Federation of European Protistological Societies

ISOP International Society of Protistologists

Under the Patronage of

Istituto Superiore di Sanità

Gordon and Betty Moore Foundation

Parco Archeologico dell'Appia Antica

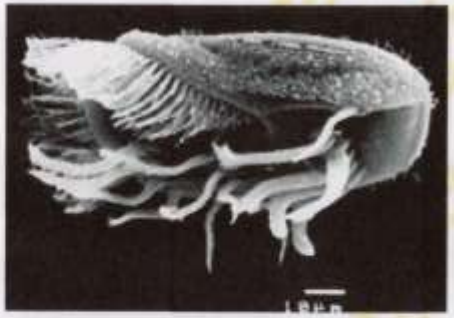
Rome 2019

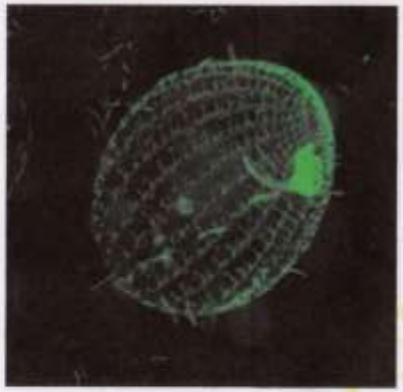
VIII ECOP - ISOP JOINT MEETING

VIII European Congress of Protistology - ISOP joint meeting

Rome • Italy

28 July • 2 August 2019





Zeiss

Gordon and Betty Moore Foundation

PROGRAM

MONDAY 29 JULY

PLENARY LECTURE ISoP Honorary Member (by ISoP)

Protozoa: Size, Shape, Structure and Functional Constraints

TOM FENCHEL[a]
[a]Institute of Biology, University of Copenhagen, Copenhagen, Denmark.

E-mail of the corresponding Author: tfenchel@bio.ku.dk.

The sizes of protozoa range by almost four orders of magnitude in terms of length and they come in a great variety of shapes and internal structure. Many also display polymorphic life histories in response to environmental heterogeneity in space and time. The talk will discuss constraints in terms of respiration, food particle acquisition particular and responses to a "feast and famine existence".

SYMPOSIUM on Ciliate in memory of Denis Lynn (by FEPS & ISoP)

The Biology and Systematics of Peritrich Ciliates: Old Concepts and New Findings.

ALAN WARREN[a]
[a]Natural History Museum, London, UK.

E-mail of the corresponding Author: a.warren@nhm.ac.uk

Peritrichs have been known for almost 340 years and were among the first protists to be observed and documented. With approximately 1,000 described species, peritrichs are one of the largest ciliate groups, accounting for almost 15% of all ciliate species. They are ubiquitous in aquatic habitats where most can be found attached, either permanently or temporarily, to a wide variety of submerged substrates, either living or inanimate, although some species are permanently free-swimming. Peritrichs play a key role in controlling populations of suspended bacteria by predation and some epibiontic forms can cause harm to their host. Despite the large body of scientific literature on peritrichs, knowledge of their origin, evolution, systematics and biogeography is scant and/or uncertain. In this talk, I will give a brief overview of some older concepts of peritrich biology (e.g. stalk and zooid contraction) and systematics. I will also report on some new findings from two recently published studies, i.e. Jiang et al. (2018) and Williams et al. (2018). The first concerns the origin, evolution and systematics of peritrichs based on phylogenomic analyses. The main findings were that: the subclass Peritrichia originated during the late Proterozoic to Cambrian, 488-820 Ma; the sister group to the peritrichs is the subclass Peniculia; and, the calcium-binding protein spasmin played a key role in peritrich evolution. The second study uses ecological niche models (ENMs) to investigate endemicity and climatic niche differentiation in three marine "flagship" ciliates, including the pelagic colonial peritrich *Zoothamnium pelagicum*, in the North Atlantic Ocean. The main findings were that our ENMs detected a clear environmental signal to the three species such that each occupies a distinct fundamental ecological niche, and that the distribution of each follows a consistent, predictable pattern that is related to climate and environmental biogeochemistry.

Amitosis and the Evolution of Asexuality in *Tetrahymena* ciliates.

HAO ZHANG[a], JOE WEST[a], RICARDO B. R. AZEVEDO[a], REBECCA A. ZUFALL[a]
[a]Department of Biology and Biochemistry, University of Houston, Houston, TX, USA.

E-mail of the corresponding Author: rzufall@uh.edu

For eukaryotes, sexual reproduction appears to be the most successful evolutionary strategy despite its many costs. While a complete explanation for sex's success remains elusive, several evolutionary benefits of sex have been identified and it is predicted that, by forgoing these benefits, asexual lineages are evolutionary dead-ends. Despite these low expectations, asexual strategies appear to be successful in some eukaryotic lineages, including the ciliate *Tetrahymena*. Here, we show that the mechanism of somatic nuclear division in *Tetrahymena*, termed amitosis, provides benefits similar to sex, allowing for the long-term success of asexual lineages. We found that amitosis, compared to mitosis, reduces mutation load deterministically, slows the accumulation of deleterious mutations under genetic drift, and accelerates adaptation. These benefits provided by amitosis are comparable to evolution under sexual reproduction, and arise from the fact that amitosis can generate substantial genetic variation among asexual progeny. Our results support the idea that the ability to persist in the absence of sex may depend on non-sexual genetic mechanisms to confer benefits typically provided by sex.

The Biology and Systematics of Oligotrichean Ciliates (Alveolata, Ciliophora): New Findings and Old Concepts

SABINE AGATHA[a]
[a]Department of Biosciences, Paris Lodron University of Salzburg, Salzburg, Austria.

E-mail of the corresponding Author: sabine.agatha@sbg.ac.at

Denis Lynn was among the first emphasizing the importance of the ciliate ultrastructure for inferring phylogenetic relationships. He continuously integrated the new findings obtained by the application of cutting-edge techniques into bigger concepts and classifications. Following his recommendations, the biodiversity of Oligotrichea was studied, combining different methods (live observation, protargol staining, scanning and transmission electron microscopy, barcoding, and phylogenetic analyses) and life cycle stages (trophonts, resting cysts, and dividers). Originating from his findings and concepts, new phylogenetic hypotheses have been established in recent years and are currently tested with the main aim to provide morphological and ultrastructural characters that allow the splitting of non-monophyletic taxa and support unexpected relationships in molecular genealogies. The Oligotrichea are mainly planktonic ciliates that comprise the monophyletic taxa Oligotrichida and Choreotrichida with the non-monophyletic aloricate choreotrichids and the monophyletic loricate tintinnids. Compared to the tintinnids, the oligotrichids and aloricate choreotrichids are cytologically well known, particularly in respect of their ciliary patterns, while the situation is inverse concerning the number of barcoded species. The recent findings and hypotheses on Oligotrichea are placed into the context of some of Denis Lynn's discoveries and conceptual ideas, e.g., the structural conservatism, the rule of excluded sectors, and the usage of the somatic kinetid's ultrastructure for proposing relationships.
The study is financially supported by the Austrian Science Fund (FWF) projects P28790 and I3268.

Ciliate Diversity and Ecological Interactions in Neotropical Environments

LAURA R. P. UTZ[a]
[a]School of Science, Pontificia Universidade Catolica do Rio Grande do Sul PUCRS, Porto Alegre, Brazil.

E-mail of the corresponding Author: laura.utz@pucrs.br

Ciliates are found in a variety of environments such as fresh and salt water, soil, and the digestive tract of animals; however, their diversity is poorly known especially in rare environments. Studies focusing on morphological or molecular data of rare habitats have highlighted Ciliophora as the most abundant phylum of unicellular eukaryotes. For example, Bromeliads are a Neotropical group of plants whose leaves often overlap allowing the entrapment of water. These rare habitats called phytotelmata harbor a diversity of organisms including bacteria, ciliates, arthropods and vertebrates. The diversity of ciliates in phytotelmata is very high, with several new species described in the last 20 years. Ciliates are also very diverse in sandy beaches; this type of ecosystem represents 2/3 of littoral zones in the world, and are shaped by complex factors such as winds, waves and sediment type. Although studies have shown a richness of interstitial ciliates as high as 40 species/cm^3, the lack of knowledge about interstitial communities regarding ecological and taxonomic aspects is still a problem, especially in the Neotropics. Likewise, periphyton is a complex community composed by bacteria, algae and heterotrophic unicellular eukaryotes that adhere to living or non-living surfaces in aquatic environments. Ciliates are an important component of periphyton, presenting a considerable diversity and reaching high abundance especially in freshwater environments. In this talk, I will provide a general overview about the diversity of ciliates in bromeliad tanks using morphological and DNA metabarcoding analyses. In addition, I will present data from a recent study comparing the diversity and abundance of ciliates in bromeliads from the Atlantic forest following an altitude gradient. Regarding sandy beaches, I will highlight the diversity of ciliates along the coast of Rio Grande do Sul state, southern Brazil, comparing their abundance with an urbanization gradient. Finally, I will present novel DNA metabarcoding data on the diversity of ciliates that compose the periphyton of a freshwater lake in Southern Brazil.

Oral Session ECOLOGY & BIOGEOGRAPHY 1

Tracking Genotypic Changes in *Paramecium* Isolates Between Ponds and Seasons in Ulster County, NY, USA

KATHERINE DOBOSH[a]
[a]SUNY New Paltz, New Paltz, NY, USA.

E-mail of the corresponding Author: katherinedobosh@yahoo.com

The numerous species of *Paramecia* can vary morphologically, functionally, and genetically. Previous biogeographical studies of *Paramecium* suggest that the cells follow the 'everything is everywhere' hypothesis and that local ecology determines the particular strains found in any given location. However, there has not been much research done on strain and species changes from season to season over short geographical distances as well as if or how *Paramecia* overwinter under ice. Over seven consecutive seasons, we have sampled five local ponds for *Paramecium* cells. We isolated single cells, created lines of culture and allowed them to grow to high density from each collected sample. We then extracted DNA, amplified specific genes by polymerase chain reaction (PCR), and sequenced them by Sanger sequencing. To determine the species, we compared the new sequences to sequences of known *Paramecium* species. We found species diversity within ponds as well as between ponds, and shared genotypes between the ponds, indicating that there has been recent migration between them. There are also preliminary indications that the abundance of certain species changes from summer to fall, hinting at possible adaptive differences between the species. Out of five ponds sampled in the winter, we were able to isolate *Paramecia* from one pond, suggesting that *Paramecia* may overwinter in this region. We are currently analyzing the specific haplotypes of the different species and further sampling to more clearly determine the patterns of strain and species changes over different seasons.

New Records of Flagship Ciliates Discovered in Soil from Florida, USA

HUNTER N. HINES[a,b], PETER J. MCCARTHY[b], GENOVEVA F. ESTEBAN[a]
[a]Bournemouth University, Bournemouth, England, UK; [b]Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, Florida, USA.

E-mail of the corresponding Author: hunter.n.hines@gmail.com

Ciliated protozoa are microbial eukaryotes common in soil environments. As grazers and members of the microbial loop, ciliates are of fundamental importance to healthy soils. Many ciliates form cysts and therefore exist in a cryptic state under adverse conditions; rewetting of natural soil samples reveals a community of ciliates which emerge as their preferred niche develops. The literature contains examples of 'flagship' soil ciliates which were described as endemic to the region in which they were discovered such as Africa or South America. We hypothesized that due to large population numbers and the ability of many soil ciliates to readily encyst, flagship ciliates that had previously been thought as restricted to other continents could be discovered in North America. Samples were collected from natural soils in Florida, USA, and cultured using a flooding technique to encourage ciliate excystment and the growth of other native organisms. Samples were enriched with sterile wheat grains, incubated at 30˚C, and periodically examined using light microscopy. Target ciliates were identified by morphology and 18S rRNA gene sequencing. Two 'flagship' ciliates described in the literature were discovered in Florida: a gold-colored species first described from, and only previously found in Africa, *Condylostomides etoschensis*, and a blue-colored ciliate first described from South America, *Condylostomides coeruleus*. These are first records for North America, and the first report of these species outside of their alleged restricted geographical ranges. Morphometrics and images matched those in the literature. The 18S rRNA gene sequences for *C. etoschensis* are the first reported, and that obtained from *C. coeruleus* closely matched the only sequence for this species deposited in GenBank. Flagship ciliates from soils can disperse beyond alleged restricted geographical ranges, and barriers to dispersal, such as distance, are apparently overcome by large population numbers and encystment, which provides resilience to environmental stress.

Structure and Molecular Composition of the Kinetocyst in the Centrohelid Heliozoan *Raphidiophrys contractilis*

TOSHINOBU SUZAKI[a], MOUSUMI BHADRA[a], MUNEHIRO KARASAWA[a], MAYUMI KOBAYASHI[b], MD SHAFIQUL ISLAM[c]
[a]Graduate School of Science, Kobe University, Kobe, Japan; [b]National Research Institute of Fisheries Science, Yokohama, Japan; [c]Bangladesh Ansar and VDP, Bangladesh.

E-mail of the corresponding Author: suzaki@kobe-u.ac.jp

The centrohelid heliozoan *Raphidiophrys contractilis* was found to recognize curdlan, an insoluble β-1,3-glucan, as food. When a suspension of curdlan gel was added to the heliozoans, the gel particles were ingested into food vacuoles. By affinity purification with curdlan gel, a protein of 100 kDa was isolated as the only β-1,3-glucan-binding protein from the detergent-extracted cell homogenate of *R. contractilis.* The protein was identified as major vault protein (MVP), which is known as the main component of "Vault complex". The *R. contractilis* MVP reacted with an antibody against human MVP, and specific binding to β-1,3-glucan was verified by a competition assay with laminarin, a soluble-type β-1,3-glucan. The heliozoans were mixed with prey flagellate *Chlorogonium capillatum* and extracellular fluid was collected during food-uptake. The fluid was then subjected to pull-down assay with curdlan gel, by which multiple protein species were detected including MVP as one of the major proteins, suggesting that MVP is secreted from the heliozoans during food uptake as a component of a large protein complex. The centrohelid heliozoans utilize kinetocyst to capture prey organisms. The kinetocyst is a type of extrusome that releases its contents to the prey organism when it contacts the heliozoan cell surface. Immunoelectron microscopy revealed that MVP is localized in the "jacket" region of the kinetocyst. Isolation of kinetocysts was performed and the molecular constituents were analyzed. As a result, it was found that the kinetocyst also contains TEP1, which is known as a minor component of the "Vault complex". The ultrastructure of kinetocysts was further investigated using cryo-electron microscopy and electron tomography techniques. As a result, no structure corresponding to "vault complex" was found in the kinetocyst. Instead, the kinetocyst was composed of a network of four layers of fibrous materials and two spherical "cores" in the center. "Vault complex" is a cell structure that exists in many eukaryotes, but its function is still unclear. In this study, MVP was shown to be directly involved in food recognition, which might suggest that the most primitive function of MVP in eukaryote ancestors was to distinguish between self and non-self.

Testate Amoebae Diversity in Tierra Del Fuego Peatlands: How Many Are We Missing?

LUCIANA BURDMAN[a,b], GABRIELA MATALONI[a], EDWARD MITCHELL[b], ENRIQUE LARA[c]

[a]University of San Martin, Institute of Environmental Research and Engineering. Buenos Aires, Argentina; [b]University of Neuchâtel, Laboratory of Soil Biodiversity. Neuchâtel, Switzerland; [c]Centro Austral de Investigaciones Científicas, CONICET. Tierra del Fuego, Argentina; [d]Real Jardin Botanico, CSIC. Madrid, Spain.

E-mail of the corresponding Author: lburdman@unsam.edu.ar

Testate amoebae are a polyphyletic group of amoeboid protists that build self-secreted shells. Because of their conspicuous aspect and relative ease in species identification, testate amoebae are frequently use for bioindication of past and present environmental conditions and have been central in the debate on cosmopolitanism in microbes. However, although research on testate amoebae has greatly increased in the last decade, less than 10 % of all publications are based in Southern Hemisphere (after SCOPUS), biasing our understanding of species (palaeo)ecology and biogeographical patterns.

Rancho Hambre and Andorra are two peatlands located in Tierra del Fuego dominated by a *Sphagnum magellanicum* moss matrix, containing clear and vegetated pond patches. We sampled 4-5 sites representing each of these 3 environmental types. In each pond, 3 communities were collected: periphyton, plankton and benthos.

A total of 79 species distributed in 25 genera were found, including 41 potentially new species. Total richness doubled previous records for Tierra del Fuego peatlands after Vucetich (1956-1980) and van Bellen *et* al. (2014) who found 44 and 32 species respectively. Our survey provided first records for the region for 65 species. Communities included well-known South American (i.e. *Certesella australis*) and Southern Hemisphere endemics (i.e. genera *Apodera* and *Alocodera*). Remarkably, this is the second record ever for 2 species: *Amphitrema paparoensis* (from New Zealand) and *Amphitrema congolense* (Republic of Congo) that might be new Southern Hemisphere biogeographic flagships. Genera *Difflugia* and *Padaungiella* presented 9 and 2 morphotypes respectively that could not be assigned to any known species. Molecular approaches will be needed to disentangle the specific diversity in the highly plastic genera *Heleopera*, *Centropyxis* and *Netzelia*. Our results illustrate the lack of knowledge on testate amoebae diversity in Tierra del Fuego peatlands, which might be the next frontier for biodiversity investigation in testate amoebae.

Bottom-Up Vs. Top-Down Control of Two Contrasting Freshwater Ciliates

XIAOTENG LU[a], THOMAS WEISSE[a]
[a]Research Department for Limnology, Mondsee, University of Innsbruck, Mondsee, Austria.

E-mail of the corresponding Authors: xiaoteng.lu@student.uibk.ac.at; Thomas.Weisse@uibk.ac.at

We investigated bottom-up and top-down control of two contrasting freshwater ciliates, *Histiobalantium bodamicum* and *Vorticella natans*. Both ciliates are common planktonic species but their abundance as well as swimming behavior are clearly different. Both species are well adapted to cold conditions, tolerating 5°C, but *H. bodamicum* cannot survive at temperature >18.5°C. In contrast, *Vorticella natans* thrived at 20°C. We analyzed bottom-up control by measuring numerical response (NR) and functional response (FR) of both ciliates over temperature ranging from 5°C to 20°C and food levels ranging from 0.02 to 7.0 mgC L^{-1}. Three main alterations were observed in the shape of NR with temperature: change in the maximum growth rate (μmax), in the initial slope (α), i.e. the affinity between the ciliate and prey, and in the threshold level (V') needed to sustain the population. Our FR data did not fit to Holling's type II curvilinear response but rather to Holling's type I rectilinear response. We compared FR of the two species in detail at moderate and high food levels typical of mesotrophic and eutrophic lakes. The relation of FR to temperature differed between the two species; in particular, we found a clear trend that ingestion rate of *H. bodamicum* decreased with temperature at high food level. Taken together, our result suggests that *V. natans* is the superior competitor to *H. bodamicum* in terms of bottom-up control. We studied top-down control of *H. bodamicum* and *V. natans* in predation experiments with three common microcrustacean predators: the cladoceran *Daphnia hyalina*, the calanoid copepod *Eudiaptomus gracilis* and the cyclopoid copepod *Cyclops abyssorum prealpinus*. For *V. natans*, the grazing rate by three predators was comparable, ~0.023 $pred^{-1}$ d^{-1}; for *H. bodamicum*, the grazing rate was lower and more variable, ranging from 0.011-0.020 $pred^{-1}$ d^{-1}. We conclude that *V. natans* is more strongly top-down controlled than *H. bodamicum*, which may explain the usually low abundance of *V. natans* compared to *H. bodamicum* and other common freshwater ciliates in many freshwater lakes.

Through the Magnifying Glass – the Global Diversity of Rhogostomidae and their Environmental Drivers

HUESNA OEZTOPRAK[a], MICHAEL BONKOWSKI[a], KENNETH DUMACK[a]
[a]Institute of Zoology, Terrestrial Ecology, University of Cologne, Köln, Germany.

E-mail of the corresponding Authors: hoeztopr@smail.uni-koeln.de; M.Bonkowski@uni-koeln.de; kenneth.dumack@uni-koeln.de

Recent environmental studies consistently report a high abundance and diversity of Rhogostomidae (Thecofilosea, Cercozoa, Rhizaria) in various aquatic and terrestrial habitats. However, since the rise of protistology only a hand full of Rhogostomidae species have been described so far. *Rhogostoma schuessleri* and R. *minus* were first described by Belar in 1921 and only very recently three new *Rhogostoma* species have been described from soil (*R. cylindrica*), freshwater (*R. micra*) and plant leaves (*R. epiphylla*). We investigated the putative cryptic diversity of Rhogostomidae, by reanalysing environmental sequencing data from marine, freshwater and terrestrial habitats around the globe, isolating and characterizing new rhogostomid species via their SSU rDNA sequence and morphology. So far we were able to detect more than 10 major clades in a comprehensive analysis of SSU rDNA phylogeny of NCBI data and approx. 450 sequences lacking any assigned described species. During these studies the previously wrongly assigned species *Sacciforma* (= *Plagiophrys*) *saccifromis* was revaluated and shifted into a new genus in the Rhogostomidae. We hypothesise that Rhogostomidae contain various cryptic species and that geographic regions are not the main factor shaping their dispersal, since preliminary data indicate a distribution of phylotypes within specific habitat types. We will show data on the relationship between species composition and their environments as well as on the morphology of different species in order to gain a better picture of the family Rhogostomidae.

The fate of SAR11 and Roseobacter by Marine Heterotrophic Flagellates

JI YEON SUNG[a], JONG SOO PARK[a]
[a]Department of Oceanography, School of Earth System Sciences, Kyungpook National University, Daegu, Republic of Korea.

E-mail of the corresponding Author: jongsoopark@knu.ac.kr

To better understand the dynamics of microbial communities in the ocean, it is important to figure out how the microbial food web interacts with the ecosystem and how it affects the changing ecosystem. The most abundant bacterium SAR11 is usually distributed in nutrient-poor oligotrophic seawater, while Roseobacter is often detected in nutrient-rich coastal seawater. However, the mortality of the most abundant bacteria, SAR11(Pelagibacterales) and Roseobacter (approximately >50% of bacterioplankton density in the ocean), by heterotrophic flagellates is still unknown, although the mortality of SAR11 infected by virus has been proposed. Here, we investigated growth responses of the dominant marine heterotrophic flagellates (i.e. *Cafeteria roenbergensis, Developayella elegans* and *Pteridomonas danica*) to the marine bacteria SAR11 and Roseobacter. It seems that the heterotrophic flagellates isolated from coastal seawater samples, preferred to grow in artificial media with Roseobacter, which is substantially distributed in coastal seawater, and is much larger than SAR11. Therefore, Roseobacter is more likely to be preferred for growth of heterotrophic flagellates. Overall, different growth responses of coastal heterotrophic flagellates to SAR11 and Roseobacter may influence on marine bacterial populations.

Oral Session DIVERSITY & SPECIATION 1

Genome Comparison in Chrysophyceae Reveal Huge Genetic Diversity

STEPHAN MAJDA[a], JENS BOENIGK[a], DANIELA BEISSER[a]
[a]University of Duisburg-Essen, Department of Biodiversity, Essen, Germany.

E-mail of the corresponding Author:stephan.majda@uni-due.de

Autotrophic and heterotrophic nutrition is scattered across the tree of life and the switch from phototrophy to heterotrophy occurred many times independently. The multifold switch of the basic nutritional mode reveals its importance for the evolution of eukaryotic diversity. The Chrysophyceae are among the most appropriate organisms to investigate the evolutionary significance of this shift of the nutritional mode since the loss of photosynthesis occurred many times independently within this group. Chrysophytes are among the numerically dominating flagellated freshwater protists in many ecosystems and play an important role in global oxygen production as well as in the transfer of bacterial secondary production to higher trophic levels. We examined genomes of 16 chrysophytes including phototrophic, mixotrophic and heterotrophic lineages. A special focus was on intraspecific variation of *Poteriospumella lacustris* to gain insights into diversification mechanisms, genome structure and genome variation. *Poteriospumella lacustris* serves as one of the first free-living non-model organism for genome-wide intraspecifc variation. The strains were sequenced with the Illumina and partly with the PacBio platform and subsequently assembled to draft genomes with a size of 50-110 Mb. We compared gene content, gene density, SNP distributions, proportion of repetitive regions, ploidy and GC content. Most surprisingly the ploidy differs between three strains of the species *P. lacustris* (JBM10: diploid, JBC07: triploid, JBNZ41: tetraploid). The ploidy in the other species ranged from diploidy to tetraploidy without correlation to nutrition or taxonomy. We show that gene mutations occur in different functional groups with varying frequencies and gene mutations arose more frequently with higher ploidy. Intraspecific genetic variation occurs predominantly in non-coding regions or genes belonging to ecological niche adaptation. The gene comparison between strains of different nutritional modes reflected varying stages of genome reduction associated with varying evolutionary selection pressure. We demonstrate several mechanisms of diversification as well as the dimension of intraspecific variation. Furthermore, the interspecific variation reveals genome changes induced by nutritional shift. These findings are exemplary for several other protist lineages with shifts in nutrition modes.

Three Novel Oxymonad Lineages from the Australian Termite *Porotermes adamsoni*

RENATE RADEK[a], KATJA MEUSER[b], ANDREAS BRUNE[b]
[a]Evolutionary Biology, Institute for Biology/Zoology, Freie Universität Berlin, Berlin, Germany; [b]Department of Biogeochemistry, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

E-mail of the corresponding Authors: renate.radek@fu-berlin.de; brune@mpi-marburg.mpg.de

The symbiotic gut flagellates of lower termites form host-specific consortia, which are composed of members of the Parabasalia and Oxymonadida. The analysis of their coevolution with termites is so far hampered by a lack of information particularly on the species colonizing the basal host lineages. To date, there are no reports on the presence of oxymonads in termites of the family Stolotermitidae. We discovered three deep-branching lineages of oxymonads in the damp-wood termite *Porotermes adamsoni*. One tiny species (6–10 µm) morphologically closely resembles the genus *Monocercomonoides* but shows a high sequence dissimilarity to recently published sequences of Polymastigidae from cockroaches and vertebrates. A second small species (9–13 µm) has a slight affinity to members of the Saccinobaculidae, which are found exclusively in wood-feeding cockroaches of the genus *Cryptocercus*, the closest relatives of termites, but shows a combination of morphological features that is unprecedented among any oxymonad family. The new lineage has scales on its surface, a protruding axostyle with a periaxostylar ring, and the proximal parts of its four flagella are twice as thick as usual. The third species is much larger (30–120 µm), very rare, and morphologically resembles members of the genus *Oxymonas*, its phylogenetic sister group. These findings significantly advance our understanding of the diversity of oxymonads in termite guts provide important cues that will help to reconstruct the evolutionary history of symbiotic digestion.

Phylogenomic Analysis of the Nucleariid Amoebae, the Earliest-Diverging Lineage of Holomycota

LUIS JAVIER GALINDO[a*], GUIFRÉ TORRUELLA[a*], DAVID MOREIRA[a], YANA EGLIT[b], ALASTAIR G.B. SIMPSON[b], ECKHARD VÖLCKER[c], STEFFEN CLAUß[c], AND PURIFICACIÓN LÓPEZ-GARCÍA[a]

[a]Unité d'Ecologie, Systématique et Evolution, CNRS, Université Paris-Sud, Université Paris-Saclay, AgroParisTech, Orsay, France; [b]Department of Biology, and Centre for Comparative Genomics and Evolutionary Bioinformatics, Dalhousie University, Halifax, Nova Scotia, Canada; [c]Penard Laboratory, Cape Town, South Africa; [*]These authors contributed equally.

E-mail of the corresponding Authors: luis.galindo@u-psud.fr; guifftc@gmail.com; puri.lopez@u-psud.fr

Nucleariid amoebae (Opisthokonta) are a group of non-flagellated, mainly free-living, phagotrophic filose amoeba found in both fresh-water and marine environments. They have been known since the 19th century but their diversity and evolutionary history remain poorly understood. Molecular 18S rRNA gene phylogenies placed *Nucleria* as a deep branch of the opisthokonts, specifically related to the Holomycota. Subsequent analyses with this marker placed *Fonticula* and *Parvularia* together with *Nucleria* and confirmed their position as sisters to the rest of Holomycota. Nevertheless, the 18S rRNA does not resolve the internal relationships between nucleariid clades and many *incertae sedis* nucleariid species await molecular characterization. To overcome these limitations, we have obtained genomic and transcriptomic data from three *Nucleria, two Pompholyxophrys and o*ne *Lithocolla* species by combining culturing and single-cell genome and transcriptome amplification methods. The phylogeny of the complete 18S rRNA sequences of *Pompholyxophrys* and *Lithocolla* confirmed their suggested evolutionary relationship to nucleariid amoebae, although with moderate support for internal splits. To improve the phylogenetic resolution, we carried out phylogenomic analyses based in two multi-gene datasets and obtained full support for the monophyly of the nucleariid amoebae, which comprise two major clades: i) *Parvularia* + *Fonticula* and ii) *Nucleria* plus the mineral scale-bearing genera *Pompholyxophrys* and *Lithocolla*. Based on these findings, the evolution of some traits of the earliest-diverging lineage of Holomycota can be inferred: The last common ancestor of nucleariids was most likely a freshwater, bacterivorous, non-flagellated filose and mucilaginous amoeba. From this ancestor, two groups evolved to reach smaller (*Parvularia-Fonticula*) and larger (*Nucleria* and related scale-bearing genera) cell sizes, leading to different ecological specialization. The *Lithocolla* + *Pompholyxophrys* clade developed exogenous or endogenous cell coverings from a *Nucleria*-like naked ancestor. Our analyses also led to the identification of probable bacterial endosymbionts in *Pompholyxophrys*, which have also been found in several *Nucleria* species, suggesting that bacterial endosymbionts might be an ancient trait in this eukaryotic phylum.

A Single-Cell Genome Reveals Diplonemid-Like Ancestry of Kinetoplastid Mitochondrial Gene Structure

JEREMY G. WIDEMAN[a,b], GORDON LAX[c], GUY LEONARD[a], DAVID MILNER[a], ALASTAIR G.B. SIMPSON[c], AND THOMAS A. RICHARDS[a]
[a]Living Systems Institute, University of Exeter, Exeter, UK; [b]Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada; [c]Department of Biology and Centre for Comparative Genomics and Evolutionary Bioinformatics, Dalhousie University, Halifax, Nova Scotia, Canada.

E-mail of the corresponding Author: jeremy.grant.wideman@gmail.com

Euglenozoa comprises euglenids, kinetoplastids, and diplonemids, with each group exhibiting different and highly unusual mitochondrial genome organisations. Although they are sister groups, kinetoplastids and diplonemids have very distinct mitochondrial genome architectures, requiring widespread insertion/deletion RNA editing and extensive *trans*-splicing, respectively, in order to generate functional transcripts. The evolutionary history by which these differing processes arose remains unclear. Using single-cell genomics, followed by SSU rDNA and multigene phylogenies, we identified an isolated marine cell that branches on phylogenetic trees as a sister to known kinetoplastids. Analysis of single-cell amplified genomic material identified multiple mitochondrial genome contigs. These revealed a gene architecture resembling that of diplonemid mitochondria, with small fragments of genes encoded out of order or on different contigs, indicating that these genes require extensive *trans*-splicing. Conversely, no requirement for kinetoplastid-like insertion/deletion RNA-editing was detected. Additionally, while we identified some proteins so far only found in kinetoplastids, we could not unequivocally identify mitochondrial RNA editing proteins. These data invite the hypothesis that extensive genome fragmentation and *trans*-splicing were the ancestral states for the kinetoplastid-diplonemid clade but were lost during the kinetoplastid radiation. This study demonstrates that single-cell approaches can successfully retrieve lineages that represent important new branches on the tree of life, and thus can illuminate major evolutionary and functional transitions in eukaryotes.

Carpediemonas membranifera has an Unusually Modified Machinery for Processing and Segregating DNA

DAYANA SALAS-LEIVA[a], BRUCE CURTIS[a], EELCO TROMER[b,c], JON JERLSTROM-HULTQVIST[a], MARTIN KOLISKO[d], ZHENZHEN YI[e], JORIS TREEP[c], GEERT KOPS[c], ALASTAIR SIMPSON[a], ANDREW ROGER[a]

[a]Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB), Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada; [b]Department of Biochemistry, University of Cambridge, Cambridge, UK; [c]Oncode Institute, Hubrecht Institute, KNAW (Royal Netherlands Academy of Arts and Sciences) and University Medical Centre Utrecht, Utrecht, The Netherlands; [d]Institute of Parasitology Biology Centre, ASCR, České Budějovice, Czech Republic; [e]Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitor, School of Life Science, South China Normal University, Guangzhou, China.

E-mail of the corresponding Authors: dayana.salas@dal.ca; andrew.roger@dal.ca

All cells must replicate and segregate their DNA with precision. In eukaryotes, replication and segregation are part of a conserved and regulated process that begins with the identification of origins of replication by the origin of replication complex (ORC) and progresses with the replication of DNA, which is subjected to continuous repair and several checkpoint controls until new cells are generated. To gain insights into the diversity of this system in eukaryotes, we carried out a comparative genomics analysis of the protein machineries involved in DNA processing and segregation in metamonads. We also generated a high-quality draft genome for the free-living metamonad *Carpediemonas membranifera* using long and short read technologies with high depth of coverage. Our analyses show that all metamonads, except *Carpediemonas,* harbor canonical protein machineries for processing and segregating DNA. *Carpediemonas* is the first known eukaryotic lineage with both an ORC-independent DNA replication system and an NDC80 complex-independent chromosome segregation mechanism, as both the ORC and NDC80 systems seem to have been secondarily lost. Our results raise the possibility of an as-yet undescribed origin of replication recognition mechanism in microbial eukaryotes.

Massive, Unprecedented Intein Content in two *Anaeramoeba* Genomes Reveals New Aspects of Intein Mobility in Eukaryotes.

LUCIE GALLOT-LAVALLÉE[a], JON JERLSTRÖM-HULTQVIST[a], COURTNEY W. STAIRS[b], IVAN CEPICKA[c], ANDREW J. ROGER[a], JOHN M. ARCHIBALD[a]
[a]Department of Biochemistry & Molecular Biology, Centre for Comparative Genomics & Evolutionary Bioinformatics, Dalhousie university, Halifax, Canada; [b]Institute for Cell and Molecular Biology, Uppsala, Sweden; [c]Department of Zoology, Cepicka lab, Prague, Czech Republic.

E-mail of the corresponding Authors: lucie.gallot-lavallee@dal.ca; john.archibald@dal.ca

Inteins are self-splicing, selfish mobile protein elements with an enigmatic origin and evolution. Inteins are found in bacteria, archaea, eukaryotes and even viruses. However, how these selfish elements spread and the factors contributing to their persistence is poorly understood, in particular in eukaryotes where they are scarce. Here we show that the genomes of the anaerobic protists *Anaeramoeba ignava* and *A. flamelloides* have 113 and 51 inteins, respectively, in stark contrast to 4 found in the most intein-rich eukaryotic genome described previously. The *Anaeramoeba* inteins belong to 2 classes and reside in a wide range of proteins, some also invaded in eukaryotes, in diverse prokaryotes or viruses. Other *Anaeramoeba* inteins are in entirely new genomic locations. Using sequence similarity-based networks and phylogenomic methods, we show that some of the *A. ignava and A. flamelloides* inteins can be traced back to their common ancestor, while others appear to have likely been acquired from viruses. Some of the *Anaeramoeba* inteins have moved intragenomically, either between ancient paralogs, or into unrelated proteins with common motifs. Virus-derived inteins are found in diverse proteins, supporting the idea that large dsDNA viruses of eukaryotes have contributed to the spread of inteins with relaxed target site specificities. Taken together, our large and novel intein dataset extends the spectrum of eukaryotic intein-containing proteins and provides insight into eukaryotic intein dynamics and evolution.

Diversity of Fornicates in the View of Environmental V4 and V9 Datasets: Contrasting Taxonomic Composition and Putative New Clades

ILYA UDALOV[a,b,c], ERIC SALOMAKI[a], MARTIN KOLÍSKO[a]
[a]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic; [b]Laboratory of Cellular and Molecular Protistology, Zoological Institute RAS, Saint Petersburg, Russia; [c]Department of Invertebrate Zoology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia.

E-mail of the corresponding Author: chlamydophrys@gmail.com

Fornicata (Excavates, Metamonada) is a monophyletic group of both host-associated and free-living flagellates that inhabit anaerobic environments, predominantly anoxic sediments or within the gut of diverse animals. This taxon unites the diplomonads and retortamonads, which are sister lineages, with the paraphyletic assemblage of "*Carpediemonas*-like" organisms which occupies a basal position in the Fornicata clade. Despite the fact that Fornicates were first documented in the 19th century and amongst them are medically and economically important parasites, their diversity remains poorly studied and online databases contain fewer than 200 18S rRNA sequences for the group. The aim of this study is to expand our knowledge of fornicate biodiversity by identifying previously unrecognized fornicates from large-scale high-throughput sequencing efforts. We have compiled data from amplicon surveys using the hyper-variable V4 and V9 regions of 18S rRNA gene of microbial eukaryotic communities living in anoxic biotopes (anoxic fresh-water and marine sediments, deep-sea, or soil), which are likely to contain our sequences of interest. Putative Fornicata sequences were searched using BLASTn and their true phylogenetic assignment was confirmed using the Evolutionary Placement Algorithm (EPA). Our analyses demonstrated strong V4 primer bias against the diplomonads and retortamonads, as we have recovered no sequences from these groups in V4 datasets, while we recovered representatives from all groups in the V9 datasets. Most of the newly identified Fornicata V4 amplicons branch with the "*Carpediemonas*-like" organisms and our analyses revealed existence of putative new lineages within Fornicata. Supported with GAČR 18-28103S research grant.

Nephromyces Represents a Diverse and Novel Lineage of the Apicomplexa that Has Retained Apicoplasts

SERGIO A. MUÑOZ-GÓMEZ[a], KEIRA DURNIN[a], LAURA EME[b], CHRISTOPHER PAIGHT[c], CHRISTOPHER E. LANE[c], MARY B. SAFFO[d], CLAUDIO H. SLAMOVITS[a]
[a]Centre for Comparative Genomics and Evolutionary Bioinformatics, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada; [b]Unité d'Ecologie, Systématique et Evolution, CNRS, Université Paris-Sud/Paris-Saclay, AgroParisTech, Orsay, France; [c]Department of Biological Sciences, University of Rhode Island, Kingston, USA; [d]Smithsonian National Museum of Natural History, Washington, DC, USA.

E-mail of the corresponding Authors: sergio.munoz@dal.ca; claudio.slamovits@dal.ca

A most interesting exception within the parasitic Apicomplexa is *Nephromyces*, an extracellular, probably mutualistic, endosymbiont found living inside molgulid ascidian tunicates (i.e., sea squirts). Even though *Nephromyces* is now known to be an apicomplexan, many other questions about its nature remain unanswered. To gain further insights into the biology and evolutionary history of this unusual apicomplexan, we have sequenced a metagenome and a metatranscriptome from the molgulid renal sac, the specialized habitat where *Nephromyces* thrives. Our phylogenetic analyses of conserved nucleus-encoded genes robustly suggest that *Nephromyces* is sister to both haemosporidians and piroplasmids (the Hematozoa). Furthermore, a survey of the renal sac metagenome revealed 13 small contigs that closely resemble the genomes of the non-photosynthetic reduced plastids (i.e., apicoplasts) of other apicomplexans. We show that these apicoplast genomes correspond to a diverse set of most closely related but genetically divergent *Nephromyces* lineages that co-inhabit a single tunicate host. In addition, the apicoplast of *Nephromyces* appears to have retained all biosynthetic pathways inferred to have been ancestral to parasitic apicomplexans. Our results shed light on the evolutionary history of the only probably mutualistic apicomplexan known, *Nephromyces*, and provide context for a better understanding of its life style and intricate symbiosis.

Waking a Sleeping Giant: Morphology, Life History Observations, and Molecular Phylogeny of *Wagnerella borealis* Mereschkowsky 1878 (Gymnosphaerida)

YANA EGLIT[a], VASILY ZLATOGURSKY[b], FABIEN BURKI[c], ALASTAIR G.B. SIMPSON[a]

[a]Department of Biology and Centre for Genome Evolution and Bioinformatics, Dalhousie University, Halifax, NS, Canada; [b]Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; [c]Department of Organismal Biology, Systematic Biology Program, Uppsala University, Uppsala, Sweden.

E-mail of the corresponding Author: yeglit@dal.ca

The tree of eukaryotes has coalesced over recent years into a handful of major groups; however, some previously-described protists with highly distinctive morphologies remain without a phylogenetic affiliation. Gymnosphaerids were lumped with desmothoracids, actinophryids, centrohelids and some other superficially similar eukaryotes with radiating pseudopodia as a part of "Heliozoa". Molecular phylogenies have placed desmothoracids in Rhizaria, actinophyrids in Stramenopiles, and centrohelids with haptophytes. Gymnosphaerids, by contrast, have been little studied in recent decades, due to absence of cultures and rarity of findings. Thus, they remain to be examined using molecular methods and placed within the eukaryote tree. *Wagnerella borealis* is a striking gymnosphaerid cell up to 3 mm long that consists of a widened base, long stem, and a spherical head, all extensively covered in large siliceous "spicules"; it was originally described as a sponge. *Wagnerella* was last extensively studied by Zülzer (1909), who reported unusual features such as nuclear migration from base to head during the life cycle. We found a source of *Wagnerella* within a marine epiphyte community in the Pacific Northwest of North America. Over two weeks we collected and examined over two hundred cells to obtain molecular data and verified Zülzer's microscopic observations. Here, we present preliminary phylogenetic data and evidence in support of the surprising nuclear migration claims, as well as a hypothesised life cycle. We hope our research brings gymnosphaerids out of their long obscurity and into the modern world of molecular biology and current microscopy methods.

SYMPOSIUM - Protist diversity and function in the dark ocean - challenging the paradigms of deep-sea ecology- (by FEPS)

Protistan Microzooplankton in the Mesopelagic Mediterranean Sea

JOHN R. DOLAN[a], MARIA CIOBANU[a], LAURENT COPPOLA[a]
[a]Laboratoire d'Océanographie de Villefranche-sur-Mer, Sorbonne Université, CNRS UMR, Station Zoologique, Villefranche-sur-Mer, France.

E-mail of the corresponding Author: dolan@obs-vlfr.fr

Our knowledge of deep sea protists is sparse. Here we present a unique data set concerning 3 taxa that are relatively large, robust to sampling, and easily identifiable to species level using light microscopy: tintinnid ciliates, phaeogromid cercozoans (e.g. Challengerids) and amphisolenid dinoflagellates. We sampled a near-shore deep water site in the N.W. Mediterranean Sea over a two-year period at approximately weekly intervals from January 2017 to December 2018. We found taxa that appear to be restricted to deep waters, distinct seasonal patterns of abundance in some taxa, and in others non-seasonal successional patterns. Based on data from intensive sampling following a flash-flood event, the Challengerid population appeared to respond positively to a pulse of terrogenous input. Some of the distinct mesopelagic tintinnid ciliates and amphisolinid dinoflagellates were also found in 2 samples from the North Atlantic mesopelagic gathered from near the Azores Islands in September 2018. We conclude that there are a variety of protist taxa endemic to the mesopelagic, that the populations are dynamic, and they are probably widely distributed in the deep waters of the world ocean

Biodiversity and Biogeography of Deep-Sea Benthic Foraminifera and their Possible Roles in Bathyal and Abyssal Ecosystems

ANDREW J GOODAY[a]
[a]National Oceanography Centre, University of Southampton Waterfront Campus, Southampton, UK.

E-mail of the corresponding Author: ang@noc.ac.uk

Much of the huge literature on benthic foraminifera is the work of geologists and therefore has focused mainly on well-known, multichambered, typically calcareous taxa that have a high fossilisation potential. However, starting in the 1960s and 70s, biologists became increasingly aware that foraminifera are an often dominant component of deep-ocean faunas down to extreme hadal depths, and spanning size classes from meiofauna to megafauna. As depth increases, the abundance of multichambered taxa declines so that abyssal and hadal faunas are dominated by highly diverse, 'primitive' single-chambered monothalamids, most of them (>90%) undescribed. Many have complex test morphologies, some occupy cryptic microhabitats or are attached to firm substrates. They include the xenophyophores (megafauna-sized foraminifera with very distinctive characteristics) and the enigmatic komokiaceans, as well as forms that are impossible to assign to higher taxa. Evidence regarding species ranges is ambiguous. Ranges spanning several oceans are common among multichambered morphospecies, particularly in the abyss, and in a few cases are supported by genetic data. Some monothalamids, however, appear to have restricted distributions, although rarity makes it difficult to distinguish endemism from undersampling. Discerning biogeographic patterns among this vast protistan diversity therefore remains a major challenge.
Their shear abundance means that benthic foraminifera are likely to be key players in deep-sea ecosystems. Observational and experimental studies point to their important role in processing labile organic carbon on the ocean floor, including in oxygen minimum zones where some calcareous species flourish despite severely hypoxic conditions. Foraminifera of all kinds are consumed, in turn, by deposit feeding invertebrates and specialist predators, providing a link between lower and higher levels of deep-sea foodwebs. The tests of megafaunal taxa, notably xenophyophores, provide habitat structure for invertebrates such as polychaetes and crustaceans as well as larger animals. Xenophyophores can grow rapidly and may colonise fresh substrates together with smaller foraminifera. Thus, in terms of their abundance, diversity, and ecological significance, deep-sea foraminifera are hard to ignore.

Global Distribution and Unique Protist Communities in the Deep Sea

ALEXANDRA SCHOENLE[a]
[a]University of Cologne, Biocenter, Institute for Zoology, General Ecology, Cologne, Germany.

E-mail of the corresponding Author: aschoenl@uni-koeln.de

The main challenge in deep-sea microbial ecology is to elucidate biodiversity patterns at a global as well as temporal scale. Genetic approaches like next-generation sequencing (NGS) have turned out to be reliable tools in identifying novel and uncultured protistan lineages in surface waters and the deep sea. Comparative analyses showed that deep-sea communities are distinct from surface water assemblages. The deep sea might harbor a specific protist community at least for several groups underlined by a low similarity to reference sequences of public databases and differences to benthic shelf communities. Several studies showed that dominant deep-sea OTUs belonged to the Discoba, Alveolata, and Rhizaria. Studies on benthic deep-sea protist assemblages mainly concentrated on assumed hot spots like hydrothermal vents, cold seeps and anoxic regions mostly from bathyal zones (1-3 km depths). Less attention has been made for the abyssal sea floor (3-6 km depths), which is covering 54% of the Earth's surface. A recent DNA analysis of 20 mainly abyssal stations in the Atlantic and Pacific showed specific protist communities with less than 1% of OTUs occurring at all sampled stations. Although protist diversities differed on a global scale, several genotypes occurred in cultures from surface waters and the deep sea and were overrepresented in NGS results indicating the ubiquitous distribution of at least several species. It is uncertain, if benthic protist communities detected by metagenomics are actually thriving in the deep sea or are rather an artifact by deposited cells from the upper water column, encysted cells or extracellular DNA. Ecological experiments under deep-sea conditions have showed for some species the potential to thrive in the deep-sea. While NGS downstream analyses highly depend on the reference database, an appropriate taxonomic description (molecular and morphological) of protists from various habitats are of paramount importance. Benthic protist communities and the environmental factors shaping the distribution are far from being completely understood and surveys of protists in the dark ocean are still at the beginning.

Flagellates, Ciliates and Amoebae May Occupy All Niches of Deep-Sea Microbial Life

HARTMUT ARNDT[a]
[a]University of Cologne, Institute of Zoology, General Ecology, Germany.

E-mail of the corresponding Author: Hartmut.Arndt@uni-koeln.de

Microbial food webs in the sunlit ocean are dominated by the activity of heterotrophic flagellates, ciliates, amoebae and foraminifera, playing a fundamental role for the matter flux. Although the deep sea comprises the largest part of the biosphere, very little is known about the structure and function of the deep-sea microbial food webs so far. This is in striking contrast to the potential importance microorganisms can have for the global carbon flux in this part of the biosphere. Recent results of next generation sequencing point to the presence of a large variety of protists present in sediments of abyssal and even hadal regions; besides the classical protistan components of the deep, the foraminifera, representatives of nearly all other protist groups of the eukaryote tree of life have been identified based on molecular barcoding. However, are these protists really vital components of deep-sea ecosystems, or were they sunken with detritus from surface waters? What role do they play for the functioning of deep-sea ecosystems? Until recently only a very few protists, except for foraminifera, have been brought alive to the surface and investigated under ambient conditions of the deep sea. The tiny cells of nanoflagellates, naked amoebae and ciliates are generally disrupted immediately after sampling and can easily be ignored. Recent live-counts of protists in deep-sea sediments revealed significant abundances though orders of magnitude lower than in surface waters. Today, there are indications of a specific deep-sea fauna consisting of specific nano-protistan communities deviating significantly from that of shallow waters. Recently, hot spots of organic carbon that contradict the idea of a food-poor deep-sea environment have been discovered and it seems that this organic matter is channeled via several up to now mostly ignored trophic levels of protistan nano- and microfauna. While methodological studies have indicated that hydrostatic pressure significantly influences activities of protists, several species have been found to survive even pressures occurring at abyssal depths. Grazing pressure of protists should have a significant influence on the fate of deep-sea bacterial production and at least 2-3 trophic levels should be hidden among flagellates, ciliates and amoebae. Ignoring these trophic levels should have fundamental effects on estimates of global carbon fluxes.

Oral Session TAXONOMY & PHYLOGENY 1

Phylogenomic Analysis Assessing the Positions of "Orphans" Including *Microheliella maris*.

EUKI YAZAKI[a], AYAKA IMAIZUMI[b], KEITARO KUME[b], TAKASHI SHIRATORI[c], TETSUO HASHIMOTO[b,d], AKINORI YABUKI[c], KEN-ICHIRO ISHIDA[b], YUJI INAGAKI[b,d]

[a]Department of Biochemistry & Molecular Biology, Graduate School & Faculty of Medicine, The University of Tokyo, Tokyo, Japan; [b]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan; [c]Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan; [d]Center for Computational Sciences, University of Tsukuba, Tsukuba, Japan.

E-mail of the corresponding Author: yuji@ccs.tsukuba.ac.jp

Deep branching lineages in the tree of eukaryotes are potentially represented by some of "orphan" species, of which phylogenetic positions remain uncertain in previous studies. In this study, we generated transcriptomic data from "orphans" including a heliozoan-like unicellular eukaryote (protist) *Microheliella maris*, and assembled a phylogenomic alignment containing 338 genes (98,904 amino acid positions in total). Maximum-likelihood (ML) analyses of "338-gene" alignment robustly reconstructed major taxonomic assemblages, such as (1) SAR, (2) Haptista, (3) Cryptista, (4) Discoba, (5) Metamonada, (6) CRuMs and (7) Amorphea. *M. maris* was placed at the basal position of the Cryptista clade (including *Palpitomonas blix*) with full statistical support. As little morphological characteristic is shared between *M. maris* and cryptists, it is not appropriate to consider *M. maris* as a member of Cryptista. Rather, *M. maris* likely hints a previously overlooked lineage that is closely related to Cryptista. Besides *M. maris*, two undescribed "orphans" were placed separately within CRuMs, and another "orphan" was united with malawimonads in 338-gene phylogeny.

Systematic Review of the Family Blepharocorythidae Hsiung (Ciliophora, Entodiniomorphida)

FRANCIANE CEDROLA[a], MARCUS VINICIUS XAVIER SENRA[a,b], FRANCISCO FAULHABER[a], MARTA D'AGOSTO[a], ROBERTO JÚNIO PEDROSO DIAS[a]

[a]Laboratório de Protozoologia, Programa de Pós-graduação em Comportamento e Biologia Animal, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil; [b]Instituto de Recursos Naturais, Universidade Federal de Itajubá, Itajubá, Minas Gerais, Brazil.

E-mail of the corresponding Authors: francedrola@gmail.com; senramvx@gmail.com; francisco.faulhaber@yahoo.com; dagosto.mar@gmail.com; rjuniodias@gmail.com

The family Blepharocorythidae includes 25 species of entodiniomorphid ciliates, which are distributed in nine genera (*Blepharocorys*, *Circodinium*, *Charonina*, *Gorilloflasca*, *Ochoterenaia*, *Pararaabena*, *Raabena*, *Spirocorys* and *Troglocorys*) and found as symbionts of a wide variety of hindgut fermenting mammals. Currently, the characters used to group the species within this family are a long vestibulum, a single contractile vacuole and ciliary zones at anterior and posterior ends of their bodies; and, as indicated by previous molecular phylogenetic studies this family might not be monophyletic. Here, a systematic review was performed aiming to elucidate inconsistencies and shed some light into the systematics of this family of ciliates. Although many representatives of this family could not be included in our phylogenetic analysis because their 18S rDNA sequences are not yet available in public repositories, after revisiting many morphology and ultrastructure works, we were able to identify morphological and morphogenetic features that allowed us to suggest a new organization scheme for this family which is in congruence with the molecular phylogenetic data. Moreover, one of the main outcomes of this work is to highlight the importance of revisiting erstwhile literature to improve the systematic of Blepharocorythidae ciliates as is for any other group of organisms.

Morphology and Phylogeny of two New Parasitoids of the Marine Dinoflagellates, *Tuberlatum coatsi* and *Parvilucifera* sp. (Alveolata, Perkinsozoa)

BOO SEONG JEON[a], MYUNG GIL PARK[a]
[a]Department of Oceanography, Chonnam National University, Gwangju 61186, Republic of Korea.

E-mail of the corresponding Author: mpark@chonnam.ac.kr

Although recent environmental rDNA sequencing studies of the phylum Perkinsozoa have revealed that this exclusively parasitic group has considerable diversity and a wide distribution, their morphological and/or taxonomical identities remain mostly unknown. During intensive samplings in Korean coastal waters in June, August, and September 2017, two different parasitoids infecting marine dinoflagellates were detected and were successfully established in culture. Based on morphological, ultrastructural and phylogenetic analyses, both of the two parasitoids were included in the family Parviluciferaceae, and had similar life-cycle stages consisting of free-living zoospore, trophocyte and sporocyte, but showed distinct morphologically differences between the two parasitoids, as well as from the previously known species within the family. The new parasitoid *Tuberlatum coatsi* was most characterized by the presence of two to four dome-shaped, short germ tubes in the sporangium. The opened germ tubes were biconvex lens-shaped in the top view and were characterized by numerous wrinkles around their openings. The other new parasitoid *Parvilucifera* sp. had a close morphological similarity with other species within the genus *Parvilucifera*, but differed clearly by both diameter and the number of apertures. In addition, the ratio of trophocyte to sporocyte in the generation time of the new *Parvilucifera* sp. was different from that of *P. infectans* under the same condition. In this study, we will present the details of morphological characteristics and phylogeny of the newly discovered two species.

Let's Get Physical: Intense Connection Between Nucleus and Mitochondria in a Bicosoecid

DAGMAR JIRSOVÁ[a], ZOLTÁN FÜSSY[a], JITKA RICHTOVÁ[a], ANSGAR GRUBER[a], MIROSLAV OBORNÍK[a]

[a]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic.

E-mail of the corresponding Author: dasa.jirsova@gmail.com

We present a newly-described heterotropic marine nanoflagellate with two smooth flagella, which was isolated from a mixed derived from material culture collected from a rock surface in the Kvernesfjorden (Norway). The organism was characterized by scanning and transmission electron microscopy, fluorescence and light microscopy. The sequence of the small subunit ribosomal RNA gene (18S) was used as the molecular marker for determining the phylogenetic position of the nanoflagellate. In addition to the 18S sequences, the whole circular mitochondrial (mt) genome was also sequenced and annotated. tRNAs encoded in the mt genome include those for most amino acids, except Ala, Gly and Thr. In comparison to other stramenopiles, the mt genome (42,797 bp) is of a similar size, but the GC content (21.3%) is lower; in addition to that, the lack of tRNA-Thr is a shared feature among all stramenopiles. Based on morphological observations the novel flagellate shares the presence of the microtubular root (R3), the key ultrastructural character of the flagellar apparatus, which is typical for the family Bicosoecida (Heterokonta). Furthermore our phylogenetic analyses of 18S corroborate the morphological data and place the sequence from our yet to be described species with other representatives from the group Bicosoecida. Intriguingly, the mitochondria of this nanoflagellate frequently associate with the nucleus through an electron-dense disc at the boundary of the two compartments. Although this unique phenomenon was observed for young cultures only (14 days and younger), the mitochondria stay in close contact with the nucleus throughout the whole cell cycle. The function of this association remains unclear. However, we suppose that the fusion between these organelles could be an energy-saving mechanism during rapid growth where the mitochondria directly supply the nucleus with ATP molecules for the energy depending nuclear transport.

