However, we know very little about the ecology of these eukaryotic microbes. One interesting aspect is that there is no further attenuation of protist concentrations with depth below the mesopelagic environment (> 1000 m). Another is that protists scale linearly with prokaryotic abundances even at very low prokaryotic concentrations despite the fact that average prey concentrations in the deep sea fall well below feeding thresholds of bacterivores. This contribution summarizes our most recent findings, explores some of the apparent contradictions, and compares several alternative hypotheses that our work group is currently involved in testing.