Phylogenomic Reconstruction of Holozoa Through the Lense of Metagenomics: The Genome of the First Filasterean Parasite Provides Insights into the Unicelular Ancestry of Animals

KONSTANTINA MITSI[a,*], ANDER URRUTIA[b,f,*], MICHELLE LEGER[a], STEPHEN W. FEIST[b] , DAVID BASS[b,c], IÑAKI RUIZ-TRILLO[a,d,e]

[a]Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain; [b]Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Weymouth, UK; [c]Department of Life Sciences, Natural History Museum London, London, UK; [d]Departament de Genètica, Universitat de Barcelona, Barcelona, Spain; [e]Institució Catalana de recerca I Estudis Avançats (ICREA), Barcelona, Spain; [f]Res Ctr Experimental Marine Biology & Biotechnology (PIE-UPV/EHU) & Zoology & Animal Cell Biology Dept., (UPV-EHU) Bilbao, Spain; [*]These authors contributed equally.

E-mail of the corresponding Authors: kcmitsi@gmail.com; d.bass@nhm.ac.uk; inaki.ruiz@ibe.upf-csic.es

The eukaryotic group Holozoa comprises animals and their unicellular relatives, namely Choanoflagellates, Filasterea and Teretosporea (Ichthyosporea+Pluriformea). Unicellular holozoans hold a phylogenetic position that is key to addressing a long-standing open evolutionary question: the transition to animal multicellularity. To expand the extant holozoan genomic dataset, here we report the morphology, nuclear and mitochondrial genomes of *Txikispora sp.*. *Txikispora sp*. is known to infect at least two amphipod genera, Echinogammarus sp. and Orchestia sp. collected from the southwest coast of United Kingdom. It is the first confirmed filasterean parasite as it triggers host response in the form of granuloma formation and melanization, reducing host motility and general fitness. Phylogenomic reconstruction based on 85 single-copy protein domains and 23,526 aa positioned this novel unicellular holozoan species as an early-branching filasterean. The genome was acquired following a metagenomic pipeline, an approach that is commonly used to describe complex prokaryotic communities but is still in limited use for studies of eukaryotes. Comparative analysis revealed that the *Txikispora sp*. genome encodes most genes involved in the flagellar toolkit as well as with the majority of genes previously identified as the multicellular toolkit. The latter include the integrin adhesome and many developmental transcription factors. In addition, genes involved in meiotic recombination were identified. Overall, our results add to our understanding of the the genomic repertoire of the last unicellular common ancestor of animals, reinforce the current holozoan phylogeny by expanding the available dataset and provide insights into the mechanisms that facilitate a parasitic lifestyle in a filasterean.

Integrative Taxonomic Study of Four Species of Extremophilic Ciliates of the *Plagiopyla* Genus, Underpinning the Revision of the *Plagiopylidae* Family (Plagiopylea: Plagiopylida)

VENKATA MAHESH NITLA[a], VALENTINA SERRA[a], SERGEI I. FOKIN[a,b], LEANDRO GAMMUTO[a], FRANCO VERNI[a], VENKATA SANDEEP BHAGAVATULA[c], KALAVATI CHAGANTI[c], LETIZIA MODEO[a], GIULIO PETRONI[a]
[a]Department of Biology, University of Pisa, Pisa, Italy; [b]Department of Invertebrate Zoology, St. Petersburg State University, St. Petersburg, Russia; [c]Department of Zoology, Andhra University, Visakhapatnam, India.

E-mail of the corresponding Author: venkatamahesh.nitla@gmail.com

The ciliate members of the *Plagiopylidae* family are cosmopolitan in distribution and have been retrieved in various oxygen-depleted and microaerobic habitats. To date, the Plagiopylidae family comprises four genera: *Plagiopyla*, *Lechriopyla*, *Pseudoplagiopyla*, and *Paraplagiopyla*. Unfortunately, detailed descriptions based on integrative taxonomic methods are lacking for many Plagiopylidae species. In this context, we applied an integrative taxonomical approach, comprising classical morphology, ultrastructural analyses, 18S rDNA sequencing, and phylogenetic tree reconstruction, in order to characterize some *Plagiopyla* members. Moreover, we investigated some morphological features not recorded in previous studies, such as length and end point of striated band with respect to longitudinal axis, number and type of micronuclei, number of contractile vacuole pores, number of cytoproct dense ciliary rows, and extrusome morphology. Indeed, these features, according to our study, appear to be of great importance in describing and identifying the *Plagiopyla* species. As a result, we have re-described two well-known, still not comprehensively studied free-living species of the genus, *P. nasuta* Stein, 1860 and *P. frontata Kahl*, 1931, and identified and described for the first time two novel species of the genus, i.e. *P. ramani* sp. nov. and *P. narasimhamurtii* sp. nov. All these *Plagiopyla* species are from the fresh water habitat of Lake Kolleru (India), except for *P. frontata*, which was isolated from slightly brackish water (2%) in the the Inkoo region, on the Baltic Sea (Finland). After a critical revision of the taxa included in the Plagiopylidae family, we propose: 1. the exclusion of the *Paraplagiopyla* genus due to the fact that it differs in the pattern of somatic kineties and lacks the striated band characteristic of plagiopylids; 2. the synonymizing of the genus *Lechriopyla* with Plagiopyla, based on their morphological and phylogenetic affinities, and the renaming of *Lechriopyla mystax* as *Plagiopyla mystax* comb. nov. A detailed explanation of this matter will be presented.

Dating the Radiation of the Euglyphid Testate Amoebae with Fossils and DNA

CLÉMENT DUCKERT[a], RALF MEISTERFELD[b], DAVID SINGER[c], EDWARD A. D. MITCHELL[a,d], ENRIQUE LARA[e]
[a] Laboratory of Soil Biodiversity, University of Neuchâtel, Neuchâtel, Switzerland; [b] Institute for Biology II, RWTH Aachen University, Aachen, Germany; [c]Department of Zoology, University of São Paulo, São Paulo, Brazil; [d]Botanical garden of Neuchâtel, Neuchâtel, Switzerland; [e]Real Jardín Botánico, CSIC, Madrid, Spain.

E-mail of the corresponding Authors: clement.duckert@unine.ch; meisterfeld@rwth-aachen.de; david.singer.bio@outlook.com; edward.mitchell@unine.ch; enrique.lara@rjb.csic.es

The family Euglyphidae (Cercozoa: Imbricatea) is composed of testate amoebae that can be found in a broad variety of soil and freshwater environments. They build their tests from small ornamented self-secreted silica scales whose shape, dimensions and arrangement are taxonomically informative. Despite being cosmopolite and very abundant, their evolution, the extent of their diversity and its triggers are mainly unknown. We characterized 17 Euglyphidae taxa by documenting their morphology with scanning electron microscopy and obtaining partial 18S rRNA sequences, and constructed a phylogenetic tree of previously and newly barcoded species. Several lineages present synapomorphies, allowing us to infer the position of previously and newly established fossils within the phylogenetic tree of the Euglyphidae to date the main diversification events. In order to assess the shifts in diversification rates while also considering taxa that are not present in our phylogeny, we compiled a list of the taxa from the literature and assigned each of them to a position within our time-calibrated phylogenetic tree based on their morphology. Here we show that the family Euglyphidae originated during the Jurassic and can be split in two monophyletic clades according to their habitat (terrestrial or aquatic). We infer that the terrestrial species radiated about 27 million years ago whereas the diversification of aquatic species did not experienced such a diversification. The synchronicity of the radiation of the terrestrial clade with the expansion of grasslands, which had a major impact on the terrestrial silica cycle, suggests a direct causal link between major biogeochemical changes and diversification rates of terrestrial micro-eukaryotes, as previously suggested for aquatic microorganisms such as nonmarine diatoms.

Integrating Traditional Descriptions with the Holobiont Concept and Genomics Analyses in the "Next Generation Taxonomy" Approach: *Euplotes vanleeuwenhoeki* sp. nov. as Case Study

VALENTINA SERRA[a], LEANDRO GAMMUTO[a], VENKATAMAHESH NITLA[a], MICHELE CASTELLI[b], OLIVIA LANZONI[a], DAVIDE SASSERA[c], CLAUDIO BANDI[b], BHAGAVATULA V. SANDEEP[d], LETIZIA MODEO[a], GIULIO PETRONI[a]
[a]Department of Biology, University of Pisa, Pisa, Italy; [b]Romeo and Enrica Invernizzi Pediatric Research Center, Department of Biosciences, University of Milan, Milan, Italy; [c]Department of Biology and Biotechnology "Lazzaro Spallanzani", Pavia University, Pavia, Italy; [d]Department of Biotechnology, Andhra University, Visakhapatnam, India.

E-mail of the corresponding Author: giulio.petroni@unipi.it

In 1991 Margulis defined holobionts as the assemblage of "two or more organisms, members of different species" which remain associated "throughout a significant portion of their life history." In recent times, holobionts have been described among many and far-related groups of living beings, such as plants, algae, insects, corals, and even humans. These studies have kindled increasing interest in different contexts, but to our knowledge, the holobiont concept has not been applied in taxonomy. Here we propose a new approach to modern taxonomy aimed to complement the classical/morphological tools traditionally used in taxonomy by integrating the holobiont concept and its genomic and bioinformatics analyses. The inclusion of symbiont morphology and of mitochondrial and symbiont genomes will allow the discipline to move towards what could become the "next generation taxonomy."
As an example of this new paradigm in the characterization of holobionts, we propose the comprehensive description of the ciliate *Euplotes vanleeuwenhoeki* sp. nov. (Euplotia, Ciliophora). This novel *Euplotes* species, retrieved in the freshwater lake of Kolleru (Andhra Pradesh, India), shows the plesiomorphic features of the genus: 10 fronto-ventral cirri and double-argyrome *eurystomus*-type. While it presents 3 deep longitudinal furrows in the dorsal region, recalling the morphology of *E. trisulcatus*, other morphological traits and molecular analyses nonetheless confirm the attribution to a novel species. *Euplotes vanleeuwenhoeki* belongs to the *E. trisulcatus* - *Euplotes* cf. *antarcticus* - *E. charon* (AF492705), *E. magnicirratus* (AJ549210), and *E. euryhalinus* (EF094968, JF903799) clade, based on 18S rDNA phylogenetic analyses. The complete mitochondrial genome (mitogenome) of this ciliate results in a single linear contig 41,682 bp long with a GC content of ~0.25%. This mitogenome shows an overall synteny with the mitochondrion of *E. minuta*, *E. crassus* and *Oxytricha trifallax* of previous studies, with the exception of the two terminal regions. A novel bacterial endosymbiont belonging to *Verrucomicrobia* is hosted in the cytoplasm of *E. vanleeuwenhoeki*, "*Candidatus* Pinguicoccus supinus" gen. nov., sp. nov. (*Opitutae, Verrucomicrobia*). Interestingly, this endosymbiont shows an extremely reduced genome (~163 Kbp), which suggests high integration with the host. Thus, we are presenting the first case of such an extreme reduction in *Verrucomicrobia* and the first case in a protist host.

Biodiversity and Adaptation of Protists to Extreme Aquatic Environments in the Atacama Desert

ALEXANDRA RYBARSKI[a], FRANK NITSCHE[a], HARTMUT ARNDT[a]

[a]Department of General Ecology, Institute of Zoology, Biocenter Cologne, University of Cologne, Cologne, Germany.

E-mail of the corresponding Authors: alexandra.rybarski@uni-koeln.de; fnitsche@uni-koeln.de; Hartmut.Arndt@uni-koeln.de

Reconstructing the phylogenetic tree that unites all lineages of eukaryotes is still a grand challenge. The difficulty to define homologous characters across the very different lineages makes it extremely difficult to resolve evolutionary processes. The incompleteness of consistent paleontological records of the delicate single cell organisms makes calibration of evolutionary time scales imprecise. To get more insights into speciation processes of heterotrophic flagellates we use the class Placididea within the phylum Stramenopiles. This recently discovered group was found as halophilic organisms characterized by their small size (2-5 µm) and their adaptability to live at extreme conditions. Before this study, only two species from marine waters in Japan and one from a brackish lake in Kenya were described. In this study, we succeeded in isolating 21 novel strains of Placididea: 15 from the Atacama in Chile and in addition two from Germany, one from Kenya, one from the Atlantic Ocean and two from the abyssal zone of the Caribbean Sea for phylogenetic comparison. Our isolated strains, together with previously described placidids, have been used for multigene analysis to investigate potential speciation of geographically separated populations. Placidids turned out to be generalists, broadly adapted to salinity and UV radiation where low mutation rates suffice to be able to adapt to extreme environments. We would like to contribute insights into the diversification processes among Placididea combining morphological-, ecological- and phylogenetical approaches.

Oral Session ECOLOGY & BIOGEOGRAPHY 2

Resolving Protistan Parasite and Host Interactions in a Coastal Tidal Pond Using a High-Resolution Time Series

TAYLOR SEHEIN[a], REBECCA GAST[a], MARIA PACHIADAKI[a], LAURE GUILLOU[b], MICK FOLLOWS[c], VIRGINIA EDGCOMB[a]

[a]Woods Hole Oceanographic Institution, Woods Hole, MA, USA; [b]Station Biologique de Roscoff, Roscoff, France; [c]Massachusetts Institute of Technology, Cambridge, MA, USA.

E-mail of the corresponding Author: trsehein@mit.edu

Protists are taxonomically diverse metabolic drivers of energy and nutrient flow. Elucidating the extent and consequences of specific protistan interactions on the marine environment is critical for understanding community structure and dynamics, and overall ecosystem function. Parasitism is widespread, albeit understudied, in marine environments, with a limited number of studies focused on the regulation of bloom-forming taxa by protistan parasites. Recent global ocean surveys of eukaryotic molecular signatures reveal parasitic protists belonging to the order Syndiniales are abundant and ubiquitous, suggesting their major role in marine food webs. To investigate the impacts of Syndiniales parasites on protist populations in coastal marine environments, a high-resolution data set was generated for Salt Pond (Falmouth, MA, USA) in 2018. Coastal ponds, like Salt Pond, are highly productive systems that support a wide variety of protist groups and are therefore ideal for studying syndinian parasites and their preferred hosts. Three depths were sampled every 2-3 days from March to October, capturing periods of peak productivity (spring and fall) and stratification (summer). Molecular 18S ribosomal RNA barcode libraries were used to characterize the diversity of the protist communities and to highlight clades of Syndiniales for which limited or no known host data were available. The general Group II Syndiniales probe was applied using CARD-FISH and visualized with epifluorescent microscopy to quantify the abundance of free-living spores, the ratios of the protist community infected, and abundant host morphotypes. Results from these analyses suggest that protist communities within Salt Pond are seasonally distinct and infections within these communities reach local maxima at approximately monthly interval. Furthermore, parasite strains and host morphotypes differed during high infection periods. These interactions will be explored further using single-cell genomic approaches in the near future. Collectively, these data inform on the broader impacts of Syndiniales on the ecology of Salt Pond by characterizing parasite diversity and specific host-parasite interactions and quantifying impacts on susceptible protist populations.

Richness and Composition of soil Cercozoan Communities Exhibit Different European Scale Biogeographical Patterns

FLORINE DEGRUNE[a], AURÉLIEN SAGHAI[b], GINA GARLAND[c], SANA ROMDHANE[d], PABLO GARCIA-PALACIOS[e], CHANTAL HERZOG[c], ANNA EDLINGER[c], LAURENT PHILIPPOT[d], SARA HALLIN[b], MARCEL VAN DER HEIJDEN[c], MATTHIAS C. RILLIG[a]

[a]Freie Universität Berlin, Institut for biology, Plant ecology, Berlin, Germany; [b]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala, Sweden; [c]Agroscope, Plant-soil Interactions, Zürich, Switzerland; [d]INRA, UMR 1347 Department of Agroecology, Dijon, France; [e]King Juan Carlos University, Biology and Geology, Mostoles, Spain.

E-mail of the corresponding Author: florine.degrune@gmail.com

Soil protists have gained much interest over the past years as they play a decisive role in the soil foodwebs. However, unlike bacteria and fungi, there is still a huge gap in the information on the factors structuring their communities. Protists encompass unicellular organisms not only distantly related (polyphyletic), but also featuring a vast array of functional traits. This has limited our ability to study their structure at the community level, until recently. Here we examine the importance of different biotic and abiotic factors as key drivers of the biogeography of protistan communities, focusing on a major group of protist, the Cercozoa. From 217 soils samples collected during an intensive survey conducted at large spatial scale in Europe (Biodiversa project), we measured 67 environmental factors (soil, geography, climate), together with the diversity and composition of cercozoan communities. Machine learning facilitated identifying the best drivers of cercozoan diversity (richness) and community composition. Our results show that Cercozoa are not randomly distributed and that richness and community composition of Cercozoa exhibit different biogeographical patterns, with cercozoan richness strongly structured by soil conditions, and community composition strongly structured by climatic and geographic conditions.

Ciliate Diversity on Heron Reef, GBR - Patterns and Changes

GAYTHA A. LANGLOIS[a]

[a]Department of Science and Technology, Bryant University, Smithfield, RI, USA.

E-mail of the corresponding Author: langlois@bryant.edu

Multiyear field and laboratory studies conducted at the Heron Island Research Station (HIRS) located in the Capricorn Region of the Great Barrier Reef have revealed complex microbial 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 extended study (2006-2019) has emphasized the diversity of ciliates and diatoms found in shallow reef sediments, including the relative abundance of different trophic groups. The interactive dynamics of opportunistic and/or potentially pathogenic ciliate species associated with damaged corals undergoing disease conditions (such as Brown Band disease) have been observed, including *Porpostoma* sp. 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. DNA extracted from sediment samples and Protargol staining of selected ciliate species provided additional information about the diversity and relative abundance of ciliates. Observations of diatoms and flagellates suggests a higher proportion of microalgae in recent as compared to earlier observations. The absence of epiphytic Protista on macroalgae was evaluated, using extracted algal exudates. The Brown Band Disease ciliate, *Porpostoma* sp., was not found in association with healthy corals, in sediment samples, algal surfaces, or plankton observed prior to bleaching in the 2016-2019 samples in late January, posing the question of their location when not acting as opportunistic pathogens. Future studies will focus on the life cycle of *Porpostoma* sp. on Heron reef in comparison with its reported incidence elsewhere on other reefs.

Comparison Between Molecular and Morphological Identifications of Foraminifera (Rhizaria) from Oxic and Anoxic Sites in the Gulf of Mexico Near the Mississippi River Delta

MAGALI SCHWEIZER[a], THÉO MARCHAND[a], SOPHIE QUINCHARD[a], LAURIE BRETHOUS[b], FRANS JORISSEN[a], JULIEN RICHIRT[a], LAURE APOTHÉLOZ-PERRET-GENTIL[c], BRUNO BOMBLED[b], JAN PAWLOWSKI[c], MARTIAL TAILLEFERT[d], CHRISTOPHE RABOUILLE[b]

[a]LPG-BIAF, UMR 6112-CNRS, UNIV Angers, Université de Nantes, Angers Cedex, France; [b]LSCE, UMR 8212-CEA-CNRS-UVSQ-IPSL, Université Paris-Saclay, CEA-Orme des Merisiers, Gif sur Yvette, France; [c]Department of Genetics and Evolution, University of Geneva, Switzerland; [d]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, USA.

E-mail of the corresponding Author: magali.schweizer@univ-angers.fr

The Gulf of Mexico near the Mississippi delta has the world greatest seasonal hypoxic zone (dead zone) linked to a river. During the summer 2017, this zone was the largest observed since measurements began in 1989. Geochemical data and foraminifers were collected at that time. A total of ten stations have been sampled, six in the neritic zone which is more impacted by river discharge and hypoxia and four in the bathyal zone which is less influenced by coastal phenomena. Among the six stations situated in the neritic zone, one was close to hypoxia and one close to anoxia.

Here, in order to investigate the diversity of live foraminifers at these different stations, we compare data obtained with traditional methods (morphological identification and quantifications of individuals) and with molecular methods (DNA barcoding with SEM images and metabarcoding).

Twenty-nine foraminiferal morphospecies have been recognised: 52% belong to rotaliids, 38% to textulariids, 7% to lagenids and 3% to miliolids, all bearing a mineral test. For the DNA barcoding, nine species have been identified: 78% represent rotaliids, 11% textulariids and 11% miliolids. For the metabarcoding, 5,894 OTUs have been obtained by High Throughput Sequencing: 82% are unassigned, 7% are identified as rotaliids, 7% as textulariids and 4% as monothalamids (organic shelled foraminifers).

We will discuss the differences between these three methods and combine the obtained results to characterise the biodiversity and to compare it to the geochemical parameters in the different sites.

Coupling Ribotypes to Phenotypic Plasticity in Ciliated Protists

JUN GONG[a], SONGBAO ZOU[a,b], RAO FU[b], HUIWEN DENG[a], QIANQIAN ZHANG[b]

[a]School of Marine Sciences, Sun Yat-Sen University, Zhuhai Campus, Tangjiawan, Zhuhai, China; [b]Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China.

E-mail of the corresponding Author: gongj27@mail.sysu.edu.cn

Ribosomal RNA genes have been commonly targeted to characterize populations and communities of unicellular eukaryotes (protists) in metabarcoding studies. However, protistan ribotypic traits have been rarely linked to phenotypic attributes (cell size, growth rate etc.) of individuals, which are known to have large morphological variability across life-cycle stages and under environmental changes. Previously, we addressed these issues using single-cell analysis of ciliated protists cultivated or treated at different temperatures as testing models, and showed that per-cell rDNA and rRNA copy numbers scaled with cell volume, and the copy number ratio of rRNA:rDNA was related to cell size of ciliates captured at the exponentially growing stage (Fu & Gong, 2017, JEM 64: 885–896).

As a new contribution, we followed further found that the copy numbers of 18S rDNA and rRNA in single cells of two soil species (*Colpoda inflata* and *C. steinii*) decreased progressively from lag, exponential, plateau to resting cyst phases. Short-term chilling treatment of *Colpoda* led to formation of unstable cysts, causing dramatic drops in cellular rDNA and rRNA copy numbers. Both per-cell rDNA and rRNA copy numbers were well correlated with body size and macronuclear size across all life-cycle stages and temperature treatments, indicating the power law scaling relationship between ribotype copy number and cell volume (biomass) should hold for environmental samples, in which protistan individuals of different growth stages usually exist at varying conditions. Fluorescence in situ hybridization assays with specific-probes identified and well separated the mixed resting cysts of two *Colpoda* species, highlighting the potential of using relevant molecular tools in assessing the "inactive" part of protistan diversity and quantity in the environment. High throughput sequencing of the two *Colpoda* species, plus two marine species *Euplotes vannus* and *Strombidium sulcatum*, revealed high intra-individual polymorphisms of both 18S rDNA and rRNA. In each species a single haplotype dominated, with a proportion ranging from 37% to 86% and varying between in rDNA vs. rRNA, resting cystic vs. exponentially growing stages, and at different temperatures. Generally, operational taxonomic unit (OTU) clustering at the identity of 97% resulted in many OTUs from a single cell, implying that many rare rDNA and rRNA variants lead to the inflation of species richness in diversity surveys.

Isolation and Evaluation of a Novel Strain of *Chlorella sorokiniana* that Resists Grazing by the Predator *Poterioochromonas malhamensis*

MINGYANG MA[a,b], YINGCHUN GONG[a], QIANG HU[a]
[a]Center for Microalgal Biotechnology and Biofuels, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China; [b]University of Chinese Academy of Sciences, Beijing, China.
E-mail of the corresponding Author: springgong@ihb.ac.cn

The protozoan flagellate *Poterioochromonas malhamensis* is one of the main contaminants in *Chlorella* culture; however, few effective and affordable methods have been developed to control it. Unexpectedly, one strain of an unknown green alga was observed to be capable of contaminating *P. malhamensis* culture and defending itself against the protozoan. Based on cell morphology and molecular information, the green microalga was identified as *Chlorella sorokiniana* CMBB-146. Feeding experiments showed that the addition of the predator *P. malhamensis* not only had no negative effect on the growth of *C. sorokiniana* CMBB-146, but also stimulated an increase in the biomass of *C. sorokiniana* CMBB-146. The grazing resistance of *C. sorokiniana* CMBB-146 against *P. malhamensis* was also verified on a pilot scale with 100-L raceway ponds. Further experiments revealed that *P. malhamensis* showed a strong ability in ingesting *C. sorokiniana* CMBB-146 cells, but the ingested cells were hard to digest. Through comparison with other species of *Chlorella* without grazing resistance, we found that the differences on cell size, cell morphology, and biochemical composition had no relation with the ability of *C. sorokiniana* CMBB-146 to resist grazing by *P. malhamensis*. Finally, it is speculated that variation in cell wall composition, especially the content of galactosamine, empowers *C. sorokiniana* CMBB-146 to resist *P. malhamensis*. Now we are parsing the grazing-resistance mechanism with genomics and transcriptomics.

Disentangling Environmental Effects in Microbial Association Networks

INA MARIA DEUTSCHMANN[a], GIPSI LIMA-MENDEZ[b,c], ANDERS K. KRABBERØD[d], JEROEN RAES[b], KAROLINE FAUST[b*], RAMIRO LOGARES[a*]

[a]Institute of Marine Sciences (ICM), CSIC, 08003, Barcelona, Spain; [b]Department of Microbiology and Immunlogy, REGA Institute, KU Leuven, Leuven, Belgium; [c]VIB, Center for the Biology of Disease, Leuven, Leuven, Belgium; [d]Department of Biosciences, Section for Genetics and Evolutionary Biology (Evogene), University of Oslo, Oslo, Norway; [*]Shared last authors.

E-mail of the corresponding Authors: ina.m.deutschmann@gmail.com, ramiro.logares@gmail.com

Ecological interactions among microbes are fundamental for ecosystem functioning. Yet, most of them remain unknown. High-throughput omics can help unveiling microbial interactions by inferring associations, which can be represented as networks. Associations in these networks can indicate ecological interactions between species or alternatively, similar or different environmental preferences, in which case the association is environmentally-driven. We developed an approach to determine whether or not two species are associated in a network due to environmental preference. We use four methods (Sign Pattern, Overlap, Interaction Information, and Data Processing Inequality) that in combination can detect what associations are environmentally-driven. We implemented our approach in a publicly available software tool called EnDED. Our program was tested on simulated networks as well as on real marine microbial networks constructed with spatial or temporal community composition data that included prokaryotes and protists. We found evidence of environmentally-driven associations in all tested datasets. For instance, in a network constructed with 10 years of monthly data, including both marine prokaryotes and protists from the Mediterranean Sea, we found that 14% of the associations were environmentally-driven. We conclude that environmentally-driven associations are ubiquitous in microbial association networks and that it is crucial to determine and quantify them in order to generate more accurate hypotheses on ecological interactions in the microbial world. In particular, our approach could be useful to determine interactions between protist hosts (environment) and prokaryotes (symbionts, parasites).

Does *Tetrahymena* Like It Hot? Evolution of Thermal Responses at a Generic Level

DAVID JS MONTAGNES[a], ZHAO LYU[b], CHEN SHAO[b]
[a]Institute of Integrative Biology, University of Liverpool, BioSciences Building, Liverpool, UK; [b]School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, China.
E-mail of the corresponding Author: dmontag@liv.ac.uk

Two, predictions exist regarding how species respond to temperature along a cross-taxa thermal landscape. The "hotter is better" or "thermodynamic constraint" hypothesis argues that biochemical reactions drive rates, with taxa occupying high-temperatures niches performing better at their thermal optima (T_{opt}). In contrast, the "hotter is not better" or "biochemical adaptation" hypothesis predicts that taxa occupying low-temperature niches evolve to compensate for their biochemical constraints; i.e. growth rates at T_{opt} of species that have adapted to low- and high-temperatures will be similar. A second evolutionary prediction proposes that trade-offs occur, where taxa that are successful at higher temperatures will sacrifice thermal flexibility, exhibiting relatively narrow thermal ranges. Although both these predictions are being explored across disparate taxa, there has been little consideration of how closely-related taxa may have evolved. Such a focus at the genus level, where related species might be expected to have similar responses, should provide insights as to how evolutionary trends arise. To this end we have used seven species of the model ciliate *Tetrahymena* to evaluate how thermal pressure has driven evolutionary changes in closely-related taxa. Specifically, we examine growth rate, as a metric of fitness, over each species' full-thermal range. We then fit an appropriate mechanistic function to the thermal-response data and use the function's parameters to explore trends and test the above hypotheses. Recognising 1) support for the "hotter is not better" hypothesis, 2) little change in thermal range with T_{opt}, but 3) the occurrence of other subtler taxon-specific trends with T_{opt}, we then assess if these differences map on to *Tetrahymena* phylogeny as revealed by cox1 and SSU rDNA.

Oral Session DIVERSITY & SPECIATION 2

Plastoquinone-Mediated Electron Transport in a Non-Photosynthetic Plastid of the Heterotrophic Green Alga *Hyalogonium* sp.

MOTOKI KAYAMA[a], JUN-FENG CHEN[a], TAKASHI NAKADA[b,c], YOSHIKI NISHIMURA[d], TOSHIHARU SHIKANAI[d], HIDEAKI MIYASHITA[a], YUICHIRO KASHIYAMA[e], RYOMA KAMIKAWA[a]

[a]Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan; [b]Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata; [c]Systems Biology Program, Graduate School of Media and Governance, Keio University, Tsuruoka, Yamagata; [e]Graduate School of Engineering, Fukui University of Technology, Fukui, Japan.

E-mail of the corresponding Author: kamikawa.ryoma.7v@kyoto-u.ac.jp

Regardless of the benefits of ATP- and NADPH-production by photosynthesis, some algae and plants have evolved to non-photosynthetic heterotrophs or parasites by losing photosynthesis. It remains unclear how the plastid electron transport chain evolves in association with the loss of photosynthesis. To gain insight into evolution of the plastid electron transport chain, we investigated a novel color-less strain of the green alga, *Hyalogonium* sp. NrCl902. A DNA sequencing has successfully determined the complete sequence of a circular plastid genome in *Hyalogonium* sp. NrCl902. Further, we performed transcriptome analyses to detect transcripts for plastid functions. Plastid-encoded and nuclear-encoded plastid-targeted proteins identified in these analyses suggest that *Hyalogonium* sp. NrCl902 lacks photosynthesis and carbon fixation, in addition to the chlorophyll biosynthesis pathway. However, most of plastid metabolic functions are highly likely retained in this non-photosynthetic green alga. Most importantly, the non-photosynthetic green alga also retains plastid biosynthetic pathways of carotenoids and plastoquinone, as well as plastid-targeted electron transport-related proteins, such as NADH Dehydrogenase 2 (NDH2), ferredoxin, Ferredoxin:NADPH Oxidereductase (FNR), and Terminal Oxidase (pTOX). HPLC analyses followed by tandem mass spectrometry analyses indicated that the non-photosynthetic green alga indeed possessed carotenoids, plastoquinone, and plastoquinol. Presence of both plastoquinone and plastoquinol indicates that these molecules act as electron carriers in the plastid. Given these findings, the non-photosynthetic green alga *Hyalogonium* sp. NrCl902 has a plastid electron transport pathway which is likely consisted of plastoquinone, NADPH, NDH2, ferredoxin, FNR, and pTOX. As some of those proteins are present also in other non-photosynthetic, distantly related algal species, such a modified pathway or similar ones might tend to be retained even after loss of photosynthesis.

Exploring the Deep Evolutionary History of Phagotrophic Euglenids with Single-Cell Transcriptomics

GORDON LAX[a], MARTIN KOLISKO[b], YANA EGLIT[a], WON JE LEE[c], PATRICK KEELING[d], ALASTAIR G.B. SIMPSON[a]

[a]Dalhousie University, Halifax, Canada; [b]Institute of Parasitology Biology Centre, České Budějovice, Czechia; [c]Kyungnam University, Changwon, South Korea; [d]University of British Columbia, Vancouver, Canada.

E-mail of the corresponding Author: gordon.lax@dal.ca

Euglenids are a ubiquitous group of flagellates that includes well-known phototrophic algae, but also osmotrophic and phagotrophic forms. The majority of phyletic diversity can be found in phagotrophic euglenids, which independently gave rise to both osmotrophs and phototrophs. The phylogenetics of osmotrophs and phototrophs are relatively well developed, but our understanding of deep euglenid evolution has been severely hampered by limited knowledge of even basic evolutionary relationships among phagotrophic euglenid taxa. Among other unresolved questions, the exact origins of phototrophic euglenids are currently unclear. Almost all prior analyses used one gene, the SSU rRNA (or occasionally *hsp90*) as the phylogenetic marker. Most of these analyses also suffered from very limited taxon sampling. Overcoming the limitations of a single gene, we employed 'omics' methods, especially single-cell transcriptomics on identified and photo-documented phagotrophic euglenids. Using this, we generated a substantial multigene dataset of euglenids, emphasising the broad range of phagotrophic euglenid diversity. This dataset demonstrates that single-cell transcriptomes from euglenids are suitable for use in phylogenomic analyses. We present the most broadly sampled multigene analyses carried out for euglenids as a whole, and discuss phylogenetic and evolutionary implications for this major group of unicellular eukaryotes.

A Novel Lineage of Predatory Protists Sheds Light on the Origin of Photosynthetic Eukaryotes: a Morphological Perspective

DENIS V. TIKHONENKOV[a,b], RYAN M. R. GAWRYLUK[c], ELISABETH HEHENBERGER[d], FILIP HUSNIK[b], ALEXANDER P. MYLNIKOV[a], PATRICK J. KEELING[b]

[a]Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Russia; [b]Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada; [c]Department of Biology, University of Victoria, Victoria, British Columbia, Canada; [d]Monterey Bay Aquarium Research Institute, CA, USA.

E-mail of the corresponding Author: tikho-denis@yandex.ru.

Integration of a photosynthetic cyanobacterium into a phagotrophic protist spurred the radiation of Archaeplastida, the 'primary' plastid-bearing eukaryotic supergroup that comprises Viridiplantae (green algae + land plants), Glaucophyta, and Rhodophyta (red algae). Archaeplastids have had an enormous impact on terrestrial and aquatic ecosystems, and multiple independent enslavements of such primary algae – yielding organisms with secondary and tertiary plastids – has since shaped the evolution of diverse eukaryotic taxa. Here we present the discovery of *Rhodelphis*, the first described representative of an ancient lineage (new phylum of arhaeplastids Rhodelphidia) that is robustly supported as sister to red algae in phylogenomic analyses. Surprisingly, the characteristics of *Rhodelphis* are nearly opposite to the defining features of red algae: they are non- photosynthetic, motile biflagellate predators that actively feed upon other microbes, along with a genome-lacking relic primary plastid. So, the common ancestor of *Rhodelphis* and red algae was mixotrophic, akin to the archaeplastid common ancestor. Here we show that *Rhodelphis* morphology is very different from Archaeplastida cells. It possess several unique ultrastructural traits and also has features shared with representatives of multiple supergroups of eukaryotes, indicating the very ancient ancestral morphological state of the *Rhodelphis* cell. The absence of *Rhodelphis*-specific features in its closest photosynthetic relatives illustrates dramatic changes in cell morphology occurred in the transition from a mixotrophic ancestor to photosynthetic organisms. The ancestors of Archaeplastida and cryptophytes (or their common ancestor) may have also resembled *Rhodelphis* cells morphologically. The discovery of *Rhodelphis* has implications for the origins of eukaryotic photosynthesis, as it demonstrates that *eukaryovory* was lost independently multiple times within Archaeplastida, and that reliance on photosynthesis alone was not an ancestral trait of the group. The study was supported by the Russian Science Foundation (grant no. 18-14-00239).

A Novel Lineage of Predatory Protists Sheds Light on the Origin of Photosynthetic Eukaryotes: a Genomic Perspective

RYAN M. R. GAWRYLUK[a], DENIS V. TIKHONENKOV[b,c], ELISABETH HEHENBERGER[d], FILIP HUSNIK[c], ALEXANDER P. MYLNIKOV[b], PATRICK J. KEELING[c]
[a]Department of Biology, University of Victoria, Victoria, British Columbia, Canada; [b]Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Russia; [c]Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada; [d]Monterey Bay Aquarium Research Institute, CA, USA.
E-mail of the corresponding Author: ryangawryluk@uvic.ca

Archaeplastida is a eukaryotic 'supergroup' united by the presence of primary plastids. Among archaeplastids, red algae are unusual in that they lack several biosynthetic pathways, and cellular structures, such as centrioles and flagella. However, the paucity of lineages closely related to red algae means that little is known about the timing of gene loss, or the genomic repertoire of the red algal ancestor. Here, we present the results of genomic and transcriptomic analyses of two species from a novel phylum (Rhodelphidia) that phylogenomic analyses robustly support as sister to Rhodophyta. Unlike red algae, which are immotile and photosynthetic, *Rhodelphis* are gene-rich, highly motile flagellated predators that retain a non-photosynthetic plastid that plays a role in heme biosynthesis. Together, our analyses indicate that the common ancestor of *Rhodelphis* and red algae was motile, mixotrophic and genetically complex, and that phagotrophy-based feeding was lost independently in distinct archaeplastid lineages.

Time Calibrated Morpho-Molecular Classification of Radiolaria

MIGUEL M. SANDIN[a], FABRICE NOT[a]
[a]Sorbonne Université, CNRS, UMR7144 - Roscoff, France.
E-mail of the corresponding Author: miguelmendezsandin@gmail.com

Radiolaria are holoplanktonic ameboid protists belonging to the Rhizaria lineage (SAR). Their elaborated opaline silica skeleton preserves well in sediments, exhibiting an exhaustive fossil record dating back to the Cambrian. In contemporary oceans, molecular-based metabarcoding surveys performed at global scale have shown Radiolaria to contribute significantly to plankton communities. Despite their importance in both modern and past ecosystems, radiolarian taxonomic classification remains controversial. Recent studies on Acantharia and Collodaria have integrated molecular (rDNA) with both optical and electronic microscopy to explore relationships within these radiolarian taxa. Here we present original results on the taxonomic classification of Nassellaria and Spumellaria, two important radiolarian taxa presenting an extensive fossil record, along with detailed analyses of their evolutionary history based on molecular clock estimates. In addition, by merging all radiolarian phylogeny datasets available to date, our analyses demonstrate that Radiolaria rose during the early Neoproterozoic. At that period, the Acantharia lineage, bearing strontium sulphate skeleton, diverge from Radiolaria exhibiting opaline silica skeletons (*i.e.* polycystines). The symmetry of polycystines skeletons was then established over the following geological periods with the appearance of Spumellaria and Nassellaria in the early Paleozoic. Although, it was not until the Triassic that the first currently living radiolarian representatives diversify. Our newly comprehensive and contextualized morpho-molecular framework contributes to improve the understanding of the evolutionary history of Radiolaria and brings a standpoint to explore their diversity and distribution in contemporary oceans.

Spotlight on Nudiform Choanoflagellates – an Evolutionary Paradox

SABINE SCHIWITZA[a], FRANK NITSCHE[a]

[a]Biological Department, Institute of Zoology, Biocenter Cologne, University of Cologne, Cologne, Germany.

E-mail of the corresponding Authors:sabine.schiwitza@uni-koeln.de; fnitsche@uni-koeln.de

Lorica-bearing choanoflagellates (Acanthoecida) are separated into two families based on their way of lorica production. In the tectiform condition, the mother cell provides a bundle of costal strips prior to cell division to the juvenile cell, whereas nudiform reproducing species have to develop the lorica after division independently. This observation could be confirmed by molecular analysis, but the ecological and evolutionary significance is still under debate. Nudiform choanoflagellates are discussed as an evolutionary paradox as the species are indeed consistent in their way of cell division and lorica production but in terms of morphological characterization they lack coherency. Considering species richness, tectiform choanoflagellates contain a multitude of species compared to nudiforms, where until now only six species were present. With our study we draw attention to the prior neglected and as minor described family of nudiform choanoflagellates. Only recently, we could discover a new sister clade within the nudiforms and described the genus *Enibas*, comprising until now the species *E. tolerabilis* and *E. thessalia*, but with high potential of a greater extent as eDNA data suggest. Interestingly, these species resemble morphologically the tectiform genus *Stephanoeca*, but show clearly the nudiform cell division and lorica production, supporting the phylogenetic classification within the nudiforms. This particular stephanoecid morphology is now present in both families. It becomes even more obvious that the genus *Stephanoeca* is in need of revision as we could additionally assign a previous only morphologically described *Stephanoeca* species to the nudiform family based on molecular data. With our study we could show that the family of nudiform choanoflagellates is broadly underestimated. The combination of molecular and morphological tools together with distinct observations regarding the condition of reproduction will lead to a revision within the Acanthoecida and will help to understand the evolutionary relationship between both conditions.

Assessing the Occurrence and the Origin of SSU rDNA Intragenomic Polymorphism in Planktonic Foraminifera

MATTIA GRECO[a], RAPHAEL MORARD[a], MICHAL KUCERA[a]

[a]MARUM - Centre for Marine Environmental Sciences, Bremen, Germany.

E-mail of the corresponding Author: mgreco@marum.de

Molecular analyses on the 18S rDNA gene in planktonic foraminifera have led to significant advances in the understanding of their phylogenetic history as well as a re-assessment of their biological diversity. At the same time, it became obvious that the application of the 18S rDNA is complicated by the existence of intragenomic polymorphism (differences among the multiple copies of the gene within single individuals). This phenomenon is especially critical for species identification in environmental metabarcoding surveys, where it cannot be established whether different sequence motives originated from within the same individual. To be able to correctly interpret metabarcoding data, we need to understand the patterns of incidence and extent of intragenomic variability among taxa and clades and assess their predictability. To this end, we use clone libraries from single-cell DNA extractions and characterise the occurrence of intragenomic polymorphism in 33 species representing all major lineages of extant planktonic foraminifera. The dataset comprises 2406 clonal sequences (2 to 183 sequences per individual) covering five hypervariable regions (37f, 41f, 43e, 45e-47f and 49e). We observe that the magnitude and incidence of intragenomic variability vary greatly among species, even within the same lineage. We compared the secondary structure of rRNA with the occurrence of the intragenomic polymorphism and found that reduced values in intragenomic variability were associated with different folding patterns of the secondary structure compared to closely related species with higher variability. We used the changes in secondary structure to calculate a measure of divergence complementing simple genetic distance. A mapping of the intragenomic distance and secondary structure divergence among species on the phylogenetic history of the group reveals that reduced intragenomic variability is a derived character, associated with long-branch lineages. This observation can be interpreted as evidence for suppression of intragenomic variability due to accelerated molecular evolution.

Mutation Rates of Choanoflagellate and Ciliate Species from the Atacama Desert Based on Molecular Clock Analysis

FRANK NITSCHE[a], SABINE SCHIWITZA[a], HARTMUT ARNDT[a]

[a]Biological Department, Institute of Zoology, Biocenter Cologne, University of Cologne, Cologne, Germany.

E-mail of the corresponding Author: fnitsche@uni-koeln.de

The Atacama Desert in North Chile is one of the harshest environments on earth. The severe conditions, in particular the high UV radiation and the highly divergent conditions in aquatic habitats like the Salars, demand high adaptations. Exemplified by the group of choanoflagellates from aquatic environments, we show that the high UV radiation may favor positive mutations which allow a fast adaptation to the extreme environment, mirrored by a high substitution rate within the studied genes. On the other hand, ciliates, which were isolated from soil, show a much more conservative substitution rate as they are not directly exposed to high UV radiation. Using a strict molecular clock, we demonstrate that the aquatic organisms, choanoflagellates, show a much higher substitution rate than predicted by previous studies. In contrast, ciliates show a substitution rate within the expected values.

SYMPOSIUM - From genomics to flagellar and ciliary structures and cytoskeleton dynamics – (by FEPS)

Genome Wide Tagging in Trypanosomes Uncovers Flagellum Asymmetries

JACK SUNTER[a]
[a]Oxford Brookes University, Oxford, UK.

E-mail of the corresponding Author: jsunter@brookes.ac.uk

Organelles have highly organised, complex internal structures that enable them to perform a diverse set of biological functions. Analysing this complex structural organisation on a genome-wide scale, using mass spectrometry is challenging and often loses fine-scale structural resolution. To address this, we have determined the localisation of every protein encoded in the *Trypanosoma brucei* genome using our high-throughput tagging methodology and the data is freely available on our website http://tryptag.org. We have shown many proteins localise to specific organelle sub-domains and this organelle asymmetry/inhomogeneity applies to all organelles, including the flagellum, endoplasmic reticulum and mitochondrion. Here, as one example, we concentrate on our comprehensive molecular cartography of the flagellum and the role of protein asymmetry within the flagellum.
Trypanosoma brucei and *Leishmania mexicana* have complex life cycles in which they encounter a variety of different environments; the ability to move and navigate in these different environments is crucial for their success as parasites. Their movement is driven by the flagellum, which has an apparently symmetrical 9+2 axoneme structure. Asymmetries along the length of motile flagella have been identified in a number of organisms, typically in the inner and outer dynein arms. Flagellum beat waveforms are adapted for different functions and they may start either near the flagellar tip or near its base (and may be symmetrical or asymmetrical). We hypothesised that proximal/distal asymmetry in the molecular composition of the axoneme may control the site of waveform initiation and direction of waveform propagation. We show that the proximal and distal portions of the flagellum contain distinct outer dynein arm docking complex heterodimers. This proximal/distal asymmetry is produced and maintained through flagellar growth by a concentration gradient of the proximal docking complex, generated by intraflagellar transport. Furthermore, this asymmetry is involved in regulating whether a tip-to-base or base-to-tip beat occurs, which is linked to a calcium-dependent switch. Our data show that the mechanism for generating proximal/distal flagellar asymmetry can control waveform initiation and propagation direction, which will be crucial for navigating complex, in vivo environments.

Deciphering the Molecular Mechanisms that Coordinate Ciliary Outer Doublet Complexes – Search for "Missing Links"

RAFAŁ BAZAN[a], MARTYNA POPRZECZKO[a], ADAM SCHROFEL[b], EWA JOACHIMIAK[a], ANNA OSINKA[a], HANNA FABCZAK[a], GAIA PIGINO[b], DOROTA WLOGA[a]

[a]Laboratory of Cytoskeleton and Cilia Biology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland; [b]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

E-mail of the corresponding Author: d.wloga@nencki.gov.pl

Some of the ciliated or flagellated protists became a model-of-choice in a cell biology research including the analyses of cilia and flagella ultrastructure, and functioning. Importantly, the vast majority of so far analyzed ciliary proteins are evolutionarily conserved and play the same role in protozoa and vertebrate motile cilia, including human. Thus, experiments conducted on ciliated or flagellated protists may enrich not only the general knowledge of cilia protein composition and molecular mechanisms that regulate cilia beating but also shed a light on the basis of human disorders caused by a dysfunction of motile cilia.

The skeleton of the motile cilium is composed of 9 microtubule doublets positioned at cilium periphery and two central microtubules. These microtubules serve as docking sites for macro- and micro-complexes that are specific either to outer doublets or central microtubules.

A full understanding of the molecular mechanisms that govern cilia beating is not possible without the identification and functional analyses of all proteins involved. While protein composition and function of major ciliary complexes (outer and inner dynein arms, nexin-dynein regulatory complex, and radial spokes) are well defined, the knowledge of minor ciliary structures that regulate the activity of macrocomplexes or participate in the signal transduction between these macrocomplexes is limited.

Using ciliate Tetrahymena as a model organism and genetic, biochemical and microscopy methods, we have recently shown that several novel ciliary proteins play a role in the regulation of the activity of the different inner dynein arms and possibly in the signal transmission between nexin-dynein regulatory complex and inner dynein arms.

Research supported by the National Science Centre, Poland (Opus13, 2017/25/B/NZ3/01609)

From Centrosomal Microtubule Anchoring and Organization to Basal Body Positioning: TBCCD1 An Elusive Protein

BRUNO CARMONA[a,b], CAROLINA CAMELO[a,f], MANON MEHRAZ[c], MICHEL LEMULLOIS[c], DAVID CARMELINO FERREIRA[a], SOFIA NOLASCO[b,d], MARIANA LINCE-FARIA[e], H. SUSANA MARINHO[a], MÓNICA BETTENCOURT-DIAS[e], ANNE MARIE TASSIN[c], FRANCE KOLL[c], HELENA SOARES[a,b]

[a]Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; [b]Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal; [c]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris Sud, Université Paris-Saclay, Gif sur Yvette, France; [d]Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal; [e]Instituto Gulbenkian de Ciência, Oeiras, Portugal; [f]present address: Luschnig lab, Münster University, Münster, Germany.

E-mail of the corresponding Author: mhsoares@fc.ul.pt

Cilia are microtubule-based organelles that protrude from the cell surface and fulfill critical motility and sensory functions being required for normal embryonic development and for homeostasis of human adult tissues. Cilia loss or dysfunction is associated with human ciliopathies. At their base cilia have a centriole/basal body (BB), which can be derived from the centrosome and assembles the ciliary axoneme. This process requires the correct positioning/anchoring of the centrosome's mother centriole/BB to the cell membrane. A clear picture of the different signals and players involved in centrosome positioning/anchoring is still not available.

Published work from our group identified a new centrosomal TBCC domain-containing human protein (TBCCD1) that is involved in centrosome correct positioning and primary cilia assembly. In mammalian cells TBCCD1 is observed at pericentriolar satellites, in basal bodies of primary and motile cilia and at primary cilia ciliopathy hot domain, the transition zone. Super resolution microscopy shows that TBCCD1 is localized at the distal region of the centrosome and its depletion dramatically affects the centrosome subdistal protein CEP170, a component of primary and motile cilia basal feet. By doing a proximity-dependent biotin identification (BioID-MS) screen for TBCCD1 interactors several well-known proteins encoded by ciliopathy genes were identified, *e.g.* the centrosomal proteins OFD1 and Moonraker/KIAA0753 associated with Digital Syndrome 1 and Joubert syndrome, respectively. OFD1 and Moonraker are required for the maintenance of centrosome structure and both proteins localization is dramatically disturbed by TBCCD1 depletion. To clarify the role of human TBCCD1 in cilia biogenesis we used the ciliate *Paramecium*. Noteworthy, in *Paramecium* TBCCD1 knockdown causes abnormal basal body associated rootlets organization, anomalous BB positioning/anchoring defects. Our data using human cells and the ciliate *Paramecium* support a role of TBCCD1 in centrosome structure maintenance and BB anchoring at the cell membrane. The *Paramecium* phenotypes confirm that TBCCD1 is a new candidate to a ciliopathic gene probably by founding the TBCCD1/Moonraker/OFD1 functional conserved module required for cilia assembly.

Central Pair Proteins in Cilia Beating Regulation

EWA JOACHIMIAK[a], ANNA OSINKA[a], HANAN FARAHAT[a], MARTYNA POPRZECZKO[a], HANNA FABCZAK[a], DOROTA WLOGA[a]
[a]Laboratory of Cytoskeleton and Cilia Biology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland.

E-mail of the corresponding Author: e.joachimiak@nencki.gov.pl

Motile cilia and flagella are crucial for cell motility of numerous protists. They are highly conserved structures composed of nine peripheral dublet microtubules and two single central pair (CP) microtubules. Ciliary microtubules are accompanied by additional macro- and microcomplexes that generate and regulate ciliary beating. Major doublet complexes include dynein arms (DA), nexin-dynein regulatory complex (N-DRC) and radial spokes (RSs), while CP microtubules form several projections, called C1a-f and C2a-e, and a connecting bridge. It was shown that regulation of ciliary movement depends RS/CP interactions. In *Chlamydomonas*, CP is built up of at least 25 proteins. To better understand the role of PC in cilia motility regulation we aimed to identify new CP proteins in *Tetrahymena thermophila*. For this purpose, we expressed Spef2A, a homologue of known CP1b projection, SPEF2/CPC1 protein, fused with BirA* ligase, that biotynylates proteins in a distance no longer that approx. 10 nm. Among identified biotinylated proteins, we found several new potential CP proteins. Functional analysis showed that four of them co-localize, have similar impact on ciliary motility and causes similar effect on cilia ultrastructure. Thus, most likely all analysed proteins form C1b or neighbouring projection.
This research was funded by the National Science Centre, Poland OPUS 15 grant no. 2018/29/B/NZ3/02443 to EJ and by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie COFUND grant agreement No-665735 (Bio4Med) and by Polish Ministry of Science and Higher Education within 2016-2020 funds for international projects implementation (agreement No-3548/H2020/COFUND/2016/2).

Oral Session PARASITISM 1

Microsporidia from Protists: Diversity, Morphology and Phylogeny

ELENA NASSONOVA[a,b], OKSANA KAMYSHATSKAYA[b,c], YULIA YAKOVLEVA[b], ELENA SABANEYEVA[b], GITA PASKEROVA[b], ALEXEY SMIRNOV[b], JULIA WALOCHNIK[d], ROLF MICHEL[e], LUIS JAVIER GALINDO[f], DAVID MOREIRA[f], PURIFICACIÓN LÓPEZ-GARCÍA[f], IRMA ISSI[g]

[a]Laboratory of Cytology of Unicellular Organisms, Institute of Cytology RAS, St. Petersburg, Russia; [b]Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; [c]Resource center "Culture Collection of Microorganisms", St. Petersburg State University, St. Petersburg, Russia; [d]Molecular Parasitology, Institute for Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria; [e]Central Institute of the Federal Armed Forces Medical Services, Koblenz, Germany; [f]Ecologie Systématique Evolution, CNRS, Université Paris--- Sud, AgroParisTech, Université Paris--- Saclay, Orsay, France; [g]All-Russian Institute of Plant Protection, St. Petersburg - Pushkin, Russia.
E-mail of the corresponding Authors: nosema@mail.ru; o.kamyshatskaya@yandex.ru; st041958@student.spbu.ru; sabaneyeva@gmail.com; gitapasker@yahoo.com; polychaos@mail.ru; polychaos@mail.ru

Among protists, infections with microsporidia and microsporidia like organisms have been reported from ciliates, paramyxeans, gregarines and amoebozoans. The number of species reported from ciliates and paramyxeans is rather limited. In the phylogenetic trees they occur in the clades 4 and 5 of higher microsporidia. Most probably, these cases represent the host switch from invertebrate hosts. Hyperparasites of gregarines from marine worms, the metchnikovellids, are deeply branching microsporidia. Amoebozoa are parasitized by poorly studied organisms belonging to the genera *Paramicrosporidium* and *Nucleophaga*.
They are considered either as microsporidia like rozellids or as 'short branched' microsporidia. The parasites from unicellular hosts have either a short polar tube, with a limited number of coils or a manubrium (which may be extended into a loosely packed tube). The latter is characteristic for the extrusion apparatus of metchnikovellids and its counterpart in the parasites from amoebae. It is very likely, that the manubrium represents an ancestral state adapted to parasitism in unicellular organisms. In contrast, for propagation in a multicellular organism, a parasite needs to have a long, coiled polar tube and an elaborated mechanism for its discharge. The ancestors of microsporidia probably evolved as intracellular parasites of single celled hosts thus bypassing the stages of tissue and intracavitary parasitism characteristic of other intracellular parasites. While retaining their intracellular localization, microsporidia might have been transferred to other groups of organisms, which closely interacted with their initial unicellular hosts. The gregarines might have facilitated dispersion of microsporidia among marine, freshwater and terrestrial annelids and arthropods. Amoebae are even better candidates for a vector role as they are well known transmitters of pro and eukaryotic organisms and viruses and are considered to be the 'melting pots' of evolution.
Supported with RSF grant 197420136.

Ultrastructural Affinities of *Nucleophaga amoebae* (Opisthokonta: Rozellomycota): from *Rozella* to Microsporidia

OKSANA KAMYSHATSKAYA[a,b], OLGA GORDETSKAYA[a], YELISEI MEZENTSEV[a], JULIA WALOCHNIK[c], DANIELE CORSARO[d], ROLF MICHEL[e], ALEXEY SMIRNOV[a], ELENA NASSONOVA[f,a]

[a]Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; [b]Resource center "Culture Collection of Microorganisms", St. Petersburg State University, St. Petersburg, Russia; [c]Molecular Parasitology, Institute for Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria; [d]CHLAREAS Chlamydia Research Association, Vandœuvre-lès-Nancy, France; [e]Central Institute of the Federal Armed Forces Medical Services, Koblenz, Germany; [f]Laboratory of Cytology of Unicellular Organisms, Institute of Cytology RAS, St. Petersburg, Russia.

E-mail of the corresponding Author: oksana.kamyshatskaya@gmail.com

Rozellomycota (Cryptomycota, Rozellosporidia) is a widespread environmental clade, an intermediate link between fungi and opisthokont protists. Most of its diversity remains cryptic, few species are studied at the organismal level. We investigated the ultrastructure of *Nucleophaga amoebae* KTq2, a parasite of *Thecamoeba quadrilineata*. Spores of the parasite were engulfed by the amoebae during phagocytosis and found inside the food vacuoles. Sporonts, the earliest life cycle stage found, were observed in the nucleolus of the host nucleus. The cell surface of sporonts was ornamented with an additional membrane associated with a complex network of tubular structures, apparently playing a role in the interaction with the host cell. Sporonts possessed a nucleus with a centrally located nucleolus and one or (rarely) more mitochondria with tubular cristae and an electron-transparent matrix (altogether rather similar to those of *Rozella* spp.). In the process of development, the sporonts transformed into multinucleate sporogonal plasmodia. The latter gradually increased in size and, at the advanced phase of proliferation, occupied almost the entire volume of the host nucleus. The surface of the plasmodia was uneven, with numerous finger-like protrusions penetrating the karyoplasm, which probably served to increase the surface area and to intensify the exchange with the host cell. The nuclei of the plasmodium divided by intranuclear pleuromitosis. In the cytoplasm of the plasmodia we perceived multiple mitochondria. Plasmodia converted into the sporophorous vesicles, containing numerous spores. Often, the simultaneous (but not necessarily synchronous) development of several parasites was observed within the same host nucleus. No evidences of phagocytosis were observed. In the spores, structures similar to elements of the extrusion apparatus of microsporidia could be identified. In the structure and development of *N. amoebae* both, microsporidia-like (tubular structures, elements of extrusion apparatus, absence of phagocytosis) and *Rozella*-like (mitochondria, finger-like protrusions) characters, can be traced. The most remarkable finding is the observation of the morphologically pronounced mitochondria. The mitochondrial genome of *N. amoebae* may be of special interest, but no genomic data are yet available. Supported with RSF grant 19-74-20136.

Investigating *Paramecium caudatum* Susceptibility to *Holospora undulata* Infection

WINIFFER CONCE[a]

[a]Department of Biology, SUNY New Paltz, New Paltz, NY, USA.

E-mail of the corresponding Author: Winifferc@gmail.com

Paramecium caudatum is a single-celled ciliate that has been shown to be susceptible to infection by the parasitic bacterium *Holospora undulata. Holospora* infects *Paramecium* by entering the cell via the oral apparatus and translocating to the micronucleus where it takes either its reproductive or its infectious form depending on resources available within the *Paramecium* cell. Previous work has shown that different *Paramecium* strains have varying susceptibility to *Holospora* but the factors that determine these differences are yet unknown. In order to determine the genes that factor into the infectious process, we would like to determine which *Paramecium caudatum* strains isolated from around the world and representing three syngens, and whose genomes are sequenced, are more susceptible to infection than others. We have developed a protocol to effectively infect naive strains of *Paramecium caudatum* and to track infection phenotypes over a week time course. We have successfully been testing the susceptibility or resistance of the respective strains and will continue to do so to then combine the infection phenotypes of these strains with comparative genomics and studies focusing on gene expression during the different infection stages. This will help us to determine the cellular factors that contribute to the symbiosis, and then to track how they have evolved to produce resistance or susceptibility.

Phylogenomics and Comparative Transcriptomics of Secondarily Free-Living Diplomonads

ERIC SALOMAKI[a], MONIKA WIŚNIEWSKA[a], AGNIESZKA PEŁESZ[a], PETR TÁBORSKÝ[a], EVA MAZANCOVÁ[b], VASANA JINATHAM[c], ELENI GENTEKAKI[c], IVAN ČEPICKA[b], MARTIN KOLÍSKO[a]

[a]Institute of Parasitology, Biologické centrum AV ČR, v.v.i., České Budějovice, Czech Republic; [b]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic; [c]School of Science, Mae Fah Luang University, Chiang Rai, Thailand.

E-mail of the corresponding Author: eric.salomaki@paru.cas.cz

Diplomonads are flagellated protists that inhabit oxygen-poor environments and lack conventional mitochondria. They are classified within Fornicata and are comprised primarily of host-associated commensals and parasites that reside in the intestinal tract of animals including humans (e.g., *Giardia intestinalis*). Additionally, free-living representatives have been described that inhabit freshwater and marine anoxic sediments (e.g., *Hexamita inflata*). The group is particularly interesting as the free-living taxa appear to be nested within a clade of host-associated species, suggesting a reversal from host-dependence to become secondarily free-living. As parasites become increasingly reliant on a host for nutrients and metabolites, reduced selective pressure often leads to the parasite losing genes that are essential for a free-living life-strategy. A previous transcriptomic investigation of the putatively secondarily free-living diplomonad *Trepomonas* sp. suggested that *Trepomonas* acquired several genes by horizontal gene transfer (HGT), widening its metabolic capacity and allowing the reversal back to free-living lifestyle. This finding was striking, however all prior studies suffer from both low taxon and gene sampling, especially within the free-living diplomonads. In this study we sequenced transcriptomes from 12 additional diplomonad isolates, (10 free-living and 2 host-associated) for phylogenomic and comparative analysis. Our phylogenomic analysis provides robust support for the evolutionary history of diplomonads providing a framework for subsequent comparative analyses. The 12 newly sequenced transcriptomes were investigated for genes identified as originating from HGT and putatively functioning in free-living lifestyle in the previously published *Trepomonas* transcriptome. Comparative analyses exploring the genomic basis enabling the transition from parasitism to a free-living life strategy is ongoing.

Host-Parasite Relationships of *Blastocrithidia raabei* in the True Bug *Coreus marginatus*

ANNA I. GANYUKOVA[a], MARINA N. MALYSHEVA[a], ALEXEI Y. KOSTYGOV[a,b], ALEXANDER O. FROLOV[a]
[a]Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia; [b]Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czechia.

E-mail of the corresponding Authors: anna.ganyukova@gmail.com; frolal@yandex.ru

Trypanosomatids are mainly known for their dixenous representatives — trypanosomes, leishmaniae, and phytomonads, many of which cause diseases in humans, domestic animals, and cultivated plants. Within this family, dixenous life cycles appeared repeatedly on the basis of monoxenous ones. This fact determines the importance of monoxenous parasites of insects as model objects in studying the evolution of host-parasite relationships between insect vectors and dixenous flagellates. However, until now the life cycles have been studied only in a handful of species and often quite fragmentarily.

We studied *Blastocrithidia raabei,* parasite of the dock bugs *Coreus marginatus*. The full life cycle of *B. raabei* takes place in the host intestine, but unlike most previously studied monoxenous trypanosomatids, it does not have a clear preference for a certain part of the host intestine. Epimasigotes are found in large numbers from the M2 midgut section to the rectum. In all intestinal sections flagellates divide intensively attaching to the intestinal epithelium with modified flagella. Furthermore, inside the M4 section, parasites attach to symbiotic bacteria of the host localized in/on the crypt epithelium, and capture them with flagella.

The development of *B. raabei* at the posterior end of the M3 section is unique. This section of the digestive system is separated from M4 by the specialized M4b section that includes a "constricted region" functioning in pentatomomorphan bugs as the host organ for selective symbiont sorting. Flagellates gathering before it form several local groups. Only one of them is associated with the entrance to the "constricted region" and proceed to the hindgut. Others are located near the intestinal walls, where they pass through the epithelium and induce separation of the basal lamina. The flagellates actively proliferate in the formed cavities and some of them attach to the inner surface of the basal lamina using flagella.

The formation of flagellar cysts begins in the M2 section. The number of epimastigotes involved in this process increases progressively towards the posterior intestinal sections and reaches its maximum in the rectum. The mechanism of cyst formation and their structure are similar to those of other blastocritidias described earlier.

This work was supported by the Russian Science Foundation grant 18-14-00134.

Revisiting an Old Affair: *Zoothamnium intermedium* (Precht, 1935) and Copepod Relationships in Chesapeake Bay

LÚCIA S. L. SAFI[a], RYAN CARNEGIE[a]

[a]Department of Aquatic Health Sciences, Virginia Institute of Marine Science, Gloucester Point, VA, USA.

E-mail of the corresponding Author: lucia.safi@gmail.com

As epibionts, peritrich ciliates are known to cause a variety of detrimental effects when colonizing

animal hosts, particularly small sized species. The effects from this ecological relationship can resonate or amplify through host population dynamics and affect their food webs. *Zoothamnium intermedium* is an obligate peritrich epibiont and has been reported on a variety of hosts and environments. Different researchers found conflicting distribution patterns and host preference, even though studies in Chesapeake Bay the species presented strong host specificity for two calanoid copepods. In this study, we aimed to answer questions on life cycle, host preference, and ecological relationships attributed to the North American *Z. intermedium* species. The York River (USA) was sampled between Fall 2014 to Summer 2015, and collections of plankton, free-living peritrichs, and bacteria in the water column were performed. Bacteria abundance in the water column followed spring and summer blooms and was accompanied by abundance and species richness of free-living peritrichs. Analyses of the ciliate 18S sequences and copepod COI sequences were used to confirm species identification. None of the analyzed zooplankton taxa was found to be colonized by *Z. intermedium*, exception being copepods *Acartia tonsa* and *Centropages hamatus*. The peritrich epibiont displayed a mixed pattern for host preference, presenting high colonization rates, particularly on *C. hamatus*, even when *A. tonsa* was by far more abundant. Populations of *C. hamatus* are considerably smaller, and hence more prone to the harmful effects of epibiosis. Interestingly, other copepods species were found to be colonized throughout Chesapeake Bay, raising questions on the use of ecological data in ciliate taxonomy.

The Salmon Pathogen *Spironucleus salmonicida* Induces Necrosis of Salmon Kidney Cells *in vitro*

COURTNEY W STAIRS[a,*], ÁSGEIR ÁSTVALDSSON[a,*], STAFFAN G SVÄRD[a], THIJS JG ETTEMA[a]

[a]Department of Cell and Molecular Biology, Uppsala, Sweden; [b]Laboratory of Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University, The Netherlands; [*]These authors contributed equally.

E-mail of the corresponding Author: courtney.stairs@icm.uu.se

Spironucleus salmonicida is an anaerobic diplomand parasite of salmonids. Unlike *Giardia intestinalis*, its close relative that infects the gastrointestinal tract of humans, *S. salmonicida* is able to cause systemic infection. After initial colonization of the anoxic intestines, the parasite can invade the blood and infect various tissues throughout the host – including the oxygen-rich skin surface and gills. We are interested in exploring how these parasites interact with host cells and tolerate oxygen during infection.

To investigate the morphological and molecular changes to both host and parasite cells during infection, we developed an *in vitro* interaction system using *S. salmonicida* and Atlantic salmon kidney cells and performed electron microscopy and gene expression analysis over 24 h. We found that after 3 h of interaction, the salmon cells did not proliferate, while the number of S. *salmonicida* cells increased. The parasites appear to attach to the surface of the salmon cells often causing deform. We also did not observe any parasites within the salmon cells. By 24 h, most of the salmon cells were destroyed.In terms of gene expression, after 3 h, we observed a shift in the expression genes encoding immune invasion strategies (e.g., cysteine-rich proteins), transcription factors and oxygen stress management. Although, most differentially expressed genes encode for proteins of unknown functions. Throughout the interaction, the salmon cells did not up-regulate genes encoding immune modulating or apoptosis functions. Instead, the host cells upregulate genes related to cell growth, strongly suggesting the cells are necrotic.

Altogether, this study has providing the first molecular analysis of how *S. salmonicida* interacts with salmon cells during infection. This data has allowed us to generate a list of candidate hypothetical proteins that might play a role in oxygen stress metabolism and even pathogenesis. Future in-depth molecular investigations of these gene products will lead to a better understanding of host:pathogen interactions in this lineage of eukaryotes.

Insight Into Phytomyxea-Host Interactions with a Combination of RNAseq and in-situ mRNA Visualization

SIGRID NEUHAUSER[a], MARTIN KIRCHMAIR[a], STEFAN CIAGHI[a], JULIA BADSTÖBER[a], ANNA LOACKER[a], ADOLF M. SANDBICHLER[b], CLAIRE GACHON[c]
[a]Institute of Microbiology, University of Innsbruck, Innsbruck, Austria; [b]Institute of Zoology, University of Innsbruck, Innsbruck, Austria; [c]Scottish Association of Marine Science, Scottish Marine Institute, UK.

E-mail of the corresponding Author: sigrid.neuhauser@uibk.ac.at

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. To address this lack of knowledge we recently generate transcriptomes of the clubroot pathogen *Plasmodiophora brassicae* in B. oleracea and the brown algal parasite *Maullinia ectocarpii* infecting the brown algal genome model *Ectocarpus siliculosus* Ec32m. Analysing these data we were able find first evidence for a pathogen induced systemic reaction in plant and brown algal hosts. We selected biologically interesting genes which were validated with single molecule FISH methods which allow to link the transcripts to specific stages of the life cycle allowing for a functional analysis of selected transcripts. Using a SABATH-type Methyltransferase which is produced by *P. brassicae* (PbBSMT) we were able to demonstrate spatiotemporal patten and to provide evidence for the function of this gene during the pathogen-host interaction. We were also able to provide proof for a fast and local pathogen response in brown algae by visualising Ec32m transcripts. Overall we provide new information on the genetics and physiology of phytomyxea and their interaction with their host.

TUESDAY 30 JULY

PLENARY LECTURE ISoP Past-President (by ISoP)

Micro-Eukaryotes in Animal and Plant Microbiomes: Ecologies of Disease?

DAVID BASS[a,b]
[a]Centre for Environment, Aquaculture and Fisheries Science (Cefas), Barrack Road, Weymouth, Dorset, UK; [b]Department of Life Sciences, The Natural History Museum, Cromwell Road, London, UK.

E-mail of the corresponding Author: david.bass@cefas.co.uk

Studies of animal and plant microbiomes are burgeoning, but the majority of these focus on bacteria and rarely include micro-eukaryotes other than fungi. However, there is growing evidence that micro-eukaryotes living on and in larger organisms (e.g. plants, animals, macroalgae) are diverse and in many cases abundant. While many groups of micro-eukaryotic parasites are recognised, myriad other micro-eukaryotes are associated with hosts as previously unknown parasites (often genetically divergent so difficult to amplify using standard PCR primers), opportunistic parasites, and other ecto- and endo-symbionts. These fulfill a wide range of roles from pathogenesis to mutually beneficial symbioses, but mostly their roles are unknown and likely fall somewhere along this spectrum, although with the potential to switch the nature of their interactions with the host under different conditions. This talk will review the current state of play in 'eukaryome' studies, present some case studies from aquatic invertebrates and macroalgae, and from fish and crustacean pond aquaculture systems in Bangladesh and south east Asia.
The composition and dynamics of host-associated microbial communities are increasingly recognized as important moderators of host health. This 'pathobiome' approach to understanding disease is beginning to supercede a one-pathogen-one-disease paradigm, which cannot sufficiently explain many disease scenarios. The second part of this talk will present the pathobiome concept: the set of host-associated organisms (encompassing eukaryotes, bacteria, and viruses) associated with reduced health status, as a result of interactions between members of that set and the host.

SYMPOSIUM ISOP ADVANCES - Ten years of metabarcoding: what have we learned and how do we move forward? - (by ISoP)

Strengths and Limitations of Metabarcoding for Assessing Protist Communities

LUCIANA SANTOFERRARA[a], GEORGE B. MCMANUS[a]

[a]University of Connecticut, Connecticut, USA.

E-mail of the corresponding Author: luciana.santoferrara@uconn.edu

High-throughput sequencing of targeted genetic markers (metabarcoding) has become nearly routine to analyze protist diversity. Dozens of studies have used this approach to explore diverse environments (water, soil, host-associated), locations (from the shoreline to some of the most extreme sites on Earth), and scales (from local to circumglobal) with high sensitivity and taxonomic resolution. Amid a multitude of exciting findings, scientists have also identified and addressed several technical and biological biases. Results can change markedly based on markers, lab protocols, bioinformatic pipelines, reference databases, etc., but most of these issues are now well known and data quality has progressively improved over the last decade. A review of studies that have evaluated metabarcoding accuracy using mock communities or parallel microscopy shows that method optimization reduces most of the errors that can potentially affect metabarcoding (false negatives, false positives, artifactual sequence variants, misidentifications), but taxon disproportions usually remain a problem. Thus, results on taxon distribution and qualitative community structure are proven solid, while conclusions on taxon prevalence may still be premature. Alternative sequencing strategies, more rigorous protocols (including replicates, controls, internal standards) and new bioinformatic approaches continue to improve metabarcoding as a tool for diversity studies and as a complement to other methods for inference of meaningful phylogenetic and ecological knowledge.

Long Metabarcoding of the Eukaryotic rDNA Operon to Phylogenetically and Taxonomically Resolve Environmental Diversity

MAHWASH JAMY[a], RACHEL FOSTER[b], LUCAS CZECH[c], ALEXEY KOZLOV[c], PIERRE BARBERA[c], ALEXANDROS STAMATAKIS[c,d], DAVID BASS[b,e], FABIEN BURKI[a]

[a]Department of Organismal Biology, Program in Systematic Biology, Uppsala University, Uppsala, Sweden; [b]Department of Life Sciences, Natural History Museum, London, UK; [c]Scientific Computing Group, Heidelberg Institute for Theoretical Studies, Heidelberg, Germany; [d]Institute of Theoretical Informatics, Karlsruhe Institute of Technology, Karlsruhe, Germany; [e]Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset, UK.

E-mail of the corresponding Author: fabien.burki@ebc.uu.se

High-throughput DNA metabarcoding has revolutionized the analysis of microbial diversity, but this approach is generally restricted to amplicon sizes below 500 base pairs. For eukaryotes, the bulk of amplicons currently generated corresponds to short hypervariable regions of the rDNA operon, such as the V4 and V9 regions in the small subunit (18S) gene or the internal transcribed spacer (ITS). These short regions contain only limited phylogenetic information, which makes it impractical to use environmental DNA in full phylogenetic inferences. However, new long-read sequencing technologies such as Pacific Biosciences may provide enough sequence lengths to overcome the poor phylogenetic resolution of short amplicons. To test this idea, we amplified soil DNA and used PacBio Circular Consensus Sequencing to obtain a ~4500 bp region of the eukaryotic rDNA operon spanning most of the 18S and the large subunit (28S). The CCS reads were treated with a novel curation workflow to retain only high-quality clusters, which were combined with available 18S and 28S reference sequences to infer a global phylogeny of eukaryotes. A total of 1019 sequences were included, of which a majority (589 sequences) corresponded to the new long environmental CCS reads. The inferred tree was generally well-resolved, demonstrating the potential of long metabarcoding to produce a robust phylogenetic framework for environmental data. In order to assign taxonomy to the long environmental reads, we also developed a phylogeny-aware approach that showed greater accuracy than available methods using shorter reads. Our results show that long amplicons can be treated in a full phylogenetic framework to provide greater taxonomic resolution and an evolutionary perspective to environmental DNA.

Hypothesis Testing and Inference of Ecological Patterns from Large Marine Metabarcoding Datasets

RAMIRO LOGARES[a]
[a]Department of Marine Biology and Oceanography, Institute of Marine Sciences (ICM)-CSIC, Barcelona, Catalonia, Spain.

E-mail of the corresponding Author: ramiro.logares@icm.csic.es

During the last 10 years, metabarcoding became a standard tool across laboratories investigating protists diversity, ecology and evolution. After an initial "far west" period, characterized by a multitude of coexisting techniques and approaches for analyzing the produced data, metabarcoding has developed into a mature technique. Many studies have used metabarcoding to document and describe the extent of protistan diversity across spatiotemporal scales, being likely one of the first tools that put many leaves in the tree of protistan life. This naturalistic era has paved the road for a new wave of hypothesis driven studies, aiming at uncovering the mechanisms that generate the patterns of protist distributions that we observe. Here, I will present our work into this direction. First, I will present a study where we quantify the relative role of *selection*, *dispersal limitation* and *ecological drift* in structuring pico-plankton communities from the surface global ocean. Then, I will present results of a study where we generate a *seasonality-index* based on null models in order to quantify microbial seasonality, and which was applied to pico- and nano-planktonic protists sampled over ten years. Our analysis of the surface global-ocean pico-plankton indicates that selection, a mechanism typically invoked to explain microbial distributions, is a relevant driver of protist community structure, but more interestingly, they indicate that dispersal limitation may be a key process affecting protist distributions in the surface global-ocean. Furthermore, our results indicate that a minor part of the protist diversity in a community may be seasonal over several years. This agrees with results obtained from association networks, which indicate that a small subset of a protist community may show recurrent associations over time. These temporal analyses raise questions on how ecosystem function is maintained over time and the amount of ecological redundancy in protists communities. I will end by arguing that metabarcoding is one of the best tools for bringing macroecology to the protist world, and that protist macroecological patterns should be analysed in the light of those found for animals, plants and prokaryotes.

Metabarcoding as Basis to Study the Diversity and Ecophysiology of Halophilic Microeukaryotes

SABINE FILKER[a]
[a]University of Technology Kaiserslautern, Molecular Ecology, Germany.

E-mail of the corresponding Author: filker@rhrk.uni-kl.de

The identification of environmental barriers that govern species distribution is a fundamental concern in ecology. Salt was previously identified as a major transition boundary for micro- and macroorganisms alike, selecting for organisms adapted to this environmental stressor and probably also preventing their dispersal. The salinities causing species turnover in protistan communities, however, remained unknown. We, thus, applied an eDNA metabarcoding approach to microeukaryote communities of various aquatic salt habitats from different geographic regions, with salinities from 0.8 % (brackish water) to > 40 % (salt saturation). Partitioning of diversity pointed to a niche differentiation, suggesting distinct salinity classes defining the boundaries for protistan community turnover. Regardless of their geographic origin, protistan communities in these salinity categories displayed different taxonomic memberships and significantly different degrees of community complexity. The results from eDNA metabarcoding provided a basis to study physiological properties that allow some protists to cross these environmental barriers while keeping others confined to a specific salinity class. Therefore, we isolated and cultured halophile ciliates to address this subject in laboratory experiments. Among others, proton nuclear magnetic resonance spectroscopy identified the compatible solute strategy to combat high-salt conditions, enabling transitions of salinity boundaries in the lower to medium salinity range. We observed significant positive correlations of different intracellular compatible solute concentrations and external salt concentrations in the medium. The finding of varying relative proportions of compatible solutes within the ciliates pointed to slight differences in haloadaptive strategies by regulatory action of the ciliates. Based on this as well as transcriptomic data, we were able to infer a time-resolved model for cellular mechanisms to combat changes in salinity. This provides an explanation for the detected eDNA metabarcoding patterns of planktonic protists along salinity gradients.

Oral Session METABARCODING

Seasonality of Benthic and Pelagic Protists in permanently–Mixed Marine Habitats: Analysed through 18S rDNA Metabarcoding

MARIARITA CARACCIOLO[a], NICOLAS HENRY[b], FABIENNE RIGAUT-JALABERT[b], ERIC THIEBAUT[a,b], NATHALIE SIMON[a,b]
[a]Sorbonne Université, Station Biologique de Roscoff, UMR 7144, ECOMAP, Place Georges Teissier, Roscoff, France; [b]Centre National de la Recherche Scientifique CNRS, Station Biologique de Roscoff, UMR 7144, ECOMAP, Place Georges Teissier, Roscoff, France.

E-mail of the corresponding Authors: mariarita.caracciolo@sb-roscoff.fr; nathalie.simon@sb-roscoff.fr

Protists are key players in the marine environment through their roles of primary producers (phytoplankton), and the transfer of organic matter to higher trophic levels (phagotrophic and osmotrophic protists). Some protists also directly regulate the proliferation of other species (parasitic protists). In coastal marine waters, protists that thrive in the water column include truly pelagic species but also organisms with a bentho-pelagic life cycle or truly benthic species that are resuspended in the water column by currents and water mixing. In this study, we investigated the seasonality of protists groups in the 3 categories using a metabarcoding approach (18S rDNA V4 Illumina sequencing), at the SOMLIT-Astan time-series station (Roscoff, Western English Channel). This site is particularly suitable because of the absence of stratification in the water column that reinforces the coupling between pelagic and benthic organisms. The participation of benthic protists leads in turn to an interannual variability in the biomass and the diversity. Our results show (1) that protists with benthic affinities are important players of the coastal microbial eukaryotic community, (2) that these protists show recurrent seasonal successions and (3) that the interaction with benthic communities also affect the dynamics of the truly planktonic protists. We suspect that benthic-pelagic coupling processes are important drivers of the seasonality for the whole community and for the timing and amplitude of the bloom of phytoplankton in permanently-mixed, temperate, near-shore habitats.

Comparative Analyses of the V4 and V9 Regions of 18S rDNA for Assessment of Eukaryotic Diversity Estimated from Field Surveys Using the Illumina Platform

JAEHO CHOI[a], JONG SOO PARK[a]
[a]Department of Oceanography, School of Earth System Sciences, Kyungpook National University, Daegu, Republic of Korea.

E-mail of the corresponding Author: jongsoopark@knu.ac.kr

Illumina sequencing is a representative tool for understanding the massive diversity of microbial eukaryotes in natural ecosystems. The V4 and V9 regions of 18S rDNA are usually used to access the diversity of microbial eukaryotes. However, this culture-independent tool is rarely applied for eukaryotic community in field samples of brackish water. Here, we investigate eukaryotic community in a saline pond (2-4‰ salinity) on Dokdo Island, Korea, using Illumina sequencing with primer sets of the V4 and V9 regions of 18S rDNA from August, 2016 to June, 2018. As previous studies have implied, the number of extant OTUs (Operational Taxonomic Units) from the V9 primer set are much higher than that from the V4 primer set. Total 1,413 OTUs and 915 OTUs are detected using the V9 and V4 primer set, respectively. Interestingly, taxonomic analyses of these OTUs at the class level reveal that some interesting groups (e.g. Karyorelictea in Alveolata, Stygamoebida in Amoebozoa, Trebouxiophyceae in Archaeplastida, Cyathomonadacea in Cryptista, Metromonadea in Rhizaria, Ochrophyta in Stramenopiles, and Heterolobosea in Excavata) fail to describe their diversity using V4 primer set, although those diversities are represented using V9 primer set. This result suggests that the diversity of eukaryotic community can be substantially varied depending on the choice of primers. Further, the molecular phylogenetic trees of the V4 region was more robust than those of the V9 region. Therefore, the Illumina sequencing data from the V9 region may be advantageous for estimating a richness of eukaryotic community, while the sequencing data from the V4 region may be suitable for understanding the molecular phylogenetic relationships in field samples.

ILLUMINAting the Deep Sea - Patterns of Protist Diversity in Abyssal Depths

MANON HOHLFELD[a], ALEXANDRA SCHOENLE[a], KAROLINE HERMANNS[a], HARTMUT ARNDT[a]
[a]Institute of Zoology, Department of Biology, University of Cologne, Germany.

E-mail of the corresponding Author: Manon.Hohlfeld@uni-koeln.de

The deep-sea floor represents the largest benthic habitat on earth. In the past, it was assumed to be a contiguous and desert-like habitat with relatively constant environmental conditions and lacking physical barriers. This led to the assumption that species have vast distribution ranges. Nowadays, it is known that deep-sea ecosystems are extremely heterogeneous at all spatial scales and frequently characterised by sudden changes. Bathymetric features such as mid-ocean ridges and fracture zones form a highly complex landscape. Canyons, seamounts, deep-water coral reefs, pockmarks or faults shape the habitat at a local scale. However, there is still a great lack of knowledge concerning patterns of species diversity and distribution in this vast environment. In this study, we analysed protist communities of three abyssal basins in the southern North Atlantic Ocean and the Caribbean Sea by using Illumina sequencing of the V9 SSU rDNA. The results of our metabarcoding approach revealed differences in the protist community composition of sediment samples taken from different cores of the same Multi-Corer deployment, being separated by less than 1m, what indicates the existence of patterns of protist diversity at a local spatial scale. Comparisons of the three sampled deep-sea basins revealed differences in the protist community structure, showing large-scale patterns of protist diversity which might be shaped by environmental gradients. A high number of reads had no close representatives in the reference database, suggesting a potentially great number of so far molecularly undescribed species.

Integrative Framework to Handle Metabarcoding Data: a Guideline Illustrated by Planktonic Foraminifera.

RAPHAËL MORARD[a], MICHAL KUCERA[a]
[a]MARUM Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany.

E-mail of the corresponding Author: rmorard@marum.de

Since the advent of high-throughput metabarcoding surveys, the planktonic realm is seen as a treasure trove of diversity, inhabited by a small number of abundant taxa, and a hugely diverse and taxonomically uncharacterized consortium of rare species. These results replicated across nearly all protists groups suggest that classical taxonomy has missed the vast majority of the diversity in surface oceanic waters, questioning the validity of morphologically based taxonomic concepts. We used planktonic foraminifera, a group of calcifying protists for which morphological taxonomy is resolved and limited as a study case to test whether the view produced by the metabarcoding approach is genuine or partly produced by technical or conceptual limitations. We developed a curated reference database of ~7000 planktonic foraminifera sequences produced from single-cell specimens and associated with a molecular taxonomy system aiming at parsing the genetic variability existing below the morphological species concept (e. g. Cryptic diversity, Intragenomic variability). We used the reference database to interpret a metabarcoding dataset generated from samples collected during the TARA Ocean expedition. The resulting diversity assessment with the metabarcoding dataset indicates that the diversity of planktonic foraminifera is modest and finite, which is congruent with the morphologically based observation and excludes the existence of a large consortium of rare taxa within this group. Our results illustrate that the correct interpretation of metabarcoding datasets requires robustly curated barcoding databases with appropriate coverage, and that barcoding efforts should continue in pace with the growing body of environmental meta–omics datasets.

Using High Throughput Sequencing DNA Metabarcoding to Assess Diversity Patterns of *Nebela* (Amoebozoa; Arcellinida) Along an Elevation Gradient

QUENTIN BLANDENIER[a,b], DAVID SINGER[a,c], RAPHAEL JAUSLIN[a], CLÉMENT DUCKERT[a], AMANDINE PILLONEL[a], MOLLY BAUR[a], EDWARD A.D MITCHELL[a,d], ENRIQUE LARA[b]

[a]Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland; [b]Real Jardín Botánico, CSIC, Madrid, Spain; [c]Department of Zoology, Institute of Biosciences, University of São Paulo, Brazil; [d]Jardin Botanique de Neuchâtel, Neuchâtel, Switzerland.

E-mail of the corresponding Authors: quentin.blandenier@unine.ch; david.singer.bio@outlook.com; raphael.jauslin@unine.ch; clement.duckert@unine.ch; amandine.pillonel@unine.ch; molly.baur@unine.ch; edward.mitchell@unine.ch; enrique.lara@rjb.csic.es

Protists are useful model organisms to test macroecological questions such as how climate contributes in shaping biotic communities. However, the acquisition of the relevant response data was historically time-consuming and expensive. The application of molecular approaches such as High Throughput Sequencing DNA metabarcoding now allows the retrieval of large amounts of genetic data in a short time at reasonable costs.

The study of diversity patterns along elevation gradients is a classical approach in ecology, but there are only limited data for soil protists. Our aim was to test to which extent climatic (elevation) or soil variables (moss pH and total N content) explained the community composition of soil protists. We collected *Sphagnum* mosses from 12 Swiss peatlands along a 1200m elevation gradient from 600 to 1800 m a.s.l. and analysed the diversity of genus *Nebela* (Amoebozoa; Arcellinida), which comprises at least eight similar-looking species with contrasted ecological preferences. We sequenced the mitochondrial Cytochrome Oxidase subunit I (COI) of genus *Nebela* by Illumina MiSeq.

The analysis of >25 million sequences reads revealed 14 phylotypes corresponding to six described species and eight unknown phylotypes (UP) corresponding to potential new species. Three species largely dominated the communities, together accounting for 94% of all sequences: *N. rotunda* (45%), *N. gimlii* (30%) and *N. collaris* (19%), whereas five UP appeared in low numbers (i.e. each < 0.1% of the community).

This study confirms that DNA metabarcoding targeting specific taxonomic groups is effective to study the diversity patterns of soil protists, thus making it possible to compare patterns between micro- and macro-organisms and assess the universality of macro-ecological theories.

Surveying Microbial Eukaryote Diversity in Phytotelmata Habitats

ROBIN S. SLEITH[a], JEAN-DAVID GRATTEPANCHE[a,b], LAURA A. KATZ[a,c]
[a]Smith College, Department of Biological Science, Northampton, Massachusetts, USA; [b]Temple University, Department of Biology, Philadelphia, Pennsylvania, USA; [c]University of Massachusetts Amherst, Program in Organismic and Evolutionary Biology, Amherst, Massachusetts, USA.

E-mail of the corresponding Author: rsleith@smith.edu

Microbial eukaryotes represent the majority of eukaryotic diversity, encompassing a spectrum of microorganisms such as fungi, protists, and algae. These eukaryotic microbes are important for ecosystem functioning, essential for monitoring and predicting environmental change, and also excellent models for understanding biological interactions and evolutionary history. However, many lineages of microbial eukaryotes remain understudied, as most are uncultivable. Recent advances in genomic techniques have allowed previously uncultivable, and therefore under-sampled, lineages to be explored. In this study we survey microbial eukaryote diversity in the freshwater habitats of pitcher plant and bromeliad phytotelmata. We focus on diversity within the SAR clade (Stramenopiles, Alveolates, Rhizaria) which includes many photosynthetic algae (e.g., diatoms, dinoflagellates, brown algae), parasites (e.g., Oomycetes), heterotrophic organisms (e.g., ciliates, Cercozoa) and many other uncultivable lineages. By using specific primers designed to amplify the 18S (SSU) rRNA gene within these groups, we describe a method that allows characterization of community diversity across these lineages. Phytotelmata were sampled from natural and built environments allowing the factors that structure microbial communities to be examined. Our analyses indicate that bromeliad communities were dominated by Alveolate taxa, specifically ciliates, while pitcher plant communities had more representation from Rhizarian taxa.

EukBank: a Community Resource to Explore the Earth Eukaryotic Diversity

CÉDRIC BERNEY[a], FRÉDÉRIC MAHÉ[b], NICOLAS HENRY[a], JEENA RAJAN[c], LUCAS CZECH[d], PIERRE BARBERA[d], ALEXANDROS STAMATAKIS[d], GUY COCHRANE[c], COLOMBAN DE VARGAS[a]

[a]Station Biologique de Roscoff, Sorbonne Université and CNRS, France; [b]CIRAD, Montpellier, France; [c]European Nucleotide Archive, EMBL-EBI, UK; [d]Heidelberg Institute of Theoretical Studies, Germany.

E-mail of the corresponding Author: cedric.berney@sb-roscoff.fr

Recent global efforts to explore the total diversity of life in planetary biomes, from viruses to animals and plants, are pointing at microbial eukaryotes (protists) as the potentially most diverse and complex life compartment on the planet, in terms of gene repertoire, genome and cell structures, and taxonomy. Protists may well be the main makers of the microbial dark matter, and play pivotal roles in the complexification, structuration, and resilience of extent ecosystems. Assessing the global diversity of protists therefore represents the first-order knowledge that we need to acquire before selecting key lineages for deeper biological, ecological, and evolutionary studies. As the protistology community

large public genetic datasets addressing their diversity can be difficult to access, and their analysis is hindered by the lack of a universal taxonomy unifying reference gene databases.

As part of UniEuk (www.unieuk.org), the international initiative to build a universal taxonomic framework for eukaryotes, we developed EukBank, a community resource hosted at EMBL-EBI, aiming at sharing, unifying, and exploring high-throughput eukaryotic DNA metabarcoding datasets under the UniEuk taxonomic framework. We started with the V4 18S rDNA marker, and the primary version of EukBank (involving 190 data contributors) contains over 150 submitted datasets. This represents about 6 billion raw DNA reads (more than three times the size of the recently published Earth Microbiome Project) from over 13,000 samples collected worldwide from marine abyssal plains, bathy- to epi-pelagic plankton, coastal sediments and water column, fresh water rivers, lakes, sewage, and soils and forests. Preliminary analyses of EukBank V1.0 demonstrate the potential of the workflow to assess eukaryotic diversity across biomes (richness, saturation, functional diversity), and detect novel eukaryotic lineages of ecological and/or phylogenetic relevance. With the implementation of additional marker genes, EukBank will represent a key resource to unveil and classify total eukaryotic diversity in the next decades.

UniEuk (universal taxonomic framework for eukaryotes): Advances and Future

CÉDRIC BERNEY[a], CHRISTIAN QUAST[b], MAKSYM PYATYGORSKYY[b], JEENA RAJAN[c], MARK HOEBEKE[a], DAVID BASS[d], FRANK OLIVER GLÖCKNER[b], GUY COCHRANE[c], COLOMBAN DE VARGAS[a]

[a]Station Biologique de Roscoff, Sorbonne Université and CNRS, France; [b]Max Plank Institute and Jacobs University, Bremen, Germany; [c]European Nucleotide Archive, EMBL-EBI, UK; [d]CEFAS, Weymouth, UK.

E-mail of the corresponding Author: cedric.berney@sb-roscoff.fr

UniEuk (www.unieuk.org) is an open, community-based and expert-driven international initiative to build a flexible, adaptive universal taxonomic framework for eukaryotes, focused primarily on protists, and implemented into the European Nucleotide Archive at EMBL-EBI. Launched in May 2016, the project aimed at becoming an indispensable community hub to centralize, safeguard and promote our current global knowledge on eukaryotic diversity and evolution, integrating morphology and ecology with key molecular information. It was carefully designed around three complementary modules maximizing direct community input: (1) EukRef, a standardized, open-source bioinformatics pipeline that allows taxonomic curation of publicly available 18S rDNA sequences, generating homogeneous sets of aligned sequences and phylogenetic trees; (2) EukBank, a public repository of high-throughput metabarcoding datasets that allows monitoring of total eukaryotic diversity (e.g. saturation, phylogeny) across biomes, and identification of ecologically relevant new lineages; and (3) EukMap, a user-friendly representation of the taxonomic framework in the form of a publicly navigable tree, fully editable by registered users, where each node/taxon is associated with standardized features (name, contextual data, links to pictures and literature, etc.).

The 2019 ECOP/ISOP meeting will mark the 4th anniversary of the first public announcement of UniEuk to the protistology research community. As we reach the end of the implementation phase of the project, we will report on the project's advances and the successful deployment of all modules. Throughout the meeting, the UniEuk coordinator and several team members will be available to answer your questions, and to provide live demonstrations of EukMap. A key aspect of the project's philosophy was to build a system with a very high potential for long-term self-sustainability via integration of the key components into permanent research structures, and a minimal community input requirement in the long run. We will present in detail our vision to achieve this self-sustainability within a few years, and how the three UniEuk modules will significantly contribute to promoting protistology research in the next decades.

Oral Session RESPONSE TO ENVIRONMENTAL STRESS

Different Sodium Tolerance of Functional Membrane Pyrophosphatase Orthologs in Freshwater and Saltwater Microalgae

JUAN M. MADROÑAL[a], AGUSTÍN HERNÁNDEZ[b], JOSÉ R. PÉREZ CASTIÑEIRA[a], AURELIO SERRANO[a]
[a]Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Seville, Spain, [b]Departamento de Parasitologia, ICB-Universidade de Sao Paulo, Sao Paulo, Brazil.

E-mail of the corresponding Author: aurelio@ibvf.csic.es

Ion-translocating pyrophosphatases (mPPases) are integral membrane proteins that use the chemical energy of inorganic pyrophosphate (PPi) for the vectorial transport of ions (H^+ or Na^+) and subsequent generation of electrochemical gradients. Nuclear genomes of plants and many microorganisms (prokaryotes and protists) encode distinct types of m-PPase paralogs, namely K^+-dependent H^+(or Na^+)-PPases and K^+-independent H^+-PPases. Although this functional diversity has been known for years, its physiological significance in connection with the apparent genetic redundancy of mPPases found in many organisms has not been clarified yet; besides, Na^+-PPases have so far only been described and characterized in prokaryotes. A comparative biochemical study on mPPases of total cell membranes from a number of freshwater and saltwater microalgae of diverse phylogenetic groups (Chlorophyceae, Bacillariophyceae, Eustigmatophyceae, Prasinophyceae, Rhodophyceae) revealed that: 1) although all analyzed microalgae exhibited substantial levels of mPPase activity and 70-kDa protein, enzyme kinetic data suggested a clade-specific prevalent expression of different mPPase types; 2) concomitant changes of mPPase activity and protein levels were observed in some species in response to stressing environmental factors (e.g. increased NaCl concentration); and 3) while mPPase activity of saltwater microalgae was *in vitro* unaffected or even activated by Na^+ and showed group-associated differences in K^+-dependency, the K^+-dependent H^+-PPase found so far in freshwater microalgae was severely inhibited by Na^+ in a non-competitive manner. Overall, the results strongly suggest a functional prevalence in saltwater microalgae of Na^+-tolerant mPPases –either Na^+-PPase (Rhodophyceae, Prasinophyceae) or K^+-independent H^+-PPase (Eustigmatophyceae, Cyanidiophyceae)– over their K^+-dependent H^+-PPase paralogs. This scenario is different from that of higher plants and freshwater microalgae in which the main functional mPPase is unvaryingly a Na^+-sensitive K^+-dependent H^+-PPase. We propose that genetic redundancy should allow functional prevalence of mPPase paralogs that would eventually facilitate microalgae ecological plasticity. Thus, these simple ionic pumps may be involved in a metabolic adaptive strategy to enhance salt tolerance which could have biotechnological applications for the design of more salt-tolerant crops.

Immune Impacts of *Cryptosporidium parvum* within a Non-Target Model, *Dreissena polymorpha*.

MAXIME LEPRÊTRE[a,b], ARNAUD SALVADOR[b], CHRISTINE ALMUNIA[c], JEAN ARMENGAUD[c], ALAIN GEFFARD[a], MÉLISSA PALOS-LADEIRO[a]

[a]University of Reims Champagne-Ardenne, UMR-I 02 INERIS-URCA-ULH SEBIO "Environmental Stress and Biomonitoring of Aquatic Environments", UFR Sciences Exactes et Naturelles, Campus du Moulin de la Housse, Reims, France; [b]University of Lyon, University of Claude Bernard Lyon 1, CNRS UMR 5280 Institute of Analytical Sciences (ISA), Villeurbanne, France; [c]CEA-Marcoule, DRF/IBITEC-S/SPI/Li2D, Laboratory "Innovative technologies for Detection and Diagnostics", Bagnols-sur-Cèze, France.

E-mail of the corresponding Author: maxime.lepretre@univ-reims.fr

While most ecotoxicological studies focus on the impact of chemical contamination on aquatic organisms, few studies assess the impact of biological contamination, such as protozoan parasites. Among contaminations of freshwater, the protozoa *Cryptosporidium sp*, can be introduced in aquatic ecosystems through effluents from waste-water and rain-waters from urban, suburban, and agricultural areas. To date, several studies have highlighted the use of bivalves, such as the zebra mussel *Dreissena polymorpha*, to reveal water contamination by protozoa. Previous studies have shown that *C. parvum* were accumulated in the hemolymph of *D. polymorpha* and phagocyted by hemocytes, the immune cells of the zebra mussel. However, the interactions between *C. parvum* and the immune system of *D. polymorpha* are still not investigated at the molecular level. In this context, this study aims to identify the protein immune responses of *D. polymorpha* challenged with *C. parvum*. For this purpose, a comparative proteomics approach was carried out on hemocytes and plasma from hemolymph non-exposed and exposed during 4 hours to *C. parvum* (5 protozoa per hemocyte) in *ex vivo* conditions. Results revealed the immune proteins modulated under protozoan exposure and highlighted immune mechanisms involved in defence against *C. parvum*. Based on this experiment, we choose to follow proteins involved in recognition, internalisation and destruction of *C. parvum* during *in vivo* challenges of zebra mussels. Five *C. parvum* per hemocyte were injected in the adductor muscle of zebra mussels. Then sampling of hemolymph were performed at 4, 8 and 24 hours post challenge. Proteins of interest were quantified by targeted mass spectrometry in hemocytes and plasma in order to monitor the dynamic of immune proteins during protozoan infection. Results indicate that infection with *C. parvum* generate an active immune response in the hemolymph of *D. polymorpha* since several proteins involved in immune signalling, phagocytosis, apoptosis, recognition and destruction of micro-organisms were modulated during both *ex vivo* and *in vivo* challenges. This study brings a better understanding of the interaction between protozoan and a non-target bivalves and emphasize the utmost importance to consider protozoan contamination of freshwater ecosystems for a better environmental and sanitary risk management.

Localization of Nutrient Transporters in Native and Ecdysing Cells of the Dinoflagellate *Prorocentrum minimum*

VERA KALININA[a], OLGA MATANTSEVA[a]
[a]Institute of Cytology Russian Academy of Sciences, St. Petersburg, Russia.

E-mail of the corresponding Authors: verakamakalinina@gmail.com; olga.matantseva@gmail.com

Dinoflagellates possess a complex cell covering called amphiesma. It consists of a continuous plasma membrane (PM), and amphiesmal vesicles localized beneath PM. In armored dinoflagellates amphiesmal vesicles contain cellulosic thecal plates. Many dinoflagellates are able to shed their cell covering. This process, called ecdysis, may occur as a stage of the life cycle or be induced by stress. During ecdysis a cell sheds its PM, thecal plates and the outer amphiesmal vesicle membrane. In this way, the inner amphiesmal vesicle membrane becomes the new PM.
Prorocentrum minimum is an armored dinoflagellate representing a good model to study ecdysis. It is able to shed its theca in response to mechanical stress, such as centrifugation. Obviously, the protein composition of the inner amphiesmal vesicle membrane is different from that of PM. In this study, we explored localization of nitrate and urea transporters within *P. minimum* amphiesma and followed their expression in the new PM - during and after ecdysis.
We obtained polyclonal antibodies against nitrate transporter NRT2.1 and urea transporter DUR3 that play a significant role in N metabolism of *P. minimum* (Matantseva et al., 2016). It was shown that both nitrate and urea transporters are localized in the PM. Confocal microscopy revealed that the periflagellar area has more bright labeling with the antibodies against NRT2.1, than the rest of PM, suggesting that this region could play an important role in dinoflagellate nutrition.
Ecdysis was induced by centrifugation at 3000g. After 0, 2, 4 and 6 hours the treated cells were fixed in 2% paraformaldehyde, stained with anti-NRT2.1 antibodies and analyzed by confocal microscopy. The results indicated that in 1-2 hours after centrifugation *P. minimum* started to actively synthetize membrane protein NRT2.1 and the cytoplasm became full of NRT2.1-containing vesicles. After 4 hours, the cells left their old thecal plates. At that time point, the cells did not contain significant amount of cellulose but their PM were already full of NRT2.1 transporters, suggesting that the protein composition of the new PM was formed. After 6 hours, the new amphiesma including the newly synthesized thecal plates developed.
This research was funded by the Russian Science Foundation, project 18-74-10093.

Transcriptome Analysis of Light Stress Condition in *Paulinella micropora,* an Amoeba with Nascent Photosynthetic Organelles

DUCKHYUN LHEE[a], KHAOULA ETTAHI[a], DEBASHISH BHATTACHARYA[b], HWAN SU YOON[a]

[a]Department of Biological Science, Sungkyunkwan University, Suwon, Republic of Korea; [b]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ, USA.

E-mail of the corresponding Author: hsyoon2011@skku.edu

In photosynthetic organisms, the production of reactive oxygen species is stimulated under high irradiance. For this reason, photosynthetic organisms have evolved photoprotective mechanisms and light can have a dramatic impact on gene expression and plastid physiology. The amoeba *Paulinella chromatophora*, which has independently gained plastids from alpha-cyanobacterium relative recently (90-140 Mya), also developed light-dependent regulatory response. Previous study using quantitative PCR showed that some of the EGT-derived nuclear high light inducible genes regained light regulation. Moreover, photosynthesis-related genes on plastid genome appear to exhibit minimal transcriptional regulation in response to changes in light intensity, implying that host cell has taken control over organelle processes.

In this study, the transcriptome analysis of *Paulinella micropora*, which is sister species to *P. chromatophora*, were performed. Seven-time points were sampled for both normal light and high-light stress conditions with each alteration of light/dark cycle. Sequencing results provide a significant change in gene expression between control versus high-light condition from each time points. Compared to photoprotective mechanisms of Archaeplastida lineages, how photosynthetic *Paulinella* deal with photo-oxidative damage is investigated in this study.

Sex Enhances Survival in *Paramecium*

AMARINDER SINGH THIND[a], VALERIO VITALI[b], MARIO R. GUARRACINO[a], FRANCESCO CATANIA[b]
[a]LabGTP (Laboratory of Genomics, Transcriptomics and Proteomics), Institute for High Performance Computing and Networking (ICAR), National Research Council (CNR), Naples, Italy; [b]Institute for Evolution and Biodiversity, University of Münster, Münster, Germany.

E-mail of the corresponding Author: francesco.catania@uni-muenster.de

Sex is commonly thought to be preserved in nature because it may create adaptive genetic variation. Here we ask a simple question: can sex enhance fitness regardless of its concomitant effect on genetic diversity? The ciliate *Paramecium* is a good model to address this question. *Paramecium* can generate fully homozygous individuals reproducing sexually via self-fertilization. Leveraging a combination of time-course transcriptomic data and phenotypic assays, we show that self-fertilizing *Paramecium* cells as well as cells that have recently become fully competent for self-fertilization are more likely to withstand a harsh heat shock. This survival advantage is coupled with distinct physiological changes. Our observations suggest that the molecular mechanisms that control self-fertilization in *Paramecium* can increase survival in stressful environmental conditions. They provide an initial framework for linking sexual reproduction to the process of adaptation in the absence of genetic variation.

Thermo-plasticity of Programmed DNA Elimination in *Paramecium tetraurelia*

VALERIO VITALI[a], REBECCA HAGEN[a], FRANCESCO CATANIA[a]
[a]Institute for Evolution and Biodiversity, University of Münster, Münster, Germany.

E-mail of the corresponding Author: fcata_01@uni-muenster.de

Background: Functional nuclear differentiation in paramecium requires the precise and reproducible elimination from the germline dna of thousands of intergenic and gene-interrupting sequences known as Internal Eliminated Sequences (IESs). This impressive feat of genome remodeling is accomplished via Programmed DNA Elimination (PDE), a natural small-RNA guided genome editing evolved to safeguard the integrity of the somatic genome in the face of transposon proliferation. Rationale: Remarkably, alternative genome rearrangement can lead to phenotypic differentiation in otherwise genetically identical cells (identical germline DNA). Exploring the environmental sensitivity of PDE could further our understanding of how genetic variability in the somatic nucleus originates and the extent of which this contributes to adaptive processes. Using the model ciliate *Paramecium tetraurelia* we are investigating the impact of the environmental temperature on the efficiency of genome rearrangement, with a special focus on IES elimination. Results: Our data suggests that the efficiency of IES elimination in *P. tetraurelia* is affected by the environmental temperature. Departure from the standard cultivation temperature of 25-27°C leads to elevated rates of incomplete IES excision. A large excess of this somatic IESs is known from previous studies to be under epigenetic control, thus potentially passed on to the sexual offspring despite its somatic origin.

Conclusions: We hypothesize that the environmental modulation of PDE may be a source of adaptive variation. Somatic IES retention could be co-opted to modulate gene expression and/or diversify coding sequences. Experiments are ongoing in our lab to examine whether the plasticity of genome rearrangement can enhance growth under unfavorable environmental conditions.

Coding Content in Complete Mitochondrial Genome of *Pleurostomum flabellatum* and Extreme-Halophiles Codon Preference.

KHAOULA ETTAHI[a], JONG SOO PARK[b], HWAN SU YOON[a]
[a]Department of Biological Sciences, Sungkyunkwan University, Suwon, South Korea; [b]Department of Oceanography, Kyungpook National University, Daegu, South Korea.

E-mail of the corresponding Authors: ettahi.khaoula@gmail.com; hsyoon2011@skku.edu.

Discoba (Excavata) is a clade of highly diverse unicellular eukaryotes that are of key importance for understanding the early evolution of mitochondrial genomes. It includes three major lineages (Jakobida, Euglenozoa and Heterolobosea). Jakobida has the most gene-rich mitochondrial genome studied to date, whereas, Euglenozoa is characterized by harboring a low number of genes in mtDNA along with gene fragmentation. Heterolobosea is the third major lineage of Discoba and disposes of most gene-rich mitochondrial DNAs (mtDNAs) outside of the jakobids. Additionally, Heterolobosea lineage includes extreme-halophiles, such as *Pleurostomum flabellatum* which can grow at more than 300‰ salinity. Formerly, the biochemistry of the mt organelle of *P. flabellatum* was uncertain.
The current study seeks at assembling the first and complete mtDNA of *P. flabellatum*. *P. flabellatum* mtDNA was assembled in one circular molecule (~57.8 kb) containing 66 genes including 45 putative protein-coding genes, 19 tRNAs and 2 rRNAs. The overall G+C content of the genome is 29%.
A phylogenomic analysis on mtDNA sequences of 14 conserved mitochondrial-encoded proteins reconstructed from 13 mitochondrial genomes, revealed that *P. flabellatum* forms a monophyletic clade with other Heterolobosea taxa with high-resolution value and *P. flabellatum* is most related to *Naegleria* spp. Furthermore, overall mt genome synteny was conserved between *P. flabellaum* and *Naegleria* spp.
Codon preference of an organism from extremophilic habitat differs from normal habitat one. The analysis of extreme-halophiles mtDNA showed different codon usage pattern and selectivity from non-halophiles where CUC codon had highest preference. Generally, extreme-halophiles preferred codons contain G/C-base with high preference of G/C-ending (80%).

SYMPOSIUM - All Roads Lead to Rome: Comparing Molecular and Cellular Paths to Eukaryotic Multicellularity - (by ISoP)

Evolution of Multicellularity in the Amoebozoan Lineage

PAULINE SCHAAP[a]

[a]College of Life Sciences, University of Dundee, Dundee, UK.

E-mail of the corresponding Author: p.schaap@dundee.ac.uk

The macroscopic world is dominated by life forms such as animals, plants and fungi that arise through repeated divisions from a cellular spore or zygote. However, in both prokaryotes and eukaryotes, multicellularity evolved many times by aggregation and colony formation of single cells. Currently, aggregative multicellularity has reached the highest level of complexity in the Dictyostelia, members of the eukaryote supergroup Amoebozoa. Up to a million amoebas can move together to form a multicellular organism, that displays light-oriented migration and construction of a spore-bearing fruiting structure with up to 5 different cell types. I will present an overview of the signaling mechanisms that are used by the model system *Dictyostelium discoideum* to build its fruiting structures and summarize comparative studies describing how these mechanisms evolved from a signaling pathway that mediates stress-induced encystation in solitary Amoebozoa. Finally, I will present recent data on the selective advantages of stress survival by sporulation in fruiting bodies over unicellular encystation, which indicate that an adaptation to colder habitats could have been the ultimate cause of Dictyostelid multicellularity.

Comparing Paths to Multicellularity in Amoeboid Protists

ALEXANDER K. TICE[a,b] , MATTHEW W. BROWN[a,b]

[a]Department of Biological Sciences, Mississippi State University, MS, USA; [b]Institute for Genomics, Biocomputing, & Biotechnology, Mississippi State University, MS, USA.

E-mail of the corresponding Author: at1422@msstate.edu

The evolutionary innovations of multicellularity have occurred many times on the eukaryotic tree of life. In Amoebozoa, two distinct, distantly related, lineages contain socially multicellular organisms, the well-studied dictyostelids and the lesser-known copromyxids. *Copromyxa protea* is a dung inhabiting sorocarpic amoeba that forms simple but macroscopic fruiting structures composed of a single cell type. The formation of sorocarps is induced by one or a few founding amoebae, which by an unknown mechanism entice nearby trophozoites to crawl upon them and subsequently encyst causing apical growth of the sorocarp. Using time-lapse microscopy as well as transcriptomic methods, we are now able to begin to unlock the developmental program in this social slime mold. In this study, we sampled mRNA from 3 distinct life stages with 3 replicates of each stage. We identified ~2,200 differentially expressed transcripts across the 3 sampled life stages using differential expression profiling techniques. Of these transcripts, ~100 represent transcripts unique to early sorocarpic formation and ~200 are uniquely expressed in the apical tips of maturing sorocarps. Further, a large set of transcripts is up regulated in both the early sorocarp and maturing sorocarp samples as would be expected given the life cycle of *C. protea.* Gene ontology enrichment analyses show a significant up regulation of transcripts associated with signal transduction, cell adhesion and cell surface activity in this set of shared transcripts between early sorocarps and sorocarp tip stages. Protein family domains associated with transcription factors and adhesion proteins are also significantly enriched in these developing multicellular stages. We also demonstrate the independent nature of copromixid multicellularity from dictyostelid multicellularity through analysis of expression level differences in one to one orthologs across developmental time in these two organisms.

The Evolutionary Origin of Animal Contractile Cells

THIBAUT BRUNET[a], TESS A. LINDEN[a], BEN T. LARSON[a], NICOLE KING[a]

[a]Molecular and Cell Biology Department, University of California Berkeley/HHMI, Berkeley, CA, USA.

E-mail of the corresponding Authors: t.brunet@berkeley.edu; nicoleking.ucb@gmail.com

Contractile cell types are universally present in animals and fundamental to animal life. Contractions of individual, scattered cells underlies amoeboid cell movement, as found e.g. in immune cells (in adult organisms) and individually migrating embryonic cells (during development), while tissue-scale collective cell contractility underlies both embryonic morphogenesis and adult motricity. However, the origin of animal contractile cell types remains obscure. As the sister-group of animals, choanoflagellates hold the promise of illuminating the evolutionary origins of animal cell biology. Intriguingly, choanoflagellate genomes encode an extensive complement of homologs to animal contractility genes, suggesting the involvement of (yet unidentified) contractile processes in their life history. Here, I report on the recent discovery of both individual and collective cell contractility in choanoflagellates. Under confinement, the model choanoflagellate *Salpingoeca rosetta* rapidly undergoes a phenotypic switch from a flagellate to an amoeboid cell phenotype that resemble animal migratory cells in both structure and function. This dramatically extends the known phenotypic repertoire of choanoflagellates and suggests an ancient origin for animal crawling cells, in line with the temporal-to-spatial transition hypothesis for the origin of animal cell types. Finally, collective cell contractility has also been recently discovered in a newly discovered colonial choanoflagellate isolated from a Carribean island, that undergoes rapid and reversible whole-colony inversion in response to external photic and mechanical stimuli.

Not All Follow the Same Road: Fungi Took a Unique Evolutionary Route to Multicellularity

LASZLO G. NAGY[a]

[a]Synthetic and Systems Biology Unit, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary.

E-mail of the corresponding Author: lnagy@fungenomelab.com

Fungi evolved complex multicellularity in two steps and comprise a variety of forms that organize into hierarchically nested complexity levels. The most widespread form is hyphae, characteristic of filamentous fungi. Hyphae are extensively modified in various groups; for example they can aggregate to form compact, 3-dimensional fruiting bodies in several independent clades, which are similar to multicellular animals and plants in complexity level. Losses or reductions in hyphal growth ability occurred in multiple lineages, giving rise to secondarily unicellular yeasts and yeast-like fungi. These morphogenetic transitions show extensive convergence in fungi and picture a shared, but unknown morphogenetic program. The emergence of both hyphae and fruiting bodies correlate with surprisingly little gene family expansion, rather, gene co-option and exaptation seem to be important sources of multicellularity-related genes. Classic culprits of multicellularity (adhesive proteins, kinases, transcription factors) only partially correlate with transitions in complexity level, suggesting as yet unknown mechanisms involved in multicellular growth in fungi. This two-step process fits neither the aggregative nor colonial ways of the evolution of multicellularity which, combined with its genetic background, suggests that fungi took a unique route to multicellularity.

Oral Session PARASITISM 2

In silico Analysis of Potential Vaccine Candidates for *Tritrichomonas foetus*, the Causative Agent of Bovine Trichomoniasis.

ELEANOR SENIOR[a] and ANDREW JACKSON[a]

[a]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK.

E-mail of the corresponding Author: senior@liverpool.ac.uk

Tritrichomonas foetus is an anaerobic flagellated protist and the causative agent of the venereal disease bovine trichomoniasis. This disease causes spontaneous abortions and, in some cases, infertility in cows and is responsible for decreased calving rates and milk production; infected animals are usually culled. Bovine trichomoniasis is therefore responsible for significant economic losses to farmers in several countries where the disease is endemic, including Australia, Brazil and the USA. Currently there is no vaccine available that can prevent reinfection.

In order to identify potential vaccine candidates for this parasite a reverse vaccinology approach was implemented. The *Tritrichomonas foetus* genome was sequenced on the PacBio platform (147Mb, N50 = 84,706), assembled using SMRTportal and then annotated using multiple automated processes including BRAKER, SNAP and BLAST2GO, integrated with transcriptomic data from both trophozoite and pseudocyst cell types, and improved through manual curation. Cell surface specific genes were identified using in silico prediction of signal peptides, transmembrane domains and GPI anchors.

In our *T. foetus* genome 84,706 genes have been identified, 1,607 of which contain a signal peptide and a transmembrane helix suggesting they are cell surface expressed and will be further examined as potential epitopes.

We have produced the first fully-annotated *T. foetus* genome as the first step in a reverse vaccinology approach to this important livestock disease. Preliminary analysis of predicted cell surface proteins has resolved diverse transmembrane proteins as potential vaccine candidates.

A Novel Parasite-Secreted Lysine and Glutamic Acid Rich Protein 2 (KERP2) from *Entamoeba histolytica* Infiltrates the Host Nucleus and Regulates the Host-Parasite Relationship.

KOUSHIK DAS[a], TOMOYOSHI NOZAKI[a]
[a]Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

E-mail of the corresponding Author: nozaki@m.u-tokyo.ac.jp

Amoebiasis caused by the protozoan parasite *Entamoeba histolytica* is one of the major enteric diseases in humans. Contact-dependent cytolysis of host cells by *E. histolytica* is an important hallmark of amoebiasis that points out the importance of molecules involved in the interaction between the parasite and the human cells. To decipher the molecular and cellular mechanisms supporting the invasion of the intestinal epithelium by amoebic trophozoites, proteins involved in the interaction of the parasite with human enterocytes *were previously analyzed. Along with some known proteins with previously demonstrated functions (such as* the Gal/GalNAc lectin and α-actinin), *two novel proteins [E. histolytica lysine (K) and glutamic acid (E) rich protein 1 (EhKERP1) and 2 (EhKERP2)] were identified. While the* significance of *Eh*KERP1 in parasite virulence and liver abscess formation have been investigated, functional implication of *Eh*KERP2 in physiology of amoebiasis remains unexplored. Present study has revealed that unlike *Eh*KERP1, which is localized in intra-cellular vesicles and on the plasma membrane of the trophozoites, *Eh*KERP2 is primarily localized in the nucleus. *Eh*KERP2, being a leaderless protein, is secreted by the parasite during interaction with human enterocytes through a novel pathway independent of the classical ER-Golgi anterograde transport. We found that the trophozoites secreted exogenous *Eh*KERP2, which then readily infiltrates both in human enterocytes and adjacent *E. histolytica* trophozoites, and translocates to the nucleus. We also found that internalization of secreted *Eh*KERP2 by host cells is a spontaneous process, independent of energy and endocytic pathways. Exogenously added *Eh*KERP2, after it was internalized and translocated to the nucleus, was biologically active and provided protection to parasites against oxidative stress. Hence, *Eh*KERP2 is a novel parasite-secreted host nucleus-infiltrating protein that may represent a previously unknown regulatory principle governing the host-parasite relationship at the molecular level.

A Lineage-Specific Mitosomal Membrane Protein Possibly Involved in Mitosome-Endosome Membrane Contact Site in *Entamoeba histolytica*

HERBERT J. SANTOS[a,b,c], YUKI HANADATE[b,c], KENICHIRO IMAI[d,e], TOMOYOSHI NOZAKI[a,b,c]

[a]Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [b]Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan; [c]Graduate School of Life and Environmental Sciences, University of Tsukuba, , Ibaraki, Japan; [d]Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan; [e]Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan.

E-mail of the corresponding Authors: hjsantos@m.u-tokyo.ac.jp; nozaki@m-utokyo.ac.jp

Membrane contact sites (MCSs) are regions where two organelles come within a distance of 30 nm, and have been observed between many organelles including the endoplasmic reticulum, endosomes, and mitochondria. However, reports of MCS involving mitochondrion-related organelles (MROs) remain lacking. *Entamoeba histolytica*, the etiological agent of amoebiasis, possesses an MRO called mitosome. We recently discovered several *Entamoeba*-specific transmembrane mitosomal proteins (ETMPs) from in silico and cell biological analyses. One of them is ETMP1, which was predicted to have one transmembrane domain and two coiled-coil regions, and was demonstrated to be integrated to the mitosomal membranes based on carbonate fractionation and immunoelectron microscopy data. ETMP1 forms a 180 kDa complex and immunoprecipitation analysis detected a candidate interacting partner, EH-domain containing protein (EHD1). We expressed epitope-tagged EHD1 in *E. histolytica* and subsequent immunofluorescence and immunoelectron microscopy data demonstrated an unprecedented MCS between the mitosome and the endosome. Live imaging analysis of GFP-EHD1 expressing strain demonstrated that EHD1 is not involved in endocytic uptake of RITC-dextran per se, but is observed in MCS between endosomes of various sizes. *In vitro* assays using recombinant His-EHD1 demonstrated ATPase activity, binding to phosphoinositide phosphates, and tubulation of liposomes. MCSs are involved in lipid transfer, ion homeostasis, organelle inheritance, apoptosis, and have been recently implicated in mitochondrial and endosomal dynamics. The serendipitous discovery of the ETMP1 interacting partner EHD1, led to the observation of mitosome-endosome contact site. It opened a new view of how the relic mitochondria of *Entamoeba* may likewise be involved in organelle crosstalk, a conserved feature of mitochondria and other organelles in general.

Comparative genomic analysis of the Rab GTPases in *Giardia intestinalis* and its Fornicata relatives

SHWETA V. PIPALIYA[a], DAYANA SALAS-LEIVA[b], ANDREW J. ROGER[b], JOEL B. DACKS[a]

[a]Division of Infectious Diseases, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Alberta, Canada; [b]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Dalhousie University, Halifax, Canada

E-mail of the corresponding Authors: pipaliya@ualberta.ca; dacks@ualberta.ca

Giardia intestinalis is a parasitic lineage within the Fornicata that is responsible for causing the gut infection Giardiasis. Giardia has undergone tremendous reduction in its endomembrane system, both morphologically and in the encoding protein machineries. One such family of proteins are the Rab GTPases. Rab GTPases, which belong to the Ras superfamily of small GTPases, act as molecular switches to regulate cargo containing vesicles formation at various points in the endomembrane system. Previous comparative analysis of the Rab family proteins across eukaryotes revealed presence of 23 Rab GTPases in the Last Eukaryotic Common Ancestor with six of these being present in Giardia AWB. Strikingly, Giardia is one of the only two lineages that lacks the endocytic Rab5 and Rab7 proteins but instead possesses few unclassified Rabs. We We carried out a comparative genomic analysis of the 23 LECA Rab GTPases in all available Fornicata genomes including in the unpublished genomic dataset of the basal lineage, *Carpediemonas membranifera,* to detect any divergent orthologs missing from previous analysis using more updated methods. Our homology searches revealed presence of at least 10 LECA Rabs in *Carpediemonas membranifera* including Rab5 and 7 which was validated by preliminary phylogenetic analysis. Overall, we observe patterns of reduction in the Rab complement with transition into parasitism. Notable, our analysis shows presence of multiple paralogs of Rab7 in *Spironucleus salmonicida* however we failed to identify any Rab5 orthologs. Similar to the previous analysis, we did not detect any orthologs of Rab5 or 7 in six assemblages of Giardia. These findings point towards a gradual loss in the endolysosomal Rab GTPases consistent with the streamlining of the late endosomal pathway as observed morphologically and in other associating molecular protein machineries in these diplomonad lineages.

Comparison of *Cryptosporidium proliferans* and *C. muris* Genomes

PETR TÁBORSKÝ[a], ERIC SALOMAKI[a], MONIKA WIŚNIEWSKA[a], MARTIN KVÁČ[a], MARTIN KOLÍSKO[a]

[a] Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budejovice.

E-mail of the corresponding Author: taborsky.1988@gmail.com

Cryptosporidium (Apicomplexa) is obligatory intracellular parasite infecting gastrointestinal tract of vertebrates, specifically the of intestine or stomach. Recently a new stomach-infecting species, *Cryptosporidium proliferans*, have been described. *Cryptosporidium proliferans* has been originally isolated from rodent *Tachyoryctes splendens*, but it has been also found in *Syncerus cafer*, *Equus africanus* and *Equus cabalus*. *Cryptosporidium proliferans* is morphological and molecularly distinguishable from other gastric *Cryptosporidium* spp. based on analysis of several genes, morphological differences and differences in course of infection, although initially it has been considered to be a strain of *Cryptosporidium muris*. *Cryptosporidium proliferans* appears to be a lot more virulent than *C. muris* and causes major morphological changes of stomach tissue compared to non-infected controls or controls infected with *C. muris*. Here we report genome sequence of *C. proliferans* obtained by combination of Illumina and Minion nanopore sequencing. Nanopore sequencing largely improved the quality assembly and we have shown that the assembled genome is comparable in size and structure to the genome of *C. muris*. Results of comparative genomic analyses aimed specifically at identification of virulence factors will also be presented.

Can Protozoa Control Water Borne Parasites?

HAZEL FARTHING[a], JIAMEI JIANG[b], DAVID MONTAGNES[a]
[a]Institute of Integrative Biology, University of Liverpool, Liverpool, UK; [b]College of Life and Fisheries, Shanghai Ocean University, Shanghai, China.

E-mail of the corresponding Authors: hlhfarth@liv.ac.uk; jm-jiang@shou.edu.cn

Globally, amphibian populations are being driven towards extinctions by chytridiomycosis (the fungal pathogen *Batrachochytrium dendrobatidis*, Bd). Common conservation strategies (e.g. culling, direct treatment of hosts) are unsuitable for controlling Bd due to the virulent nature of the disease and ethical issues associated with the conservation status of amphibian hosts. An alternative solution to controlling the pathogen may be to target Bd's dispersal stages: ~3 μm flagellated zoospores that transmit the disease between amphibians. These free-swimming spores are thought to be nutritious and poorly protected, making make them vulnerable to consumption by protozoa. Furthermore, if the spores are nutritious, then exponential growth of protozoan populations around amphibian hosts may provide a rapid mechanism to control spore dispersal at their source. Initial studies by others suggest that *Paramecium* consumes Bd spores. However, the growth of *Paramecium* and other protozoa on Bd spores has not been assessed. Here we test the ability of several species of ciliates to grow on Bd spores and compared their survival rates to starvation rates when no food is available. To do so, ciliates were grown monoxenically on Bd, with antibiotics to prevent bacterial contamination. Small populations of ciliates were transferred every two or three days to fresh Bd, to maintain Bd abundance and quality. Parallel to the Bd-treatment, ciliates were maintained in water without Bd, to determine mortality due to starvation. This procedure was continued for up to 16 days, and change in abundance was measured to assess growth or mortality of the ciliate population. Ciliates exhibited five responses: 1) increased mortality rate in the presence of Bd, compared to that in water alone (*Stentor coeruleus*); 2) similar mortality rate compared to that in water alone (*Urocentrum turbo*); 3) reduced mortality rate when Bd was present (*Oxytricha* sp.); 4) initial positive growth in the presence of Bd over 3 to 9 days with a slow decline, leading to mortality (*Paramecium aurelia, P. caudatum*); and 5) continued growth on Bd (*Tetrahymena pyriformis*). These results indicate that not all ciliates grow on Bd alone, but some such as *Tetrahymena*, may be promising candidates for further exploration of Bd control.

Blastocystis hominis and _Cryptosporidium_ spp Infections among Immunocompromised and Immunocompetent Individuals in Guilan-Iran

MOHAMMAD REZA MAHMOUDI[a], PANAGIOTIS KARANIS[b]
aDepartment of Medical Parasitology and Mycology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran; [b]University of Cologne, Medical Faculty and University Hospital, Cologne, Germany.

E-mail of the corresponding Authors: mrmahmoodi2002@yahoo.com; panagiotis.karanis@uk-koeln.de

Background & Aims: *Blastocystis hominis* is one of the most common intestinal protozoan parasites in human. *Cryptosporidium* spp. are highly prevalent in patients with immunodeficiency but also common cause of diarrhea in immunocompetent people it is considered to be one of the most important foodborne and waterborne parasites. The present study was performed to investigate the prevalence of both parasites in Guilan – Iran in the years 2017-2018.
Materials & Methods: In this descriptive cross-sectional study, the stool samples of 631 immonocompromised patients and 383 control group were examined for parasitic infections. Stool samples from each of the cases were examined using direct examination with saline and lugol, formalin-ethyl acetate concentration method and stained with Ziehl-Neelsen for *Cryptosporidium* spp. detection.
Results: *B. hominis* was detected in 57 (8.9%) of immunocompromised and 16 (4%) of the immunocompetent individuals. This difference was statistically significant ($\chi 2=17.3$, P=0.001) between patient and control groups. It was found higher in hemodialysis (10.1%) than cancer patients (8%), but no statistically significant difference was seen among these groups (p=0.1). The examination of the stool specimens with formalin-ether concentration and Ziehl – Neelsen staining, revealed no positive *Cryptosporidium* oocyst in examined people.
Conclusion: *B. hominis* infection was the most common parasitic infection. Systematic stool examinations in specialized parasitological laboratories should be included as a part of routine medical care in such groups of patients as we reported. Regarding the negative findings for *Cryptosporidium*, this may be a consequence of the better living conditions of the patients in the area but also the techniques applied, which are unable to find the parasites, when excreted in low numbers. More sensitive methods are required to improve the diagnosis and our current understanding of the epidemiology and real significance of *Cryptosporidium* in the area.

Survey of Free Living Amoeba, the Genus of *Acanthamoeba* in Ophthalmology, Organ Transplant and Chemotherapy Wards in Educational Hospitals of Guilan-Iran -2018

MOHAMMAD REZA MAHMOUDI[a], NASTRAN SHIROUDI[a], FARSHID SAADAT[a], PANAGIOTIS KARANIS[b]
[a]Department of Medical Parasitology and Mycology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran; [b]University of Cologne, Medical Faculty and University Hospital, Cologne, Germany.

E-mail of the corresponding Authors: mrmahmoodi2002@yahoo.com; panagiotis.karanis@uk-koeln.de;

Introduction: *Acanthamoebae* are free-living amoebae (FLA) that cause life-threatening granulomatous amoebic encephalitis in immunocompromised patients and amoebic keratitis (AK) in contact lens wearers. It can harbor pathogenic agents too. The present work aimed to determine the presence of *Acanthamoeba* species isolates in dust of different hospital wards in Guilan, Iran.
Material and Method: A total of 106 dust samples collected from Ophthalmology, Organ transplant and Chemotherapy wards in Guilan, Iran in 2018. All samples followed by cultivation on non-nutrient agar plates pre-seeded with *E. coli*. All positive samples for *Acanthamoeba* were subjected to thermo-tolerance assays as criterion for evaluation of the pathogenicity.
Results: Out of 106 samples, 36.5% were positive for *Acanthamoeba* spp.. Temperature tolerance test indicate that four (3.7%) of samples could be potentially pathogenic because grew at high temperature (40 ^{o}C & 42 ^{o}C). The detection rate of free-living amoebae in transplant, chemotherapy and ophthalmology wards was 41.1%, 33.3% and 39% respectively.
Conclusion: The high distribution of *Acanthamoeba* species in different hospital wards represents a health risk for patients refry to these hospital wards. Therefore, improving the methods of disinfection to reduce the risk for these parasites is recommended.

Oral Session ECOLOGY & BIOGEOGRAPHY 3

PIDA: The Planktonic Protist Interaction Database

MARIT F. M. BJORBÆKMO[a], ANDREAS EVENSTAD[a], LINE L. RØSÆG[a], ANDERS K. KRABBERØD[a], RAMIRO LOGARES[b]

[a]University of Oslo, Department of Biosciences, Evogene, Oslo, Norway; [b]Institut de Ciències del Mar (CSIC), Barcelona, Catalonia, Spain.

E-mail of the corresponding Authors: a.k.krabberod@ibv.uio.no; ramiro.logares@icm.csic.es

Microbial interactions are crucial for aquatic ecosystem functioning. Although much work has been done on interactions over the last few centuries, the information is scattered and not easily accessible. To aid the protist community we have surveyed the literature, searching specifically for interactions among planktonic protist and gathered the information in a comprehensive, manually curated *Protist Interaction DAtabase* (PIDA; DOI: doi.org/10.1101/587352). In total, we have registered ~2,500 ecological interactions from ~500 publications, going back to the late 1800's. The literature was dominated by studies based on direct observation of interactions such as light microscopy (82%), and only 38% of those were combined with molecular methods. Symbiosis was the most common type of interaction (43% of all records), followed by predation (39%) and parasitism (18%). The majority of interactions (82%) were from marine or brackish waters, while studies from freshwater systems accounted for a smaller fraction of the interactions (18%). All major protistan groups are represented in PIDA as either hosts, symbionts, parasites, predators, or prey. The SAR supergroup (Alveolata, Stramenopiles and Rhizaria) dominated with ~92% of the total entries.

To explore the structure of PIDA we calculated the specialization index *d*' (Kullback-Leibler distance) for each type of interaction, symbiont-host, parasite-host and prey-predator independently. Although all three kinds of interactions had a mixture of both specialists and generalists, we found that parasites and symbionts are more specialised than the other interactors.

We also compared the SAR records in PIDA with one of the most well-known recent environmental diversity campaigns, Tara Oceans, in order to uncover biases in the scientific literature. The groups that seem to be largely overlooked in the literature were Labyritnthulomycetes and MAST among the Stramenopiles, and Syndiniales in Alveolatea.

PIDA constitutes an expandable resource to investigate the protist interactome and test hypotheses deriving from omics tools.

Everything Is Everywhere? Comparison of Ciliate Communities in Seven Different German Rivers and Their Role in the Elimination of Potentially Pathogenic Bacteria

ANJA SCHERWASS[a], ALEXANDRA SCHÖNLE[a], EIKE LÜCKERATH[a], BIANCA HENNIGS[a], PATRICK HEIDBÜCHEL[a], ALEXANDRA JEUCK[a], HARTMUT ARNDT[a]

[a]University of Cologne, Biocenter, Institute for Zoology, General Ecology, Cologne, Germany.

E-mail of the corresponding Authors: anja.scherwass@uni-koeln.de; alex.jeuck@gmail.com

The percentage of pathogenic and antibiotic resistant bacteria released from water treatment plants is increasing nowadays. Therefore, effective mechanisms regarding the reduction of bacteria and viruses are needed. We studied the ciliate fauna using morphological and metabarcoding techniques to compare seven rivers in Germany (Spree, Havel, Rhine, Moselle, Ruhr, Isar and Ilz), which differed concerning their morphological and physico-chemical conditions during a one-year-study in the spring and summer season.

Taking the data of the monitoring of bacteria, viruses and ciliates into account we assessed the role of ciliates for the elimination of bacteria and viruses with special focus on pathogens. Moreover, grazing experiments under semi-natural conditions were run.

The results of the monitoring revealed a different taxonomic structure of the microbial food webs in the rivers investigated, which especially depends on different hydrological properties of each river. Moreover, the analysis of the protozoan fauna with molecular tools underlined a high diversity of potentially bacterivorous protozoans, which was indicated by analysis of morphological taxa.

The subsequent calculation of grazing rates on bacteria showed that in some rivers (e.g. Rhine and Ruhr) protozoans may play an important role regarding the elimination of bacteria (and thereby also pathogenic bacteria). This was also supported by the results of the grazing experiments.

Single-cell Genomics Illuminates the Eco-Evolution of a Cosmopolitan Lineage Of Tiny Ocean Grazers

FRAN LATORRE[a], AURELIE LABARRE[a], INA M. DEUTSCHMANN[a], ALEIX OBIOL[a], ANDERS KRABBEROD[b], ERIC PELLETIER[c,d], MICHAEL SIERACKI[Ee], OLIVIER JAILLON[c,d], RAMON MASSANA[a], RAMIRO LOGARES[a]

[a]Department of Marine Biology and Oceanography, Institute of Marine Sciences (ICM)-CSIC, Pg., Barcelona, Spain; [b]Department of Biosciences, Section for Genetics and Evolutionary Biology (Evogene), University of Oslo, Oslo, Norway; [c]Metabolic Genomics, Genoscope, Institut de Biologie François Jacob, CEA, CNRS, Univ Evry, Université Paris Saclay, Evry, France; [d]Research Federation for the study of Global Ocean Systems Ecology and Evolution, FR2022 / Tara; [e]National Science Foundation, Alexandria, VA USA.

E-mail of the corresponding Authors: latorre@icm.csic.es, ramiro.logares@icm.csic.es

Marine protists have a paramount importance for the functioning of the ocean ecosystem. In particular, marine heterotrophic flagellates have a crucial role in the microbial loop, channeling organic matter to upper trophic levels. Understanding the specific role of the different flagellate species in marine food webs, as well as their adaptations to the environment, requires comprehending their spatiotemporal distributions and genomes. Given that most species are unculturable, Single-Cell genomics represents a useful approach to access their genomes. Here we investigate four clades of an uncultured cosmopolitan lineage of marine picoeukaryotic grazers (MAST-4 A/B/C/E). We found contrasting spatial distributions in the four MAST-4 clades in the global surface ocean (Malaspina & Tara Oceans data) suggesting adaptive divergent evolution leading to niche differentiation. Subsequently, we investigated the genomes of these species using Single-Cell Genomics in order to determine whether niche differentiation could explain species co-occurrence and co-exclusion patterns. Analyses from 69 single-cells allowed recovering 66-83% of the MAST-4 A/B/C/E genomes, showing that they have diverged considerably in evolutionary terms. Yet, the functional characterization of these genomes indicated that they are substantially similar, but also that each clade features specific functions, suggesting the acquisition or loss of functional features. In particular, the four MAST-4 differed in their glycoside hydrolases (GHs). Different MAST-4 displayed different lysozyme protein families, which are possibly used for the degradation of bacterial-prey cell walls. We analyzed the expression of specific functions across the global ocean, which suggested that MAST-4 clades A & C, featuring similar composition of GHs, may co-exclude each other, while clades B & C, featuring a different set of GHs, may co-occur. In sum, our results show that MAST-4 lineages have diverged substantially in terms of DNA-sequence composition, while maintaining similar functional repertoires. Yet, we found that MAST-4 lineages differ in specific functions, which may be related to the adaptation to certain niches and their prey choice.

Periphytic Ciliate Colonization in two Tufa-depositing Systems

RENATA MATONIČKIN KEPČIJA[a], VESNA GULIN[a], MIRELA SERTIĆ PERIĆ[a]
[a]Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia.

E-mail of the corresponding Author: rmatonic@biol.pmf.hr

Tufa is a freshwater calcium-carbonate deposit, creating a myriad of microhabitats for periphyton. While increasing habitat heterogeneity, it also generates a certain stress for periphytic biota. Periphyton development was studied using glass slides in two tufa-depositing environments characterised by barrage lakes: National park Krka and National park Plitvice Lakes (Croatia). NP Krka is under the influence of Mediterranean climate, whereas NP Plitvice Lakes has continental climate. The slide carriers were seasonally exposed on tufa barriers between barrage lakes during a one month period. The seasonal differences were pronounced for tufa deposition and chlorophyll *a* accrual, especially within NP Plitvice Lakes. During one year, 72 ciliate morphospecies were determined in NP Krka, and 71 were found in NP Plitvice Lakes. Ciliate assemblages were mostly influenced by the position within the lake system. Peritrichs and suctorians strongly dominated in assemblages on tufa barriers below lakes. Both groups probably prospered from the overflowing plankton-rich water. Flow velocity likely structured periphyton, as there was a clear relationship between ciliate biomass and taxa frequency within certain velocity range. Microhabitats with slow flow supported up to 50% more diverse ciliate assemblages than fast flowing microhabitats. In both hydrosystems, folliculinids *Lagotia dinaridica*, endemic to Dinaric hydrosystems, and *Ascobius lentus* colonized substrates under slow flow conditions. High tufa deposition during summer had negative influence on ciliate diversity, probably due to strong sedimentation. High discharge events mostly led to flushing of the attached taxa, whereas few taxa resisted the hydrological stress. Our findings suggest that the seasonal and microhabitat variations of tufa deposition processes, productivity and flow conditions likely play a dominant role in generating distributional patterns of periphyton community assemblages within karst and tufa-precipitating hydrosystems.

A Biosilica-Related Protein Newly Identified from a Rhizarian Testate Amoeba: a Key Enzyme of Eukaryotic Silica-Biomineralization?

MAMI NOMURA[a], TAKURO NAKAYAMA[b], SHIN-ICHI MIYAMURA[c], MASAKI YOSHIDA[c], KAZUO INABA[d], RYOMA KAMIKAWA[a], KEN-ICHIRO ISHIDA[b]

[a]Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan; [b]Faculty of Life Environmental Sciences, Tohoku University, Sendai, Japan; [c]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan; [d]Shimoda Marine Research Center, University of Tsukuba, Shimoda, Japan.

E-mail of the corresponding Author: nomura.mami.36x@st.kyoto-u.ac.jp

Formation of biological structures from silica is called silica-biomineralization. Two silicic acid transporter families (the SIT/SIT-L and Lsi2) are known to be responsible for pre-concentration of silicic acids. Analyses of these transporters have contributed to gaining insight into evolution of silica-biomineralization in eukaryotes. However, pre-concentration of silicic acids is only the first step of silica-biomineralization and no protein involved in the other processes of silica-biomineralization has been identified. To gain further insight into evolution of silica-biomineralization, identification and phylogenetic analyses of additional proteins involved in silica-biomineralization are needed.

In this study, we identified cellobiose phosphorylase (CBP) as a component of the silica shell of *Paulinella micropora*, a rhizarian testate amoeba. Blast search of CBP against genomes and transcriptomes from a wide variety of eukaryotes detected its homologues from almost all the silicified organisms belonging to Rhizaria, Stramenopiles, Alveolata, and Haptophyta, suggesting that CBP takes a role in silica-biomineralization of those silicified organisms. Given that vase-shaped microfossils, analogous to modern rhizarian testate amoeba, were found in the rock of the Tonian period in the Neoproterozoic, emergence of CBP in eukaryotes might have been traced back to that era. On the basis of the broad distribution and the early emergence of CBP in eukaryotes, the enzyme could be an important protein to understand the evolution of eukaryotic silica-biomineralization as well as the SIT/SIT-L and Lsi2.

Oral Session GENOMIC & TRANSCRIPTOMIC 1

Genome and Transcriptome Drafts of the Heterotrophic Euglenoid *Rhabdomonas costata* Provides First Insights Into its Mitochondrial Metabolism

PETR SOUKAL[a], ŠTĚPÁNKA HRDÁ[a], ANNA KARNKOWSKA[b], JANA SZABOVÁ[a], RAFAL MILANOWSKI[b], MILUŠE HROUDOVÁ[c], HYNEK STRNAD[c], ČESTMÍR VLČEK[c], IVAN ČEPIČKA[d] AND VLADIMÍR HAMPL[a]

[a]Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic; [b]Department of Molecular Phylogenetics and Evolution, University of Warsaw, Warsaw, Poland; [c]Institute of Molecular Genetics, Academy of Science, Prague, Czech Republic; [d]Faculty of Science, Charles University, Prague, Czech Republic.

E-mail of the corresponding Author: vlada@natur.cuni.cz

Euglenoids represent a group of protists with diverse modes of feeding including phagotrophy, osmotrophy, phototrophy and mixotrophy. Until today, there is not available any complete or partial nuclear genome sequence of heterotrophic euglenoid. In our work we are trying to partially fill this gap by presenting genomic and transcriptomic drafts of a 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 gene prediction, nevertheless, the comparison of transcriptomic and genomic data allowed us to manually inspect and describe features of its introns, including noncanonical intron known from phototrophic euglenids. The set of 39,585 putative *Rhabdomonas* proteins was predicted from the decontaminated transcriptome. Only 26,052 predicted proteins have any homologue in GenBank database and their function was automatically annotated; 16% of these bear recognizable splice leader sequence at their 5‘terminus. Products of ~550 transcripts were predicted to localize into the mitochondrion. Functional annotation of this set provided the first data on *Rhabdomonas* mitochondrion, which resembles in many respects the mitochondrion of *Euglena gracilis*, but exhibits some interesting differences, for example the absence of pyruvate dehydrogenase complex (functionally substituted by pyruvate: NADP+ oxidoreductase) and the absence of alternative oxidase in the electron transport chain.

Evolution of Meiosis and Sex in Eukaryotes

PAULO G. HOFSTATTER[a] ,DANIEL J. G. LAHR[a]

[a]Universidade de São Paulo, São Paulo, Brazil.

E-mail of the corresponding Authors: paulogh@usp.br; daniel.lahr@gmail.com

Sexual processes are inherent to eukaryotes and involve basically cell fusion (plamogamy/karyogamy) and meiosis. Sex is hypothesized to be ancestral to eukaryotes, but this process has not been observed in many groups yet. The occurrence of meiosis-specific proteins may be used for detection of yet unknown sexual cycles in organisms with available molecular data. We analyzed a broad range of genomes and transcriptomes of representatives of the eukaryotic diversity in search for specific plasmogamy and meiotic machineries. Afterwards, we refined this approach to focus on organisms recognized to be asexuals by morphological observation, among them the bdelloid rotifers, some fungal groups, and some intracellular protists. Our results show that the meiotic machinery evolved by events of gene duplication from DNA repair genes that occur in Archaea. This fact provides another instance of the importance of gene duplication events in the evolution of eukaryotes. The meiotic machinery was found to be ubiquitous in extant major eukaryotic lineages, supporting the notion that the eukaryotic ancestor was sexual and also suggesting the maintenance of sexual cycles in all those groups. There was no difference in the patterns of distribution of the meiotic machinery between fully sexual groups and lineages with unknown sexual cycles. However, the lack of meiotic proteins in some genomes suggests that sex (or at least, meiosis) has been secondarily lost in some minor groups, namely the fungal genus *Malassezia*, which lacks the meiotic machinery. Additionally, bdelloid rotifers maintain an incomplete meiotic machinery with highly diverging sequences, an indication of either neofunctionalization or pseudogenization processes of their meiotic machinery components. Overall, our results point to a widespread maintenance of ancestral sexual processes in eukaryotes with rare, isolated occurrences of loss of sex in some minor lineages.

Evolution of Cell Death Machinery in Holozoan Protists

MICHELLE M. LEGER[a], IÑAKI RUIZ-TRILLO[a, b, c]

[a]Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain; [b]Department of Genetics, University of Barcelona, Barcelona, Spain; [c]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.

E-mail of the corresponding Author: inaki.ruiz@ibe.upf-csic.es

Regulated cell death involves the death of specific cells in a controlled manner, in response to extrinsic or intrinsic triggers. While regulated cell death in response to extrinsic factors has been reported in eukaryotes such as *Chlamydomonas* or *Dictyostelium*, its molecular basis in these organisms is poorly understood. However, it is an essential function in animals, where several different cell death pathways regulate responses to cell damage, cell turnover, and – most importantly - normal development. Accordingly, the cell death machinery present in the unicellular ancestors of animals were likely critical to the appearance of complex multicellularity in animals.

Holozoan protists - filastereans, ichthyosporeans, Pluriformea and choanoflagellates - are the closest unicellular relatives of animals; some members of each clade exhibit simple multicellularity during some life stages. As a result, these lineages present an excellent opportunity to understand the elements of the cell death 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 cell death-related proteins and protein domains in holozoan protists. We show that the best-known animal cell death pathway, apoptosis, is a true animal innovation, but that proteins and domains implicated in cell death pathways are more widely present in holozoan protists.

Investigation of the Effect of Bacterial Endosymbionts on Life Strategy Transitions Within Phylum Apicomplexa

ELIZABETH HUNTER[a], CHRISTOPHER PAIGHT[a], CHRISTOPHER E. LANE[a]

[a]Department of Biological Sciences, University of Rhode Island, Kingston, RI, USA.

E-mail of the corresponding Author: lizhunter@uri.edu

Apicomplexa is a diverse phylum of metazoan-infecting parasites, containing numerous clinically significant genera, including *Plasmodium* (malaria), *Toxoplasma (*toxoplasmosis), and *Cryptosporidium* (cryptosporidiosis). Recently, a single apicomplexan genus, *Nephromyces*, has been described as mutualistic. The sister genus of this mutualist, *Cardiosporidium*, contains typical parasitic species. These two systems exist in parallel, not only from an evolutionary standpoint, but also from a life history perspective. Species from both genera inhabit closely related host species, and also host their own endosymbiotic bacteria, making them ideal for life strategy comparisons. The term symbiosis encompasses a sliding spectrum of prolonged, intimate associations between species ranging from parasitism to mutualism, and numerous life history traits can tip the scales in either direction. Bacterial endosymbionts are rare in apicomplexans, but genomic data indicates an Alphaproteobacterial symbiont has been retained since *Nephromyces* and *Cardiosporidium* speciated from their last common ancestor. The presence of bacterial endosymbionts in *Nephromyces* may have contributed to the transition from parasitism to mutualism, as well as the low virulence of *Cardiosporidium* species. Characterizing the metabolic capabilities of *Cardiosporidium's* endosymbiotic Alphaproteobacterium is critical to evaluating this hypothesis, and understanding apicomplexan interactions with the host tunicate. By sequencing the genome of this bacterial endosymbiont we provide context to better understand life strategy transitions along the symbiotic spectrum and the role of endosymbiotic bacteria in this process.

Genes Differentially Expressed Between *Cryptocaryon irritans* Theront Cells Derived From 1 And 10 hrs Post-Encystment

HONGSHU CHI[a], MICHAEL GOLDSTEIN[b], WEI-JEN CHANG[b], HUI GONG[a]

[a]Biotechnology Institute, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian, China; [b]Department of Biology, Hamilton College, Clinton, NY, USA.

E-mail of the corresponding Authors: wchang@hamilton.edu; ghxfjm@163.com

The ciliated protozoan *Cryptocaryon irritans* infects a wide range of marine fish and causes the highly lethal white spot disease. This parasite possesses three morphologically and physiologically distinct life stages: an infectious theront, a parasitic trophont, and an asexually reproductive tomont stages. During the theront stage, the spindle-shaped *C. irritans* actively seeks for host fish, and after establishing successful infection in the subcutaneous layer of skin of the host fish, the parasite will transform into round-shaped trophont cells and grow into significantly larger, eye-visible ones (hence the white spots). Mature trophont cells detach from host fish, and develop into the tomont cells where rounds of asexual divisions would occur to give rise to theront cells and the infectious cycle repeats. It was reported that infectivity of theront cells declined 6 - 8 hours post-encystment, but what causes the decline remains unclear. Here we use RNA-seq and compare gene expression profiles of theront cells collected by one and 10 hrs post-encystment. We show that genes related to energy production and consumption are both downregulated, suggesting that theront cells might be running out of reserve energy 10 hrs post-encystment. Other findings will also be discussed.

Microtubule Dynamics in the Haptonema of Haptophyte Algae

RYUJI YANASE[a], MAMI NOMURA[a, b], KOGIKU SHIBA[a], KAZUO INABA[a]

[a]Shimoda Marine Research Center, University of Tsukuba, Shimoda, Japan; [b]Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan.

E-mail of the corresponding Authors: yanase@shimoda.tsukuba.ac.jp; kinaba@shimoda.tsukuba.ac.jp

The haptonema is an elongated microtubule-based motile organelle uniquely present in haptophytes. The most notable and rapid movement of a haptonema is the "coiling", which occurs within a few milliseconds by a mechanical stimulation. The coiling occurs in a Ca^{2+}-dependent but motor-independent mechanism. Here, we analyzed the coiling process in detail by a high-speed filming and showed that haptonema coiling was initiated by left-handed twisting, followed by writhing to form a helix from the distal tip. In the recovery process, called "uncoiling", the helix slowly uncoiled from the proximal region. Electron microscopy showed that the seven microtubules were arranged in a haptonema mostly in parallel but that one of the microtubules often wound around the others in the extended state. A microtubule stabilizer, paclitaxel, inhibited coiling and induced right-handed twisting of the haptonema in the absence of Ca^{2+}. The paclitaxel-induced bend propagated toward the proximal region by the addition of Ca^{2+}. Then, we carried out an immunocytochemical study and showed that centrin-related proteins were localized along the whole length of haptonema, with often concentrated at the tip. In addition, immunoblot analysis revealed that the anti-centrin monoclonal antibody recognized at least two centrin-related proteins with the molecular masses of 23 and 18 kDa. The lower molecular weight centrin band showed electrophoretic mobility shift in SDS-PAGE depending on Ca^{2+}. These results suggest that switching microtubule conformation with the aid of Ca^{2+}-binding microtubule-associated proteins, possibly such as the centrin-related proteins, is responsible for haptonematal coiling. Currently, we are trying to determine the detailed localization and function of these proteins in the haptonema.

SYMPOSIUM ISOP ADVANCE - Applications of Genetic Tools for Advancing Research on Marine Protists (by ISoP/Moore Foundation)

Diplonema papillatum, a Representative of the Highly Diverse and Abundant Marine Microeukaryotes, Can Be Genetically Manipulated

BINNYPREET KAUR[a,b], DRAHOMÍRA FAKTOROVÁ[a,b], MATÚŠ VALACH[c], GERTRAUD BURGER[c], JULIUS LUKEŠ[a,b]

[a]Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic; [b]Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic; [c]Robert Cedergren Centre for Bioinformatics and Genomics, Département de Biochimie, Université de Montréal, Montréal, Canada.

E-mail of the corresponding Authors: binny@paru.cas.cz; jula@paru.cas.cz

The marine pelagic ecosystems represent by far the world's largest ecosystem on the Earth. Recently, an obscure marine group termed as diplonemids was found to be 2nd to 3rd most species-rich and 6th most abundant marine protists in the world ocean. Diplonemids belong to the phylum Euglenozoa, together with their extensively studied sister groups - kinetoplastids and euglenids. However, until now, only few diplonemid species have been formally described and we know close to nothing about their diversity and ecological functions in the world ocean. To fill this gap in our knowledge, we are studying their morphology, life cycle, endosymbionts and ecological significance. Our main aim is to develop methods for the genetic modifications of diplonemids, using *Diplonema papillatum* as their model representative, and turn it into a genetically tractable system suitable for functional studies of its genes. We have already transformed *D. papillatum* by integrating various constructs into either random locations in its genome, or by homologous recombination into targeted loci. Moreover, we have shown that the electroporated genes are being transcribed and translated.

Transforming Dinozoa: Steps Forward and Steps Back

ROSS F WALLER[a]
[a]Dept of Biochemistry, University of Cambridge, Cambridge, UK.
E-mail of the corresponding Author: rfw26@cam.ac.uk

Dinoflagellates are single-celled plankton that dominate the world's oceans, both in abundance and species richness. As photosynthetic organisms they contribute a substantial fraction of global carbon fixation, and as essential symbionts of corals they maintain and protect coastlines and reef ecosystems throughout the tropics. But they can also form toxic algal blooms hundreds of kilometres long, and some have evolved as parasites, affecting both fish stocks and landed catches with significant impact to food security and economies. Dinoflagellates are also truly amazing eukaryotic cells. They have near abandoned the use of histones in their nuclei, and have expanded their nuclear genome sizes to up to 80-fold that of humans. On the other hand, their organelle genomes are the most heavily reduced in gene content of any eukaryote, although these genes are scattered on multiple small elements and require complex processes such as RNA trans-splicing and editing to decipher. Further, many dinoflagellates have entered into new endosymbiotic partnerships creating novel photosynthetic organelles and providing opportunities for studying the process of organellogenesis. A major limitation to studying the biology and evolution of dinoflagellates is their recalcitrance to genetic manipulation. We are spearheading efforts to overcome this technical barrier, using a wide range of approaches, including: CRISPR/Cas9-mediated breaks; modified dinoflagellate RNA viruses; and transformation of organelle genomes. Using the basal dinozoan, and important mollusc pathogen, *Perkinsus marinus* as a successful starting point for several of these approaches, we are also expanding these strategies into the diversity of dinoflagellates.

Tools for Stable Integrative Transfection of *Bodo saltans*: A Micro-Eukaryote with Polycistronic Peptide Coding Genes

FATMA GOMAA[a,b], ZHUHONG LI[c], NOELIA LANDER[c], MIGUEL A. CHIURILLO[c], HEBA ALZAN[d], ROBERTO DOCAMPO[c], VIRGINIA P. EDGCOMB[b]

[a]Harvard University, Department of Organismic and Evolutionary Biology, MA, USA; [b]Woods Hole Oceanographic Institution, Department of Geology and Geophysics, MA, USA; [c]University of Georgia, Department of Cellular Biology, GA USA; [d]Washington State University, Pullman, WA USA.

E-mail of the corresponding Authors: fatmagomaa@fas.harvard.edu; zhli@uga.edu; noelia2309@hotmail.com; mchiurillo@yahoo.com; heba@vetmed.wsu.edu; rdocampo@uga.edu; vedgcomb@whoi.edu

Bodo saltans is a free-living kinetoplastid that shares similar genome structure with trypanosomes and *Leishmania*. Genomic and transcriptomic data show that *B. saltans* has unique biological characteristics, including osmoregulatory adaptations for living in both fresh and marine environments, the ability to switch between aerobic and anaerobic glycolytic pathways, and potentially, the ability to survive desiccation. Our goal has been to develop stable transfection protocols to study gene functions and regulation in *B. saltans*. We first optimized many electroporation parameters and variables, and we constructed promoter-less plasmids, relying on the endogenous elements for transcription. These plasmids were designed to target 3 specific loci of the *B. saltans* genome: elongation factor-1 alpha (EF-1 alpha), paraflagellar rod 2 (PFR2), and 18S ribosomal DNA (rDNA). *B. saltans* cells were electroporated using a square-wave electroporator (Nepa21, Bulldog Bio, Inc.). G418 resistant cells were obtained from transfection with the EF- 1 alpha plasmid, and cells have been growing in the G418 selection medium for around 6 months. Genotyping shows the presence of the complete sequence of the Neo gene and at least partial plasmid sequence. However, we are working to determine if homologous recombination of the plasmid into the *B. saltans* genome has occurred. The application of CRISPR/Cas9-based approaches is being explored.

Successful Genetic Manipulation of the Thraustochytrid *Aurantiochytrium limacinum* and Challenges Extending to Other Labyrinthulomycetes

JACKIE L. COLLIER[a]

[a]School of Marine and Atmospheric Sciences (SoMAS), Stony Brook University, Stony Brook, New York, USA.

E-mail of the corresponding Author: jackie.collier@stonybrook.edu

Labyrinthulomycetes are abundant and ubiquitous osmoheterotrophic marine protists (basal stramenopiles) thought to function ecologically as fungus-like decomposers. There are four distinct groups of cultivated labyrinthulomycetes – labyrinthulids, aplanochytrids, oblongichytrids, and thraustochytrids. The first three of these groups are so far monogeneric, while there are at least a dozen genera of thraustochytrids. Some thraustochytrids have the ability to synthesize carotenoids, which is uncommon among heterotrophic eukaryotes, and may be associated with protecting the large amounts of essential omega-3 polyunsaturated fatty acids they store in lipid droplets from oxidative damage. To gain better understanding of carotenoid function in thraustochytrids, we have produced mutants of *Aurantiochytrium limacinum* ATCC MYA-1381 in which the trifunctional gene encoding the first three carotenogenesis-specific reactions (phytoene synthase, phytoene desaturase, lycopene cyclase) has been interrupted by double homologous recombination with a construct containing a zeocin resistance (*shble*) expression cassette. As predicted, the knockout mutants lack the carotenoid pigmentation found in the wild-type. Complementation with the wild-type gene to confirm that this phenotype is due to the knockout is in progress. Differences between the wild-type and knockout mutants in growth rate and biomass yield, lipid content, survival in stationary phase, and response to oxidative stress are being evaluated under growth conditions that induce different amounts of carotenoid accumulation in the wild-type. While *A. limacinum* ATCC MYA-1381 is a good strain to work with in the lab, it does not represent the most environmentally relevant labyrinthulomycetes, so we are attempting to extend our methods into both other genera of thraustochytrids and into the other groups of labyrinthulomycetes. Challenges we are facing include a surprising diversity of antibiotic resistance phenotypes, different growth forms and life histories, uncertainties about whether marker expression constructs driven by exogenous promoters will function in each strain, and technical uncertainties in the introduction of exogenous DNA.

Transfection and Reverse Genetics in Marine Ciliates with Highly Amplified Nanochromosomes.

ANGELA PIERSANTI[a], FRANCESCA PAPI[a], RACHELE CESARONI[b], LAWRENCE A. KLOBUTCHER[c], ESTIENNE C. SWART[d], SANDRA PUCCIARELLI[a], PATRIZIA BALLARINI[a], MATTEO MOZZICAFREDDO[a], MARIUSZ NOWACKI[b], CRISTINA MICELI[a]

[a]School of Biosciences and Veterinary Medicine, University of Camerino, Italy; [b]University of Bern, Switzerland; [c]Department of Molecular Biology and Biophysics, University of Connecticut, Farmington, USA; [d]Max Planck Institute for Developmental Biology, Tubingen, Germany.

E-mail of the corresponding Author: cristina.miceli@unicam.it

The study of marine ciliates of the *Euplotes* genus has provided significant insights into microbial ecology. These organisms have an unusual genetic organization with two genomes, that is, a micronuclear genome representing the germ line, and a macronuclear genome containing single gene nanochromosomes amplified to thousands of copies for their somatic life. Improved understanding of this unique organization has been beneficial in elucidating universal principles in the biology of telomeres. However, the application of transfection and reverse genetics techniques remains a challenge. The development of such techniques would contribute to a better understanding of the complex transposition mechanisms occurring in the new macronucleus after each sexual cycle.

In this context, we worked on different methods of transfection and gene silencing by RNAi for *Euplotes crassus* and *E. focardii.* The former is mesophilic and has a cosmopolitan distribution, as it has been collected from most of the Earth's oceans, while the latter is a strictly psychrophilic Antarctic species and survives at temperatures from -2 °C to 15 °C. 1. We generated plasmids and artificial nanochromosomes ending with telomeres that contain either a GFP reporter and/or a drug selection marker. 2. We found that *E. crassus* and *E. focardii* (which has a slower growth rate) display naturally high resistance to a number of drugs/antibiotics that are typically used for selection in transfection experiments. However, they are much more sensitive to G418, paromomycin and puromycin when grown in a culture medium of 10% artificial seawater and 90% of 0.3 M glucose. 3. We demonstrated the delivery of Cy3-labelled control plasmid to *Euplotes* by lipofection, electroporation, and microinjection. 4. In order to set up the RNAi technology, we analysed all the steps involved in RNAi by feeding with silencing bacteria. We obtained the silencing of the telomerase gene in *E.focardii,* which showed a phenotype with visible changes in nuclear structure and with characteristics of senescence. (We would like to acknowledge the Moore Foundation for its financial support.)

Oral Session TAXONOMY & PHYLOGENY 2

Phylofisher: A Phylogenetically Aware Pipeline for Phylogenomic Dataset Construction

DAVID ŽIHALA[a,b], ALEXANDER K. TICE [a,c], TOMÁŠ PÁNEK[a,b], SERAFIM NENAROKOV[d], ANDREW J. ROGER[e], MARTIN KOLÍSKO[d], FABIEN BURKI[f], LAURA EME[g], MAREK ELIAS[b], MATTHEW W. BROWN[a,c]

a. Department of Biological Sciences, Mississippi State University, MS State, MS, USA; [b]University of Ostrava, Ostrava, Czechia; [c]Institute for Genomics, Biocomputing, & Biotechnology, Mississippi State University, MS State, MS, US; [d]Institute of Parasitology Biology Centre, ASCR. České Budějovice, Czechia; [e]Centre for Comparative Genomics and Evolutionary Bioinformatics, Dalhousie University, Halifax, Canada; [f]Systematic Biology program, Uppsala University, Sweden; [g]Unité d'Ecologie, Systématique et Evolution, Université Paris-Sud, France.

E-mail of the corresponding Author: matthew.brown@msstate.edu

Molecular phylogenetic practices have rapidly evolved over the last decade. To deduce phylogenetic relationships amongst taxa, single to few gene phylogenies have led way to use of many more genes for inference, termed phylogenomics. While phylogenomics has proven to be indispensable for inferring the deepest relationships within the tree of life, there is considerable variation in dataset construction approaches. Several key considerations must be addressed to ensure quality dataset inputs for subsequent phylogenomic inferences. These include dealing with paralogy (in-, mid-, and deep-), contamination, and phylogenetic signal in gene trees. As methodologies are quite different between research groups, we have developed a program that takes into account all above considerations in an easy to obtain, install, and use software package. We term this program Phylofisher. This program ships with a dataset of 250+ proteins from 250+ eukaryotic taxa across the tree of Life. Our methodology is highly adaptable to other input dataset also. Our approaches will be discussed in depth providing an outline of our program, including a phylogenetically aware method that aids in the selection of the correct sequence within input data. Additionally, we will illustrate the effectiveness of our methods through inference of a deep tree of eukaryotic life.

Species Identification and Phylogenetic Analysis of *Leishmania* Isolated from Patients, Vectors and Hares in Xinjiang Autonomous Region, The People's Republic of China

YUN-FU CHEN[a], LI-FU LIAO[b], DE-HUA LAI[a], ZHAO-RONG LUN[a]

[a]Center for Parasitic Organisms, Skate Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen University, Guangzhou, China; [b]Center for Disease Control and Prevention of Xinjiang and Research Center for Laboratory Animal of Xinjiang, Urumqi, Xinjiang, P.R. China.

E-mail of the corresponding Authors: chenyunfu@21cn.com; liaolif@21cn.com; laidehua@mail.sysu.edu.cn; lsslzr@mail.sysu.edu.cn

Visceral leishmaniasis (VL) is declared as one of the six major tropical diseases by the World Health Organization. This disease has been successfully controlled in China, except some of western areas. Xinjiang Autonomous Region is currently endemic with both anthroponotic VL (AVL) and desert type zoonotic VL (DT-ZVL). Here, we further assessed *Leishmania* species isolated from patients and/or sand-fly vectors of both VLs with special emphasis on Tarim hares (*Lepus yarkandensis*) as the potential reservoir animal for DT-ZVL. Phylogenetic analysis of sequences of ITS1, *hsp70* and *nagt* (encoding *N*-acetylglucosamine-1-phosphate transferase) genes PCR-amplified from *Leishmania* isolates placed them all into the same clade of *L. donovani* complex, unexpectedly, AVL isolates were close to *L. infantum* while DT-ZVL isolates were close to *L. donovani*.b. The differences seen between DT-ZVL isolates and all *L. donovani* examined suggest geographic isolation of the former for independent evolution from all *L. donovani* examined. The sequence identity of isolates from patients, vectors and Tarim hares from the DT-ZVL sites provides strong evidence for this animal as a potential reservoir.

Recent Contributions to the Morphology, Ecology and Molecular Phylogeny of Ciliates (Alveolata, Ciliophora) from the Southern Brazil

ROBERTO JÚNIO PEDROSO DIAS[a], FRANCIANE CEDROLA[a], ROBERTO MARCHESINI[a,c], SILAS JÚNIOR MUNIZ COSTA[a], ELEN FURTADO[a], PRISCILA FREGULIA[a], VÍTOR HALFELD[a], BIANCA SARTINI[a], JÉSSICA VILAS BOAS[a], YASMINE COSTA MOREIRA[a], MARIANA FONSECA ROSSI[a], MARCUS VINICIUS XAVIER SENRA[b], INÁCIO DOMINGOS DA SILVA-NETO[c], MARTA D´AGOSTO[a]
[a]Laboratório de Protozoologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Minas Gerais, Brazil; [b]Instituto de Recursos Naturais, Universidade Federal de Itajubá, Minas Gerais, Brazil; [c]Laboratório de Protistologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

E-mail of the corresponding Author: rjuniodias@hotmail.com

In the last ten years, the Laboratory of Protozoology, Federal University of Juiz de Fora (Brazil), in collaboration with other research groups in Brazil and around the world, has directed efforts to improve the knowledge on the diversity of ciliates in several ecosystems in southern Brazil. During this time, we investigated ~170 morphospecies of free-living, symbionts, commensals and parasitic ciliates, sampled in streams, lakes, bromeliads, brackish waters, mangroves, lichens, gastrointestinal tract of a wide range of domestic and wild herbivorous mammals and the integument of invertebrates, fishes and tadpoles. The main contributions on alfa-taxonomy, evolution and ecology of these ciliates were: (1) description and re-characterization of ciliate species for which little morphological information is available; (2) checklist and morphological characterization of ~90 ciliate species ascribed to the subclass Trichostomatia, endosymbiotic of herbivorous mammals (domestic horse, cattle, sheep and capybara), as well as ~20 new 18S-rDNA sequences; (3) systematic revision and molecular phylogeny of the families Blepharocorythidae, Cycloposthiidae and Ophryoscolecidae (Trichostomatia); (4) morphological characterization of ~50 ciliate species ascribed to the subclass Peritrichia, as well as ~30 new 18S-rDNA sequences; (5) contributions to the evolution of Peritrichia (18S-rDNA), with particular interest in Epistylididae and Operculariidae; (6) ecological aspects of the epibiotic relationship between peritrich ciliates and several groups of aquatic hosts, as copepods, molluscs, annelids, and insect larvae; (7) morphological characterization of ~20 ciliate species from bromeliads, with 10 new 18S-rDNA sequences; (8) morphological characterization of ~15 ciliate species associated to lichenized fungi, which represents a poorly known microcosms, with three new 18S-rDNA sequences; (9) characterization of prockaryotic symbionts of freshwater and brackish water ciliates; and (10) contributions to the study of ciliates as indicators of water quality in lotic systems and sewage treatment plants.

The Evolutionary History of Photosynthetic Stramenopiles Is Revealed by Transcriptomics of Undersampled Photosynthetic Lineages

KRISTINA X TERPIS[a], MATTHEW W. BROWN[b], HEROEN VERBRUGGEN[c], J. CRAIG BAILEY[d], CHRISTOPHER E LANE[a]

[a]University of Rhode Island, Kingston, RI, USA; [b]Mississippi State University, Mississippi State, MS, USA; [c]University of Melbourne, Parkville, VIC, Australia; [d]University of North Carolina-Wilmington, Center for Marine Science, Wilmington, NC, USA.

E-mail of the corresponding Author: kristina_terpis@uri.edu,

Stramenopiles are a major eukaryotic clade, branching with Rhizaria and Alveolata to form the supergroup SAR. Members of this diverse clade of protists are found in marine, freshwater, and terrestrial habitats and contain large numbers of both photosynthetic and non-photosynthetic species. There are currently 17 described classes of photosynthetic stramenopiles. Of these classes much is known about Bacillariophyceae due to their contribution to global biogeochemical cycles, and how large kelps create entire ecosystems. However, little is known about the rest of this diverse group. Historically, studies have used single gene or multigene phylogenies to investigate the evolutionary history of stramenopiles leaving most deep evolutionary relationships unresolved. Recent technological advances has made the generation of transcriptomic datasets fairly easy and cost effective, allowing for the alignments of hundreds of genes to be used for phylogenomic analyses. In an effort to establish a robust phylogeny for photosynthetic stramenopiles (Ochrophyta), 24 novel transcriptomes were constructed to investigate evolutionary relationships within this diverse group. For this research, we cultured photosynthetic stramenopiles from 8 different classes (Chrysophyceae, Chrysomerophyceae, Dictyochophyceae, Eustigmatophyceae, Phaeothamniophyceae, Pinguiophyceae, Raphidiophyceae, and Xanthophyceae), of which some are undescribed species and others are of uncertain taxonomic affinity. Our phylogenomic dataset was built from 246 conserved eukaryotic genes representing more than 50 photosynthetic stramenopiles. Our phylogeny robustly places taxa among the ochrophytes, which were previously considered *insertae sedis*. Novel resolved relationships among the Ochrophyta will be discussed.

The Net-Like Heterotrophic Amoeba *Leukarachnion* sp. PRA-24 (Ochrophyta, Stramenopiles) has a Cryptic Plastid

KARIN JAŠKE[a], TOMÁŠ PÁNEK[a], ANEŽKA ELIÁŠOVÁ[a], MAREK ELIÁŠ[a]
[a]Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic.

E-mail of the corresponding Authors: karin.jaske@gmail.com, marek.elias@osu.cz

In 1942 Geitler described *Leukarachnion batrachospermi*, a colourless amoeboid organism forming large anastomosing networks and walled cysts. Its phylogenetic affinity remained uncertain until Grant et al. (2009) studied strain PRA-24 (maintained at ATCC) identified as a potentially new species of the genus *Leukarachnion*. Phylogenetic analysis of four nuclear gene sequences obtained from *Leukarachnion* sp. PRA-24 revealed that it is a stramenopile related to the amoeboid algae *Chlamydomyxa labyrinthuloides* and *Synchroma grande* within Ochrophyta, and thus can be classified as a secondarily non-photosynthetic representative of Synchromophyceae. This raises a possibility that it has retained a colourless plastid, but no candidates for it were found by transmission electron microscopy. We employed an alternative approach and generated transcriptomic and genomic data from *Leukarachnion* sp. PRA-24. Initial analyses of our transcriptome assembly revealed transcripts encoding proteins with N-terminal regions that fulfill the definition of characteristic ochrophyte bi-partite plastid-targeting presequences. Among them are enzymes of the C5 pathway of haem biosynthesis, suggesting that *Leukarachnion* sp. PRA-24 has a plastid-derived organelle supplying the whole cell with haem. We also identified candidates for plastid-localized components of the translation apparatus, suggesting that the hypothetical plastid has retained a genome. Searches of an assembly of DNA sequencing reads from the *Leukarachnion* sp. PRA-24 culture indeed retrieved two contigs that appear to represent parts of the expected plastid genome. These contigs include genes for components of the transcription and translation machineries and the ClpC protein involved in protein turnover, whereas genes related to photosynthesis are missing. Altogether, our results demonstrate that *Leukarachnion* sp. PRA-24 has a cryptic plastid, whose more detailed characteristics are being investigated and will be presented.

Tintinnid's First Top Model: Ultrastructural Insights into the Oral Ciliature of *Schmidingerella meunieri* (Alveolata, Ciliophora)

MICHAEL GRUBER[a], SABINE AGATHA[b]

[a]Department of Biosciences, Paris Lodron University of Salzburg, Salzburg, Austria; [b]Department of Biosciences, Paris Lodron University of Salzburg, Salzburg, Austria.

E-mail of the corresponding Author: michael.gruber2@sbg.ac.at

The Oligotrichea are mainly marine planktonic ciliates with a globular or obconical cell shape and an apically located membranellar zone employed in both locomotion and feeding. Their closest relatives, the euplotids and hypotrichs, are dorsoventrally flattened benthic ciliates crawling on the substrate by means of their ventral cirri. For feeding, they use their ventrally located C-shaped adoral zone of membranelles. In contrast to the oligotrichids, which still possess the ancestral C-shaped membranellar zone, the choreotrichids have the membranelles arranged in a circle. Some aloricate choreotrichids obtained the ability to jump obviously only by means of their membranellar zone, and the about 1,000 tintinnid species (loricate choreotrichids) are attached inside a cumbersome lorica. Accordingly, the membranellar zone changed its position, function, and shape during the evolution of the Oligotrichea likely with effects on its ultrastructure, i.e., the links between the basal bodies of a polykinetid and the links between polykinetids. For a comparative study on the oral ciliature, ultrastructural data from euplotids and hypotrichs are available in the literature, whereas information is insufficient concerning the Oligotrichea. *Schmidingerella meunieri* (Kofoid & Campbell, 1929) Agatha & Strüder-Kypke, 2014 was chosen here as tintinnid representative because it has been investigated in various, primarily ecological respects, making it a suitable model organism. The findings on *S. meunieri* are the first detailed ones on tintinnid ciliates and Oligotrichea in general. Thus, the current analysis focused on the detection of apomorphies by comparing the situation in *S. meunieri* with that in the outgroup taxa, the euplotids and hypotrichs. Beyond some plesiomorphies, supposedly derived character states are actually discovered. However, more data are required from other tintinnids, aloricate choreotrichids, and oligotrichids for placing the apomorphies correctly in the cladograms and on the gene trees. The study was financially supported by the Austrian Science Fund (FWF Project P 28790).

Oral Session PROTIST AS MODEL AND BIOIDICATORS

Exploring Signatures of Spatial Cell Type Differentiation in *Capsaspora owczarzaki*, a Close Relative of Animals

SEBASTIÁN R. NAJLE[A,B], ARNAU SEBÉ-PEDRÓS[C], LINAS MAŽUTIS[D], IÑAKI RUIZ-TRILLO[E,F]

[a]Institut de Biología Evolutiva (CSIS-Universitat Pompeu Fabra), Barcelona, Spain; [b]Instituto de Biología Celular y Molecular de Rosario and Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR-CONICET), Rosario, Argentina; [c]Center for Genomic Regulation, Barcelona, Spain; [d]Institute of Biotechnology, Vilnius University, Vilnius, Lithuania. [e]Departament de Genètica, Microbiología i Estadística, Universitat de Barcelona, Spain; [f]*ICREA*, Barcelona, Spain.

E-mail of the corresponding Author: sebastian.najle@upf.edu

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. However, it is not clear whether spatial cell-type differentiation is a consequence of multicellularity or if it was already present in the most recent common ancestor of animals. To elucidate this, we setup single-cell RNA-Seq in the filasterean amoeba *Capsaspora owczarzaki*, one of the closest unicellular relatives of animals. The temporal differentiation cycle of *C. owczarzaki* include a stage of aggregative multicellularity. These aggregates, composed from few hundred to tens of thousands of cells, are assumed to be without spatial cell differentiation. However, at the moment there was no molecular evidence proving that all the cells in the aggregates are identical. Preliminary analyses of differential gene expression at single-cell resolution showed an unexpected molecular heterogeneity in the individual cells forming the aggregates. The characterization of these putative cell-types, as well as the molecular mechanisms underlying programs of cell differentiation will allow us to better understand the origin of cell-type differentiation during the evolution of animal multicellularity.

The Great "Life Cycle" Scam

FREDERICK SPIEGEL[a], MADISON JENNINGS[a], LORA SHADWICK[a]

[a]Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA.

E-mail of the corresponding Author: fspiegel@uark.edu

Since the mid 19th Century, biologists have been using the concept of "life cycle" to discuss and illustrate the states a particular kind of organism manifests as it progresses from a selected starting state through a series of changes until the next manifestation of that state. Presentations of "life cycles" can be highly detailed, or they can be quite simple. They are used as a metaphor for communicating with other specialists, and they are also used extensively to introduce newcomers to biology to the progression of organisms through various life stages. In either case, the "life cycle" metaphor introduces a number of severe limitations to our understanding of comparative developmental biology and evolutionary biology. The "life cycle" metaphor is especially problematic with regard to eukaryotes. Since the time of LECA, the stages an organism goes through has, of necessity, included portions of the life trajectories of more than one individual (here defined as a genetically and/or historically distinct entity). An individual goes through a life trajectory from its origin/birth to its death. Only part of its life trajectory is involved in participating in the production of a subsequent state or states in its "life cycle". In our discussion of "life cycles", we are prone to forget that the subsequent manifestation of the initial state is a new and unique individual, that such new individuals may be produced in great numbers, that they may arise from the interaction among several progenitors in previous states, and that significant amounts of time will pass between the initial manifestation of a trait and its subsequent appearance. These shortcomings can lead to all sorts of biological misinterpretation. We will present a number of more complex, but realistic approaches that biologists should consider using to replace the outdated "life cycle" scam.

Reactivated Channels Along Tufa Barrier as Favorable Habitat for Colonization of Microfauna

VESNA GULIN[a], RENATA MATONIČKIN KEPČIJA[a], MIRELA SERTIĆ PERIĆ[a]
[a]Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia.

E-mail of the corresponding Author: vesna.gulin@biol.pmf.hr

Uncontrolled growth of invasive tree species *Ailanthus altissima* (Mill.) Swinge, commonly known as tree of heaven, caused drying of water channels along the longest tufa barrier (Skradinski buk) located within the National Park Krka, Croatia. Removal of *A. altissima* resulted in reactivation of five channels, which have been dry for decades. Our goal was to investigate the impact of the invasive species removal on the periphytic microfauna colonization. To reach the goal, the invasive species was removed in several repetitive campaigns during 2017 and 2018. Periphyton samples were collected monthly from October 2017 to December 2018 at seven sites including five newly formed channels and two previously present channels. Periphyton was sampled on both natural (tufa) and artificial (glass slide) substrates. Additionally, water physico-chemical characteristics, organic matter content and tufa deposition rate at each site were measured. Water physico-chemical characteristics showed no significant difference among the newly formed and previously present channels, but they reflected a pronounced seasonal variability. Reactivated channels showed higher nitrite concentrations, but lower pH and dissolved oxygen concentrations, which was likely associated to higher organic matter content originating from forest soil developed on tufa barrier during dry phase. Previously present channels demonstrated denser moss cover and higher tufa deposition rate in comparison to the reactivated channels. Periphyton community in tufa substrate showed higher diversity in reactivated channels, presumably due to lower competition and predatory rates and/or intensive organic matter decomposition processes. Artificial substrate supported diverse community dominated by peritrichs, while vagile periphytic taxa dominated on tufa substrate. The latter could be partly explained by differences in sampling technique: artificial substrate allowed direct examination and is considered as a less destructive sampling method. Environmental data and protozoan assemblage patterns demonstrate that reactivated channels along the tufa barrier represent favorable habitats for microfauna colonization. Our results emphasize the need for multidisciplinary approach in monitoring tufa environments and a continuous and regular sampling in order to make a proper assessment of the complex substrate colonization processes.

Inter-Annual Variability of Summer Phytoplankton Community at Oil Spill Coasts Including the Hebei Spirit Oil Spill Site Near Taeanhaean National Park, Korea

WONHO YIH[a], HYUNG SEOP KIM[a], SOO-GUN JO[a], JAE SEONG KIM[b], YEONG DU YOO[a], KYEONG AH SEONG[a]
[a]Department of Marine Biotechnology, Kunsan National University, Kunsan, Korea; [b]Water&Eco-Bio Corporation, Gunsan, Korea.

E-mail of the corresponding Authors: ywonho@kunsan.ac.kr; kimjs90@kunsan.ac.kr; ydyoo77@kunsan.ac.kr

Right after the 2007 Hebei Spirit Oil Spill phytoplankton ecosystems were investigated for 11 years based on the seasonal monitoring of the composition and abundance of phytoplankton species. Comparable time-series data from the 1989 Exxon Valdez or the 2010 Deepwater Horizon Oil Spill sites were not available. It was suggested that the ecological healthiness of phytoplankton ecosystems at EVOS sites had recovered after 10 years following the oil spill based on chlorophyll concentrations even though these concentrations only represented phytoplankton communities in most cases. Chlorophyll concentration can only reflect limited aspects of highly complex phytoplankton ecosystems. During the last 11 years following the 2017 HSOS, extreme variabilities were met in the seasonally averaged ratios of diatoms to phototrophic flagellates including dinoflagellates based on the microscopic cell countings. Summer phytoplankton communities exhibited some cyclic inter-annual changes in dominant groups every 2−4 years. During the early years (2008−2010) cryptophytes or raphidophytes (*Chattonella* spp.) dominated alternately each year, which was repeated again in 2014, 2015 and 2017. Two thecate dinoflagellates, *Tripos fusus* and *Tripos furca*, together accounted for 52.5% and 50.0% of all organisms in the summers of 2011 and 2012, respectively, which was repeated again in 2018. Summer occurrence and dominance by the phototrophic flagellates including HABs (Harmful Algal Blooms) species as well as their inter-annual variabilities in the oil spill sites could be utilized as markers for the stable and long-term management of healthy ecosystems. For this type of scientific ecosystem management monitoring of chlorophyll concentrations may sometimes be insufficient to gain a proper and comprehensive understanding of phytoplankton communities located in areas where oil spills have occurred and harmed the ecosystem.

Detection of Potential Low-Oxygen Adaptations in Free-Living Testate Amoeba Using a Transcriptomic Approach

GIULIA M. RIBEIRO[a,b], XYRUS X. MAURER-ALCALÁ[b,c,d], LAURA A. KATZ[b], DANIEL J.G. LAHR[a]

[a]Department of Zoology, Biosciences Institute, University of São Paulo, São Paulo, Brazil; [b]Department of Biological Sciences, Smith College, Northampton, USA; [c]Program in Organismic and Evolutionary Biology, University of Massachussetts, Amherst, USA; [d]Current Address: Institute of Cell Biology, University of Bern, Bern Switzerland.

E-mail of the corresponding Authors: giuliamagriribeiro@gmail.com; dlahr@ib.usp.br

Low oxygen environments represent a challenge for organismic survival. Nevertheless, all eukaryotic groups have members that have developed adaptations to survive these kinds of environments. The anaerobic ability is attributed to the acquisition of genes for new metabolic pathways related to energy generation with little or no oxygen. Testate amoebae inhabit a wide variety of contrasted environments. One lineage of testate amoebae, that is a good bioindicator is *Arcella sp.*. There are many records of *Arcella* sp. in low oxygen environments, but the mechanism involved in the resistence of their cells in such stressful conditions remain undescribed. We obtained several transcriptome of *Arcella intermedia* and we used a bioinformatic pipeline that allowed us to annotate in detail the *Arcella intermedia* transcriptome. We were able to identify metabolic pathways and study their evolutionary history through phylogenetic reconstructions. In this work, we demonstrate evidence that *Arcella intermedia* and other two testate amoeba species have components of energy production pathways related to resistance in low oxygen environments. We also highlight that occupation of low oxygen environments by *Arcella* may be related to acquisition of new genes. Our results reveal the presence of anaerobic related pathways in the Tubulinea group, and should be considered in ecological studies.

Dinoflagellates Can Like It Cool and Mixed

SOLENN MORDRET[a], ROBERTA PIREDDA[a], MARINA MONTRESOR[a], ADRIANA ZINGONE[a], WIEBE H.C.F. KOOISTRA[a], DIANA SARNO[b]
[a]Integrative Marine Ecology (EMI), Stazione Zoologica Anton Dohrn, Naples, Italy; [b]Research Infrastructures for Marine Biological Resources (RIMAR), Stazione Zoologica Anton Dohrn, Naples, Italy.

E-mail of the corresponding Authors: solenn.mordret@szn.it, robpiredda@gmail.com

Dinoflagellates are important components of marine planktonic food webs, acting as primary producers, consumers, symbionts or parasites. Environmental metabarcoding assessments have demonstrated that dinoflagellates dominate marine planktonic communities both in terms of abundance and diversity. However, little information is available on dinoflagellate species dynamics and their occurrence in time. In this study, we investigated the temporal composition of the dinoflagellate community at the Long Term Ecological Research station LTER-MC in the Gulf of Naples (Mediterranean Sea) using the V4 barcode region of the 18S rRNA gene for 48 dates, sampled between 2011 and 2013. Dinoflagellates were 32.7% of reads and 5.7% of ribotypes of total protists. A total of 96 different genera belonging to 23 dinoflagellate Superclades were retrieved throughout the samples. Results of multivariate analyses revealed three principal seasonal clusters associated to winter, spring-summer and late summer-autumn conditions. LEfSe (LDA Effect Size) analyses made it possible to identify a series of biomarker taxa that characterized the three seasonal clusters: 30 taxa for winter, 18 for spring-summer and 19 for late-autumn. Extended local similarity network-based analysis (eLSA), performed on a reduced dataset (547 ribotypes representing 1.6% of the ribotypes but 74.5% of the reads at 97% similarity to a reference), displayed a single connected network structure. Within the network, three statistically supported modules were detected based on the topology. The strong seasonal pattern was confirmed by the observation that these modules matched with winter, spring-summer and late summer-autumn communities when compared to seasonal specialization annotations of each ribotype (i.e. nodes). Heterotrophic dinoflagellates dominated the winter module, while chloroplastidic dinoflagellates were more abundant in spring-summer. Overall, the network topology corresponded to the "small-world" property, which indicates robust communities with stable dinoflagellate successions and functional redundancy over three year seasonal cycles. This result also highlights the important role of biotic interactions in structuring assemblages in time as well as identifying highly connected "hub" nodes that are keystone dinoflagellate taxa which, if removed, would destabilize the seasonal community dynamic.

Oral session MOLECULAR & CELL BIOLOGY 1

Unexpected function of cytosolic Fe-Fe hydrogenase from *Trichomonas vaginalis*

TAMARA SMUTNÁ[a], ALENA DOHNÁLKOVÁ[a], JAN TACHEZY[a], IVAN HRDÝ[a]

[a]Department of Parasitology, Faculty of Science, Charles University, Prague, Czechia.

E-mail of the corresponding Authors: smutna2atur.cuni.cz; hrdy@natur.cuni.cz

Hydrogenases are specific group of ancient enzymes that evolved in anaerobic ecosystems. They usually catalyze reduction of protons and formation of hydrogen gas but under certain conditions can also function in hydrogen oxidation, utilizing it as the source of reducing power.

Trichomonas vaginalis belongs to microaerophilic protists that harbor hydrogenosomes, modified, anaerobic form of mitochondria. These small organelles participate in energy metabolism and accommodate several hydrogenase paralogues that release molecular hydrogen in a well-documented, ferredoxin-dependent pathway.

In our study we focused on a so-far undescribed cytosolic paralogue of Fe-Fe hydrogenases. Based on the experimental data we speculate that physiological function of this enzyme could be hydrogen consumption rather than release under conditions of increased need for reducing equivalents. Despite the notorious oxygen sensitivity of Fe-Fe hydrogenases, we were able to successfully isolate this enzyme from *T. vaginalis* cytosol. We also identified unexpected proteins that could function as electron acceptors.

Cyclic Polyploidy and Chromatin Extrusion in *Amoeba proteus* and Related Species

MARIIA BERDIEVA[a], SERGEI DEMIN[a], YULIA PODLIPAEVA[a], ANDREW GOODKOV[a]

[a]Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russia.

E-mail of the corresponding Authors: maria.berd4@yandex.ru; pelgood1@gmail.com

Asexually reproducing organisms accumulate mutations in series of sequential generations and need efficient mechanisms for zeroing their negative effect. One such mechanism may be cyclic ploidy, an alternation of polyploidization and depolyploidization during the life cycle (Lahr et al., 2011). Here we show that agamic amoebae *Amoeba proteus* and related species have a special type of cyclic polyploidy. It is known that the cell cycle of *A. proteus* and related species has two unusual features: the absence of G1 phase and an intensive DNA hyperreplication (Ord 1968; Makhlin 1987, 1993). We confirmed these data by microscopic and cytometric analysis of nuclei in the individual amoeba cells. Moreover, study of amoebae chromosome number revealed that their nucleus has an euploid status only for a small fraction of the cell cycle, during metaphase and early telophase. The rest of the time it has an aneuploid status, which is a consequence of hyperreplication. Chromatin extrusion – stretching and ejection of the chromatin material from the nucleus into the cytoplasm – in the late interphase and early prophase provides the depolyploidization. The circumnuclear actin filaments could carry out this ejection mechanically. We studied the extrusion process using fluorescent staining and confocal microscopy. Individual chromatin fibrils or their bundles and peripheral diffuse material could be eliminated. The chromatin extrusion was observed in six studied *A. proteus* strains and related species. The changes in the nucleus ultrastructure during extrusion process was described. The features of eliminated material were studied. A generalized scheme of the life cycle of *A. proteus* and related species, which includes polyploidization and depolyploidization stages has been proposed.

A Peculiar Plastid in a Novel Lineage of Non-Photosynthetic Chlamydomonadales

TOMÁŠ PÁNEK[a], KRISTÍNA ZÁHONOVÁ[a], SEBASTIAN C. TREITLI[b], NAOJI YUBUKI[c], TEREZA ŠEVČÍKOVÁ[a]; ELIŠKA ZADROBÍLKOVÁ[c], MARTIN SOKOL[a], VYACHESLAV YURCHENKO[a], IVAN ČEPIČKA[c], MAREK ELIÁŠ[a]

[a]Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic; [b]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic; [c]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic.

E-mail of the corresponding Author: mistrpanek@seznam.cz

Colourless chlamydomonadalean green algae constitute at least three unrelated lineages representing independent losses of photosynthesis. We investigated two new strains of non-photosynthetic flagellates (AMAZONIE, MBURUCU) isolated from microoxic freshwater sediments in South America. According to the rDNA region the two strains constitute a separate deep clade of Chlamydomonadales and represent two different species, consistent with their morphological differences. Using a combination of Illumina and Oxford Nanopore technologies, we are sequencing the plastid genome of the AMAZONIE strain (> 360 000 bps in length) that is nearly twice as large as the size of the largest non-photosynthetic plastid genome sequenced so far, that of the unrelated chlamydomonadalean *Polytoma uvella*. The even more extreme inflation of the AMAZONIE plastid genome is due to longer repeat-rich intergenic regions, but partially reflects a higher number of genes, including those for ATP synthase subunits completely absent from *P. uvella*. To understand the actual cellular function of the plastid of the AMAZONIE strain, we reconstructed the putative plastid metabolic network using transcriptome data. The plastid proved to house a rich set of biosynthetic pathways, including those for producing starch (in agreement with evidence from the electron microscope), amino and fatty acids, haem, isoprenoid precursors, purines and pyrimidines. Most surprising is the presence of the plastoquinone synthesis pathway, type II NADH dehydrogenase and plastiquinol terminal oxidase, indicating that chlororespiration takes place in the AMAZONIE plastid. The physiological significance of the latter process is under investigation.

Expression of Heterologous Genes in Dinoflagellates. Can the Nut Be Cracked?

CLAUDIO SLAMOVITS[a], RONIE HARO[a], PIA ELUSTONDO[a], SUSANA BREGLIA[a]
[a]Dalhousie University, Halifax, Nova Scotia, Canada.

E-mail of the corresponding Author: Claudio.slamovits@dal.ca

Dinoflagellates constitute a major clade of unicellular eukaryotes with vast ecological relevance that boast unique cellular and molecular characteristics resulting from a rich and intriguing evolutionary history. However, research on these important protists has been hampered by the lack of reliable and accessible molecular tools and methods such as genetic transformation. Recently, efforts to achieve genetic transformation and expression of heterologous proteins in other lineages of protists resulted in new genetically tractable model organisms including diatoms, choanoflagellates and related protists, diplonemids, et cetera, while dinoflagellates proved especially resilient to transformation. This presentation describes our efforts to transform the basal dinoflagellate *Oxyrrhis marina* through various methods, and analyzes and discusses our results in the light of the unusual cell and nuclear biology of dinoflagellates. While stable genetic transformation may prove hard and take a long time to achieve, we explored the alternative of using RNA encoding suitable marker proteins and present evidence for successful transient expression of heterologous proteins in O. marina via introduction of artificial mRNAs, which were designed to mimic mature mRNAs from dinoflagellates and synthesized in vitro. Due to its design, this method could be used in diverse dinofagellates with minimal modifications. This approach has the potential to enable experimental avenues such as probing protein targeting and localization which were previously unattainable.

Basal body Protein BBP1 in *Trypanosome brucei* Contributes to the Recruitment of Tyrosinated α-tubulin in Basal Bodies and Assembly of Pro-Basal Body

CONG-XI ZHANG[a], XIAO-KUN HONG[a], ZHAO-RONG LUN[a], DE-HUA LAI[a]

[a]School of Life Sciences, Sun Yat-sen University, China.

E-mail of the corresponding Author: laidehua@mail.sysu.edu.cn

Abstract: Basal bodies in *Trypanosome brucei*, which is the pathogen of human African trypanosomiasis (HAT), share similar architecture with centrioles in mammal cells. Basal bodies are formed in pair of two parts: the mature basal body as the root of flagellum and the pro basal body as the precursor of mature basal body. BBP1 is identified from a proteome of trypanosome basal bodies as an unknown protein. Firstly, we confirm BBP1‘s duel localization of both mature basal body and pro basal body via co-location with SAS6. BBP1 depletion by RNAi in *T. brucei* shows growth inhibition and results cells of multiple kinetoplasts and multiple nucleus, in addition the 9+2 structure in flagellum is also interrupt. Then, we carefully looked at early events occurs during beginning of RNAi BBP1 depletion to access possible mechanism. 8 hours after RNAi induction, the assembly process of pro basal body was interrupted, as the assemble of SAS6 was impeded in the new pBB and the newly assembled flagellum failed to attach to cell body also appeared after BBP1 RNAi. 24 hours after RNAi induction, the recruitment of tyrosinated α-tubulin in basal bodies was failed, however, the localization and expression of recruitment associated protein, TbRP2, were not affected. In reverse, in TbRP2 RNAi cells, both the location of BBP1 and the recruitment of tyrosinated α-tubulin in BBs were affected. By the evidences above, we here demonstrate BBP1 an important protein during cell division of *Trypanosoma brucei*, which may play roles in assembly of pro basal body and flagellum.

Supported by: the National Sciences Foundation of China (#31772445) and the Natural Science Foundation of Guangdong Province (2016A030306048)

Non-Photosynthetic *Cryptomonas* Conducts Inorganic Carbon Fixation?

GORO TANIFUJI[a], AKINORI YABUKI[b], MOTOO ITO[c], YUKI MORONO[c], HIDETAKA NOMAKI[d], YOSHIYUKI ISHITANI[e], RYOMA KAMIKAWA[f], YUICHIRO KASHIYAMA[g]

[a]Department of Zoology, National Museum of Nature and Science, Tsukuba, Japan; [b]Marine Biodiversity and Environmental Assessment Research Center, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Japan; [c]Kochi Institute for Core Sample Research, JAMSTEC, Kochi, Japan; [d]Super-cutting-edge Grand and Advanced Research Program, JAMSTEC, Yokosuka, Japan; [e]Center for Computational Sciences, University of Tsukuba, Tsukuba, Japan; [f]Graduate School of Global Environmental Studies, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan; [g]Graduate School of Engineering, Fukui University of Technology, Fukui, Japan.

E-mail of the corresponding Author: gorot@kahaku.go.jp

The majority of organic carbon on modern-day Earth is believed to be the product of photosynthesis. RuBisCO is the key enzyme responsible for carbon fixation in the photosynthetic machinery. Nevertheless, a part of photosynthesis-lacking eukaryotes retains RuBisCO-related genes. The retention of RuBisCO in non-photosynthetic organisms could be explained by recent losses of photosynthesis (i.e., on the process of gene losses), or alternative functions of RuBisCO such as involvement in lipid biosynthesis. However, experimental surveys on RuBisCO in non-photosynthetic organisms are limited in a few cases, resulting in controversy in functions of RuBisCO in non-photosynthetic organisms. *Cryptomonas paramecium* is an organism that has secondarily lost photosynthesis but still retains RuBisCO-related genes including *rbc*L and *rbc*S in the plastid genome. However, functional analyses of *C. paramecium* regarding carbon fixation have never been conducted. Here, we tested the possibility whether *C. paramecium* can fix the inorganic carbon by using genome/transcriptome as well as stable carbon isotope tracer ($^{13}C/^{12}C$) experiments. Genome/transcriptome analyses revealed that *C. paramecium* possesses other protein components necessary for the Calvin Benson cycle along with RuBisCO. While all protein genes for photosystem I,

1. and cytochrome *b*6/*f* complex are missing, the redox cofactor (i.e., plastid genome encoded ferredoxin) and biosynthetic pathway for the electron carrier (i.e., plastoquinone) are intact. The carbon isotope-labelling experiment on axenic cultures of *C. paramecium* exhibited an enrichment of ^{13}C in the cellular organic carbon, where bulk cellular organic carbon isotopic ratio was determined; $^{13}C/^{12}C$ ratio was elevated in cells grown in a $H^{13}CO3^-$-labelled medium without any organic nutrition, while the ratio was not significantly elevated in cells grown in the medium with an organic nutrition and $H^{13}CO3^-$-labelling. The carbon isotope imaging of the cells using a nano-scale secondary ion mass spectrometry (NanoSIMS) further revealed that the ^{13}C enrichment was localized in particular regions of the cells. These observations above suggest that *C. paramecium* possibly retains an ability of inorganic carbon fixation. We discuss on the possible mechanism for the carbon fixation in non-photosynthetic *C. paramecium*, including still uncovered mysteries such as the source of energy utilized in the carbon fixation.

Oral session TAXONOMY & PHYLOGENY 3

Systematic Assessment of Congruence in Multi-Gene Data

CAESAR AL JEWARI[a], SANDRA L. BALDAUF[a]

[a]Program in Systematic Biology, Uppsala University, Uppsala, Sweden.

E-mail of the corresponding Authors: caesar.aljewari@ebc.uu.se; sandra.baldauf@ebc.uu.se

Phylogenomics uses multiple genetic loci to construct evolutionary trees, usually as a single concatenated data set. One of the core premises in this approach is the assumption of congruence, i.e. similar, or approximately similar evolutionary history for the concatenated loci. Violation of this assumption can give unsupported results, or even highly supported erroneous results. Thus, assessing the congruency within a multigene dataset is a critical task. However, it is also a difficult task, and various attempts to implement solutions to this problem have shown uncertain degrees of success. Mostly, incongruence is still assessed primarily by single gene trees (SGTs). However, especially for deep phylogenetic nodes, SGTs lack sufficient clean phylogenetic signal to reveal congruence or incongruence.

The primary sources of incongruence are when paralogs or xenologs are mistakenly classified as orthologs, and to a lesser extent, when genes or taxa evolve in substantially different patterns. The first problem is not addressed by current tree building methods, which assume orthology. Parameter-rich evolutionary models should have the flexibility to cope with the second problem, but at a very high computational cost and risk of overfitting or, even worse, fitting more to artifact than signal.

We have developed a new algorithmic approach to assess incongruence in multigene data. This combinatorial statistical learning method uses repetitive subsampling (jackknifing) and group testing to rank incongruence per taxon per gene. The ranking is measured by quantifying topological inconsistencies and taxon placement deviation in data subsamples to extract taxon-gene stability ranks. Transforming the problem into a combinatorial analysis task also overcomes the problem of missing data by using concatenated subsets of genes, where each combination is very likely to have full taxon representation. This also reduces the dimensionality of the problem by reducing tree space. Results of testing of this method on deep nodes in the eukaryote tree will be presented.

Born to Crawl – New Phylogenomic Tree of Amoebozoa Based on Genome Wide Data Analysis

YONAS I. TEKLE[a], ALEXEY SMIRNOV[b], FIONA WOOD[a], O. ROGER ANDERSON[c]

[a]Dept. of Biology, Spelman College, Atlanta, Georgia, USA; [b]Dept. of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, Russia; [c]Dept. of Biology, Lamont-Doherty Earth Observatory of Columbia University, New York, USA.

E-mail of the corresponding Author: ytekle@spelman.edu

The supergroup Amoebozoa was assembled based on molecular phylogenetic analyses defying long held views of traditional taxonomy of amoeboid eukaryotes. The group unites a diversity observed in almost all cellular life forms and encompasses enigmatic lineages recalcitrant to modern phylogenetics. Our understanding of the group has substantially improved with recent analysis of phylogenomic studies. However, deep divergences, taxonomic placement of some key taxa and character evolution in the group largely remain poorly elucidated or controversial. Here we surveyed available Amoebozoa genomes to mine highly conserved single copy genes, which were used as a reference framework to enrich gene sampling from genomes/transcriptome data of amoebozoans spanning all known taxonomic diversity. This approach generated the largest supermatrix (1559 genes with over a half million amino acid sites) in the group to date. We recovered a well-resolved and supported tree of Amoebozoa with previously enigmatic taxa finding stable homes. In our analysis the deepest branching group is Tubulinea; the Tevosa hypothesis is not supported. Our results do not support the Conoza/Lobosa dichotomy; instead they support the idea of the subsequent branching of Tunbulinea and Discosea from the main stem of Amoebozoa evolution. This finding has important evolutionary implications, suggesting the amoeboid nature of the amoebozan Last Common Ancestor (LCA).

A Peculiar Trypanosomatid Lineage with Biflagellated Cells.

ALEXEI KOSTYGOV[a,b], ALEXANDER FROLOV[b], MARINA MALYSHEVA[b], ANNA GANYUKOVA[b], VIKTORIA SPODAREVA[a,b], DIEGO MACEDO[a], JANA KRÁLOVÁ[a], JULIUS LUKEŠ[c,d], DARIA TASHYREVA[c], VYACHESLAV YURCHENKO[a,e]
[a]University of Ostrava, Faculty of Science, Life Science Research Centre, Ostrava, Czech Republic; [b]Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia; [c]Biology Centre ASCR, v.v.i., Institute of Parasitology, České Budějovice, Czech Republic; [d]University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic; [e]Martsinovsky Institute of Medical Parasitology, Tropical and Vector Borne Diseases, Sechenov University, Moscow, Russia.

E-mail of the corresponding Author: kostygov@gmail.com

Trypanosomatids are well known owing to the dixenous representatives such as trypanosomes and leishmaniae causing severe diseases in humans and domestic animals. However, insect-restricted monoxenous trypanosomatids are also of great interest not only as models to study human pathogens, but also for their peculiarities such as bacterial symbionts, non-canonical genetic code etc. We turned our attention to a monoxenous flagellate that was reported to have a high proportion of biflagellated cells in the culture. Previously this phenomenon did not appear important and was explained by delayed division. This trypanosomatid was originally described as *Herpetomonas muscarum ingenoplastis.* Using molecular phylogenetic analysis we revealed that it does not belong to any of the trypanosomatid genera but represents a separate lineage. In addition, we isolated another biflagellated trypanosomatid with similar morphology and being related to "*H. m. ingenoplastis*". Using light, electron and fluorescent microscopy we demonstrated that both in culture and in the intestine of the insect host these trypanosomatids keeped their biflagellate state in the interphase and usually the two flagella adhered tightly to each other as if they were glued together with something. Monoflagellate state was transient and could be seen only right after the division. We speculate that double flagella appeared in these trypanosomatids in response to the need to thicken the motile part of the cell. In other trypanosomatids, this is achieved by attaching flagellum to the lateral surface of cell body with the formation of either epi- or trypomastigotes.
This work was supported by the Russian Science Foundation grant 18-14-00134 and European Regional Funds (grant 16_019/0000759).

New Classification of Cyanidiophyceae (Rhodophyta) Based on Comparative Analysis of Organelle Genomes.

SEUNG IN PARK[a], CHUNG HYUN CHO[a], CLAUDIA CINIGLIA[b], HWAN SU YOON[a]
[a]Department of Biological Sciences, Sungkyunkwan University, Suwon, Korea; [b]Department of Environmental, Biological and Pharmaceutical Science and Technologies, University of Campania "L. Vanvitelli", Caserta, Italy.

E-mail of the corresponding Authors: marine376@naver.com; hwansu@gmail.com

Cyanidiophyceae is a class of unicellular red algae that thrives in acidic (pH 0.5-3.0), high temperature (50-55°C) and heavy-metal rich extreme environments in volcanic hot springs around world. These algae are primarily photo-autotrophic but some of these species (i.e. *Galdieria sulphuraria*) have heterotrophic or mixotrophic growth. Boundary of genus within the Cyanidiophyceae is hard to determine based only on morphological feature due to its very simple morphology. In addition to morphology, physiological and molecular data are needed to find its real identity. To better understand its evolutionary history and clarify relationship within the Cyanidiophyceae, five complete mitochondria and plastid genomes (*Galdieria maxima* 8.1.23, *Cyanidium caldarium* ACUF 063, Mesophilic *Cyanidium* sp., *Galdiria sulphuraira* SAG 108.79, *Galdieria sulphuraria* DBV 011) were constructed in this study. With published Cyanidiophyceae genome data, we compared genome size, GC contents, number of protein coding genes and genome structure in organelle genomes. We found a clear separation between the Galdieriales clade (*Galdieria sulphuraria* and *G. phlegrea*) and the Cyanidiales clade (*Cyanidium caldarium,* Mesophilic *Cyanidium, Cyanidioschyzon merolae*, and *Galdieria maxima*). Based on these organelle genome data, we propose new classification system in this presentation.

Born to Crawl – the Origin and Evolution of Amoebozoa

ALEXEY SMIRNOV[a], YONAS TEKLE[b], FIONA WOOD[b], O. ROGER ANDERSON[c]

[a]Dept. of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; [b]Dept. of Biology, Spelman College, Atlanta, Georgia, USA; [c]Dept. of Biology, Lamont- Doherty Earth Observatory of Columbia University, New York, USA.

E-mail of the corresponding Author: alexey.smirnov@spbu.ru

It was accepted for more than two decades that Amoebozoa are split in two large clades – the Lobosa, comprising Tubulinea and Discosea and the Conosa, the latter include Eumycetozoa, Variosea and Archamoebea. However, recent multigene phylogeny does not support this branching, instead it suggests subsequent divergence of Discosea and Tubulinea lineages from the main stem of amoebozoan tree. Thus the model that suggests the origin of Amoebozoa from a flagellated ancestor must presume independent and complete loss of kinetosomes and flagella in two of three their major evolutionary lineages and numerous modifications and losses in the remaining one – Evosea (Cutosea plus Conosa). The reason for such massive losses remains unclear. In the present talk we propose a non-flagellated origin of Amoebozoa exemplified by basal lineages belonging to Discosea or Tubulinea. We suggest a hypothesis, with evidence from morphology and genetics, that the ancestral amoebozoan might be an amoeboid organism, not a flagellate. In this model the origin of Amoebozoa was triggered with the loss of flagellum and kinetosomes in amoebozoan Last Common Ancestor (LCA). To retain the motility, LCA of Amoebozoa had to develop an acto-myosin system, which gave rise to the amoeboid movement. Crawling way of life provided this organism with certain adaptive advantages, the major one probably was an ability to increase body size. The ability to build the kinetosomes, was only reemerged in the crown of the amoebozoan tree that is at the base of Evosea clade and gradually reappearing within various groups of Evosea. A reappearance of biflagellated cells is also observed but only in the very crown group – Eumycetozoa and only as a part of their complex life cycle. It seems that reemerging of kinetosomes was an event that launched the formation of complex life cycles that are entirely absent in Tubulinea and Discosea, but present in Variosea, Archamoebea and reached top complexity in the Eumycetozoa. Supported with RSF grant 17-14-01391 (AS) and NSF Grant #1831958 (YT).

Further Investigations on the Phaeothamniophyceae Using a Multigene Phylogeny, with Descriptions of Five New Species

LOUIS GRAF[a], ROBERT A. ANDERSEN[b], HWAN SU YOON[a]

[a]Department of Biological Sciences, Sungkyunkwan University, Suwon, Korea; [b]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, USA.

E-mail of the corresponding Author: louis.graf@gmail.com

The heterokont algae are an exceptionally diverse group of protist that includes a wide range of morphological types such as amoeboid, capsoid, coccoid, flagellates, brown seaweeds, and silica-walled diatoms. Recent phylogenetic analyses show that there are three major clades of photosynthetic heterokont algae, termed the SI, SII and SIII clades. Within the SI clade, the class Phaeothamniophyceae regroups freshwater filamentous genera such as *Phaeothamnion* and nonfilamentous genera (e.g. *Phaeoschizochlamys*, *Stichogloea*). These genera have been reported from around the world. Because their cell walls are tough and it is difficult to obtain DNA from cells, there are limited reports for this class from environmental DNA sequences. Consequently, the number of phaeothamniophytes investigated using molecular techniques has remained small.

In this study, we examined 12 strains representing eight species classified in the algal class Phaeothamniophyceae. Based upon a five-gene molecular phylogeny (nuclear-encoded SSU rRNA and plastid-encoded *psa*A, *psb*A, *psb*C and *rbc*L) and light microscopic observations, we describe five new species: *Phaeoschizochlamys santosii* sp. nov., *Phaeoschizochlamys siveri* sp. nov., *Phaeothamnion wetherbeei* sp. nov., *Stichogloea fawleyi* sp. nov. and *Tetrachrysis dopii* sp. nov. Molecular phylogenetic analyses proved more reliable evidences for distinguishing species. The Phaeothamniophyceae, as delimited here, form a monophyletic group that is sister to the Aurearenophyceae. Evolutionary trends within the Phaeothamniophyceae and in the SI clade are discussed.

WEDNESDAY 31 July

PLENARY LECTURE

Adaptation Strategies of the Invasive Bloom-Forming Dinoflagellates in Brackish Waters

SERGEI SKARLATO[a]
[a]Department of Cytology of Unicellular Organisms, Institute of Cytology RAS, St. Petersburg, Russia.

E-mail of the corresponding Author: sergei.skarlato@mail.ru

Many planktonic dinoflagellates play a crucial role in the marine coastal waters by forming harmful algal blooms (HABs) or "red tides" that misbalance the flows of matters and energy in the ecosystems and negatively impact aquatic flora, fauna, water quality and human health. In Europe, invasion of the semi-closed brackishwater Baltic Sea by dinoflagellates *Prorocentrum minimum* and their HAB-formation history therein are of the utmost ecological and economic importance for human population of nine countries located along the Baltic shores. Currently, *P. minimum* is one of the five dinoflagellate species from the genus *Prorocentrum* and the only one phytoplankton species which can be considered truly invasive in the Baltic Sea. In this study, the data on biology and peculiar invasive history of the potentially toxic dinoflagellate *P. minimum*, its reaction to abrupt external stresses, cellular and molecular adaptation strategies, ecological niche dimensions, population heterogeneity and mixotrophic metabolism that empower distribution of this harmful species in the Baltic coastal waters are presented. *P. minimum* has a great potential for mixotrophy, which is a key to understanding its success in eutrophic coastal regions that experience drastic fluctuations of nutrient loads (in terms of both concentration and chemical composition). Intrapopulation variability represents another effective mechanism to maintain dinoflagellate population under changing environmental conditions which ensures their survival, even after the exposure to severe stress. Mixotrophic metabolism and cell-to-cell intrapopulation heterogeneity are of particular importance for *P. minimum* flourishing in the fluctuating brackishwater environment. This research was carried out within the frames of the newly emerged research discipline – Translational Aquatic Ecology, which implies close linkage of cellular biology, molecular ecology and bioinformatics with the classical ecological theories and practices. Topicality of such synthesis is defined by the exceptional ecological and socio-economic importance of harmful dinoflagellates for humans and their environment since these protists may negatively impact ecosystem health, fisheries, aquaculture, recreation and tourism. Funded by the Russian Science Foundation, project 19-14-00109.

SYMPOSIUM - Free-living amoeba and neglected pathogen protozoa: health emergency signals? (by FEPS)

A Comparative 'Omics Approach to Pathogenicity in the Brain-Eating Amoeba, *Naegleria fowleri*

EMILY K. HERMAN[a], FRANCINE MARCIANO-CABRAL[b], NORBERT MÜLLER[c], TOM WALSH[d], GEOFFREY PUZON[d], JOEL B. DACKS[a]
[a]Division of Infectious Disease, Department of Medicine, University of Alberta, Edmonton, Canada; [b]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, Virginia, USA; [c]Institute of Parasitology, University of Bern, Bern, Switzerland; [d]The Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia.

E-mail of the corresponding Author: dacks@ualberta.ca

Of the 40 described *Naegleria* species, only *N. fowleri* can establish infection in humans, killing almost invariably within two weeks. In the brain, the amoeba performs trogocytosis of brain material causing massive inflammation. Its non-pathogenic sister species Naegleria gruberi is used as a laboratory model organism and has a fully sequenced genome. The exact pathogenicity factors distinguishing *N. fowleri* from its harmless relatives are unclear. We have here taken an -omics approach to understanding *N. fowleri* biology and infection at the system level. We provide the first estimate of strain diversity at the full genome level, finding little conservation in synteny but high conservation in protein complement. We also demonstrate that the *N. fowleri* genome encodes a similarly complete cellular repertoire as was found in its sister species *Naegleria gruberi*. This complement includes up to ~500 proteins as potential pathogenicity factors, being absent from the harmless *N. gruberi* but present in *N. fowleri* strains. Futhermore our transcriptomic analysis of low versus high pathogenicity *N. fowleri* cultured in a mouse infection model allowed us to identify over 300 differentially regulated genes. Overall, these studies have allowed us to develop the first systems-level model for the cellular basis of pathogenicity in this enigmatic organism.

Pathogenicity in *Acanthamoeba*

JULIA WALOCHNIK[a]
[a]Institute for Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria.

E-mail of the corresponding Author: julia.walochnik@meduniwien.ac.at

Acanthamoeba spp. are ubiquitous free-living amoebozoans and do not need a host. They can, however, cause disease upon accidental contact with a potential host. In humans, they are the causative agents of a painful inflammation of the cornea, the so-called *Acanthamoeba* keratitis (AK), and of several disseminating infections potentially resulting in granulomatous amoebic encephalitis (GAE) in the immunocompromised host. The first cases of AK were reported in the 1970ies and in the mid-eighties an association between AK and contact lens wear was established. Today, acanthamoebae, besides pseudomonads and staphylococci, are regarded as the most important causative agents of keratitis in contact lens wearers. AK often shows a severe progression, which is due to a lack of awareness but also to the lack of specific treatment. The metabolically inactive cysts pose a particular problem, as they may reside within the tissue and lead to reinfection after termination of treatment. In industrialised countries the annual incidence of AK lies between 0.1-1 cases per 100,000 inhabitants, with a marked regional variation depending on contact lens wear habits and mode of water supply. In contrast to the early years, today, males and females are equally affected and the most vulnerable group are the 21-30-year-olds. For GAE, less than 500 cases have been published worldwide since its discovery. The pathogenicity of *Acanthamoeba* depends on cell-cell contact and is characterised by contact-mediated cytolysis, the contact being established via lectin-like amoebic adherence molecules. The ability of acanthamoebae to lyse cells is mainly based on hydrolases and phospholipases, whereby the release of a 133-kDa serine protease termed mannoseinduced protein (MIP) 133 seems to be a crucial step in the pathogenic cascade. Moreover, acanthamoebae may induce host cell death and overcome host defence by the release of adenosine diphosphate (ADP) and they also can express a pore-forming protein, the so-called acanthaporin.

Free Living Amoebae as Pathogens and as Vectors of Endocytobionts

PATRICK SCHEID[a]
[a]*Laboratory of Medical Parasitology, Central Military Hospital Koblenz, Department XXI Medical Microbiology, Koblenz, Germany.*

E-mail of the corresponding Author: patrickscheid@uni-koblenz.de

Free-living amoebae (FLA) can be detected in specimen, both, in the natural aquatic environment and in artificial, man-made aquatic environments. Some strains of these FLA can be pathogenic to humans and animals. As etiological agents of the so -called Acanthamoebiasis, some strains of the genus *Acanthamoeba* can cause several specific disease symptoms in humans. The most well known disease is the *Acanthamoeba* keratitis (AK). The AK is not necessarily associated with an immune suppression, but rather with a trauma, exposure to contaminated water or, particularly, the improper handling of contact lenses, which promotes infection. The Acanthamoebiasis of the central nervous system is called granulomatous amebic encephalitis (GAE). The GAE differs from the primary amoebic meningoencephalitis (PAM), a very progressive and dangerous encephalitis, which is caused by *Naegleria fowleri* (the "brain eating amoeba"). The clinical picture of GAE by *Balamuthia mandrillaris* is characterised by a chronic progress with headache and neck stiffness.
The prevalence of FLA in water networks is associated with biofilms, where they live sympatric within a biocoenosis together with other microorganisms. In such a biocoenosis there are multiple interactions between FLA and other microorganisms:
In addition to their role as pathogens, FLA are known to serve as hosts of and vehicles for the transfer of various intracellular organisms (fungi, viruses, bacteria, other eucaryonts), some of them being natural human pathogens. They act as reservoir or vector of microorganisms like *Legionella* sp., *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Mycobacterium* sp., *Cryptosporidium* sp. and members of the Parachlamydiaceae. In the cyst-stage of the FLA, these intracellular organisms (endocytobionts) are protected to a high degree against any adverse environment (FLA as "Trojan horse"). This may lead to risks to health in terms of the development of pathogenicity/virulence and antibiotic resistance (FLA as "Trainings ground").
In conclusion, it can be stated that FLA pose a considerable risk regarding Environmental Health and Public Health significance, acting as human parasites and as vectors of for phylogenetically diverse microorganisms. Environmental and climatic changes are affecting the FLA abundance, which may lead to an increase of infectious diseases associated with FLA or their endocytobionts.

Intestinal protozoa searching for a disease or for a doctor?

ADRIANA CALDERARO[a]
[a]Department of Medicine and Surgery, University of Parma, Parma, Italy.

E-mail of the corresponding Author: adriana.calderaro@unipr.it

Although highly prevalent in developing countries, intestinal parasitoses frequently afflict subjects in industrialized ones as well. However, besides the agents with a well-known clinical relevance and those with an open debate about it (*Blastocystis hominis* and *Dientamoeba fragilis*), limited information is available about neglected protozoan infections such as those caused by *Cyclospora cayetanensis*, *Isospora belli*, *Enteromonas hominis*, *Iodamoeba buetschlii, Balantidium coli*, and free-living amoebae. In our laboratory, from 2011 to 2018, conventional diagnosis of intestinal parasitosis (microscopic examination of fresh/concentrated faeces and cultivation in Robinson's medium) was performed on 20,978 faecal samples belonging to 13,596 patients, all presenting with the clinical suspicion of intestinal parasitosis; real-time PCR assays for the differentiation of *Entamoeba histolytica* and *E. dispar* and for the detection of *D. fragilis* were also used when clinical manifestation and/or risk factors for parasitic infections were reported, and/or when diagnostic stages of intestinal parasites were detected by microscopy. Intestinal parasitosis was diagnosed in 2062 patients (15.2%), about half of whom were immigrants from developing countries; of these cases, 1957 caused by protozoa (14.4%). The most common intestinal protozoan detected was *B. hominis* (2047 cases), followed by *D. fragilis* (467 cases) and *G. intestinalis* (195 cases). Interestingly, only one case of infection by *I. belli* together with *B. hominis* was diagnosed in an HIV-positive patient with fever and diarrhea and documented absence of enteropathogenic agents (bacteria, parasites, viruses). The data presented confirm the importance of suspecting protozoan infections even in non-endemic areas, particularly in cases in which no pathogens other than protozoa are present, and indicates the value of adopting adequate diagnostic methods. Data regarding the detection of enteropathogenic agents other than parasites and data regarding cases of clinical symptoms resolved only after therapeutic intervention and eradication of the infection caused by *B. hominis* and/or *D. fragilis* will be discussed. Knowledge of the epidemiology of intestinal parasitosis is important for health care authorities all over the world, so that they can understand the role of an infectious agent in causing disease, and thus adopt appropriate control measures and provide adequate patient care.

SYMPOSIUM - Host-Parasite Interactions in Vectorborne Protozoan Infections (by FEPS)

Babesia species of Domestic Dogs – Where do they Fit in the Piroplasmid World?

GAD BANETH[a]
[a]Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel.

E-mail of the corresponding Author: gad.baneth@mail.huji.ac.il

Babesia are tick-borne protozoan parasites from the phylum Apicomplexa, class Piroplasmea and order Piroplasmida which infect erythrocytes of animals and humans. The main effects of babesiosis are related to anemia, tissue anoxia, hemolytic toxins and inflammatory mediators produced during infection. The babesiae are divided into several lineages as assessed by 18S RNA gene phylogeny. The babesial species that infect dogs are separated into those that present with a large merozoite stage in erythrocytes (5 x 2 µm) termed "large" canine *Babesia* which belong to the *Babesia* sensu stricto and those which have distinctly smaller merozoites (0.3 x 3 µm) termed "small" and classified as *Babesia* sensu lato. The clinical manifestations of babesiosis are mainly dependant on the infecting species and host-related factors. *Babesia rossi, B. canis* and *B. vogeli* have large merozoites and are identical morphologically but differ in the severity of clinical manifestations which they cause, their tick vectors, genetic characteristics, and geographic distributions. Another yet unnamed large *Babesia* sp. most closely related to *B. bigemina* infects immunocompromised dogs in North America. The small *Babesia* spp. that infect dogs include *B. gibsoni*, *B. conradae* described from California, and *B. vulpes*. None of the *Babesia* species that infect dogs is known to be zoonotic. The transmission of babesiae occurs through the bite of a vector tick. However, *Babesia* infection has also been demonstrated to be transmitted via blood transfusion and transplacentally. Furthermore, several studies have provided evidence that *B. gibsoni* is likely transmitted directly from dog to dog via bite wounds, saliva, or ingested blood. As *Babesia* spp. are transmitted by blood product transfusions, it is recommended to screen canine blood donors for infection. Non-vectorial dog to dog transmission of babesiae by fighting can be responsible for the spread of babesiosis into non-endemic areas. The differences between *Babesia* spp. that infect dogs are also reflected in their susceptibility to drugs. Accurate detection and species recognition are important for the selection of the correct therapy and predicting the course of disease. While large form *Babesia* spp. are usually susceptible to certain drugs, small form *Babesia* are often resistant and treatment requires the use of other drugs and their combinations. Vaccines against some canine *Babesia* species are commercially available in some countries in Europe.

New *Leishmania* Parasites and New Vectors

PAUL BATES[a]
[a]Biomedical and Life Sciences, Lancaster University, Lancaster, UK.

E-mail of the corresponding Author: p.bates@lancaster.ac.uk

Leishmania are protozoan parasites that are transmitted by the bites of phlebotomine sand flies, small blood-feeding dipteran insects. Recent work has provided evidence for a new phylogenetic grouping of *Leishmania* parasites, raised to subgenus level as *Leishmania* (*Mundinia*), in addition to the three existing subgenera *Leishmania*, *Viannia* and *Sauroleishmania*. Members of *Leishmania* (*Mundinia*) include *L. enriettii* (from guinea pigs in Brazil), *L. martiniquensis* (from humans in Martinique, but now known to have a wider distribution), *L. macropodum* (from kangaroos in Australia), *L. orientalis* (from humans in Thailand), and two unnamed *Leishmania* species from Ghana and Namibia. Some of these parasites are known human pathogens, whilst others appear to be confined to wild animals. The very wide geographical spread of the *Leishmania* (*Mundinia*), wider than any of the other subgenera, together with their branching from the base of the *Leishmania* phylogenetic tree, both suggest an early branching and ancient lineage. The insect vectors of the *Leishmania* (*Mundinia*) have not been established with certainty for any member. It has been speculated that *L. enriettii* has a phlebotomine sand fly vector, however, this has not been proven, and *L. enriettii* is unable to establish mature infections in *Lutzomyia longipalpis*, a widely used permissive sand fly host. In the case of the *L. macropodum* evidence to date supports the vectors to be day-biting midges of the genus *Forcipmyia*, although transmission by midge-bite has not been demonstrated. *Leishmania* from Ghana, Thailand and *L. enriettii* are able to develop better in experimental midge vectors (*Culicoides*) than in *Lu. longipalpis* under laboratory conditions, and some species of *Sergentomyia* sand flies have been found PCR-positive in Ghana and Thailand. Therefore, the current evidence indicates the *Leishmania* (*Mundinia*) have non-conventional vectors, and likely not to be *Lutzomyia* or *Phlebotomus* species, the usual vectors. It appears that some of the *Leishmania* (*Mundinia*) may have non-sand fly vectors. This remains to be proven or disproven, however, if true, this would require a re-definition of the genus *Leishmania* to include non-sand fly transmission or the creation of a new genus to accomodate the non-sand fly transmitted parasites

Plasmodium Development in the Mosquito Midgut. A Molecular and Cellular View

TOMASINO PACE[a], FELICIA GRASSO[a], GRAZIA CAMARDA[a,d], CATHERINE SUAREZ[b], MICHAEL J. BLACKMAN[b,c], MARTA PONZI[a] AND ANNA OLIVIERI[a].
[a]Dipartimento di Malattie Infettive, Istituto Superiore di Sanità, Rome, Italy; [b]Malaria Biochemistry Laboratory, The Francis Crick Institute, London, UK; [c]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK; [d]current address: Parasitology Department, Liverpool School of Tropical Medicine, Liverpool, UK.

E-mail of the corresponding Author: anna.olivieri@iss.it

Malaria is a devastating disease caused by unicellular protozoa belonging to the *Plasmodium* genus. In 2017, WHO reported 216 million malaria cases globally and 445 thousand lethal outcomes, mainly in children under five years of age. *Plasmodium* asexual stages are responsible for the clinical manifestations of the disease, while the sexual stages, called gametocytes, are essential for parasite transmission by the mosquito vector. As drug resistant *Plasmodium* strains periodically emerge, available therapies are becoming vulnerable and the development of novel therapeutic targets is today a research priority. In particular, drugs targeting gametocytes, used in combination with drugs against the asexual stages, have the dual advantage of lowering parasite transmission and preventing the spread of drug-resistant parasites.
The *Plasmodium* subtilisin-like serine protease SUB1 is expressed in both asexual and sexual parasite stages. SUB1 was shown to be essential for egress of parasite asexual stages from the host cell, but its subcellular localization, function and potential substrates in the sexual stages are still unknown. Here we have characterized the expression profile and subcellular localization of SUB1 in *Plasmodium berghei* sexual stages. We show that the protease is selectively expressed in mature male gametocytes and localizes to secretory organelles known to be involved in gamete egress, called male osmiophilic bodies. We have investigated PbSUB1 function in the sexual stages by generating *P. berghei* transgenic lines deficient in PbSUB1 expression or enzyme activity in gametocytes. Our results demonstrate that PbSUB1 plays a role in male gamete egress. We also show for the first time that the PbSUB1 substrate PbSERA3 is expressed in gametocytes and processed by PbSUB1 upon gametocyte activation.
Taken together, our results strongly suggest that PbSUB1 is not only a promising drug target for asexual stages, but could also be an attractive malaria transmission-blocking target.

Oral Session- MOLECULAR & CELL BIOLOGY 2

Characterizing the DNA N^6-adenine Methyltransferase AMT1 in the Unicellular Eukaryote *Tetrahymena thermophile*

YUANYUAN WANG[a,b*], YALAN SHENG[a,b*], YONGQIANG LIU[a,b*], WENXIN ZHANG[c], TING CHENG[a,b], LILI DUAN[a,b], SHAN GAO[a,b,c]

[a]Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao, China; [b]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China; [c]School of Life Science, Anhui Agricultural University, Hefei, China; [d]College of Marine Life Sciences, Ocean University of China, Qingdao, China; [*]These authors contributed equally.

E-mail of the corresponding Author: shangao@ouc.edu.cn

DNA N^6-adenine methylation (6mA) is newly rediscoveredas a potential epigenetic mark across a more diverse range of eukaryotes than previously realized. Despite continuous reports of its presence in various eukaryotes, the identity of the adenine specific DNA methyltransferase (MTase) has long been a puzzle in the field and the molecular mechanisms underlying the deposition of this mark remain poorly understood in eukaryotes. In the unicellular eukaryote *Tetrahymena thermophila*, 6mA accumulates at robust levels (> 0.6%) and shows a highly specific pattern of distribution as an integral part of the chromatin landscape. These argue for its biological significance and indicate that it is deposited by a specific methyltransferase(s), rather than random uncatalyzed methylation. We here report that AMT1 (adenine methyltransferase 1) catalyzes DNA 6mA deposition in *Tetrahymena*. DNA 6mA levels are globally reduced and methylation pattern was dramatically changed in *AMT1* cells. *AMT1* cells showed growth defects and compromised development, which are underpinned by a large number of differentially expressed genes. The discovery of AMT1 as a physiologically relevant DNA methyltransferase strongly supports 6mA as an epigenetic marker important for regulating eukaryotic cell growth and development.

Diversity of Endosymbiotic Bacteria in Thecofilosea (Cercozoa, Rhizaria)

MARCEL D. SOLBACH[a], MICHAEL BONKWOSKI[a], KENNETH DUMACK[a]
[a]Institute of Zoology, Terrestrial Ecology, University of Cologne, Köln, Germany.

E-mail of the corresponding Authors: msolbach@smail.uni-koeln.de; m.bonkowski@uni-koeln.de; kenneth.dumack@uni-koeln.de

Endosymbiotic bacteria are frequently found within different groups of amoebae, especially amoebae in the Opisthokonta have been investigated for such. Still however, novel clades are described and their specific distribution is poorly understood. To fill this gap of knowledge strains of thecofilosean amoebae (Cercozoa, Rhizaria) have been isolated, cultivated and investigated for endosymbiotic bacteria. Presence of bacteria within multiple amoeba strains has been verified by fluorescence in situ hybridization (FISH) and sequencing of bacterial 16S rDNA. Various closely related, but undescribed Gammaproteobacteria have been found in multiple strains of *Fisculla terrestris* and one strain of *Lecythium hyalinum*. In one strain of *Fisculla terrestris*, a *Chryseobacterium* (Flavobacteriia) species was found, implying that closely related host species do not necessarily contain closely related endosymbionts. In one strain of *Rhogostoma* sp. a *Legionella* (Gammaproteobacteria) species was found, indicating Thecofilosea as potential vectors for human pathogens. Multiple strains of various species within the Thecofilosea will be analyzed and their potential endosymbionts sequenced. Specific FISH probes will be used to elucidate whether the obtained sequences actually belong to bacteria within the amoeba cells. Phylogenetic analysis of the bacteria and amoeba sequences will be performed to get insight into the coherence of endosymbiont and host species, as well as their diversity. Since this study is still ongoing and unpublished, no publication could be uploaded.

Splicing Pathway of Nuclear Transcripts in *Euglena gracilis*

NATALIA GUMIŃSKA[a], MAGDALENA PŁECHA[a], BOŻENA ZAKRYŚ[a], RAFAŁ MILANOWSKI[a]
[a]Department of Molecular Phylogenetic and Evolution, Faculty of Biology, University of Warsaw, Warsaw, Poland.

E-mail of the corresponding Author: milan@biol.uw.edu.pl

Nonconventional introns found in nuclear genes of euglenids and marine diplonemids exhibit some peculiar features, such as: (I) variable, non-conserved border(s), which do not follow the GT/C-AG rule, typical for spliceosomal introns and (II) a slightly conserved, stable secondary structure which brings together intron ends, placing adjacent exons in close proximity. Nevertheless, this arrangement does not resemble the conformation of self-splicing or tRNA introns. Nonconventional introns are excised from pre-mRNAs by an unknown, nonautocatalytic and probably spliceosome-independent mechanism. To gain insight into this process, and to determine the order of events during pre-mRNA maturation in euglenids, we have studied the order of intron removal from transcripts of model organism *Euglena gracilis*. For this purpose we have selected *tubA* and *gapC* genes, which concurrently harbor introns of both types (nonconventional and spliceosomal). The relative splicing pathway was first examined using a RT-PCR strategy: in an experiment based on pairwise comparison of molecules that contain one intron and have either still present or excised adjacent one (regardless of the intron type). In addition, all intermediate products of splicing were reverse transcribed and analyzed using the PacBio Next Generation Sequencing platform. It turned out that nonconventional introns are removed in a rapid way but later than spliceosomal ones. What is more, the accumulation of molecules with conventional introns removed and nonconventional still present may suggest the existence of a temporal and/or spatial gap between the two types of splicing.

The Pheromone Genes of the Ciliate *Euplotes* Generate Multiple Transcripts by Alternative Intron Splicing and Sense-Antisense Transcription

ADRIANA VALLESI[a], PIERANGELO LUPORINI[a]
[a]Laboratory of Eukaryotic Microbiology and Animal Biology, University of Camerino, Camerino, Italy.
E-mail of the corresponding Authors: adriana.vallesi@unicam.it; piero.luporini@unicam.it

In *Euplotes*, cell-type distinctive protein pheromones control a self/non-self recognition mechanism responsible for the cell switching between the vegetative and sexual stages of the life cycle. Their coding genes are expressed in the cell somatic macronucleus, where they represent the transcriptionally active versions of transcriptionally silent alleles at the micronuclear mating-type (*mat*) locus. Significant numbers of full-length pheromone-gene sequences have been determined from species that occupy different positions in the *Euplotes* phylogenetic tree. Except in *E. petzi*, which occupies the basal position in the tree, pheromone genes show a 5'-leader region which is markedly more extended than the coding region and lack canonical regulatory sequences for the gene transcription. We analyzed the expression of pheromone genes in *E. raikovi* and *E. crassus* which represent an early and a late branch, respectively, in the phylogenetic tree. In both species, in addition to the most abundant pheromone-specific transcript, these genes synthesize other transcripts through the activity of two distinct transcription start sites and a mechanism of alternative intron splicing. However, the introns of the *E. crassus* pheromone genes appeared to be unique with respect to their analogs in *E. raikovi* and other *Euplotes* species. They can be distinguished between 'matryoshka' introns (residing one inside the other like Russian Dolls) and 'non-matryoshka' introns, and some possess non-canonical CTA/TAC splicing sites complementary to canonical GTA/TAG sites suggesting that both DNA strands can be used as template for transcription. Strand-specific RT-PCR analyses confirmed this hypothesis by showing that multiple transcripts are synthesized from both sense and anti-sense strands.

Mitochondria of Some Protist Lineages Have Retained the Bacterial Signal Peptide Recognition Particle-Based Protein Targeting Machinery

JAN PYRIH[a], TOMÁŠ PÁNEK[b], KRISTÝNA CIMRHANZLOVÁ[a,c], VENDULA RAŠKOVÁ[a,c], EVA KRIEGOVÁ[a], ANASTASIOS TSAOUSIS[d], MAREK ELIÁŠ[b], JULIUS LUKEŠ[a,c]

[a]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic; [b]Faculty of Science, Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic; [c]Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic; [d]School of Biosciences, University of Kent, Canterbury,UK.

E-mail of the corresponding Authors: jan.pyrih@gmail.com; jula@paru.cas.cz

The bacterial signal peptide-based targeting machinery is comprised of signal recognition particle-docking protein FtsY, signal peptide recognition particle protein Ffh, and RNA component. It targets proteins into, or across, the inner bacterial membrane via SecYEG and/or YidC protein complexes. Although mitochondria are of bacterial origin, up till now no such targeting system was identified in this organelle. Surprisingly, we identified homologs of both bacterial Ffh and FtsY in the four protist taxa representing distantly related deeply diverged lineages: Heterolobosea, Alveida, Hemimastigophora and the genus *Goniomonas* in Cryptista. Most of the retrieved are strongly predicted as mitochondrion-targeted and the nucleus-encoded Ffh and FtsY from the heterolobosean *Naegleria gruberi* show mitochondrial localization when expressed in a heterologous system. Phylogenetic analysis confirmed that the monophyly of the mitochondrial Ffh and FtsY proteins among their respective bacterial homologs, and at least in case of Ffh α-proteobacterial origin can be inferred with confidence. It indicates common origin of this pathway (named mtSRP) in all four lineages and its presence in the last eukaryotic common ancestor (LECA). Furthermore, we have analysed the N-termini of several proteins encoded by the *N. gruberi* mitochondrial DNA in search for possible substrates of mtSRP. We found out that only the hydrophobic ones carry a signal peptide resembling sequence. These signal peptides targeted a reporter mNeonGreen fluorescent protein into the endoplasmic reticulum of *Trypanosoma brucei*, and hence were likely recognized by the cytosolic (i.e. eukaryotic) signal recognition particle. In summary, we have identified an ancestral mitochondrial protein targeting system that further enriches the spectrum of original bacterial traits that have survived in modern mitochondria.

Corallochytrium limacisporum, a Newly Emerging Model System to Address Questions of Animal Origin

ALEKSANDRA KOŻYCZKOWSKA[a], SEBASTIAN R. NAJLE[a], EDUARD OCAÑA PALLARÉS[a], ELENA CASACUBERTA[a], IÑAKI RUIZ-TRILLO[a,b,c]
[a]Institut de Biologia Evolutiva, CSIC-Universitat Pompeu Fabra, Barcelona, Catalonia, Spain; [b]Departament de Genètica, Microbiologia i Estadística, Universitat de Barcelona, Barcelona, Catalonia, Spain; [C]ICREA, Barcelona, Catalonia, Spain.

E-mail of the corresponding Authors: sebastian.najle@upf.edu; elena.casacuberta@ibe.upf-csic.es; inaki.ruiz@ibe.upf-csic.es; ed3716@gmail.com

How animals emerged from a single-celled ancestor is one of the most pivotal events in the history of life. Recent genome data from the unicellular relatives of animals have revealed the presence of some genes involved in multicellularity and animal development previously thought to be animal-specific. 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. One of the earliest branching lineages among unicellular relatives of animals is the Corallochytrea, with only two taxa described so far, *Corallochytrium limacisporum and Syssomonas multiformis. C.limacisporum* is a understudied marine free-living walled saprotroph, that in addition to its key phylogenetic position, has other features that make it relevant to be developed as a model organism: a peculiar and still uncharacterized life cycle that goes through rounds of binary divisions forming duets or tetrads, a well annotated genome which contains both, syntenic regions with other unicellular eukaryotes and several conserved homolog genes from animals. *C.limacisporum* can be cultured in axenic conditions, in both, liquid and solid medium facilitating the isolation of clonal lines. In order to perform functional studies on this taxa we need to develop genetic tools. We here present a reliable transfection protocol for Corallochytrium for both transient and stable transfection. We have also characterized four independent transformed lines. Our data shows that stable transfection occurs by integration into the genome in multiple copies and presumably episomes can be inherited in a stable manner. We have also developed vectors with different endogenous promoters in order to regulate the expression of desired genes, as well as a battery of cassettes tagging different cellular proteins, that will serve for a better understanding of its life cycle. Progress and the potential implications of our research will be presented and further discussed.

Mitochondrial Metabolism of Anaerobic Amoeba *Pelomyxa schiedti*

KRISTÍNA ZÁHONOVÁ[a], SEBASTIAN CRISTIAN TREITLI[a], ANNA KARNKOWSKA[b], JOEL B. DACKS[c], IVAN ČEPIČKA[a], VLADIMÍR HAMPL[a]
[a]Faculty of Science, Charles University, BIOCEV, Prague, Czech Republic; [b]Faculty of Biology, University of Warsaw, Warsaw, Poland; [c]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.

E-mail of the corresponding Author: vladimir.hampl@natur.cuni.cz

Pelomyxa schiedti (Mastigamoebaea, Archamoebae, Amoebozoa) is a flagellated amoeba living in anaerobic environments, and most likely bearing an anaerobic derivate of mitochondria, similarly to its relatives *Mastigamoeba balamuthi* and *Entamoeba histolytica*. We have generated genome and transcriptome reads by Illumina HiSeq and MiSeq and Oxford Nanopore platforms, and the genome was assembled by SPAdes assembler. After separation of prokaryotic sequences and improving the assembly using the P_RNA_scaffolder tool, we obtained a genomic assembly of 52.3 Mb (5,340 contigs, N50=51,552). Our initial analyses revealed the presence of two sets of NIF system subunits to mediate FeS cluster assembly. In contrast to *M. balamuthi*, in which one set localizes in mitochondria and one in the cytosol, both sets of *P. schiedti* are predicted to be cytosol-localized. We found a few proteins involved in translocation across the mitochondrial membranes (Tom40, Sam50 and Sam37) and chaperonins Cpn60 and Cpn10, the latter containing predicted mitochondrial targeting signals (MTS). Complete set of proteins of the mitochondrial glycine cleavage system were also predicted to possess MTS. Taken together, these results suggest that *P. schiedti* indeed possesses an anaerobic derivate of mitochondrion.

On the Chemical Nature of Intracellular Inclusions in the Eustigmatophyte – *Vacuoliviride crystalliferum* Using *In-Situ* Analysis by Raman Microspectroscopy

JANA PILÁTOVÁ[a], PETER MOJZEŠ[b], KAMILA HURKOVÁ[c], KATEŘINA SCHWARZEROVÁ[a]

[a]Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic; [b]Institute of Physics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic; [c]Department of Chemistry and Analysis, Institute of Chemistry and Technology, Prague, Czech Republic.

E-mail of the corresponding Authors: jana.pilatova@natur.cuni.cz; peter.mojzes@karlov.mff.cuni.cz; hurkova@vscht.cz; katerina.schwarzerova@natur.cuni.cz

The eustigmatophyte algae are widely used in biotechnology, while being also a fascinating object for basic research. Compared to other Stramenopiles, eustygmatophyte algae possess many distinct ultrastructural features. For example, extraplastidial stigma in zoospores, reddish globule and lamellate vesicles in vegetative cells that were described in 1972 (Hibber and Leedale, 1972). Another example is a highly-regular structure coined as crystalline body that has been newly observed in vacuoles of *Vacuoliviride crystalliferum* NIES-2860 (Nakayama et al. 2015). The composition, physiology, and biogenesis of this unique subcellular structure are currently unknown.Herein we show *in vivo* and *in situ* identification of the chemical nature of various intracellular inclusions present in *V. crystalliferum* via confocal Raman microspectroscopy – some of them for the very first time. The crystalline body is composed of saturated fatty acids (or their derivatives). The composition of the autofluorescent reddish globule seems to be complex, probably containing unspecified polysaccharides (or related hydrocarbons) and sitosterols. Interestingly, neither carotenoids nor considerable amounts of neutral lipids were detected by Raman microscopy in contrast to lipid bodies stored in the cytoplasm. Small crystalline structures reported by Nakayama et al. (2015) have been identified as anhydrous crystalline guanine (Moudříková et al. 2017). Finally, we encountered polyphosphate granules and lipid droplets, as well. For independent confirmation and refinement of the chemical analysis provided by Raman microscopy we are proceeding with the isolation of above-mentioned inclusions by gradient ultracentrifugation followed by LC-MS. This work was supported by the Charles University Grant Agency (grant No. 796217), the Ministry of Education (grant NPUI No. LO1417) and the Czech Science Foundation (grant No. 17-06264S).

SYMPOSIUM - Waterborne Infections of Protozoan Origin: How Much Do We Really Know (by FEPS)

Waterborne Protozoan Infections: Health Problem Dimension Worldwide

PANAGIOTIS KARANIS[a]
[a]University of Cologne, Medical Faculty and University Hospital, University of Cologne, Germany.

E-mail of the corresponding Author: panagiotis.karanis@uk-koeln.de

Parasitic infections are a major global health burden. Waterborne protozoan infections, with the dominance of *Cryptosporidium* and *Giardia* infections, constitute the leading causes of infectious diseases worldwide and can infect a wide range of vertebrate hosts. The pathogenic species generating significant morbidity and mortality in both the developing and developed world. Transmission occurs following direct or indirect contact with the transmissive stages of the parasites through the fecal-oral route by a variety of mechanisms, including person-to-person, zoonotic, and consumption of contaminated water and food. These infections were considered neglected because relatively little attention has been devoted to their surveillance, prevention, and/or treatment. It was thought that waterborne parasitic infections are typically associated with poor and often marginalized communities in low-income countries, however, such infections are also present in the industrialized and developed countries. The waterborne protozoan parasitic infections are also called neglected parasitic infections that are now targeted by the governments as priorities for public health action based on the number of people infected, the severity of the illnesses and the ability to prevent and treat them. Monitoring of water to define the characteristics of waterborne protozoan presence has a significant history with initial efforts for their detection in water being reported as early as the 1970's. This review is intended to trace the evolution of health problems worldwide regarding waterborne protozoan infections as well as the related technological developments and progress to fundamental objectives in light of information available.

Mollusc Bivalves as Indicators of Contamination of Water Bodies by Protozoan Parasites

MELISSA PALOS LADEIRO[a], D. AUBERT[b], A. BIGOT-CLIVOT[a], S. LA CARBONA[c], I. VILLENA[a], A. GEFFARD[a]

[a]UMR-I 02 INERIS-URCA-ULH SEBIO "Environmental Stress and Biomonitoring of Aquatic Environments", University of Reims Champagne-Ardenne, UFR Sciences Exactes et Naturelles, Reims, France.; [b]EA 7510 ESCAPE, Université de Reims Champagne-Ardenne, Reims, France; [c]ACTALIA, Food Safety Department, Saint-Lô, France.

E-mail address of corresponding Author: melissa.palos@univ-reims.fr

Human health, water, and environmental conditions are inextricably connected. Indeed, natural water bodies render important ecosystemic services as resources (drinking and irrigation waters), but also for recreational or commercial (shellfish farming) ends. Consequently, the quality of water bodies is an issue at European levels to ensure the sustainability of resources and brings significant societal expectations. Aside from the direct exposure of humans through the consumption of raw shellfish, the ability of protozoa to accumulate in coastal and continental bivalves could represent an alternative choice as an evaluation tool of water quality. Such diagnostic tool avoids the limitations associated both to the sporadic nature of water sampling and to potent high dilution of microorganisms in the water matrix. Bioaccumulation is defined as the accumulation of a target in an organism relative to its concentration in the surrounding environment. It depends on several intrinsic and environmental factors which have to be characterized prior to use in bioaccumulation assessment. Since several years, our research groups develop methods for protozoan detection and quantification based on the capacities of invertebrates to accumulate protozoa as an integrative tool. In a set of laboratory experiments, the continental mussel, the zebra mussel *Dreissena polymorpha*, were exposed to *Cryptosporidium parvum*, *Giardia duodenalis* and *Toxoplasma gondii* (oo)cysts followed by a depuration time in protozoa-free water. Interesting results showed not only that mussels accumulate protozoa proportionally to water contamination but also that protozoa were still present in mussel tissues at the end of the depuration step, reflecting the integrative character of *D. polymorpha*. Protozoa bioaccumulation in invertebrate was also assessed during environmental campaign and prove that aquatic organisms could be a new tool to point out biological contamination of watercourses by protozoan parasites.

New Directions in Parasite Detection: Micro-and Macrofluidic Platforms

JUDIT PLUTZER[a], MÁRTON HARTDÉGEN[b], BIANKA FABINYI[b], ANDRÁS J. LAKI[b]
[a]National Public Health Center, Budapest, Hungary; [b]Pázmány Péter Catholic University, Budapest, Hungary.

E-mail of the corresponding Author: plujud@yahoo.com

A Lab-on-a-Chip (LoC) is a theorem that integrates several laboratory functions on a single portable or handheld device to achieve automation and high-throughput screening. LoCs can handle small amount of liquids down to picoliters using microfluidics, which deals with the precise control and manipulation of fluids. Microfluidics is generally used in many life science and food safety applications, but in DNA analysis is particularly suitable. Due to the complex procedures required for DNA analysis, microfluidics enabled to integrate all the different steps on-chip and significantly reduce the amount of required reagents and the analysis time, making reactions more efficient and reducing chemical waste.
Nowadays, PCR based DNA amplification methods are the basics and cost-effective ways to identify the DNA of *Giardia*, moreover the specificity and sensitivity of the DNA amplification can be further increased by touchdown loop-mediated isothermal amplification (LAMP). In this work we tested and compared all published *Giardia* LAMP protocols, furthermore we optimized them for microfluidic environment. Optimization included the careful selection of temperature range and adjusting reaction components concentration in the reaction mixture in order to obtain the lowest detection limit and shortest reaction time. In case of positive LAMP reaction, the turbidity change can be detected by the naked eye or adding fluorescent dye produce a color change in the solution enabling endpoint detection. The evaluated LAMP protocol is rapid, reliable and cost-effective for LoC platforms to identify *Giardia* species in different origin of samples.
Standardized and commonly used methods for *Giardia* and *Cryptosporidium* detection are EPA1623 and ISO 15553 microscopic techniques, which give morphological and quantitative information about the protozoa and certainly have advantages, but also limitations. The generally used EPA1623 method consists of preconcentration, immunomagnetic labelling and magnetic trapping (IMS) and microscopic detection steps. These steps can be fully implemented in a serial modular macrofluidic LoC system. Here a magnetic filtration module (MFM) of the IMS of macrofluidic platform using cutting-edge 3D printing technology was presented, which can simplify and reduce the number of steps of the IMS and significantly increase the efficiency of the parasite separation.

Waterborne Protozoan Infections in the Era of Climatic Changes

MARIA CRISTINA ANGELICI[a]
[a]National Institute of Health, Department of the Environment and Health, Rome, Italy.

E-mail of the corresponding Author: mariacristina.angelici@iss.it

There is a dearth of information on how extreme climatic events can influence the risk of pathogen transmission through the environment. Extreme climatic events such as droughts, cyclones and floods increase human exposure to waterborne pathogens, not only through direct contact with water, but also through the ways these events modify human behavior. For example, when the temperature increases, people tend to drink more water and to freshen up by bathing more often, but when disastrous events have provoked major water contamination, these activities can prove deleterious. Moreover, extreme environmental conditions induce people to reduce practices of hygiene. Such dramatic environmental changes also affect pathogen protozoa survival. When water sources are contaminated by sewage waters, cysts/oocysts are suspended in the surface, interstitial and groundwater network. Temperature increase causes the concentration of cysts/oocysts concentration to move down the water column to the sediment level, where they remain alive for a long time. These events also lead to the extension of the transmission phase, passive transportation by occasional hosts, and invasion of new environmental and even geographical areas. The relationship between extreme climatic events and waterborne diseases merits further investigation. Several authors have produced systematic reviews of the scientific literature to summarize the available information on direct cause-effect relationships between climate change and WB infection risks. Some of these publications stress a direct dynamic However, not all pathogens are indicated and, again, waterborne parasitic diseases are neglected. Nevertheless, a systematic review of 741 publications on water-transmitted infections in Europe highlighted a correlation between the outbreak of enteric diseases and four specific indicators: temperature, rainfall, floods and droughts. The review noted that in 24 papers, half documented a positive correlation between increased rainfall and waterborne infections; over half of 87 such outbreaks in 29 countries occurred after heavy rainfall and floods. Among these outbreaks, 53% were associated with the contamination of drinking water. It is vital to plan prevention and resilience measures to counter the negative effects of climatic change on human health.

Oral Session GENOMIC & TRANSCRIPTOMIC 2

Genome Analyses of the New Model Protist *Euplotes vannus* focusing on Genome Rearrangement and Mating Type Determination

XIAO CHEN[a,b], YAOHAN JIANG[a], FENG GAO[a], YUNYI GAO[a], RUITAO GONG[a], WEIBO ZHENG[a], NAOMI A. STOVER[c], CHAO LU[b], LAURA A. KATZ[d], WEIBO SONG[a]
[a]Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao, China; [b]Department of Genetics and Development, Columbia University Medical Center, New York, NY, USA;[c]Department of Biology, Bradley University, Peoria, IL, USA; [d]Department of Biological Sciences, Smith College, Northampton, MA, USA.

E-mail of the corresponding Author: gaof@ouc.edu.cn

Both the characteristics of dual genomes (the presence of both germline and somatic genomes within each cell) and special sexual reproduction (conjugation) have made ciliates as model organisms in a wide range of disciplines including cytology, evolutionary biology, genetics, epigenetics, etc. As a model organism for studies of cell and environmental biology, the free-living and cosmopolitan ciliate *Euplotes vannus* shows intriguing features like "gene-sized" macronuclear chromosomes, stop codon reassignment, programmed ribosomal frameshifting (PRF) and high-multiple mating system. However, the molecular mechanisms that account for these remarkable traits remain largely unknown. In order to reveal these molecular mechanisms, we first provided the time-course analysis of the nuclear events occurring during and after conjugation in *E. vannus* strains collected along the Yellow Sea coast of Qingdao, China, and compare them with those of other species. We then report a combined analysis of *de novo* assembled high-quality macronuclear (MAC; i.e. somatic) and partial micronuclear (MIC; i.e. germline) genome sequences for *E. vannus*, and transcriptome profiling data under varying conditions. The results include: 1) the MAC genome contains more than 25,000 complete "gene-sized" nanochromosomes (~85 Mb haploid genome size) with the N50 ~2.7 kb; 2) though there is a high frequency of frameshifting at stop codons UAA and UAG, we did not observe impaired transcript abundance as a result of PRF in this species as has been reported for other euplotids; 3) the sequence motif 5'-TA-3' is conserved at nearly all internally-eliminated sequence (IES) boundaries in the MIC genome, and chromosome breakage sites (CBSs) are duplicated and retained in the MAC genome; 4) *E. vannus* possesses two Type-I and four Type-II pheromones, including two novel alleles Ev-4 and Ev-beta, based on the genome investigation of six mating types; 5) different mating types of *E. vannus* have mating type-specific chromatin and expression profiling of Type-II pheromone loci. Together with the genome resources generated in this study, which are available online at *Euplotes vannus* Genome Database (http://evan.ciliate.org), these data provide molecular evidence for understanding the unique biology of the highly adaptable microorganisms.

Four genomes, three different structures: a study of plastid genomes of Dictyochophyceae reveals unusual variability in their organization

KACPER MACISZEWSKI[a], KWIYOUNG HAN[b], LOUIS GRAF[b], ROBERT A. ANDERSEN[c], ANNA KARNKOWSKA[a], HWAN SU YOON[b]
[a]Department of Molecular Phylogenetics and Evolution, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Poland; [b]Department of Biological Sciences, Sungkyunkwan University, Suwon, Korea; [c]Friday Harbor Laboratories, University of Washington, Friday Harbor, USA.

E-mail of the corresponding Authors: macisz@biol.uw.edu.pl; ankarn@biol.uw.edu.pl

Dictyochophyceae (silicoflagellates) are unicellular, predominantly marine algae, belonging to the clade of secondary plastid-bearing stramenopiles (Heterokontophyta). The scientific interest in this group has been developing in recent years, mainly due to its potential ecological significance and abundance in global oceans; still, no full nuclear or organellar genome of any of its representatives has been sequenced so far. Having obtained complete ptDNA sequences from four species of Dictyochophyceae: *Dictyocha speculum, Florenciella parvula, Pseudopedinella elastica* and *Rhizochromulina marina*, we observed that although their size and genetic contents are similar, there is a prominent variability in their structure. While the plastid genomes of *F. parvula* and *P. elastica* possess typical quadripartite structure with two inverted repeats encompassing rDNA, the plastid genome of *R. marina* contains a pair of repeats with identical orientation, and in case of *D. speculum,* there are no repeats whatsoever.

Our analyses show that despite the aforementioned variability of structure and overall low extent of gene order conservation in dictyochophycean ptDNA, all of the investigated genomes contain two large gene clusters with almost identical contents and order as their counterparts in some of the other lineages of Heterokontophyta. Moreover, a multitude of standard plastid genes were found to be missing from the examined plastid genomes, most probably due to extensive plastid-to-nucleus gene transfer, which has taken place as well in Pelagophyceae – a sister lineage to Dictyochophyceae. Interestingly, we also observed that the ptDNA of *D. speculum* exhibits a number of distinctive traits, such as presence of two non-identical copies of the gene encoding the large subunit of RuBisCo (*rbcL*), expansion of intergenic regions which accounts for this genome's increased size compared to its closest relatives, or a single group II intron of uncertain evolutionary ancestry in one of the photosystem I-related genes (*psaA*). We are convinced that the results presented herein provide important insights into the evolutionary history of the lineage of Dictyochophyceae; however, the events behind certain unusual traits found in these organisms require further investigation to be fully understood.

Comparative Genomics Sheds Light on the Evolution of Metabolism and Molecular Features in Euglenozoa

ANZHELIKA BUTENKO[a,b], OLGA FLEGONTOVA[a], ALEŠ HORÁK[a], VLADIMÍR HAMPL[c], PATRICK KEELING[d], RYAN GAWRYLUK[e], DENIS TIKHONENKOV[d,f], PAVEL FLEGONTOV[a,b,g], JULIUS LUKEŠ[a,g]

[a]Institute of Parasitology, Biology Centre, České Budějovice, Czech Republic; [b]Faculty of Science, University of Ostrava, Ostrava, Czech Republic; [c]Faculty of Science, Charles University, Prague, Czech Republic; [d]Department of Botany, University of British Columbia, Vancouver, Canada; [e]Department of Biology, University of Victoria, Victoria, Canada; [f]Institute for Biology of Inland Waters, Russian Academy of Sciences, Yaroslavl Region, Borok Russian Federation; Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

E-mail of the corresponding Authors: anzhelika.butenko@paru.cas.cz; jula@paru.cas.cz

Unicellular eukaryotes belonging to the group Euglenozoa (Excavata: Discoba) comprise four main lineages: Kinetoplastea, Diplonemea, Euglenida and Postgaardea. Kinetoplastids include free-living bacteriovorous protists, as well as medically and veterinary important obligate parasites of the family Trypanosomatidae (genera *Leishmania*, *Trypanosoma*). Diplonemids have recently emerged as the most specious group of marine planktonic eukaryotes and yet, virtually no sequencing data is available for these potentially major players in marine ecosystems. Euglenids, widely known by the photosynthetic Euglenophyceae (e.g., *Euglena gracilis*), include various heterotrophic species (e.g., osmotrophs like *Rhabdomonas*). Postgaardians represent understudied protists covered by epibiotic bacteria. The availability of sequencing data for euglenozoan lineages is instrumental for understanding the evolution of metabolic and molecular features, which are considered unique for the family Trypanosomatidae.

We have sequenced the transcriptomes of three diplonemids, three kinetoplastids (including two free-living basally-branching prokinetoplastids) and two euglenids and combined our data with 10 publicly available genomes and transcriptomes. The genome-wide comparative analysis of metabolism revealed that trypanosomatids and bodonids, except for free-living prokinetoplastids, are metabolically less versatile than diplonemids and euglenids. Important enzymes of amino acid, vitamins and cofactors' biosynthesis, and nucleotide metabolism were lost in all kinetoplastids or gradually within the kinetoplastid tree. The evolution of NADPH-dependent disulfide reductase systems is a clear example of gradual loss of metabolic capabilities in kinetoplastids, with trypanosomatids possessing only a trypanothione-based system apparently already present in the ancestor of Euglenozoa. Among molecular machineries known to be highly derived in trypanosomatids are the pre-replication complex (pre-RC) and kinetochores. Our results suggest that the pre-RC of euglenids resembles the classic eukaryotic structure the most, while diplonemid pre-RC is highly divergent. The situation with kinetochores is similar, with diplonemids apparently possessing a divergent machinery yet to be described, potentially indicating an existence of a highly unusual chromosome segregation and/or cell division process.

Rewinding the Metabolism of Diplomonads

ALEJANDRO JIMÉNEZ-GONZÁLEZ[a], JAN O. ANDERSSON[a]
[a]Department of Cell and Molecular Biology, Molecular Evolution program, Uppsala University, Uppsala, Sweden.

E-mail of the corresponding Author: alejandro.jimenez@icm.uu.se

Diplomonads are a group of flagellated protists found in oxygen-poor environments. They are classified within the group Fornicata (Metamonada, Excavata). They are well known as parasitic species. Giardia intestinalis, the causative agent of giardiasis in humans, is the most widely known diplomonad, but members of this group can also colonize other mammals, as well as fishes, amphibious, and birds. In diplomonads we can also find free-living species. One of these species, Trepomonas sp. PC1, has been described as a secondary free-living. Recently, new genomes and transcriptomes have been published from diplomonads and close relatives. Interestingly, only one of these relatives is considered a parasite. This lead us to the main question: Was the last diplomonads common ancestor already a parasite? And if so, which host was it associated with?

To answer these questions we reconstructed the metabolic capacities of five diplomonads species (and one diplomonads sister group), both parasites and free-livings. We combined the results of different metabolic prediction tools with what it is already known by experimental data. We observed a high similarity within the group in the main metabolic pathways, with some relevant exceptions (e.g. nucleotide metabolisms). The majority of the differences are in reactions that do not belong to central pathways (e.g. detoxification and proteases). We used a clustering approach to classify reactions as putatively present in the last diplomonads common ancestor or gained and lost during the evolution of the different lineages. The identification of patterns in the metabolic adaptations will allow us to understand lineages specific adaptations to different hosts and to a secondary free-living lifestyle.

Vertebrate Retortamonads Share Features of Anaerobic Metabolism with Relative Diplomonads

ZOLTÁN FÜSSYa, ZUZANA VAITOVÁa, TOMÁŠ PÁNEKb, IVAN ČEPIČKAc, VLADIMÍR HAMPLa,c
aFaculty of Science, BIOCEV, Charles University, Prague, Czech Republic; bFaculty of Science, University of Ostrava, Ostrava, Czech Republic; cFaculty of Science, Charles University, Prague, Czech Republic.

E-mail of the corresponding Author: zoltan.fussy@natur.cuni.cz

Although the mitochondria of extant eukaryotes share a single origin, functionally these organelles diversified to a great extent, reflecting lifestyles of the organisms that host them. In anaerobic excavates of the group Metamonada, mitochondria evolved into organelles with very limited functions (also termed hydrogenosomes or mitosomes) and an amitochondriate eukaryote, Monocercomonoides exilis, has also been reported recently. Retortamonads dwelling in intestines of vertebrates form a sister group to parasitic diplomonads, Giardia and Spironucleus, and have also been hypothesized to completely lack mitochondria. In the pilot transcriptomic data from Retortamonas dobelli, we searched for the enzymes of the core energy metabolism and for the hallmark mitochondrial proteins. Among the recovered glycolytic enzymes, we found pyrophosphate-dependent analogues of phosphofructokinase and pyruvate kinase capable of reversible ATP-independent reaction steps reminiscent of the pathway arrangement in other metamonads. We found potential traces of the mitochondrial metabolism, represented by pyruvate:ferredoxin oxidoreductase, [FeFe]-Hydrogenases, a Tim14/Pam18 protein and, importantly, two hydrogenase maturase homologs (HydF and HydG), suggesting a remnant mitochondrion is still present. A more in-depth analysis is ongoing to investigate other aspects of the adaptations of retortamonads to microaerobic environments.

Phylogenetic reconstruction Reveals Expansions of the Rab GTPase Gene Family in Amoebozoa

ALFREDO L. PORFÍRIO-SOUSAa,b, ALEXANDER K. TICEb, MATTHEW W. BROWNb, DANIEL J. G. LAHRa
aDepartment of Zoology, University of São Paulo, São Paulo, Brazil; bDepartment of Biological Sciences, Mississippi State University, Mississippi State, USA.

E-mail of the corresponding Authors: alfredo.sousa@usp.br; at1422@msstate.edu; mbrown@biology.msstate.edu; dlahr@ib.usp.br

Rab GTPase is a paralog-rich gene family involved in control of the formation and traffic of intracellular vesicles. It is characteristic of Eukaryotes and its evolutionary story has been studied based on genomic and transcriptomic data of key traditional model organisms. The last eukaryotic common ancestor (LECA) possessed around 23 Rab paralogs, while derived taxa such as Saccharomyces cerevisiae have 11, Homo sapiens have over 60 and Arabidopsis thaliana have 57. Thus, it is evident the Rab family presents a complex pattern of evolution through the eukaryotes. To date, Rab GTPases in Amoebozoa have been studied only in a few species and their evolution in this eukaryotic supergroup remains to be determined. Here we present a phylogenetic study of the Rab GTPases based on genomic and transcriptomic data, focusing in 22 representative lineages of the three major groups in Amoebozoa (Tubulinea, Evosea, and Discosea). We describe that the whole group of Amoebozoa retained most of the ancestral Rab paralogs present in LECA. These data reveal the emergence of Amoebozoa exclusive Rabs, including putative members of the exocytic and endocytic pathways, demonstrated by their phylogenetic relationships. Thus, the Rab GTPase family is thoroughly expanded in Amoebozoa and has a diverse repertoire of paralogs.

Using Single-Cell 'Omics to Study Population Genetics and Genome Evolution of Arcellinida.

AGNES K.M. WEINERa, KETTY MUNYENYEMBEa, AND LAURA A. KATZa,b
aSmith College, Department of Biological Science, Northampton, Massachusetts, USA; bUniversity of Massachusetts Amherst, Program in Organismic and Evolutionary Biology, Amherst, Massachusetts, USA.

E-mail of the corresponding Author: aweiner@smith.edu

Testate (shell-building) amoebae of the order Arcellinida (Amoebozoa) are highly abundant in freshwater ecosystems, such as bogs, fens and lakes. Within the microbial community inhabiting these ecosystems these amoebae represent top predators. Because of their abundance, their sensitivity to abiotic environmental factors and the preservation of their tests in the fossil record, testate amoebae serve as excellent bioindicators of past and present climate change. Although Arcellinida are well studied from a morphological perspective, our knowledge on their population genetics and genome evolution remains very limited. This is mostly due to the fact that they cannot be cultivated in the laboratory. To address these challenges, we have successfully developed protocols for single-cell genomics and transcriptomics. We have by now characterized transcriptomes and genomes from multiple individuals (~50 transcriptomes and ~15 genomes) of Arcellinida, which we assemble and analyze using our custom-made pipeline PhyloToL. We are using bioinformatic approaches to make inferences on patterns of molecular evolution in these lineages, and to map the transcripts to the genome to explore the structure and distribution of genes. In addition, we are using our 'omics data to study population genetic variability of populations sampled across time and space in New England bogs. Our preliminary results show surprisingly low levels of intra-population genetic variability, suggesting either high rates of dispersal or a small effective population size. For example, periodic extinction and subsequent repopulation events (i.e. boom-bust cycles) may explain these population data.

The Integrin-Mediated Adhesome Complex, Essential to Multicellularity, Is Present in the Most Recent Common Ancestor of Animals, Fungi and Amoebae.

SEUNGHO KANGa,b, ALEXANDER K. TICEa,b, DAVID ŽIHALAc, DANIEL J. G. LAHRd, MATTHEW W. BROWNa,b
aDepartment of Biological Sciences, Mississippi State University, USA; bInstitute for Genomics, Biocomputing & Biotechnology, Mississippi State University, USA; cDepartment of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic; dDepartment of Zoology, University of São Paulo, São Paulo, Brazil.

E-mail of the corresponding Authors: sk1476@msstate.edu; matthew.brown@msstate.edu

Amoebozoa is the eukaryotic supergroup sister to Obazoa, the group containing animals, fungi, and several microbial eukaryote lineages. Even though Amoebozoa represents the closest outgroup to Obazoa, little genomic-level data and attention to gene inventories has been given to the supergroup. Amoebozoa appears to have split from the last common ancestor of Obazoa and Amoebozoa somewhere around 1.5 billon 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 entire 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 integrin adhesion proteins that 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 cellular differentiation. Our results highlight that many of these proteins appear to have evolved earlier in eukaryote evolution than previously thought.

THURSDAY 1 August

PLENARY LECTURE (by FEPS)

Prof. H.-D. Görtz and his Contribution to our Knowledge of Protozoan Symbiosis

SERGEI I. FOKIN[a,b,c]
[a] Department of Biology, University of Pisa, Pisa, Italy; [b] Department of Invertebrate Zoology, St. Petersburg State University, St. Petersburg, Russia; [c] St. Petersburg Branch of the S. I. Vavilov Institute of History of Science and Technology, Russian Academy of Sciences, St. Petersburg, Russia.
E-mail of the corresponding Author: sergey.fokin@unipi.it

This lecture is based on my personal impressions of meetings and teamwork with Prof. H-D. Görtz (1945–2019) over a period of 29 years. His mother's side the family had a long business tradition, and Hans-Dieter, the eldest child, had four sisters and one brother. He started his higher education in 1966 (biology, chemistry and physics) at the University of Münster, and in 1970 completed a study on metabolism in *Formica polyctena* (Hymenoptera). In the same year he changed his scientific field to protozoology under the supervision of Prof. K. Heckmann. He received his PhD degree from the University of Münster in 1975 for the study of the fine structure of *Euplotes minuta*, and became an assistant to Prof. Heckmann. In 1983 he got habilitation in Zoology already in symbiosis field with the study "The nuclei of *Paramecium caudatum* after infection with bacteria of the genus *Holospora*", and in 1985 began employment at the University as an Associate Professor. In 1993, the centre of *Holospora* investigations in Germany, which he had created, moved to the Biological Institute of Stuttgart University, where he was hired as a full professor, following competition involving tens of applicants. From the beginning of the 1990s the *Paramecium–Holospora* system became an important model object and a subject of international scientific cooperation in many aspects, including the diversity of symbionts, adaptations and interactions between partners in the systems, the ecological and evolutionary importance of the symbiosis, mechanisms of infection, and recognition by host. Prof. H.-D. Görtz played a particularly important role in all of these studies, especially in fostering international collaboration and interdisciplinary research in the field. In 2012 he retired and returned to Münster. The author of three books, a number of chapters and reviews in different microbiological and protistological editions, and close to 80 articles in peer-reviewed journals; the former Vice-President and President of German Society for Protozoology (1999–2004) and honorary member of it (2016); the managing Editor of the European Journal of Protistology (1995–1999) is no longer with us. However, Prof. H.-D. Görtz leaves us with a deep and extremely positive legacy, both in terms of science and in the sense of human relationships.

SYMPOSIUM – Symbiosis in Ciliates: H. – D. Görtz and His Legacy (by FEPS)

Life in Cooperation: a Win-Win Situation for Single Cells and Scientists

BETTINA SONNTAG[a]

[a]Research Department for Limnology, Mondsee, University of Innsbruck, Mondsee, Austria.

E-mail of the corresponding Author: bettina.sonntag@uibk.ac.at

Yet unknown insights into ciliates that cooperate with algae and bacteria were discovered when three curious scientists, Hans-Dieter Goertz, Thomas Posch and I came together and occasionally carried out 1-2 days 'pond expeditions' ('Tuempeltour'). We collected samples from diverse water bodies and focused onto the discovery of ciliate species that lived in cooperation with bacterial and/or algal partners. The 'pond expeditions' led us to Austria, Germany and Switzerland and were more or less successful, however, we finally isolated, e.g., several mixotrophic *Coleps* strains that we cultivated and which were recently involved into highly interesting results on their morphology and phylogeny. We discovered that some common freshwater *Coleps* species are no longer distinguishable from morphology only and that the possession of algal symbionts is facultative. In our unfortunately last trip in 2017, we three visited the 'Heiliges Meer' in Germany prior to the 'Science Pub' held in Muenster. During this last meeting, Hans-Dieter gave me some green-algal bearing *Paramecium bursaria* strains with and without *Holospora* bacteria and one natural sample containing among other ciliates *Euplotes daidaleos.* We characterised all these strains from morphology and molecular sequences including their algal symbionts and in case of *E. daidaleos* also their bacterial endocytobionts by FISH (fluorescence in situ hybridisation). Additionally, in *E. daidaleos*, we discovered a green algae yet unknown as endosymbiont. In my presentation, I will focus on our new findings in the model ciliate genera *Coleps, Euplotes* and *Paramecium*, elucidate their morphology and molecular phylogeny as well as of their algal symbionts and the bacterial endosymbionts of *E. daidaleos*. The cooperation among these ciliates and their endocytobionts have been investigated by many scientists covering various aspects and bacteria and protists involved and clearly resulted in an interdisciplinary win-win situation for each one of us. Last but not least, I would like to mention that each meeting with Hans-Dieter was a fruitful exchange of thoughts, views, ciliates, their endocytobionts and was of course a lot of fun.

These studies are funded by the Austrian Science Fund FWF: projects P28333-B25 and I2238-B25.

About Escaping and Staying: a Lab Model for Studying Endosymbiotic Origins

LISA SIEGMUND[a], JOHANNES WÖSTEMEYER[a]
[a]Department of Microbiology, Friedrich-Schiller-University Jena, Jena, Germany.

E-mail of the corresponding Author: Lisa.Siegmund@uni-jena.de

Endosymbiosis is a common phenomenon in nature and occurs among almost all groups of organisms, but especially within protists these associations are found in considerable frequency and variety. Inspired by the work of Hans-Dieter Görtz and after his personal encouragement, we started our research on endosymbiosis with a completely different approach. Instead of investigating naturally occurring associations, we developed a laboratory model to examine the early steps of endosymbiotic establishment, in particular those mechanisms leading to infection of host cells. Due to evolution, looking at existing interactions reveals highly derived states rather than primary mechanisms.

Our model consisted of *Tetrahymena pyriformis* and a non-pathogenic strain of *Escherichia coli*. By fluorescence and transmission electron microscopy we show that *E. coli* is able to escape from phagosomes of *T. pyriformis* without further manipulation. To understand how *E. coli* is capable of infection, the bacterial 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. Bacteria with increased surface hydrophobicity or alkalinity exhibit higher chances to escape from phagosomes of *T. pyriformis*. In addition, an artificial oligopeptide designed for mediating membrane transport, enhances the frequency of bacterial escape events. F-pili seem to contribute to these processes too. Therefore, we conclude that bacterial surface traits play an important role in this very initial step of establishing endosymbiosis.

In a complementary approach, we investigated the ability of bacterial persistence within the ciliate and how this new lifestyle influences both partners. By offering a strain expressing a neomycin resistance gene and adding the chemically related aminoglycoside antibiotic paromomycin to the culture environment, *T. pyriformis* and *E. coli* can be "forced" into a symbiotic interaction. The ciliate benefits from intracellular bacteria, since it is more resistant to paromomycin in comparison to differently cultured organisms. *E. coli* is also affected by the intracellular lifestyle. After a few days they are not culturable outside their host and undergo morphological changes by becoming smaller and, thus, share the fate of almost all known endosymbionts.

H.-D. Gortz: a Study to Be Continued, or An Emerging Human Pathogen Occurring in Ciliates

ELENA SABANEYEVA[a], GALINA VIZICHKANICH[a], NATALIA LEBEDEVA[b], YULIYA YAKOVLEVA[a]

[a]Department of Cytology and Histology, Saint-Petersburg State University, Russia; [b]Core Facility Center for Cultivation of Microorganisms, Saint-Petersburg State University, Russia.

E-mail of the corresponding Author: e.sabaneeva@spbu.ru

Ciliates demonstrate remarkable biodiversity of their endosymbionts. Alongside with a wide range of prokaryotic invaders, ciliate cells can occasionally host some eukaryotic microorganisms in their cytoplasm. In 1982 H.-D. Gortz reported on accidental infection with yeasts of an aposymbiotic laboratory culture of *Paramecium bursaria*, which had been previously experimentally freed of their usual partner, cytoplasmic *Chlorella*. According to its morphology, the endosymbiotic yeast was at the time identified as *Rhodotorula rubra*. In 2014 we isolated from nature a strain of *P. bursaria* bearing yeasts in the cytoplasm. A host cell free culture of the endosymbionts could be maintained on the YPD agar plates, the yeast forming specific smooth spherical mucoid colonies, coral in color. On the basis of morphological and molecular analyses, the endosymbiont was identified as *Rhodotorula mucilaginosa*, which is the present day name for *Rh. rubra*. Although morphology and fine structure of the yeast corresponded to the characteristics of the formerly described isolate, it demonstrated somewhat different behavior in experimental infections. *Chlorella*-bearing strains of *P. bursaria* did not get infected with *Rh. mucilaginosa*, however, the number of symbiotic algae decreased with time. The yeast-bearing strain could not be infected with *Chlorella* in our experiments, while *Chlorella*-free (naïve) strains of *P. bursaria* got infected with *Rh. mucilaginosa*, but tended to lose the yeasts in the course of time. Double infection of naïve cells with yeasts and algae was never observed. *Rh. mucilaginosa* is a saprophytic fungus belonging to Basidiomycota, which for a long time has been regarded as a harmless one. However, nowadays, this yeast species is considered an emerging opportunistic pathogen causing severe infections in immunosuppressed humans and economically important animals. Occurrence of *Rh. mucilaginosa* in ciliates is an additional argument in support of the hypothesis claiming ciliates to serve as natural reservoirs for potential human pathogens.

Supported by RFBR grant 18-04-00562.

Comparative Genomics of Endosymbionts of Ciliates Offers New Perspectives on *Rickettsiales* Evolution

MICHELE CASTELLI[a]

[a]Pediatric clinical "Romeo and Enrica Invernizzi" Research Center, Department of Biosciences, University of Milano, Milano, Italy.

E-mail of the corresponding Author: michele.castelli@unimi.it

Rickettsiales are conventionally defined as an order of obligate intracellular bacteria in eukaryotic cells. Best studied representatives are vector-borne pathogens (e.g. *Rickettsia* spp., *Ehrlichia* spp.). However, most of the currently known phylogenetic diversity of *Rickettsiales* is formed by non-pathogenic bacteria, which are hosted prevalently by aquatic organisms, in particular protists, including ciliates, amoebas, and chlorophytes. These "non-model" *Rickettsiales* and the interaction with their hosts are still poorly investigated and little is known about their genomics and role in the host biology.

According to earlier studies, *Rickettsiales* present small genomes (typically 1-1.5 Mbp) due to reductive genome evolution, similarly to other obligate intracellular bacteria, and are consistently highly metabolically dependent on their hosts, with which they engage complex interactions, also thanks to peculiar protein secretion systems, putatively involved in pathogenesis.

Thanks to the few recent studies on "non-model" *Rickettsiales,* our current knowledge on the diversity and evolution of the whole order has greatly increased. Indeed, it is now evident that the overall functional variability of extant *Rickettsiales* is much larger. Among the most notable novel finding was the one of flagella, which may have a role in locomotion, both within host cells and, perhaps, during horizontal transmission, as well as in constituting a potential additional secretion apparatus. Moreover, several new metabolic biosynthetic pathways were found, in particular for many amino acids in "*Candidatus* Deianiraea vastatrix". These pathways likely enable this bacterium to be less dependent on its host *Paramecium primaurelia*, and may correlate with its so-far unique condition of fully extracellular *Rickettsiales* bacterium.

In sum, while the genome size and genomic complexity of each newly analysed representative taken alone is not higher than other previously studied *Rickettsiales*, the *Rickettsiales* pan-genome results significantly enlarged. Thus, given the consistence of phylogenetic inference with a putative vertical inheritance of these characters, it is intriguing to speculate that the last *Rickettsiales* ancestor was equipped with many of these features, being a host-associated, but still highly versatile bacterium, and to wonder about the subsequent paths of evolution of extant *Rickettsiales*.

First Complete Genome of *Holospora*-like Bacterium *Gortzia yakutica*

MARIA RAUTIAN[a], ALEXANDRA BELIAVSKAIA[a,b]

[a]Department of Invertebrate Zoology, St. Petersburg State University, Russia; [b]Institute of Integrative Biology, University of Liverpool,UK.

E-mail of the corresponding Authors: mrautian@mail.ru; alex.beliavskaia@gmail.com

Paramecium ciliates can host many different intracellular symbionts, of which the most frequently studied are *Holospora*, the obligately intranuclear bacteria affiliated with class *Alphaproteobacteria* and order *Holosporales*. Eleven *Holospora*-like bacteria species have been morphologically characterized and named so far; however, only four of them (*H. obtusa, H. undulata, H. elegans*, and *H. curviuscula*) have been whole genome sequenced. No finished genome sequences were available at the NCBI genome database as of April 2019.

We characterised a novel bacterium *Gortzia yakutica* which is a *Holospora*-like macronuclear symbiont of the ciliate *Paramecium putrinum*. These gram-negative bacteria have a complex life cycle presented by small reproductive forms 1-2 μm long and large infective forms up to 20 μm. The infective cells have a specific structure that allows them to survive in ambient conditions and then to infect new host cells.

In order to obtain a finished genome of *G. yakutica* we combined short-read Illumina approach and Oxford Nanopore long-read technology. Assembled genome is a circular chromosome of 1.18 Mb with the GC-content of 32%, which is typical for *Holospora*-like bacteria and other symbiotic *Alphaproteobacteria;* no plasmids were detected. We found a single rRNA operon and 1076 protein-coding genes.

Using previously published genome assemblies, we performed pan-genome analysis and defined core and accessory genomes of *Holospora*-like bacteria, and compared it with pan-genome of other symbiotic *Alphaproteobacteria* to understand genomic determinants of their complex lifestyle and intranuclear localisation. To our knowledge this is the first finished genome sequence of a *Holospora*-like bacterium allowing novel insights into their biology and genome architecture.

Identification of Factors Involved in the Early *Paramecium* Host Response to *Holospora* Infection through Expression and Evolutionary Analysis

CATHERINE KAGEMANN[a], JEAN-FRANCOIS GOUT[b], WINIFFER CONCE ALBERTO[c], OLIVER KALTZ[d], MICHAEL LYNCH[b], LYDIA BRIGHT[c]

[a]Indiana University, Bloomington, IN, USA; [b]Biodesign Institute, Arizona State University, Tempe, AZ, USA; [c]Department of Biology, State University of New York at New Paltz, Coykendall Science Building, New Paltz, NY, USA; [d]Institut des Sciences de l'Evolution (ISEM), Montpellier, France.

E-mail of the corresponding Author: brightl@newpaltz.edu

Certain *Paramecium caudatum* strains are commonly infected by *Holospora undulata,* a bacterial symbiont that is able to specifically infect the micronucleus. With the goal of identifying the precise genetic and cellular factors that determine susceptibility to *Holospora* infection in *Paramecium* cells, we have undertaken a multi-pronged approach. First, we collected and sequenced *P. caudatum* RNA at three timepoints early in the infection process. Following inoculation of naïve cells with infectious forms of *H. undulata,* we found more than twice as many differentially-expressed genes (DEGs) at the 10-minute timepoint vs the 30-minute timepoint, with 75% of the DEGs at the 30-minute timepoint also being differentially expressed 10 minutes after inoculation. A minority of genes at both timepoint have functional annotations, which is an unexpectedly small number, given that genome-wide, ~50% of genes have functional annotations. This may mean that *Paramecium*-specific, or more rapidly evolving genes are involved in very early interactions with *H. undulata.* The ways in which *H. undulata* moves into the cell and infects the nucleus suggest that proteins involved with signaling and membrane trafficking mediate this infection, and the gene expression analysis supports this, with both signaling-related genes and membrane trafficking related genes contained in the set of most highly upregulated genes post-inoculation.

In a complementary approach, we are also analyzing 6 strains of *Paramecium caudatum* that have been whole-genome sequenced, in order to determine their infection phenotype. Having both an association between infection phenotype and evolutionary patterns in genes of interest (as well as genome-wide) is allowing us to detect evolutionary patterns consistent with coevolution with a pathogen or parasite. We are focusing in particular on fast-evolving genes, to detect both positive signatures of selection and signatures of purifying selection in the differentially susceptible lines.

Occasional PASSENGERS or Functional Consortia? *Terra incognita* of Free-Living Ciliates Microbiomes

ALEXEY POTEKHIN[a], OLIVIA LANZONI[b], YURI KHLOPKO[c], DARIA POSHVINA[c], GIULIO PETRONI[b], ANDREY PLOTNIKOV[c]

[a]Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia; [b]Department of Biology, University of Pisa, Pisa, Italy; [c]Center of Shared Scientific Equipment, Institute for Cellular and Intracellular Symbiosis, Ural Division of the Russian Academy of Science, Orenburg, Russia.

E-mail of the corresponding Authors: alexey.potekhin@spbu.ru; oli.lanzoni@gmail.com; giulio.petroni@unipi.it; protoz@mail.ru

Protists themselves are not only part of microbiomes, but can also host their own microbiomes. The roles of protists in the maintenance of prokaryotic diversity in nature, as well as interactions between non-symbiotic bacteria with protists, remain unknown. We studied prokaryotic communities associated with several species of ciliates by NGS of 16S rRNA gene amplicons obtained by single cell PCR. We analyzed the bacterial consortia associated with cells of different strains of more than ten ciliate species. The species belonged to different taxonomic and ecological groups of *Ciliophora*. A variety of bacteria of different phyla (with prevalence of *Bacteroidetes, Proteobacteria* and *Firmicutes*) were identified in ciliate microbiomes. We found that numerous representatives of several dozen bacterial genera were associated with each ciliate cell, and that the ratio between these "cohabitants" might vary from cell to cell even if all ciliates belonged to the same species and originated from the same environment. However, the microbiomes of cells of the same strain were more similar than the communities associated with cells of different strains of the same species, while representatives of different species had very different sets of associated bacteria. At the same time, all microbiomes of ciliates had almost nothing in common with prokaryotic communities of their environment. While dominant bacterial genera identified in the "controls" - environmental communities - included only free living species, it was surprising to observe that the microbiomes of ciliates were composed mostly by representatives of bacterial genera that also contain opportunistic or pathogenic species. Thus ciliates can be viewed in a different light, as natural reservoirs of animal and human pathogens. The specificity and the role of microbiomes in ciliate biology have yet to be elucidated, though it also seems possible that ciliates simply provide an occasional shelter and an environmental niche for those bacteria that prefer to propagate in association with their eukaryotic hosts.

Supported by RFBR 19-04-01256a.

Unravelling the Ecology of Microbial Symbioses by Culture-Independent Single-Cell Microbiomics.

VITTORIO BOSCARO[a,b], ALESSIA ROSSI[b], ALESSIO BELLONE[b], VITTORIA MANASSERO[b], SERGEI FOKIN[b,c,d], CLAUDIA VANNINI[b]

[a]Department of Botany, University of British Columbia, Vancouver, Canada; [b]Department of Biology, University of Pisa, Pisa, Italy; [c]Department of Invertebrate Zoology, St.-Petersburg State University, St. Petersburg, Russia; [d]St. Petersburg Branch of the S.I. Vavilov Institute of History of Science and Technology, Russian Academy of Sciences, St. Petersburg, Russia.

E-mail of the corresponding Author: claudia.vannini@unipi.it

Symbioses between prokaryotes and microbial eukaryotes, particularly ciliated protists, have been studied for a long time. Nevertheless, researchers have focused only on a few cultivable host genera and species, usually considering a single symbiont at a time. Therefore, it is likely that a great number of yet-undiscovered microbial associations, involving uncultivable or difficult-to-cultivate ciliate hosts, is present in the natural environment. Almost nothing is known about complex prokaryotic consortia associated with individual ciliate hosts. Moreover, field investigations are lacking, and almost nothing is known concerning the epidemiology of even the most studied symbioses. Here, we present a pilot study using a single-cell microbiomic approach that couples reliable characterizations of the hosts to environmental surveys, through ciliate isolation followed by simultaneous amplification of eukaryotic and prokaryotic markers. The method gave reliable and satisfactory results on all ciliate specimens, regardless of their original habitat (marine or freshwater) and taxonomic affiliation. Prokaryotic microbiomes of ciliate cells differ from prokaryotic communities in the same site and habitat, suggesting that, as already assessed for many macro-organisms like plants and metazoans, ciliated protists harbor specific microbiomes. This study identified for the first time several novel prokaryotic taxa potentially associated with ciliates, and also observed known prokaryotic symbionts in association with unexpected hosts. Preliminary data on the prevalence of symbiotic prokaryotes in natural populations of the ciliate *Euplotes* have also been collected. To our knowledge, this is the first epidemiological screening on associations between protists and prokaryotes, and takes the first step towards an understanding of the distribution and ecological drivers of these symbioses. Single-cell microbiomics can now be applied to more studies aiming to unravel the evolutionary and ecological meaning of these symbiotic systems.

Oral Session TAXONOMY & PHYLOGENY 4

Shedding Light on Chaotic Shells: Reassessing Biodiversity in Genera *Centropyxis* and *Arcella* (Amoebozoa: Arcellinida) Based on Phylogenetic and Morphometric Data

RUBÉN GONZÁLEZ-MIGUÉNS[a], CARMEN SOLER-ZAMORA[a], MILCHO TODOROV[b], FERRY SIEMENSMA[c], ENRIQUE LARA[a]

[a]Department of Micology, Real Jardín Botánico (RJB-CSIC), Madrid. Spain; [b]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria; [c]independent researcher, Kortenhoef, Netherland.

E-mail of the corresponding Authors: rgmiguens@rjb.csic.es; csoler96@gmail.com, enrique.lara@rjb.csic.es

Species delimitation are subject to debate in all taxonomic groups, including protists. Therefore, a combination of approaches (i.e. morphological and phylogenetic) that integrate different species concepts is often favoured. Indeed, speciation processes are not always followed by morphological diversification, and these can be masked by phenotypic variation, making necessary the use of a combination of morphometrics and molecular approaches for species delimitation. Species diversity evaluation has often been an issue in Arcellinid testate amoebae, and a rational integration of multiple species concepts has revealed high levels of unknown diversity. Genera *Centropyxis*, Stein and *Arcella*, Ehrenberg are particularly problematic in this sense, with many poorly defined species and almost no genetic data. These organisms are present worldwide in a wide variety of environments, from more pristine environments to new environments created as a result of human activities. A reassessment of the taxonomy of these groups should create a knowledge baseline with implications in conservation of natural resources and also evolutionary biology. Here, we performed an integrative approach using the cytochrome oxidase subunit I (COI) in *Arcella* and the gene coding for the small subunit ribosomal RNA (SSU rRNA) in *Centropyxis*, together with a morphometric analysis, to infer the phylogenetic relations within these genera. The results allow the delimitation of new independent taxonomic units, towards a revision of the genera *Arcella* and *Centropyxis*.

Phylogeny and Evolution of Thecamoebid Amoebae (Amoebozoa, Discosea, Thecamoebida) YELISEI MESENTSEV[a], OKSANA KAMYSHATSKAYA[a,b], KIRILL LOTONIN[a], ELENA NASSONOVA[a,c], ALEXEY SMIRNOV[a]

[a]Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; [b]Core facility center "Culturing of microorganisms", St. Petersburg State University, St. Petersburg, Russia, [c]Laboratory of Cytology of Unicellular organisms, Institute of Cytology RAS, St. Petersburg, Russia.

E-mail of the corresponding Authors: limobestia@yandex.ru; oksana.kamyshatskaya@gmail.com; kirilllotonin@yandex.ru; nosema@mail.ru; polychaos@mail.ru

Amoebae of the family Thecamoebidae are easily recognizable and until recently we believed that most of them could be determined to species even at the light-microscopic level. The family comprises 19 species unified in four genera – *Thecamoeba*, *Sappinia*, *Stenamoeba*, and recently established *Stratorugosa*. Of them, genera *Stenamoeba*, *Sappinia* and *Stratorugosa* are rather well studied with modern methods. However, the central and the largest genus of the family – the genus *Thecamoeba* remains very poorly covered both with the ultrastructural and the molecular data.

We isolated 80 strains of thecamoebids, including no less than 15 species possessing pronounced distinctive characters even at the light-microscopic level. Most strains were isolated from terrestrial habitats, in particular – from the leaf litter, which appears to be the "hotspot" of thecamoebid diversity. We have shown the presence of sibling species within this genus and motivated the need in molecular data for the identification of isolates. By some reasons, which remain not yet clear, it is very hard to amplify the SSU rRNA gene of *Thecamoeba*, so we applied single -cell genomics to obtain data. The Cox I gene was found to be much easier to study. Basing on these data we have shown that the genus *Thecamoeba* consists of no less than four phylogenetically distinct groups. Three of them show a clear correlation with the type of the nuclear structure (vesicular nucleus, nucleus with small peripheral nucleoli and nucleus with large ribbon-like peripheral nucleoli, respectively). Of special interest is the clade, formed by the largest *Thecamoeba* species, possessing diverse and complex nuclear structure. The diversity of new morphological species and detection of sibling species show that diversity of thecamoebid amoebae remains underexplored and each known morphological species potentially may represent a new species group.

Supported with RFBR 19-04-01147 project.

The Cryptic Biodiversity of the Genus *Paramoeba* (Amoebozoa, Dactylopodida)

EKATERINA VOLKOVA[a]

[a]Laboratory of Cellular and Molecular Protistology, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

E-mail of the corresponding Author: arcellinidae@gmail.com

Paramoeba are marine amoebae of the order Dactylopodida which together with the members of the genus *Neoparamoeba* possess an eukaryotic symbiont – *Perkinsela*-like organism (PLO). In contrast to well-known amphizoic *Neoparamoeba*, the genus *Paramoeba* is poorly studied. Today there are only two described species, *P. eilhardi* and *P. atlantica*. I sampled different coastal and sublittoral biotopes in several localities of the White and Mediterranean Seas and isolated five new strains assigned to the genus *Paramoeba*. At the first sight, all these strains are very similar in morphology and difficult to distinguish from the type species *P. eilhardi*. However, a closer examination revealed subtle differences in sizes and shapes of locomotive forms between different strains. Light microscopic study including DAPI staining showed that three new strains were PLO-free. The molecular study based on COI and SSU rRNA genes showed that the sequences of different strains differed from each other and formed separate clades. Phylogenetic analysis of both markers, with addition of a more comprehensive dataset on the COI genes of *Neoparamoeba* spp. showed that all new *Paramoeba* species are grouped together in a single clade including *Paramoeba eilhardi* but excluding *Paramoeba atlantica*. Molecular analysis of the new strains confirms that they belong to new species, with the exception of one aposymbiotic amoeba isolated from the Italian Mediterranean coast that could not be distinguished from *Paramoeba eilhardi* CCAP 1560/2 based on sequence data. This finding suggests that we are dealing with the secondary loss of PLO in an independent strain assignable to *Paramoeba eilhardi*. This study demonstrates a significant biodiversity of morphologically similar species hidden inside the genus *Paramoeba*. A considerable number of these species can be found in fairly well accessible habitats suggesting a potential for a broad expansion of the biodiversity of this clade of amoebae and a refinement of its phylogeny. The data reported provide a challenge for the point of view that all *Neoparamoeba* and *Paramoeba* contain a PLO. I conclude that the loss of PLO in the evolution of *Paramoeba* occurred repeatedly in different lineages. This event may probably take place even in separate populations of the same nominal species, as I show for *P.eilhardi*.

Supported with the RFBR grant 18-34-00726; utilized equipment of St. Petersburg State University Research Park.

Capsules – Unique Organelles Characterising Specific Tintinnid Ciliate Clades (Alveolata, Ciliophora)

MAXIMILIAN GANSER[a], SABINE AGATHA[a]
[a]Department of Biosciences, University of Salzburg, Salzburg, Austria.

E-mail of the corresponding Author: maximilian.ganser@sbg.ac.at

The main feature of tintinnid ciliates is their lorica, which was used to describe and classify the more than 1,000 species. Current phylogenies demonstrate, however, that the present classification does not reflect the evolutionary relationships inferred from gene sequences in this monophyletic group. Pivotal for understanding the genealogies of tintinnid taxa as revealed by SSU rDNA gene trees is the cell morphology, for which the body of literature is comparatively scarce. The somatic ciliary pattern is of particular importance. Its development from a simple to a complex pattern by the successive addition of ciliary fields and specialised kineties generally reflects the evolutionary inferences derived from the molecular data. Nevertheless, many tintinnid species can currently not properly be assigned to particular families and/or their evolutionary relationships remain unresolved. A promising feature complex for supporting molecular evolutionary hypotheses is the capsule. These nanoscale organelles are primarily located directly underneath the cell membrane in cytoplasmic extensions that form beaded strands (striae) and/or pin-shaped organelles (tentaculoids) between or closely attached to the adoral membranelles of the tintinnids. The ultrastructure of the capsules suggests a new type of extrusive organelle unique for the tintinnid ciliates. Various capsule morphotypes possessing a similar basic structure are known. Different capsule types are supposed to be characteristic for certain genera or even families. In the present study, ultrastructural investigations on capsules from seven tintinnid families and clades allowed for verifying previous data and for expanding the taxon coverage. Taxa clustering together despite different lorica structures (hyaline, agglutinated), thus, often share the same capsule type. These preliminary data have to be confirmed by a broader taxon sampling, but might finally provide characters for diagnosing particular branches in the tintinnid phylogeny.
This study is supported by the Austrian Science Fund (FWF Project I3268).

Improving the Phylogeny of Amoebozoa: New Hypothesis on the Position of *Stygamoeba* and *Vermistella* (Discosea)

KIRILL LOTONIN[a], ALEXEY SMIRNOV[a]

[a]Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia.

E-mail of the corresponding Authors: kirilllotonin@yandex.ru; polychaos@mail.ru

Phylogenetic and phylogenomic approaches contribute a lot to resolving systematics of Amoebozoa, however, positions of some evolutionarily important lineages still remain unclear. Our recent finding shed light on the phylogenetic position of *Stygamoeba* – *Vermistella* group among amoeboid protists of the class Discosea. During the study of naked amoebae diversity in the bottom sediments of Nivå Bay (Baltic Sea, The Sound), we have found a new species morphologically resembling *Stygamoeba*. Nevertheless, 18S rRNA gene sequences revealed that this species belongs to the genus *Balamuthia* and represents a new species closely related to *Balamuthia mandrillaris.* The morphology of this organism is intermediate between *Stygamoeba* and *Balamuthia*; this allows creating a morphological row showing strong resemblance of these genera. We analyzed available data on the ultrastructure of mitochondria in *Balamuthia* and found that they are similar to those of *Stygamoeba* and *Vermistella* by the presence of flattened, ribbon-like cristae. The alignment of *Stygamoeba* SSU rRNA gene with that of *Vermistella* and *Balamuthia* shows the close structure similarity of this gene in all three genera. These species group together as a part of Centramoebia in SSU trees, when the tree contains a limited number of related taxa and a high number of sites is used in the analysis. However, with the increment of taxon sampling and the decrement of the number of sites used for the phylogeny reconstruction this grouping tends to dissapear. We have observed similar pattern in our multigene analysis. Thus, we suggest that amoebae genera *Balamuthia*, *Stygamoeba* and *Vermistella* are closely related and belong to Centramoebia in the phylogenetic tree. Supported with the RSF grant 17-14-01391.

Copemetopus mystakophoros sp. nov., a New Representative of the Ciliate Genus from a Brackish Water Pond on the Ligurian Sea Coastline (Tuscany, Italy)

SERGEI I. FOKIN[a,b], VALENTINA SERRA[a] ILARIA ANDREOLI[a], FRANCO VERNI[a], GIULIO PETRONI[a], LETIZIA MODEO[a]

[a]Department of Biology, University of Pisa, Pisa, Italy; [b]Department of Invertebrate Zoology, St. Petersburg State University, St. Petersburg, Russia.

E-mail of the corresponding Author: sergey.fokin@unipi.it

Ciliates of the genus *Copemetopus* Villeneuve-Brachon, 1940 are a brackish water protistan community adapted to live in almost anoxic environments. Up to now, only the *C. subsalsus* species has been described: Villeneuve-Brachon (1940) found it in samples from Sète district, Languedoc, France (salinity: <33‰) and assigned it to the Heterotrichea class. More recently, Al-Rasheid (2001) described this species again, in samples from the Al-Hassa Oasis, on the Arabian Gulf coastline of Saudi Arabia (salinity: >130‰). We discovered *Copemetopus* sp. in samples from the mouth of the Serchio River, in the area of Pisa, in Italy (salinity: 15‰). According to the main morphological characters (i.e. cell sizes, nuclear apparatus features, and some kinetom peculiarities) our isolate was similar to previously studied populations: size 200-450 x 90-150 ($\overline{x}$=260x100) µm; 85-103 ($\overline{x}$=92) somatic kineties; 6 dorsal brush kineties; a single dumbbell-shaped macronucleus; and 7-16 ($\overline{x}$=10) micronuclei. However, by combining different techniques (light microscopy, TEM, SEM, Feulgen staining, silver-nitrate impregnation, and FISH), it became clear that the Italian population diverged, as specimens showed somatic ciliature organized by sets of 3-6 ($\overline{x}$ =4) cilia (*vs* 1 in *C. subsalsus*) and a paroral membrane with 2 ciliary rows (*vs* 3). In addition, 8-20 ($\overline{x}$=12) (*vs* 9-10), very long sets of cilia (whips) decorated the anterior part of oral ciliature, and were clearly separated from oral cilia; this feature was not reported by Al-Rasheid (2001). It is probable that their position has been overlooked by previous authors. Based on this information, we consider our population to be a new species of the genus, and have named it *Copemetopus mystakophoros* sp. nov. FISH, SEM and TEM analyses indicated that both ecto- and endo- bacterial symbionts are associated to *C. mystakophoros* sp. nov., but, unfortunately, they were only morphologically characterized. Interestingly, while TEM data supported ciliate inclusion in Heterotrichea, phylogenetic analysis based on 18S rDNA sequencing showed that *C. mystakophoros* belongs to an early arising subclade that is independent from the main branch of the class. The present research, together with other work on anoxic brackish water environments that has been conducted in our lab for several years, suggests that some of these environments likely hide yet-unknown lineages of ciliated protists.

Ciliate Life in River Yamuna

KOMAL KAMRA[a], HARPREET KAUR[a], SHASHI[a]

[a]Ciliate Biology lab, SGTB Khalsa College, University of Delhi, Delhi, India.

E-mail of the corresponding Author: komal@sgtbkhalsa.du.ac.in

Studies of microbial biodiversity in rivers present a challenging task as a lotic body is constantly affected by natural and artificial changes. River Yamuna, the largest tributary of River Ganges, originates from Yamunotri glacier in the Himalayas and harbours diverse microbial life. It traverses 1370 Km through 5 states of Northern India before joining River Ganges at Allahabad. In the present study, ciliated protists were isolated, identified and catalogued from select sites along the River Yamuna flowing through the Delhi, India, stretch over a period of two years using an integrative approach. Eighty ciliate species belonging to 14 orders were identified. Out of these, 34 species belonging to the Order Hypotrichida included species of *Bakuella*, *Neogastrostyla*, *Oxytricha*, *Notohymena*, *Aspidisca* and *Euplotes*. The dominant non-hypotrichs were species of *Coleps*, *Dileptus*, *Paramecium*, *Frontonia*, *Spirostomum*, *Blepharisma*, *Cyclidium*, *Vorticella*, *Chilodonella* and *Colpoda*. Diversity index was calculated to estimate the ciliate diversity in the region. Morphological, morphometric, morphogenetic and phylogenetic analyses of some of the dominant ciliate species will be discussed.

Towards a New Phylogeny of the Order Trichiales (Myxomycetes, Amoebozoa)

IVÁN GARCÍA-CUNCHILLOS[a], JUAN CARLOS ZAMORA[b], MARTIN RYBERG[c], CARLOS LADO[a]

[a]Real Jardín Botánico, CSIC. Madrid, Spain; [b]Museum of Evolution, Uppsala University. Uppsala, Sweden; [c]Department of Organismal Biology, Systematic Biology, Uppsala University. Uppsala, Sweden.

E-mail of the corresponding Author: igcun@rjb.csic.es

The systematics of the Myxomycetes have been largely based on the morphological features of the fruiting bodies developed at the end of their life cycle. Since 15 years ago the use of molecular tools has been applied to the study of Myxomycetes' systematics and phylogeny, resulting in the recognition of two big clades: bright- and dark-spored Myxomycetes. The first phylogenies of the bright-spored clade, which includes the orders Trichiales and Liceales, are considered preliminarly because although they showed the delimitation of the two orders did not correspond to current classifications, unfortunately, they only included a limited number of species. The objective of this work is to deepen the knowledge of the evolutionary relationships among the species of the Trichiales order, increasing the sampling of the taxa and carrying out a multigene approach. To achieve this goal, a total of 67 species belonging to 17 genera of the order Trichiales were analysed and four independent DNA regions were studied: 18S ribosomal RNA, eukaryotic translation elongation factor 1 alpha 1, 12S ribosomal RNA (small subunit of the mitochondrial ribosome) and cytochrome c oxidase I. Phylogenetic analysis of the obtained data were conducted under both maximum likelihood and bayesian inference approaches. The results obtained in this study revealed a strong conflict between the current classification of the order and the recovered phylogenetic relationships. In this way, while some of the genera were splitted into two or more different independent clades, some new clades appeared clustering different species from different genera. In light of this new discovered relationships different morphological features of the fruiting bodies were studied. Increasing the taxon sampling in the Trichiales phylogeny sheds light on the evolutionary relationships of the species and challenges the current classification of the order, suggesting that a major systematic revision is needed.

Cultivation and Phylogenetic Diversity of Colponemids, a Crucial Assemblage for Inferring Alveolate Evolution

ANDREA GIGEROFF[a], YANA EGLIT[a], ALASTAIR GB SIMPSON[a]
[a]Department of Biology, Dalhousie University, Halifax, Canada.

E-mail of the corresponding Author: alastair.simpson@dal.ca

Alveolates represent one of the most diverse groups of eukaryotes and they are of tremendous importance in ecological terms, as well as for human and animal health. They include the ciliate protozoa, plus the apicomplexan parasites and dinoflagellate algae (together with their relatives, the colpodellids, chromerids and perkinsids). Recently, it was confirmed that colponemids, an assemblage of small-to-medium-sized biflagellated eukaryotrophs, also belong to the alveolate clade: molecular phylogenies including two *Colponema* species, one *Acavomonas*, and an SSU rDNA sequence from a crude enrichment containing *Palustrimonas*, place them as up to three different clades branching near the divergence of ciliates from other classical alveolates (myzozoans). We isolated four strains of colponemid-like organisms from hypersaline or alkaline habitats. Each was successfully established as a di-eukaryotic culture or cultures with a single prey species, either a kinetoplastid or a heterolobosean with a flagellate stage (*Pharyngomonas* or *Percolomonas*). Where documented, prey capture is raptorial via adhesion of a region near the base of the posterior flagellum. Further morphological features will be discussed. SSU rDNA phylogenies confirm that all new strains are deep-branching alveolates. One strain represents the first stable culture of *Palustrimonas*, while the others likely represent two new genera. The relationships amongst ciliates, myzozoans and the (now) five lineages of colponemids are extremely unstable in SSU rDNA phylogenies. This study confirms the critical importance of colponemids for understanding both the deep level phylogeny of alveolates, and the evolutionary history within the group, including, for example, the origins of the apical complex and the plastids of (many) myzozoa. Our new isolations therefore represent an important resource to allow future taxon-broad –omic-level examinations of these questions.

WORKSHOP Protistological Sciences dissemination

(by SIP/ISOP Grant-in-aid)

Protists are for Everyone: a Personal Overview of Knowledge Dissemination and Promoting Public Awareness

ALAN WARREN[a]
[a]Department of Life Sciences, Natural History Museum, London, UK.
E-mail of the corresponding Author: a.warren@nhm.ac.uk

With the possible exception of tertiary- and school-level education, knowledge dissemination and the promotion of public awareness of protists tends to be somewhat uncoordinated and relies on the (often heroic) efforts of individual protistologists. Such efforts usually go undocumented and are therefore difficult to summarize. Based largely on information provided by fellow protistologists, I will give a personal overview with examples of best practice and highlight some areas of neglect (i.e., a wish-list of things I would like to see). This will include: the role of natural history museums in exhibiting protists and making their collections accessible; public engagement via talks, science festivals and citizen science projects; educational materials such as videos and slide presentations; protist-inspired artworks and commercial products; and, last but not least, protist books for children.

Microscope Madness – the Excitement of Public Engagement with Protists

GENOVEVA F. ESTEBAN[a]
[a]Department of Life and Environmental Sciences, Bournemouth University, Poole, UK.

E-mail of the corresponding Author: gesteban@boournemouth.ac.uk

From young children to nonagenarians (and beyond!), people go mad when they look down the microscope and see living protists. Watching a multitude of *Euglena* in a small drop of water, the delicate shape of the colonial diatom *Asterionella*, the cytoplasm of *Metamoeba* flowing into its large emergent pseudopodia, or the majestic pink of *Blepharisma* catching small flagellates with its oral cilia, is all too exhilarating for them to witness.
My presentation will showcase successful examples of interacting with the general public through protists. One of the challenges is for children and their families to understand how to deal with something that is living but invisible to the naked eye. To solve this, I run hands-on, interactive activities at events and workshops where children, the general public and students are directly responsible of the event's success. After a short briefing, they are fully in charge of handling harmless living protists, microscopes, slides, pipettes. They also learn how to identify protists by following simple identification keys
I will also focus my presentation on how I promote protistology and aquatic sciences in schools, and how I organise public events in the local communities, both in the UK and abroad. Most recently, I have led the first Family Science Festival in the heart of Dorchester (a county town in southern England) in collaboration with the County Museum, the Town Council and the local schools, which saw over 2,000 people attending.

Introducing the Fascinating World of Ciliates to Undergraduate Students

KOMAL KAMRA[a]

[a]Department of Zoology, SGTB Khalsa College, University of Delhi, Delhi, India.

E-mail of the corresponding Author: komal@sgtbkhalsa.du.ac.in

My tryst with ciliated protists began soon after my masters in cell biology, having joined a lab where students pursuing their PhD program were exploring different aspects of these tiny microbes. At the same time, I began teaching undergraduates in a college of the University of Delhi. After completion of my PhD, I was unofficially supervising two students when it occurred to me that undergraduates (UG) should be exposed to the fascinating world of ciliates. Setting up a research laboratory in the college was the first step where students could pursue PhD programs and the UG students could do summer research projects. This paid rich dividends. In fact, two UG students came back to do their PhD in the lab. While the lab is funded by research projects I apply for, the UG students join the PhD students to do small projects. A strong Delhi group emerged when two students, now teaching in different colleges, also established a lab similar to mine in one of their colleges. The University recognized the effort and funded projects for UG students and the colleges followed. We have also organized two editions of 'International Symposium on Ciliate Biology' largely meant for UG students and teachers, giving them a platform to showcase the work they have done, interact with protistologists around the globe, and enthuse them to pursue this field later for research. Media attention followed and, in recent times, we have had two articles published in the No 1 daily newspaper of India. What catches the eye of the general public is the fact that pollution is affecting biodiversity, including those of ciliated protists, and this will be inimical because of the valuable role they play in the ecosystem.

Cell-fies: Using Instagram to Share Microbiology with a Global Audience

HUNTER HINES[a]

[a]Bournemouth University, USA.

E-mail of the corresponding Author: hunter.n.hines@gmail.com

Instagram is a smartphone based application dedicated to sharing of image content from an unlimited array of areas. Since its launch in 2010, Instagram has amassed over 1 billion users world-wide covering an extensive range of interests. Scientists often generate images as a part of their research and many could find an interested following for their content. An account @microbialecology was created in 2017 to share images of the microbes encountered during the research for a PhD. Images and short videos were produced and posted several times a week with a brief caption explaining the interesting features of the organism's biology and ecology. Within one year the account had amassed over 50,000 global followers. Instagram analytics revealed that the profile received over 1.4 million 'impressions', the total number of times all posts are viewed, in a single week at the end of 2018. While some followers initially have familiarity with classic examples of microbes (e.g. protozoa such as ciliates), many had never seen or considered microbes as the living, moving, and amazing organisms that they are. Instagram allows the free exchange of images and ideas with a global audience on a potentially unlimited array of subjects. This has powerful implications for inspiring interest in science to areas which previously may not have been exposed to such content. It has now become a reality that underprivileged regions and nations can increasingly receive free content, including science such as microbiology. Instagram is an excellent and rapid way for scientists to share content to both amateurs and professionals, perhaps even by the very experts who define their field.

Protists as Model Organisms for Biology Teaching

FEDERICO BUONANNO[a], GABRIELE ACHILLE[a], CLAUDIO ORTENZI[a]
[a]Laboratory of Protistology and Biology Education, Department of E.C.H.T., Università degli Studi di Macerata, Macerata, Italy.

E-mail of the corresponding Authors: federico.buonanno@unimc.it; claudio.ortenzi@unimc.it

Protists are one of the best models of single-celled organisms for use in teaching biology. Free-living species are very common in all fresh- and salt water environments, as well as in soils, whereas some species can be found as parasites or symbionts in large groups of eukaryotes.

In this context, we focused on heterotrophic protists, usually known as "protozoa," which are simple to collect, grow and manipulate. The various protozoa are extraordinarily different in form and activity. These unicellular microscopic organisms carry out all the functions of life within the single cell. They obtain food, digest it, and release indigestible matter from the cell body. They have no nervous system, yet respond to environmental stimuli. They grow, differentiate, have sex, and reproduce. They have no muscles, yet they move in very complex ways. Protozoa perform all of these activities within the small space of the single cell that forms their body.

Since all life is made up of cells, the study of protozoa may therefore help students to understand how larger forms of life survive and maintain their health.

Using protozoa in the classroom could be a particularly good strategy for explaining and modeling macroscopic processes and systems. What students view through microscope could then be extrapolated into an understanding of their everyday experience.

The Hidden World of Diatoms-Inspiring Children to Learn About the Wonders of Nature

EMANUELA SAMARITANI[a]
[a]The hidden world, Sheffield, UK.

E-mail of the corresponding Author: emanuela.samaritani@gmail.com

A double passion like science and reading can only increase exponentially when becoming a parent, biologically or by proxy. Discovering children literature is a very exciting experience, but what a big disappointment it can be to find out that the innumerable sides of protistology are largely unexplored, if at all, in the younger section of children's books. An avid reader myself, and having had the chance to peek briefly into the microworld, I decided I was going to fix this gap. I spent a year writing, crowdfunding and self-publishing, creating a website and expanding connections and followers on social media, in order to create The hidden world of diatoms, the first book of a series aiming at teaching children about protists existence, importance, relevance and beauty. In this talk I will tell you more about my journey from the idea to holding my book and how I am encouraging children to discover protists, one organism and one book at a time.

Promoting Protists Using Websites and Social Media to reach the General Public

JOHN DOLAN[a]
[a]Laboratoire d'Océanography de Villefranche-sur -Mer, CNRS Sorbonne Université Station Zoologique, Villefranche-sur-Mer, France.

E-mail of the corresponding Author: dolan@obs-vlfr.fr

These days many people follow current events and investigate unfamiliar subjects exclusively through the web. Consequently, if we protistologists want to reach people outside of our academic "bubble" we need to engage them through the media they use. In this short presentation, based on my personal experience, I will review the pros and cons of the use of a personal website, Facebook pages and groups, Instagram, Twitter, participation in group-based websites such as Wikipedia contributing to art and photography websites.

Examples:

http://www.obs-vlfr.fr/~dolan/
http://gallery.obs-vlfr.fr/gallery2/v/Aquaparadox/
http://aquaparadox.obs-vlfr.fr/html/ClassicMonographs.php
https://en.wikipedia.org/wiki/Tintinnid
https://www.flickr.com/photos/56879865@N08/
https://twitter.com/JohnDol84297193
https://fineartamerica.com/artists/7+john+dolan
http://www.marinespecies.org/photogallery.php?p=search&term=dolan&search.x=0&search.y=0
https://www.facebook.com/JournalPlanktonResearch

Introducing the Amazing World of Protists through Photography and Videography

ANUSH KOSAKYAN[a]

[a]Institute of Parasitology, Biology Center, Czech Academy of Sciences, Czeske Budejóvice, Czech Republic.
E-mail of the corresponding Author: anna.kosakyan@gmail.com

Protists are everywhere and consist of the vast majority of eukaryotic biodiversity. They are involved in numerous biochemical and ecological processes at the global scale, yet are often forgotten and neglected. Fortunately, nowadays the development of modern techniques give us the possibility to observe and document the invisible world of protist in a manner that was not possible before.

I will share my view on how high-quality photography and videography can become a powerful and inspiring tool to introduce this unique group of organisms to the non-scientific community. Additionally, I will discuss my experience of popularization of other organisms that could be applied for protists.

SYMPOSIUM - Mixotrophic Planktonic Protists: Living with The "Perfect Beast (by ISOP)

Mixotrophs – Food Webs and Harmful Algal Blooms

DIANE STOECKER[a]
[a]Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge MD, USA.

E-mail of the corresponding Author: stoecker@umces.edu

Mixotrophs that combine photosynthesis and ingestion have long been recognized by protistologists as common in the plankton. Only relatively recently has the quantitative importance of mixotrophy been recognized in biological oceanography. Many phytoflagellates, are constitutive mixotrophs (CM) (they have their own plastids but can ingest prey). Small mixotrophic phytoflagellates can be responsible for much of the primary production (C fixation) in nutrient (N, P or Fe) poor seas and in coastal waters during summer stratification. Mixotrophy in many harmful algal (HA) species contributes to their ability to form blooms which are disruptive to ecosystems and, in some cases, toxic. At least in some harmful dinoflagellate and haptophyte species, toxicity is linked to their ability to capture and subdue prey. Among the microzooplankton, non-constitutive mixotrophy (NCM) (plastid retention or algal endosymbiosis) is common in some taxa of oligotrich ciliates and in *Mesodinium rubrum*, a few dinoflagellates (primarily *Dinophysis* and *Amylax* spp.) and some rhizaria (Foraminifera, Polystinea and Acantharea). Biogeographical studies of mixotrophy are in their infancy, but it is evident that different types of mixotrophs are important in different regimes; for example small mixotrophic phytoflagellates and Rhizaria with endosymbionts in nutrient poor subtropical seas and ciliates with plastids in polar, temperate and Mediterranean waters. Mixotrophic ciliates in particular are good foods for metazoan zooplankton and fish larvae and are thus an important part of food webs supporting fisheries. Mixotrophic Foraminifera are important in formation and deposition of calcite and mixotrophic Polysystinea in that of opal (SiO_2) in the oceans. Mixotrophy, by alleviating nutrient stress in phytoplankton and by boosting growth efficiency in microzooplankton, increases over-all production in the sunlit upper ocean and plays an important role in biogeochemical cycling.

Mixotrophs and Challenges for Experimental Studies

GEORGE B. MCMANUS[a], LUCIANA SANTOFERRARA[b]

[a]Dept of Marine Sciences, University of Connecticut, Storrs CT, USA; [b]Dept of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT, USA.

E-mail of the corresponding Author: george.mcmanus@uconn.edu

Simultaneous possession of phagotrophic and photosynthetic capability has been documented in many protists, including flagellates, rhizarians, and ciliates. Among these organisms, flagellates have been most amenable to cultivation and experimentation. Only a few ciliate mixotrophs have been cultivated for any length of time, so we know less about them, especially the ones that retain plastids from ingested food. In recent years, we have isolated an oligotrich ciliate, *Strombidium rassoulzadegani* from multiple locations and cultivated it for up to 7 years. It can retain and use plastids from chlorophytes and cryptophytes, but appears to grow wholly heterotrophically on dinoflagellates. The boost in growth efficiency due to phototrophy is only significant when food is limiting, and higher growth in the light is countered by worse growth in the dark, suggesting a "cost" to mixotrophy. New methods in transcriptomics are making experimental approaches for studying mixotrophy more tractable. To date no unique genes for plastid retention have been identified but the cultivability and metabolic flexibility of *S. rassoulzadegani* suggest that these approaches may be fruitful in future.

Mixotrophs and Challenges for Molecular Biology

FABRICE NOT[a]

[a]CNRS & Sorbonne University Station Biologique de Roscoff, UMR7144 Adaptation and Diversity in Marine Environment (AD2M) Laboratory, Ecology of Marine Plankton (ECOMAP) team, Roscoff, France.

E-mail of the corresponding Author: Fabrice.not@sb-roscoff.fr

This contribution presents a brief overview of the opportunities and challenges for the study of mixoplankton through molecular biology approaches. In the oceans, planktonic protists exhibiting both photo(auto)trophy and phago(hetero)trophy (aka mixoplankton), are widespread and key players for the functioning of oceanic ecosystems. Mixotrophy can occur in some species through the adaptation of trophic abilities according to environmental settings or *via* the endosymbiosis of a photosynthetic cell by a heterotrophic one (i.e. photosymbiosis). The recent advent of molecular biology approaches and more particularly of high throughput technologies for nucleic acids (DNA and RNA) sequencing has revolutionized the study of protist biology (e.g. transcriptomic) and ecology (environmental genomic) opening up new avenues for the study of mixoplankton. Yet, the scarcity of cultured representatives, the low starting amount of biological material, the paucity of reference data to compare with and the lack of dedicated bioinformatics tools available, are current challenges for appropriate data acquisition and interpretation. Thanks to the rapid progresses in these fields, one can assume most of the current obstacles will be overcome soon and pave the way for a true multidisciplinary approach, integrating physiological measurements (i.e. experimental and *in situ* studies) and genetic responses to reach an accurate understanding of plankton mixotrophy.

Challenges for Modelling the Perfect Beast

ADITEE MITRA[a], KEVIN J FLYNN[a]

[a]Swansea University, UK.

E-mail of the corresponding Author: A.Mitra@swansea.ac.uk

The bulk of the "phytoplankton" and half the "microzooplankton" are photo-phago-mixotrophs – they are mixoplankton. Getting the description of mixoplankton right is important for biogeochemical and fisheries models. The lack of hard physiological data requires that we exploit phenomenological data to at least ensure our modelled mixoplankton do not behave badly. Models of mixoplankton have often deployed trait trade-off descriptions, while in reality there is little evidence for such trade-offs. The same is true of allometric scaling rules; the evidence is contrary and actually mixoplankton range over the entire spectrum of protist plankton. Models typically fail to describe the variety of mixotrophic formats, the relative roles of different nutritional modes operating under different conditions. What we do know is that the complexity of the control of physiology within a given species (responses to light, nutrients, prey etc.), and differences in physiology between different mixotroph groups, argue strongly against attempts to deploy simple descriptions. To get to where we need to be will take a significant and coordinated effort. How we can simulate the enigmatic perfect beasts will form the basis of this presentation.

Oral Session SYMBIOSIS & ENDOSYMBIOSIS

Novel Proteins and Reorganized Protein Import in the Secondary Plastid of Euglena gracilis.

ANNA M. G. NOVÁK VANCLOVÁa, MARTIN ZOLTNERb, KRISTÍNA ZÁHONOVÁa,c, ZOLTÁN FÜSSY1a, MAREK ELIÁŠc, JOHN M. ARCHIBALDd, MARK C. FIELDb, VLADIMÍR HAMPLa

aFaculty of Science, Charles University, BIOCEV, Vestec, Czech Republic; bSchool of Life Sciences, University of Dundee, Dundee, UK; cFaculty of Science, University of Ostrava, Ostrava, Czech Republic; dDepartment of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada.

E-mail of the corresponding Authors: vanclova@gmail.com; vladimir.hampl@natur.cuni.cz

Euglena gracilis is a well-studied and biotechnologically exploitable phototrophic flagellate harbouring secondary green plastids surrounded by three membranes. Several transcriptomic datasets of this organism were generated to this date, the most recent one coupled with a draft assembly of its ~0.5 Gbp genome and high-performance liquid chromatography/mass spectrometry-based proteomes of its organellar fractions. The thorough analysis of the plastid proteome revealed that the machinery for protein import across the envelope membranes has undergone major reorganization. TOC complex is either completely missing or diverged beyond recognition and TIC complex is highly reduced, possibly to the degree of constituting of only one conserved subunit (Tic21) in multiple isoforms. Strangely enough, the transit peptides of the plastid-targeted pre-proteins retain their characteristics shared with plant and other algal transit peptides (and are recognizable in a heterologous system by plant TOC), but also exhibits certain unique features which were previously overlooked. Meanwhile, the mechanism for protein import across the additional third membrane, as well as the middle one devoid of TOC, must employ novel or re-purposed translocases. We identified plastid-localized paralogs of ER-associated proteins: cpGOSR1 and cpRab5 which likely mediate the transport vesicle fusion, and two derlin-like proteins which might represent a part of a system analogous to the symbiont-specific ERAD-like machinery (SELMA) known from secondary plastids of various "chromalveolates". Additionally, an unusual homolog of the alpha subunit of F-type ATP synthase was noticed in the plastid proteome and its phylogeny and distribution pattern in eukaryotes suggests its significance and association with plastids, and secondary plastids in particular. New insights and results regarding the evolutionary history and function of these novel plastidial proteins will be presented on the conference.

Insights into the Evolution of Plastids of Euglenophyta

KACPER MACISZEWSKIa, NADJA DABBAGHb, ALEKSANDRA BOKUSa, MATTHEW S. BENNETTc, ANGELIKA PREISFELDb, RICHARD E. TRIEMERc, ANNA KARNKOWSKAa

aDepartment of Molecular Phylogenetics and Evolution, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Poland; bZoology and Didactics of Biology, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Germany; cDepartment of Plant Biology, Michigan State University, USA.

E-mail of the corresponding Author: ankarn@biol.uw.edu.pl

Euglenids are unicellular marine and freshwater algae, which acquired plastids by secondary endosymbiosis with a chlorophytean alga. Despite the common origin of their plastids, a vast variability of gene order can be observed in the plastid genomes, indicating their highly dynamic evolution. The quadripartite structure of plastid genomes, with two inverted repeats comprising rDNA, has been lost in euglenids but the number and nature of those losses are debatable. To expand the current state of knowledge on the evolution of euglenid plastid genomes, we obtained nine new plastid genomes form Phacaceae and three new plastid genomes of Eutreptiales. We observed the presence of quadripartite structure in several of the investigated lineages, indicating at least three independent losses of this typical structure in Euglenophyta. Additionally, we detected unusual ptDNA structures, such as a gene-deprived small single-copy region in Eutreptiales, which might help to understand the process behind the loss of inverted repeats.

Several times euglenids secondarily lost their ability of photosynthesis. It has been shown for one of the bleached species, *E. longa*, that it kept reduced plastid genome, with the RuBisCo large subunit gene retained as the sole remnant of the plastid-encoded part of the photosynthetic apparatus. For several other secondarily non-photosynthetic euglenid species, the evolutionary processes affecting their plastids remain unknown. To understand the reductive evolution of plastids we investigate *E. gracilis* var. *bacillaris* W3BUL – a bleached mutant that permanently lost its photosynthetic capabilities as a result of ultraviolet irradiation and naturally non-photosynthetic *E. quartana*. The analysis of their genomic DNA has shown no trace of the plastid genome, but in case of W3BUL the nuclear-encoded, plastid-targeted genes were abundant suggesting possible presence of genome-less remnant plastid. We expect to reach a conclusion on the fate of the W3BUL strain's plastid once its transcriptome is sequenced.

Path Towards Amitochondriality: Comparative Genomics of Preaxostyla flagellates

LUKAS V. F. NOVAKa, SEBASTIAN C. TREITLIa, ONDREJ BRZONa, LAURA EMEa, ANNA KARNKOWSKAa, SHWETA PIPALIYAa, PETR SOUKALa, VOJTECH VACEKa, JOEL B. DACKSa, MAREK ELIASa, VLADIMIR HAMPLa
aDepartment of Parasitology, Faculty of Science, Charles University, BIOCEV, Prague, Czechia.

E-mail of the corresponding Author: niun42@gmail.com

The first known eukaryotic organism completely devoid of the mitochondrial compartment, *Monocercomonoides exilis*, belongs to Preaxostyla, a metamonad group consisting of 1) paraphyletic free-living trimastigids with excavate morphology and mitochondria-like organelles resembling hydrogenosomes, and 2) a monophyletic group of morphologically divergent, endobiotic, putatively amitochondrial oxymonads inhabiting guts of cockroaches, termites and some other metazoans. We hope to gain better insight into the radical evolutionary changes this group of protists went through (from free-living to endobiotic, from reduced mitochondria to no mitochondria) by comparative analysis and annotation of genomes and transcriptomes of selected representatives. In addition to the already available transcriptomic and genomic sequences of *Trimastix marina* and *Monocercomonoides exilis*, we have sequenced and assembled genomic sequences of *Paratrimastix pyriformis*, *Blattamonas nauphoetae*, and *Streblomastix strix*. Annotations of multiple cellular systems (including mitochondrion biogenesis and metabolism, amino acid metabolism, metabolite transporters, autophagy, and endomembrane transport) in the 5 organisms, as well as lateral gene transfer to Preaxostyla, is discussed in the context of endobiosis and amitochondriality evolution.

Searching for Rare Protists: Evaluating Different Strategies for Finding Hidden Symbiotic Biodiversity in Arthropoda.

ALEXANDRA BELIAVSKAIAa, ALISTAIR DARBYa
aInstitute of Integrative Biology, University of Liverpool, UK.

E-mail of the corresponding Author: a.beliavskaia@liverpool.ac.uk

Eukaryotic part of symbiotic communities is hugely under-investigated. Most previous research on unicellular eukaryotic microbes has used culture-based methods or barcoding and metagenomic approaches. Eukaryotic symbionts are often sparse and hard to discover using SSU rDNA sequencing because of the similarities between the host and symbiont 18S genes. This, together with the sparsity of such symbionts, defines a need for an easy and efficient initial screening method. Naïve PCR-based methods are not sensitive enough and cannot detect low abundance species, and DNA of rare symbionts tends to be missed in the early stages of the amplification.
Several methods have been reported to block host DNA and make selective screening possible: restriction of the 18S gene of host DNA, amplification with annealing blocking primers, amplification with elongation arrest blockers, and blocking host sequences with artificial nucleic acid oligonucleotides, such as peptide-nucleic acids (PNA). There is little data on using these approaches with *Arthropoda*, so there is a need to test and evaluate them using a model system. In our work, we have undertaken an experimental comparison of these approaches in order to find the most efficient method of novel rare eukaryote discovery. We screened several populations of blood-feeding arthropods such as tsetse flies *Glossina* spp., kedflies *Lipoptena* spp., and *Ixodidae* ticks for the presence of eukaryotic symbionts. Results of selective amplification were evaluated with Oxford Nanopore high-throughput sequencing.

Mexican Euplotes euryhalinus Cyanobacteria-Grazing Vs. Possible Symbiont Acquisition

MIROSLAV MACEKa, XIMENA SÁNCHEZ MEDINAb, GLORIA VILACLARA FATJÓa, JUAN ANTONIO ARELLANO POSADASb, RENÉ SÁNCHEZ MENDOZAa, GRAZIANO DI GIUSEPPEc
aGrupo de Investigación en Limnología Tropical, UNAM, FES Iztacala, Tlalnepantla, México; bPosgrado en Ciencias del Mar y Limnología, UNAM, FES Iztacala, Tlalnepantla, México; cDepartment of Biology, University of Pisa, Pisa, Italy.

E-mail of the corresponding Authors: mmacek@live.co.uk; gdigiuseppe@biologia.unipi.it

A comprehensive analysis was performed with the 2003-2019 data from an athalassohaline maar-crater lake in Alchichica, Mexico (18°10'N; 93°10' W, 2340 m a.s.l.). Ciliate numbers were obtained using Quantitative Protargol Stain; the molecular identification of *Euplotes euryhalinus* (Valbonesi & Luporini 1990) was proven. Ingested ciliate food was analysed in DAPI stained preparations, while feeding rates were based on an *in situ* ingestion of Fluorescently Labelled Bacteria (Sherrs´ FLB method), prepared from *Synechococcus* sp. During 2018, dilution experiments were performed with 20 µm screen-harvested ciliates and 0.2 µm filtered water with low dissolved oxygen, DO and enriched carbon dioxide content ("candle-jar" treatment).
E. euryhalinus used to be found throughout the water thermal stratification-period with the anoxic hypolimnion. Surprisingly, most of the ciliate were located either around the oxycline and/or in the well illuminated layers including the surface itself. During the lake mixing, the ciliate was not recorded. *E. euryhalinus* was observed either with ingested minute diatoms (*Cyclotella choctawhatcheeana*), green algae (*Oocystis parva*, *Monoraphidium minutum*) or other chloroplast-bearing eukaryotes in different levels of pigment degradation that nullify the previous hypothesis of ciliate mixotrophy in the lake. Statistical tests are being conducted to explore the possibility of a direct relation to the nanophytoplankton peaks as a food source. However, the ciliates were not almost observed in the layers with abundant photosynthetic anoxygenic bacteria.
We had to re-interpret previously measured feeding rates upon picocyanobacteria, Pcy, although the values were confirmed in recent experiments. Furthermore, published "picocyanobacteria in feeding vacuoles" were observed *in vivo* being ingested as microcolonies. This explained why observed filtration rates were not related directly to the Pcy numbers and why the Pcy uptakes were far beyond the ciliate growth needs. In both environmental samples and in the dilution experiments, Pcy / FLB were not included in observable vacuoles, which could be related to the known acquisition of endosymbiotic bacteria by *Euplotes* spp.
It was confirmed that Pcy observed inside the ciliate cells were obtained through two different mechanisms: (i) ingestion of colonies placed in vacuoles and, as a hypothesis, (ii) filtration feeding / acquisition of potential symbionts towards the cytoplasm.

Multidisciplinary Characterization of a Novel Ciliate Endosymbiont: "Candidatus Pinguicoccus supinus," a Verrucomicrobial Species with Highly Reduced Genome

LEANDRO GAMMUTOa, VALENTINA SERRAa, VENKATAMAHESH NITLAa, MICHELE CASTELLIb, OLIVIA LANZONIa, DAVIDE SASSERAc, CLAUDIO BANDIb, BHAGAVATULA V. SANDEEPd, LETIZIA MODEOa, GIULIO PETRONIa
aDepartment of Biology, University of Pisa, Pisa, Italy; bRomeo and Enrica Invernizzi Pediatric Research Center, Department of Biosciences, University of Milan, Milan, Italy; cDepartment of Biology and Biotechnology "Lazzaro Spallanzani", Pavia University, Pavia, Italy; dDepartment of Biotechnology, Andhra University, Visakhapatnam, India.

E-mail of the corresponding Authors: leandro.g@hotmail.it; valentinasrr@gmail.com; letizia.modeo@unipi.it

Members of the *Euplotes* genus (Euplotia, Ciliophora) are known to frequently host endosymbionts, including eukaryotes (such as green algae or trypanomes), or, more commonly, one or even multiple bacterial symbionts belonging to diverse lineages. In this study, we characterized the morphology, phylogeny and genome features of "*Candidatus* Pinguicoccus supinus" gen. nov., sp. nov. (*Opitutae, Verrucomicrobia*), a bacterial endosymbiont of the novel species *Euplotes vanleeuwenhoeki*. This bacterium shows several intriguing features. The 16S rRNA gene based phylogeny, and the phylogenomic analysis, collocate this endosymbiont as a member of the *Puniceicoccaceae* family (*Verrucomicrobia*, *Opitutae*) clustering with sequences from uncultured organisms, and forming a clade related to the *Coraliomargarita*, "Fucophilus", *Cerasicoccus*, and *Ruficoccus* genera. We suggest that the long branch of "Ca. Pinguicoccus supinus" may imply a higher evolutionary rate than that of related Verrucomicrobia. "Ca. Pinguicoccus supinus" is located in the host cytoplasm, usually in clusters beneath the cortex, and sometimes in close contact with mitochondria or lipid droplets. It presents an irregular roundish-ovoid shape and is not enclosed in a host symbiosome. The genome of "*Candidatus* Pinguicoccus supinus" was found to be extremely small (163,218 Kbp), being, to our best knowledge, the fourth smallest bacterial genome sequenced to date. It is also the endosymbiont with the smallest genome found in a protist host, and the first case of such an extreme reduction in *Verrucomicrobia*. Compared to other bacterial genomes of comparable size, the genome of this endosymbiont shares the same metabolic core of basic gene expression and chaperons. However, it possesses some peculiarities: it lacks biosynthetic pathways of nutrients or metabolites potentially useful for the host, and lacks several genes considered essential for bacteria, in particular any catalytic subunit involved in DNA replication. On the other hand, it retains some genes involved in lipid metabolism, which might possibly correlate with the occasionally recorded "Ca. Pinguicoccus supinus" subcellular localization in close contact with lipid droplets. All these aspects taken together, in particular the extreme genome reduction, suggest high integration with the *Euplotes* host, although the nature of this relationship has not yet been revealed.

More than the Sum of its Parts: Uncovering the 'Killer Trait' Symbiosis Between Paramecium and its R-body Producing Endosymbionts

KATRIN GROSSERa, MARCELLO PIRRITANOb,c, FELICITAS E. FLEMMINGa, LARS KOEHLERa, GIULIO PETRONId, MARTIN SIMONb,c, MARTINA SCHRALLHAMMERa
aMicrobiology, Albert Ludwig University of Freiburg, Freiburg, Germany; bMolecular Cell Dynamics Saarland University, Saarbrücken, Germany; cMolecular Cell Biology and Microbiology, Wuppertal University, Wuppertal, Germany; dDepartment of Biology, University of Pisa, Pisa, Italy.

E-mail of the corresponding Author: martina.schrallhammer@biologie.uni-freiburg.de

The symbiosis between *Paramecium* and certain bacterial symbionts, previously grouped together as "*Caedibacter*", is infamous for the so-called 'killer trait': symbiont-free paramecia die when exposed to killer cells harboring *Caedibacter*. A central structure in the killer trait is the symbiont derived R-body (refractile body), a tightly coiled protein ribbon which can elongate and then forms a long hollow cylinder. It is crucial for killing but it is not the lethal agent. Likely, the R-body acts as delivery mechanism for an unidentified toxin. Combining genomic and transcriptomic analyses with functional experiments, we aim to unravel the interaction between *Paramecium* and its symbionts.

Although R-body and killer trait are highly specialized characteristics, we recently demonstrated that they are not indicative for a close phylogenetic relationship among their bacterial producers. Likely, horizontal gene transfer mediated by mobile genetic elements is responsible for its distribution among Gamma- and Alphaproteobacteria. R-body producing symbionts depend for their exclusively vertical transmission completely on their host. They provide no nutritional benefits to *Paramecium* and some studies observed a parasitic effect on host fitness. The only apparent benefit *Paramecium* gain from the symbiosis is the killer trait that provides a competitive advantage. However, it has a narrow effective range killing only *Paramecium* species while other ciliates do not suffer from harmful effects. Thus, we further investigated mechanisms potentially stabilizing this association by analyzing the differential gene expression of *Paramecium* infected with *Caedibacter* and symbiont-free cells. Comparative transcriptomics revealed that infected and aposymbiotic cells differ significantly in their gene expression. Apparently, the presence of the symbiont causes the up-regulation of host metabolic pathways mobilizing storage energy. This finding was supported by fitness analysis revealing increased cell densities of *Caedibacter*-harboring paramecia when compared to symbiont-free cells. At present we analyze *Caedibacter*'s genome and transcriptome for the presence of secretion systems and their effectors potentially involved in mediating the changes of host gene expression.

While we have uncovered several novel aspects, the killer trait symbiosis is a fascinating example for an interaction which is more than just the sum of its parts.

Sunbathing Paramecia - How Much Influence Have Its Algal Endosymbionts on Photoaccumulative Behaviour

FELICITAS E. FLEMMING[A], MARTINA SCHRALLHAMMER[A]
aInstitute of Biology II, Albert Ludwig University of Freiburg, Freiburg, Germany.

E-mail of the corresponding Author: felicitas.flemming@gmx.de

Paramecium bursaria lives in a facultative, mutualistic symbiosis with *Chlorella*-like algae ('green' paramecia). The ciliate profits from this interaction by gaining access to photosynthesis products and the algae benefit by increasing their motility. Green paramecia accumulate in the light. Studies exploring this photoaccumulative behaviour reveal contradicting results. While some report that algae-free *P. bursaria* ('white' paramecia) lose this ability indicating a crucial role of the symbiont, others still observe photoaccumulation even after elimination of the algae.

We combined quantitative photoaccumulation assays including green and white paramecia with molecular phylogeny of several *P. bursaria* strains and their symbionts to shed light on the impact of the algal symbionts on their host's behaviour. Specifically, we asked if there is a correlation between algal species identity and photoaccumulation of the host and to which extent the algae are responsible for *P. bursaria* behaviour.

We detect statistically significant accumulation of green paramecia in illuminated areas regardless of the endosymbiont species. Such clear pattern is missing for white paramecia. Still, more white cells accumulate in the light but to a lesser degree than green cells. Thus, our data extend previous results: the *Chlorella*-like endosymbionts influence *P. bursaria*'s photoaccumulative behaviour but they do not cause it. Currently, we are investigating a potential correlation between the duration of the symbiont-free cultivation and their affinity to accumulate in the light.

Oral Session MISCROBIOME

Eukfinder: a Specialized Pipeline for Identifying and Assembling Eukaryotic Genomes from Metagenomic Data

DANDAN ZHAO[a], DAYANA SALAS-LEIVA[a], ANDREW J. ROGER[a]

[a]Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB), Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada.

E-mail of the corresponding Authors: andrew.roger@dal.ca, d.zhao@dal.ca

Microbial eukaryotes are essential components of natural communities and play important roles in their ecosystems. Whole-genome shotgun (WGS) metagenomic sequencing of such microbial communities has the potential to inform us about the functions, physiologies and evolutionary histories of microbial eukaryotes in these ecosystems. However, unlike metagenomic studies of prokaryotes, few analyses have managed to assemble good-quality microbial eukaryote genomes from metagenomic data. To address this problem, we have developed a bioinformatics pipeline, Eukfinder, to recover and assemble nuclear and mitochondrial genomes of eukaryotic microbes from WGS metagenomics data. Several tools and databases were created to separate metagenomic reads into taxonomic groups from which genomes can be then recovered by assembling and binning. We applied Eukfinder to human gut microbiome WGS metagenomic sequencing samples to recover genomes from the protistan parasite *Blastocystis* sp. genomes. We tested two approaches in Eukfinder (assembling-first then classifying contigs vs. classification-first then assembling) and compared the efficacy of this pipeline to two other published workflows. Our preliminary results show that Eukfinder can efficiently generate high-quality near-complete nuclear and mitochondrial genomes from diverse *Blastocystis* subtypes. We anticipate that Eukfinder will be a useful tool for reference-independent and cultivation-free eukaryotic microbial genome recovery from environmental WGS metagenomic sequencing samples.

Investigations Into Toxic Dinoflagellate Relationships: A Tale of *Gambierdiscus*

BRITTANY M. OTT[a], R. WAYNE LITAKER[b], WILLIAM C. HOLLAND[b], CHARLES F. DELWICHE[a]

[a]University of Maryland-College Park, College Park, Maryland, USA; [b]National Oceanic and Atmospheric Administration, Beaufort, North Carolina, USA.

E-mail of the corresponding Author: bott1@umd.edu

Dinoflagellates are an important group of protists, found in both freshwater and marine environments, that serve as crucial constituents of microbial food webs. Although often beneficial, this group is best known for the small fraction of species that produce toxins and form harmful algal blooms, which cause major ecological, economic and health-related problems. Dinoflagellate species can produce different toxins or levels of toxin, even within the same genus. Bacterial symbionts may also affect toxin production. Consequently, it is of interest to have efficient methods to determine what species are present within a sample, including both dinoflagellates and their bacterial cohorts. Traditionally, dinoflagellates have been identified based on morphological characters, which, unfortunately, have often proved too variable to be reliable in identifying closely-related species. More recently, single gene phylogenies based on ribosomal, cytochrome, and other gene sequences have been used, but were criticized as perhaps representing the evolution of the individual genes rather than true species-level relationships. To overcome the limitations posed by single genes, we generated deep transcriptomes, which are known to be useful in determining phylogenetic relationships at the genus level and above, from putative dinoflagellate species in the genus *Gambierdiscus*. Species within this genus have previously been identified based on morphology and D1-D3 LSU phylogenies. Utilizing these transcriptomes, we: 1) examined the usefulness of multi-gene phylogenies at determining phylogenetic relationships within *Gambierdiscus*; 2) addressed the utility of the D1-D3 region of the LSU in identifying a dinoflagellate species and compared the species-specific terminal clusters with those from the multi-gene phylogenies; and 3) investigated the organismal composition and activity of bacterial travelers living within these *Gambierdiscus* cultures to search for patterns regarding the toxicity of their *Gambierdiscus* associates. Results from this study will help us better understand the inter- and intraspecies relationships within a toxic dinoflagellate genus.

An Influence of Protists on a Structure of the Halophilic Prokaryotic Community

ELENA SELIVANOVA[a], YURI KHLOPKO[a], ANDREY PLOTNIKOV[a]

[a]Center of Shared Scientific Equipment "Persistence of Microorganisms" Institute for Cellular and Intracellular Symbiosis, Ural Branch of Russian Academy of Sciences, Orenburg, Russia.

E-mail of the corresponding Author: selivanova-81@mail.ru

Protists play a crucial role in structuring natural prokaryotic communities. Phototrophic protists are the main producers of organic matter, whereas heterotrophic protists are the consumers in microbial food webs. Also they can change the bacterial community composition and diversity by production of antagonistic substances. In hypersaline environments the community structure is simplified up to mainly microbial assemblage. So halophilic microbial communities represent a good model to study interactions between halophilic phototrophic protists and prokaryotes. The aim of the study was to estimate an influence of heterotrophic and phototrophic protists on the halophilic prokaryotic community composition and diversity in uniprotist cultures. The uniprotist cultures with associated prokaryotes were isolated from an ephemeral pond near the Solyanka River with salinity 100 g/L and from the Malaya Samoroda River with salinity 110 g/L (Volgograd region, Russia). The cultures of heterotrophic protists were incubated for 14-21 days and fed by heat-killed *Pseudomonas fluorescens*. The cultures of phototrophic protists were grown under illumination. The ciliate *Fabrea salina* was fed by *Dunaliella parva* culture. After five passages the total DNA was extracted from the samples by phenol–chloroform method. 16S DNA libraries were prepared according to Illumina workflow with primers targeting the V3 and V4 regions of the SSU rRNA gene and sequenced in MiSeq (Illumina) using v3 2×300 bp paired-end reagent kit. Bioinformatic analysis was conducted using USEARCH v8.0.1623_win32. A total 38 cultures of heterotrophic and 25 cultures of phototrophic halophilic and halotolerant protists belonged to 14 different genera from 5 high rank taxa were isolated. There were representatives of Discoba, Stramenopiles, Holozoa, Tubulinea, Chloroplastida, and Alveolata. Taxonomic composition and relative abundance of associated prokaryotes was analyzed. Prokaryotic associations of the halophilic protists seem to have species dependent composition because rare OTUs were shared. This study was conducted in the Center of Shared Scientific Equipment "Persistence of microorganisms" of the Institute for Cellular and Intracellular Symbiosis, Ural Branch of Russian Academy of Sciences. The work was supported by RFBR №17-04-02079, 17-04-00135.

Genome Size Evolution in Parabasalia

GILLIAN H. GILE[a]
[a]School of Life Sciences, Arizona State University, Tempe, AZ, USA.

E-mail of the corresponding Author: ggile@asu.edu

Parabasalia is a phylum of primarily endobiotic, anaerobic protists. Some are small flagellates that opportunistically colonize diverse hosts, while others are large, morphologically complex obligate symbionts of termites. Because nuclear DNA content correlates with cell size, the larger parabasalids are expected to contain more DNA in their nuclei, and hence have larger genomes. To date, genome size has been estimated for only a handful of small parabasalids, and each falls within the range of 80-180 Mbp. Here we present DNA content measurements of large parabasalids from termite guts using flow cytometry. By mapping genome sizes on the phylogeny of Parabasalia, we find that genome size has increased in multiple parabasalid lineages independently. This hints at a strong evolutionary pressure to increase cell size, or conversely a loss of evolutionary constraint on genome size, acting in the termite hindgut environment. The largest genomes belong to protists in the class Trichonymphea, with several species harboring more than 10 pg DNA per nucleus. This represents a 100-fold increase relative to the smaller Trichomonadea flagellates. The ploidy of these large parabasalids is currently unknown, but descriptions of mitosing cells indicate only a few chromosomes per nucleus, and the smaller parabasalid flagellates are known to be haploid. If the largest parabasalids are also haploid, their genome sizes would be greater than 10 Gbp, more than 3 times the size of the human genome.

Characterising the Diversity of Microeukaryotes Associated with Seaweeds (Phaeophyceae)

MARIT F. M. BJORBÆKMOa, DAVID BASS[b,c], JANINA FUSS[a,d], RAMIRO LOGARES[a,e], ANDERS WOLD[a], STEIN FREDRIKSEN[f], ANDERS K. KRABBERØD[a], KAMRAN SHALCHIAN-TABRIZI[a]

[a]University of Oslo, Department of Biosciences, Evogene, Oslo, Norway; [b]The Natural History Museum, London,UK, [c]Centre for Environment Fisheries and Aquaculture Science (Cefas), Weymouth, UK; [d]Christian-Albrechts University Kiel, Institute of Clinical Molecular Biology, Kiel, Germany; [e]Institut de Ciències del Mar (CSIC), Barcelona, Catalonia, Spain; [f]University of Oslo, Department of Biosciences, Aqua, Oslo, Norway.

E-mail of the corresponding Authors: maritmbj@ibv.uio.no; david.bass@cefas.co.uk

Some of the most prominent and eye-catching groups of sessile organisms in marine temperate coastal areas are the large brown seaweeds (Phaeophyceae). Although coastal ecosystems encompass relatively small areas on a global scale, these are some of the most productive habitats worldwide, where seaweeds are primary producers and important drivers of carbon fluxes. Seaweeds also have important roles as ecosystem engineers that provide complex habitats for a wide diversity of marine life depending on them for nurseries, shelter and nourishment. Consequently, seaweeds are crucial for safeguarding coastal ecosystems. However, the knowledge about the diversity and functioning of microeukaryotes as part of the seaweed holobiont is limited. In this study we used general 18S rDNA eukaryotic primers and Illumina MiSeq to investigate the diversity of microeukaryotes associated with seven common brown algal species. We also investigated their potential functional roles, specificity and host-range, and whether the parasites detected are known to infect other marine organisms. To assess whether the largely unexplored brown algal-associated microeukaryote communities could function as host reservoirs for novel diversity of marine microeukaryotes, we evaluated the taxonomic assignment against sequence reference databases and constructed phylogenetic trees. We detected a wide range of microeukaryotes associated with brown algae, representing all main branches in the eukaryotic tree of life. Approximately a third of the OTUs represented novel diversity, including a broad diversity of putative parasites. Our findings demonstrate that microeukaryotes should be considered as an integral part of the seaweed holobiont.

Marked Changes in Diversity and Activity of Picoeukaryotes with Depth in the Global Ocean

CATERINA R. GINER[a,b], VANESSA BALAGUÉ[b], MASSIMO C. PERNICE[b,c], CARLOS M. DUARTE[d], JOSEP M. GASOL[b,e], RAMIRO LOGARES[b], RAMON MASSANA[b]

[a]Aquatic Ecosystems Research Laboratory, Institute for the Oceans and Fisheries, University of British Columbia, Vancouver, BC, Canada; [b]Institut de Ciències del Mar (ICM-CSIC), Barcelona, Catalonia, Spain; [c]Stockholm University, Stockholm, Sweden; [d]Red Sea Research Center (RSRC) and Computational Biosciences Research Center (CBRC), King Abdullah University of Science and Technology (KAUST), Saudi Arabia; [e]Centre for Marine Ecosystem Research, School of Science, Edith Cowan University, Joondalup, WA, Australia.

E-mail of the corresponding Author: c.giner@oceans.ubc.ca

Microbial eukaryotes are key components of the global ocean microbiome, playing fundamental roles in ecological and geochemical processes. Yet, despite their importance, our understanding of their community composition and activity in different water layers of the ocean is limited, particularly for picoeukaryotes (0.2-3 µm cell size), which are relatively cryptic under the microscope. Here, we analyzed the picoeukaryotic community structure and metabolic activity inhabiting different vertical zones of the tropical and subtropical global ocean: surface, deep chlorophyll maximum, mesopelagic (including the deep scattering layer and oxygen minimum zone) and bathypelagic. The communities of a total of 91 globally distributed samples were analyzed by high-throughput sequencing of the 18S rRNA gene, as represented by DNA (i.e. community structure) and RNA (i.e. metabolic expression), followed by delineation of 99% similarity Operational Taxonomic Units (OTUs). We found a clear stratification of the picoeukaryotic communities along the water column, with two differentiated assemblages corresponding to the sunlit and dark ocean. Individual taxonomic groups showed marked changes along the vertical gradient, with groups either increasing (e.g. Chrysophyceae or Bicosoecida) or decreasing (e.g. MALV-I or MAST-3) their abundances with depth. Using the rRNA:rDNA ratio for each individual OTU allowed us to estimate its metabolic activity. Interestingly, the highest relative activity was found in the mesopelagic layer for most taxonomic groups, whereas the lowest was in the bathypelagic. Furthermore, the oxygen minimum zone (OMZ) communities differed significantly from the mesopelagic samples, with some taxonomic groups (e.g. Ciliophora, Dinoflagellata, Acantharia) showing higher abundances and relative activity in the OMZ, whereas the relative activity of most taxonomic groups was lower at the deep scattering layer. In sum, our results characterize the change in community structure and activity of picoeukaryotes in the global-ocean water column, suggesting that the mesopelagic layer is a hot-spot for the activity of these important, and yet overlooked, components of the plankton communities.

Molecular Identification of Kleptoplastic Foraminifera (Rhizaria) and their Microbiome from Photic and Aphotic Habitats

MAGALI SCHWEIZER[a], THIERRY JAUFFRAIS[a,b], CHARLOTTE LEKIEFFRE[a], JOAN M. BERNHARD[c], SOPHIE QUINCHARD[a], HELENA L. FILIPSSON[d], EMMANUELLE GESLIN[a]

[a]LPG-BIAF, UMR 6112-CNRS, UNIV Angers, Université de Nantes, Angers Cedex, France; [b]Ifremer, RBE/LEAD, Nouméa, New Caledonia; [c]Woods Hole Oceanographic Institution, Geology & Geophysics Department, Woods Hole, MA, USA; [d]Department of Geology, Lund University, Sölvegatan 12, 223 62 Lund, Sweden.

E-mail of the corresponding Author: magali.schweizer@univ-angers.fr

Assimilation, sequestration and maintenance of foreign chloroplasts inside an organism is termed "chloroplast sequestration" or "kleptoplasty". This phenomenon is known in certain benthic foraminifera, in which such kleptoplasts can be found both intact and functional, but with different retention times depending on foraminiferal species. Kleptoplasty has been observed in foraminifera from photic habitats, and, surprisingly, also from aphotic habitats. For foraminifera, the main source of kleptoplasts has been shown to be diatoms.

Here, we study foraminiferal species from photic and aphotic habitats. The photic habitat is represented by intertidal mudflats situated on the east Atlantic coast (Isle of Ré and Bourgneuf Bay, France) and the aphotic habitat by bathyal sites in the east Pacific (Santa Barbara Basin, USA) and the Skagerrak (Gullmar Fjord, Sweden). The aims of this study are to identify the foraminifera and the kleptoplast donor organisms as well as to investigate the different kleptoplastic strategies and utilization of the kleptoplasts. The following methods have been used: DNA sequencing, scanning electron microscopy (SEM), transmission electron microscopy (TEM), nanometer-scale secondary ion mass spectrometry (nanoSIMS) and respiration rate measurements. As previously shown, foraminifera from the photic habitat are able to use the kleptoplasts to produce oxygen, i.e., the photosynthetic pathways are functional. Nevertheless, further studies are needed regarding mechanisms developed by these kleptoplastidic foraminifera for carbon partitioning and storage. Conversely, the photosynthetic pathways are not functional in the foraminifera studied to date from the aphotic habitat; the role of these kleptoplasts in the metabolism of the foraminifera is deserving of additional dedicated investigations.

Blastocystis In The Human Gut Microbiome: Italian Cases Study

MARIA CRISTINA ANGELICI[a], PAOLA ADE[a], SARA MONTECCHINI[b], MIRKO BUTTRINI[b], SABINA ROSSI[b] and ADRIANA CALDERARO[b]
[a]National Institute of Health, Rome,Italy, [b]Department of Medicine and Surgery University of Parma, Italy.
E-mail of the corresponding Author: mariacristina.angelici@iss.it

Since some years le scientific biomedical community has asserted that intestine, with his microbiome, is much more than an organic district but a whole ecosystem in which prokaryotic and eukaryotic microbes influence one others with symbiotic/predatory/parasitic relationships. It is well understood also that intestine share a very high synergy with the immune system, conditioning it. It is in communication with brain and other internal districts, as well. How the gut microbiome influences these properties is showed by the pathological status immediately arising when an alteration or an inflammatory status is present. New knowledge are now available on the physiological presence of different species of protists in human gut which possible role is not yet discovered. The interior microbiome behavior, in short, replicate the environmental one, and closed relationships among prokaryotic and eukaryotic cells are notable. They are considered mostly as not pathogens for humans (*Endolimax* sp, *Chilomastix* sp, *Entamoeba coli*, ecc.) but recent epidemiological acquisitions have revealed that many different degree of virulence may be associated to some of this protists (*Blastocystis, Dientamoeba*). There is already a plethora of publications on *Balstocystis* biology as pathogen or not pathogens, depending on different genetic pathways and association of this protozoa with chronic infections and serious chronical inflammation outcome, well known as Irritable Bowel Syndrome (IBS) and other gastrointestinal disorders. We are carrying out a wide study on Italy on this parasite both from environmental and human specimens, to characterize its genetic and pathological trend on stochastic or only symptomatic human study groups. Preliminary results show the prevalence of ST3 *Blastocystis* genotype, followed by ST1, ST4 and ST2, according to results obtained by same studies in other Mediterranean and Middle Est Countries and differently by many other Countries in which ST1 is mostly isolated. A possible role of the intestinal human microbiome on the Blastocystis pathogen genotypes colonization is here hypothesized.

Oral Session TAXONOMY & PHYLOGENY 5

Some Insights into the Structure of Genotype T4 in *Acanthamoeba*

STAS MALAVIN[a], LYUBOV SHMAKOVA[a]

[a]Soil Cryology Lab, Institute of Physicochemical and Biological Problems in Soil Science RAS, Pushchino, Russia.

E-mail of the corresponding Author: stas.malavin@gmail.com

Based on pairwise nucleotide difference and unique sequence signatures in 18S (Rns) gene, *Acanthamoeba* genus was divided into 22 sequence types (genotypes), named T1—T22. Among both environmental and clinical isolates, type T4 is predominant. Recently, the subdivision of T4 into seven groups designated $T4_A$ through $T4_E$ and $T4_{Neff}$ was declared, yet not much evidence supporting this subdivision was published. Thus, there are different levels of genetic heterogeneity inside *Acanthamoeba*, and it is not clear which level corresponds to the "species". Hypervariable regions inside the *Acanthamoeba* 18S gene represent a problem as (1) they do not align unambiguously, and (2) the obtained alignment is always gap-rich, masking the considerable share of the observed diversity from traditional methods of phylogenetic inference. To overcome this, we coded gaps as a fifth state and calculated a phylogeny from conservative and hypervariable regions as two separate partitions. This approach gave us the well-supported $T4_A$ —$T4_{Neff}$ subtypes with resolved relationships among them. At the same time, mitochondrial 16S (rns) and Cox1 gene phylogenies gave well-supported monophyletic $T4_D$, $T4_E$, and $T4_{Neff}$, and monophyletic but blended $T4_{A/B}$ and $T4_{C/F}$.
Recently, we isolated six new *Acanthamoeba* strains from Arctic permafrost sediments. All of them belong to T4 18S sequence type, three to $T4_A$ and three to $T4_B$. At the same time, five of them turn out to have identical 16S and Cox1 sequences, while the sixth (SCL-14-12) is different and steadily fall into different cluster. The detailed morphometrics of trophozoites and cysts, the rate of growth at different temperatures, and observed biological peculiarities all demonstrate the species-level diversification of SCL-14-12.
These results suggest that clusters inside mitochondrial $T4_{A/B}$ group are different species, and so are $T4_{C/F}$, $T4_D$, $T4_E$, and $T4_{Neff}$. $T4_A$ and $T4_B$ sequences, as well as $T4_C$ and $T4_F$, may represent 18S variants coexisted and evolved together in the same cells.
The study was conducted in the framework of the Governmental Program AAAA-A18-118013190181-6 and supported by RFBR grants 18-04-00824 and 17-54-150003.

Neglected Beauty: Do "Heliozoa" Deserve More Attention?

VASILY ZLATOGURSKY[a], DARIA DRACHKO[a,c], YEGOR SHISHKIN[a] , FABIEN BURKI[b]

[a]Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; [b]Department of Organismal Biology, Systematic Biology Program, Uppsala university, Uppsala, Sweden; [c]Laboratory of Cellular and Molecular Protistology, Zoological Institute RAS, St. Petersburg, Russia.

E-mail of the corresponding Author: v.zlatogursky@gmail.com

The term "heliozoa" became colloquial when the polyphyly of heliozoan taxa became obvious. Today, it refers to several unrelated clades of eukaryotic microbes with spherical amoeboid cells, mostly shaped by microtubular cytoskeleton. Even among poorly studied protists, the heliozoans represent a prime example of neglected organisms with poor taxonomy and few researchers working on improving the situation. However, heliozoans are ubiquitously distributed, often can grow in cultures and relatively simple approaches are necessary to describe or reliably identify species. Usually 18S sequence, light microscopy and, in case of forms with species-specific skeletons, electron microscopy (without sectioning or critical-point drying) are enough to sufficiently characterize any given strain. Here, we will discuss two recent projects that exemplify the hidden importance of Heliozoa. First, we show that in just one random marine sample of about 100 ml, three new species, of which two also represent new genera and families were found. Second, we show that the enigmatic marine *Meringosphaera*, which for about 100 years has been considered a genus of algae with uncertain phylogenetic affiliation, is in fact a centrohelid heliozoan. The fact that 18S of many heliozoan clades is problematically amplified with general eukaryotic primers suggests that their diversity is underestimated in metabarcoding studies of natural samples. But even the few existing metabarcoding data are hard to interpret due to the lack of strains, parallely characterized phenotypically and genetically. The ecological role of "heliozoans" is likely important, since together with ciliates they often play the role of top predators at the microscopic scale, consuming microbial preys from bacteria to microscopic animals. The international effort towards increasing the amount of research focused on "heliozoa" is necessary and especially species descriptions provided with both genetic and morphological data are critical. Otherwise our view of the protist ecology, biodiversity and evolution will remain biased against at least one important type of eukaryotic microbes.

Invention, Expansion, and Theft, the Evolution of Multicellularity in *Acrasis kona*

SANEA SHEIKH[a], CHENG-JIE FU[a], SANDRA L. BALDAUF[a]

[a]Program in Systematic Biology, Uppsala University, Sweden.

E-mail of the corresponding Author: sanea.sheikh@ebc.uu.se

Acrasids (Heterolobosea, Discoba) are large aggressive soil amoebae that undergo aggregative multicellularity when food becomes scarce. This culminates in small nearly macroscopic sorocarps protruding from the soil surface. Acrasids were long considered the primitive sister group to the well-studied sorocarpic amoebae of Dictyostelia (Amoebozoa), but these are now known to be only very distant relatives. Therefore, acrasids represent independent evolution of aggregative multicellularity and a unique system in which to study this phenomenon. Acrasids are also the only multicellular members of supra-kingdom Discoba (Excavata), within which they form a small, molecularly shallow clade. Thus, acrasids have probably evolved multicellularity relatively recently.

We have sequenced and annotated the nuclear genome of *Acrasis kona*, including three developmental stage-specific transcriptomes. The ~44 Mb genome has 15,868 predicted proteins, of which 4,987 (~31%) are novel. Of the non-novel genes, many appear to be shared only with very distant relatives, especially Bacteria, Amoebozoa and Fungi. This suggests relatively recent acquisition by horizontal gene transfer (HGT), which in many cases can be confirmed by phylogeny. A surprising number of these HGT candidate genes have been further modified in *A. kona,* including expansion into multigene families, acquisition of membrane targeting sequences (signal peptides) and/or strong developmental stage-specific expression. We focused primarily on the transition from solitary ("asocial") feeding cells to aggregating ("social") cells, one of, if not the key, transformation in aggregative multicellularity. Genes strongly up-regulated in aggregation versus growth include three semi-overlapping categories: *Acrasis*-unique genes (inventions), single members of *Acrasis*-specific multigene families (expansions), and HGT-acquisitions (thefts). Thus, evolution of multicellularity in *A. kona* involved a cobbling together of components from disparate sources. Much of this gene cocktail seems to be focused on the cell membrane, which is expected to play a key role in some of the most important aspects of aggregative development such as cell signaling, kin recognition and adhesion.

Where Oxygen Is Not Popular – Anaerobiosis in SAL Super-Group (Ciliophora)

JOHANA ROTTEROVÁ[a], WILLIAM BOURLAND[b,] VIRGINIA P. EDGCOMB[c], ROXANNE BEINART[d], MARTIN KOLÍSKO[e], PETR TÁBORSKÝ[e], ERIC SALOMAKI[e], DAVID ŽIHALA[f], IVAN ČEPIČKA[a]

[a]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic; [b]Department of Biological Sciences, Boise State University, Boise, Idaho, USA; [c]Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA; [d]Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island, USA; [e]Institute of Parasitology, Biology Centre Czech Academy of Sciences, České Budějovice, Czech Republic; [f]Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic.

E-mail of the corresponding Author: johana.rotterova@natur.cuni.cz

Although ciliates are one of the most studied protists in general, much remains unknown about the phylogenetic relationships among the major lineages, as well as about their mitochondrial metabolism and the nature of their symbioses, albeit their known diversity and distribution are striking. As our study reveals, anaerobiosis is greatly common among ciliates and particularly within the SAL (Spirotrichea, Armophorea, Litostomatea, Odontostomatea, and other lineages) super-group. After the recent discovery of several novel lineages within SAL, we selected several representatives to sequence their metagenomes and transcriptomes in order to expand our knowledge of the evolution of ciliates, phylogenetic relationships within lineages of SAL, the mechanisms of adaptation to life in anoxia, including the mitochondrial metabolism and various symbioses. Analyses of metagenomic and transcriptomic data from metopids and two newly discovered anaerobic ciliate lineages revealed traces of the classical mitochondrial pathways including amino-acid metabolism, fatty acid metabolism, and Fe-S cluster biogenesis. However, partial mitochondrial genomes, containing genes of mitochondrial Complex I, were also detected among some. Representative of one of the newly discovered lineages shows remarkable metabolic capacity, as several genes for the structure and assembly of respiratory complexes II and III and a complete F1F0 ATP synthase (Complex V) were retained. Additionally, a putatively mitochondrial-targeted pyruvate:ferredoxin oxidoreductase (PFO), which is likely involved in the production of hydrogen for ATP-synthesis, was identified. Interestingly, most of the studied taxa host various ecto- and endosymbionts that persist in our long-term ciliate cultures. To determine their identity, we applied specific autofluorescence of F420 coenzyme, FISH and CARD – FISH methods, as well as both 16S rRNA gene and metagenomic sequencing of various strains of multiple species.

Rooting the Eukaryotic Radiation with New Models of Genome Evolution

CELINE PETITJEAN[a] GERGELY J. SZOLLOSI[b], DAVID BASS[c,d], TOM A. WILLIAMS[a]

[a]Palaeobiology departement, Life Sciences Building, University of Bristol, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK; [b]MTA-ELTE Lendület Evolutionary Genomics Research Group, 1117 Budapest, Hungary; [c]Department of Life Sciences, The Natural History Museum London, Cromwell Road, London SW7 5BD, UK; [d]Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK.

E-mail of the corresponding Authors: celine.petitjean@bristol.ac.uk; tom.a.williams@bristol.ac.uk

The deep relationships among the main lineages of eukaryotes, and in particular the root of the eukaryotic tree, remain debated. Two main rooting hypotheses are actively discussed. The first, the Unikont/Bikont hypothesis (UB), places the root between the Unikonts, including Metazoa, Fungi, Amoebozoa and some related protist lineages, and the bikonts, i.e. all the other lineages, including 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. Moreover, the monophyly of the "excavate" group is also debated, increasing the complexity of this question. Each of these hypotheses has major implications for the nature of the last eukaryotic common ancestor (LECA), as its complexity level, gene content or the evolution of the main features of each lineage. In order to solve this fundamental evolutionary question, we are exploring the use of concatenation, multispecies coalescent, and recently-developed approaches to gene tree-species tree reconciliation that allow species trees to be rooted without an outgroup, as ALE. Our analyses make use of a broadly-sampled dataset of 97 complete genomes and largely-complete transcriptomes of Eukaryotes. We particularly include new important lineages for these questions concerning Eukaryotes early evolution, as several free-living Excavates, or recently published transcriptomes of orphan lineages. We present ongoing work on the topology and root of the eukaryotic tree and the metabolic capabilities of the last eukaryotic common ancestor.

Evolution of Mitochondria and Endosymbionts in Anaerobic Protists

ANDREW J. ROGER[a], JON JERLSTRÖM HULTQVIST[a], DAYANA SALAS-LEIVA[a], COURTNEY STAIRS[b], LAURA EME[c], MARTIN KOLISKO[d], PETR TÁBORSKY[e], TOMÁŠ PÁNEK[e], IVAN ČEPIČKA[e]

[a]Dept. of Biochemistry and Molecular Biology, Dalhousie University, Halifax N.S., Canada; [b]Department of Cell and Molecular Biology, Uppsala, Sweden; [c]XX, Ecology, Systematics and Evolution Department, Université Paris-Sud, Orsay, France; [d]Institute of Parasitology Biology Centre, Budejovice, Czech Republic; [d]Department of Zoology, Charles University, Prague, Czech Republic.

E-mail of the corresponding Author: Andrew.Roger@Dal.Ca

Anaerobic protists are abundant in diverse low-oxygen environments ranging from marine sediments to animal guts. Comparatively little is known about the diversity in free-living anaerobic protists with respect to their genomes, metabolisms, organelles and endosymbionts. With the development of inexpensive long-read sequencing technologies, we can now characterize the genomes of diverse anaerobic protists to understand the mechanisms by which they have evolved to inhabit low oxygen. We can also obtain the full genome sequences of associated microbes, including endosymbiotic bacteria and predict their host-symbiont interactions. Unexpectedly, we have found that symbiotic prokaryotes have played an important role in influencing the physiology and evolutionary trajectories of anaerobic protists. I will discuss these findings and their implications for understanding the origin and evolution of anerobic eukaryotes.

A Multigene Timescale and Diversification Dynamics of Ciliophora Evolution

NOEMI M. FERNANDES[a], CARLOS G. SCHRAGO[b]
[a]Laboratório de Protistologia, Departamento de Zoologia, Universidade Federal do Rio de Janeiro, Brazil; [b]Laboratório de Biologia Evolutiva Teórica e Aplicada, Departamento de Genética, Universidade Federal do Rio de Janeiro, Brazil.
E-mail of the corresponding Author: noemi.mfernandes@gmail.com

Ciliophora is one of the most diverse lineages of unicellular eukaryotes. Nevertheless, a robust timescale including all main lineages and employing properly identified fossils as primary calibrations is lacking. Here, we present a time-calibrated multigene phylogeny of Ciliophora and we used this timetree to investigate the rates and patterns of lineage diversification through time. We implemented a two-step analytical approach that favored both gene and taxon sampling. Firstly, we performed Bayesian estimation of divergence times with a dataset of 11 nuclear and mitochondrial gene alignments for 41 ciliates species. Then, the posterior probabilities of divergence times obtained in the first analysis were used to inform the prior densities of node ages for a tree obtained with three ribosomal gene and 276 species. Therefore, the final result was retrieved by combining nuclear, mitochondrial and ribosomal genes, all relying on primary ciliate fossils as calibrations. The early radiation of ciliates was dated at 1,143 Ma in the end of Meso-proterozoic, with 95% confidence interval ranging from 1,061 to 1,242 Ma, which is substantially younger than previously proposed ages (1,980−2,200 Ma). Intramacronucleata was the earliest lineage to diversify from the last common ancestor (LCA) of ciliates ca. 977 Ma. Among the current groups recognized as classes, Spirotrichea diverged earlier and its origin was dated at ca. 850 Ma. Protocruziea was the younger, with crown age estimated at 56 Ma. By expanding the number of taxon and calibrations priors, we reduced the error associated with the posterior time estimates, yielding narrower credibility intervals on the ribosomal-derived chronogram. Macroevolutionary analysis detected a significant rate shift in diversification dynamics in the spirotrichean clade Hypotrichia + Oligotrichia + Choreotrichia, which had accelerated speciation rate ca. 570 Ma, during the Ediacaran-Cambrian transition. For all crown lineages investigated, speciation rates declined through time, whereas extinction rates remained low and relatively constant throughout the evolutionary history of ciliates.

Symposium - Bioactive molecules from protists: perspectives in Biotechnology (by FEPS)

Life Under Stress: Ice Binding Proteins and Superoxide Dismutases from an Antarctic Ciliate

MARCO MANGIAGALLI[a], SANDRA PUCCIARELLI[b], ALEKSEI KALEDA[c,d], FEDERICA CHIAPPORI[e], CRISTINA MICELI[b], IDO BRASLAVSKY[c], MARINA LOTTI[a]

[a]Department of Biotechnology and Biosciences, University of Milano Bicocca, Italy; [b]School of Biosciences and Veterinary Medicine, University of Camerino, Italy; [c]Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel; [d]Department of Chemistry and Biotechnology, School of Science, Tallinn University of Technology, Tallinn, Estonia; [e]Institute of Biomedical Technologies – CNR, Segrate, Italy.

E-mail of the corresponding Author: m.mangiagalli@campus.unimib.it

Polar organisms are constantly exposed to stress conditions induced by low temperatures and reactive oxygen species. *Euplotes focardii* is an Antarctic ciliate isolated in Terra Nova bay and associated with a bacterial consortium. How *Euplotes focardii* responds to stress conditions induced by extreme cold is at the centre of our investigations.

SODs are involved in the defence against oxidative damage induced by reactive oxygen species. These metalloenzymes catalyze the dismutation of superoxide anion into molecular oxygen and hydrogen peroxide. The genes encoding for two Cu,Zn SOD (named *Ef*-SOD1a and *Ef*-SOD1b) and one Mn-SOD (*Ef*-SOD2) were identified in the genome of *E. focardii*. *Ef*-SODs genes are expressed at different levels and regulated by cold-induced stress conditions. From a biochemical point of view, the three SODs combine cold activity with overall structural robustness.

IBPs are characterized by unique ability to bind ice crystals and control their growth. The main activities of IBPs consist in freezing point depression and in inhibition of ice recrystallization, which prevents the formation of large and harmful ice crystals. The bacterial IBP, *Efc*IBP, was identified by metagenomics analysis of the bacterial consortium associated to *E. focardii*. *Efc*IBP presents one of the best ice recrystallization inhibition activity described to date and an unusual pattern of ice binding.

The characterization of these proteins contributes to our knowledge about the adaptation of *E. focardii* and its bacterial consortium to extreme environments. Overall, this organism represents a source of cold-active proteins which can be exploited for biotechnological purposes.

Function and Biotechnological Research of Metabolites from Marine Protists

ANGELO FONTANA[a], CARMEN GALLO[a], EMILIANO MANZO[a], GIULIANA D'IPPOLITO[a], GENOVEFFA NUZZO[a], RAFFAELE DE PALMA[b]
[a]Bio-Organic Chemistry Unit, CNR-Institute of Biomolecular Chemistry, Pozzuoli, Napoli, Italy; [b]Second University of Naples, Dept. of Precision Medicine, c/o II Policlinico (Bd. 3), Napoli, Italy.

E-mail of the corresponding Author: afontana@icb.cnr.it

Access to advanced molecular approaches and the trespassing of the chemical methods to biology make possible to study cells systems better than just a few years ago. Traditional research fields have drawn new life from these advances that have opened up new possibilities and applications. Our research concerns the identification and characterization of basic biochemical processes and bioactive molecules in marine diatoms. Within this frame, projects in my laboratory move along two research lines related to (a) the chemical mediators that control growth and death of these protists, and (b) the discovery of novel drug candidates.
Here we present a brief overview of these studies with a specific emphasis on their biotechnological applications.[1–5] In particular, we will focus on lipids and lipid pathways in regard to both their eco-physiological role and potential use in massive cultivation and biomass production. The second part of the presentation will deal with the description of our research platform in drug discovery with a specific example on the development of a new class of diatom-derived immunomodulators that exert specialized functions in the initiation of the adaptive immune responses and are under investigation as vaccine adjuvants and for the treatment of degenerative diseases.

Structural Modification of the Protozoan Toxin Climacostol for Biotechnological Applications

FEDERICO BUONANNO[a], ELISABETTA CATALANI[b], DAVIDE CERVIA[b], FRANCESCA PROIETTI SERAFINI[b], SIMONA PICCHIETTI[b], ANNA MARIA FAUSTO[b], SIMONE GIORGI[c], GABRIELE LUPIDI[c], FEDERICO VITTORIO ROSSI[c], ENRICO MARCANTONI[c], CLAUDIO ORTENZI[a].
[a]Laboratory of Protistology and Biology Education, Department of E.C.H.T. Università degli Studi di Macerata, Macerata, Italy; [b]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy; [c]School of Sciences and Technologies, Section of Chemistry, Università degli Studi di Camerino, Camerino, Italy.

E-mail of the corresponding Authors: federico.buonanno@unimc.it, d.cervia@unitus.it, enrico.marcantoni@unicam.it, claudio.ortenzi@unimc.it

Climacostol (5-[(2*Z*)-non-2-en-1-yl]benzene-1,3-diol) is a resorcinolic lipid produced by the fresh-water ciliate *Climacostomum virens* for chemical defense against predators. The new diastereoselective chemical synthesis of the toxin allowed us to test the antimicrobial activity of climacostol as well as its cytotoxic and pro-apoptotic effects in multiple human and rodent tumor cell, with promising results. In this study, we synthesized some novel analogues of climacostol, in order to increase the activity of the native toxin, and evaluated their effects on prokaryotic and eukaryotic microorganisms, as well as on mammalian tumor cell lines. The data collected demonstrate that the introduction of a methyl or a hydroxyl moiety to the aromatic ring of climacostol can effectively regulate both its biological activity and its mechanism of action. In addition, the choice of phenol group protection based on the methoxymethyl ether (MOM), which can easily be removed in weakly acidic environment, allowed us to synthesize a great amount of climacostol in Z-configuration, the most biologically active form. This prompted us to use the non-toxic MOM-protected molecule directly in toxicity tests, in order to identify a new strategy for the generation of efficient small organic molecules as pharmacologically active agents.

WORKSHOP Carrers for young scientist

Funding and Career for Scientists – Opportunities at the European Research Council

GIULIANO SCALZI[a]
[a]European Research Council, Belgium.

E-mail of the corresponding Author: Giuliano.Scalzi@ec.europa.eu

ERC funds the very best researchers from around the world to investigate topics of their own choosing at the frontier of knowledge. Projects supported by ERC should have the potential to substantially transform their scientific area. Scientists with a PhD older than 2 years and willing to work for part of their time at a host institution in the EU or countries associated to the Framework Programme H2020 (e.g. Switzerland, Norway, Israel, Turkey etc.) can apply. There are no restrictions on research topic, researcher nationality, current affiliation or age as ERC would like to attract the best researchers to come to Europe for projects of up to 5 years with a funding level between 1.5 and 3.5 million EUR (depending on call).

Amitochondriates – ERC Consolidator Grant

VLADIMÍR HAMPL[a]
[a]Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic.

E-mail of the corresponding Author: vlada@natur.cuni.cz

The informal presentation on the aims of the ERC consolidator grant awarded in 2018 including the brief description of the process of writing, defending and the evaluation of the proposal in ERC. Finally, I will mention the administrative part related to the actual start of the grant and its first results. Discussion interrupting the speech is welcome.

My First ERC: How To Manage Joy and Pain

IRENE RICCI[a]
[a]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy.

E-mail of the corresponding Author: irene.ricci@unicam.it

The mission of the European Research Council (ERC) is to promote the highest quality research in Europe through competitive funding, that supports investigator-driven frontier research, on the basis of scientific excellence.

The history of the 'SymbioVec' project (ERC Starting Grant, 2012) and its prosecution in 'LaunTenaBio' (ERC Proof of Concept, 2018) are examples of successful application of grant funding from the EU for career development in research.

The experience of coordinating an ERC-StG gave me the possibility of transition towards autonomy and independence, which represents the career turning point of every young scientist. 'Symbiovec' was a 5-year biotechnology research project started in 2012 and finished in 2017, entitled 'Yeast symbionts of malaria vectors: from basic research to the management of malaria control'. Specifically, we studied innovative methods based on the use of biocides to control malaria. Thanks to the funding, it was possible to recruit a team of young researchers, and to equip the laboratories with new tools (https://www.unicam.it/symbiovec/). Not only were many of the proposed objectives achieved, but the team also started a spinoff at the University of Camerino to market biocides against insect-borne diseases. This initiative has become the main topic of the ongoing 'LaunTeNabio', ERC Proof of Concept project, funded in 2018, entitled 'Launch Test of Natural Biocides for the Control of Insect Borne Diseases'.

Thus, the research line has moved from the bench to the field, and finally to market, when the starting project turned into a technology transfer project. This route produced a 'frontier research' project, as defined from the emerging areas of science and technology that often aim at covering substantial elements of both, overcoming the distinction between 'basic' and 'applied' research and combining 'Research and Innovation'.

The other side of the coin is that the management of these projects requires an almost full time commitment of the coordinator and needs not only scientific skills. In fact, the researcher comes into contact with administrative aspects that require knowledge of legislation and finances, as well as the ability to succeed in an entrepreneurial role.

Grant Proposals – When to Start and What to Think about while Writing

DOROTA WLOGA[a]
[a]Laboratory of Cytoskeleton and Cilia Biology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland.

E-mail of the corresponding Author: d.wloga@nencki.gov.pl

How to obtaining funding for the research and how to prepare a successful grant application? These are the questions that are frequently asked by young scientists. Important things are: start thinking of the interesting projects leaving yourself enough time to obtain some preliminary data and to find collaborators, discuss your ideas and methodology with colleagues, prepare the first draft as early as possible and ask somebody to critically read your grant proposal. The good news is that with some effort and work everyone can master writing.

Funding opportunities at the Gordon and Betty Moore Foundation, a part of the constellation of science philanthropies based in the U.S.

JONATHAN Z. KAYE

Gordon and Betty Moore Foundation, Palo Alto, USA, jon.kaye@moore.org

Private funders of science in the United States seek opportunities for impact by identifying areas of research where their level of potential funding can make a significant contribution. Following extensive analysis and discussion with the scientific community, the Gordon and Betty Moore Foundation launched a new funding effort in February this year: the Symbiosis in Aquatic Systems Initiative. The initiative will fund internationally, and over the next nine years it will invest $140 million to support research on the origin, evolution, physiology, natural history and ecology of symbiotic associations of marine and freshwater organisms. I will share information about the initiative's current funding opportunities, which focus on developing aquatic symbioses as model systems and on the origin of the eukaryotic cell. The latter opportunity is a joint endeavor with the Simons Foundation. I will also share perspectives on the network of private philanthropies in the U.S. that support organismal and environmental biological sciences.

FRIDAY 2 August

PLENARY LECTURE

Equating OTUs with Species Diversity

MICAH DUNTHORN[a]

Department of Eukaryotic Microbiology, University of Duisburg-Essen, Germany.

E-mail of the corresponding Author: micah.dunthorn@uni-due.de

Species are fundamental biological units used in ecological and evolutionary analyses. The discovery and enumeration of species thus forms the basis for our understanding of the functioning and stability of communities and ecosystems. Because protistan diversity is so large and their morphological characters are so small, molecular methodologies are the only way that we can attempt to uncover protistan species in complex environmental samples. One step in the sequencing-bioinformatics pipeline of molecular methods is clustering reads into operational taxonomic units (OTUs). These OTUs are used in the place of species in downstream analyses. Here I will discuss the underlying assumptions that different clustering methods make in how they handle metabarcoding, metatranscriptomic/metagenomic, and single-cell genomic data. I will then discuss how these differently constructed OTUs may or may not equate with our concepts of species and with the operational criteria that we use to delimitate species in nature.

SYMPOSIUM - Systematic of amoeboid protist

« Shallow » (Species-Level) Diversity in Arcellinida, a Ground for Ecological Research

ENRIQUE LARA[a], QUENTIN BLANDENIER[a, b], DAVID SINGER[b, c], RUBÉN GONZÁLEZ MIGUÉNS[a], LEONARDO D. FERNÁNDEZ[d], ANUSH KOSAKYAN[e]

[a]Real Jardin Botanico de Madrid, Departamento de Micologia, CSIC, Madrid, Spain; [b]Université de Neuchâtel, Faculté de Biologie, Laboratoire de Biodiversité du Sol, Neuchâtel, Switzerland; [c]Laboratory of Evolutionary Protistology, Department of Zoology, Biosciences Institute, University of São Paulo, São Paulo, Brazil; [d]Centro de Investigación en Recursos Naturales y Sustentabilidad (CIRENYS), Universidad Bernardo O'Higgins, Santiago, Chile; [e]Institute of Parasitology, Biology Center, Czech Academy of Sciences, Czeske Budejóvice, Czech Republic.

E-mail of the corresponding Authors: enrique.lara@rjb.csic.es; anna.kosakyan@gmail.com

Environmental surveys on protist diversity based on high throughput sequencing are currently showing a huge and unsuspected diversity, especially in soils. In the meanwhile, genomics and transcriptomics shed light on the earliest nodes of the eukaryotic tree. While these data are revealing how far life on Earth is predominantly microbial, they do not explain how this diversity is generated.

Community ecology and biogeography are two disciplines that aim at explaining the causes and consequences of the distribution of diversity. These disciplines are based on species and have been first developed for macroscopic organisms and, in order to benefit from the strong theoretical framework that has been built for over a century, we need to build our research on identical units. In this presentation, I will discuss the notion of species in the testate amoeba order Arcellinida (Amoebozoa, Tubulinea), giving mostly examples based on family Hyalospheniidae. I will summarize the new advances in their phylogeny and systematics, and show some examples on how the diversity of these organisms is distributed over geographical distances and ecological gradients. These patterns are strongly reminding macroscopic organisms, which advocates for a unified vision of ecology including both microbes and macroscopic organisms.

Deep Evolution of Arcellinid Testate Amoebae: Challenges and Promises

ANUSH KOSAKYAN[a],ENRIQUE LARA[b]

[a]Institute of Parasitology, Biology Center, Czech Academy of Sciences, Czeske Budejóvice, Czech Republic; [b]Real Jardín Botánico de Madrid, Agencia Estatal del Consejo Superior de Investigaciones Científicas, Madrid, Spain.

E-mail of the corresponding Author: anna.kosakyan@gmail.com

Testate amoebae are a diverse group of terrestrial and freshwater microbial amoeboid eukaryotes enclosed in test (shell). Described by naturalists in the eighteenth century, testate amoebae are frequently used in biomonitoring. Yet, in order to use their full potential, a good taxonomy that highlights deep relationships between the groups is needed.

It was shown that classical taxonomy based on morphology alone leads to serious misinterpretations of environmental data. Thus, to study testate amoebae diversity two main approaches were developed: (1) deep phylogeny, aiming at determining the relationships between groups at order or family level; and (2) fine-level phylogeny, aiming for genus to infra-specific level.

Traditionally SSU rRNA gene was used to resolve deep phylogeny of this group. However, this turned out to be particularly challenging in the case of arcellinid testate amoebae, whose main extant lineages had already radiated in the Proterozoic (730 MYA), thus being separated by huge genetic distances from each other and preventing the use of single marker genes.

To overcome these difficulties, a single cell transcriptomics approach was used resulting in a robust phylogeny that allowed the development of a new taxonomy. New genetic database was created with a huge potential for investigation in the biology of Arcellinida, setting the base for exploring gene expression profiles under different environmental disturbances, with possible further applications to bioindication by tracking functional genes.

Shedding Light on the Taxonomy of Elusive Rhizarian Taxa Illuminates Rhizarian Evolution And Ecology

KENNETH DUMACK[a]

[a]Terrestrial Ecology, University of Cologne, Cologne, Germany.

E-mail of the corresponding Author: kenneth.dumack@uni-koeln.de

Rhizaria are a very diverse group of protists with no so far known unifying morphological distinctive characters; however based on their genetics their monophyly is undisputed. Especially the Cercozoa are of high morphological diversity. Many described taxa still lack molecular data and based on morphology it might be difficult if not impossible to find the right taxonomical placement of such. In this talk I will discuss the last years of my research. I will focus thus on the Cercozoa; the talk will cover the evolution of cell coverings in Ventrifilosa (here: Thecofilosea+Imbricatea). This includes novel insight into scale evolution of the Imbricatea and the secondary loss of scales in some of its taxa. I will present a novel hypothesis how to differentiate imbricatean taxa from thecofilosean taxa, even with low resolution light microscopy. I will further give an overview on the autecology of many ventrifilosan taxa. Interestingly terrestrial thecofiloseans were until recently basically unknown, but as we show they are actually highly abundant, sometimes even representing the taxa with most protist sequence reads in environmental amplicon sequencing studies. Most thecofiloseans are eukaryovorous, mostly feeding on algae. Their high abundance and co-occurence with terrestrial algae indicate certain ecological significance in terrestrial ecosystems.

Disentangling the Systematics of the Order Physarales (Myxomycetes, Amoebozoa): an Integrated Approach Using Morphology and Multiple Gene Data

JOAQUINA MARÍA GARCÍA-MARTÍN[a], JUAN CARLOS ZAMORA[b], CARLOS LADO[b]
[a]Department of Mycology, Real Jardín Botánico-CSIC, Madrid, Spain; [b]Museum of Evolution, Uppsala University, Uppsala, Sweden.

E-mail of the corresponding Author: kina@rjb.csic.es

The class Myxomycetes (Amoebozoa) is a diverse assemblage of amoeboid protists characterized by a complex lifecycle, which includes macroscopic spore-bearing structures (sporophores) and free living amoebae feeding on soil microorganisms. Given that the latter do not present easily observable distinguishing characters for species delimitation, the taxonomy of Myxomycetes largely relies on sporophore morphology. Among others, the spore color and ornamentation, the features of sterile structures such as the capillitium, stalk, peridium or columella, and the presence and type of lime deposits in the sporophores are considered taxonomically important characters. However, some species present trait combinations that bridge the gap among different genera and even orders, making it difficult to establish their identity and phylogenetic affinities. In this work, we tested previous hypotheses of relationships among the five Myxomycete orders, based on SSU rRNA phylogenies, using the first and only comprehensive collection of transcriptomic data generated to date. The monophyly of Myxomycetes and three of its orders has been proved, which confirms the validity of their respective synapomorphies to define high-level relationships. At a finer taxonomic level, *i. e.* order Physarales (the largest clade of Myxomycetes), we show, using a four-gene dataset and an expanded taxa sampling, that most genera are not monophyletic. Although molecular synapomorphies have been identified for most clades, some internal relationships remain unknown, especially in the family Physaraceae. Furthermore, phenotypic homoplasy seems to be exceedingly common, making morphology-based taxonomy challenging. Our results indicate that the pre-molecular subdivision of Myxomycetes and, in particular, Physaraceae, is somehow flawed. Likewise, they show that transcriptomic analyses are a promising tool for establishing the phylogenetic relationships within this group. We hope that, together with thorough phenotypic studies (*e. g.*, ultrastructure), and an increased taxa sampling, transcriptomics will help us to disentangle the systematics of these elusive protists.

What's in a Name? – Importance of Morphology and Taxonomic Accuracy for the Evolutionary Studies in Naked Lobose Amoebae (Amoebozoa)

ALEXANDER KUDRYAVTSEV[a,b]

[a]Laboratory of Cellular and Molecular Protistology, Zoological Institute Russian Academy of Sciences, St. Petersburg, Russia; [b]Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia.

E-mail of the corresponding Author: alexander.kudryavtsev@zin.ru

Naked lobose amoebae (currently part of the Amoebozoa) always posed one of the most difficult challenges for protist taxonomists. Many structural characters in amoebae have lost traces of phylogenetic signal, therefore, classifications created before wide application of molecular phylogeny turned out to be artificial in many taxonomic levels. With the introduction of molecular phylogenetics and phylogenomics, the classification has been significantly reshaped, making it necessary to search for the new morphological criteria that may serve as apomorphies for the emerging taxa. Currently available research tools provide significant opportunities for understanding of the molecular basis of morphological innovations in various branches of amoebae. However, given the decreasing costs of the phylogenomic data, the tendency emerges, to significantly rely on molecular data, and neglect the morphological evidence when the latter requires much more time-consuming investigations than sequencing. In my talk, I will show several examples where this approach may significantly reduce the diversity of taxa visible to us and available for our research. One of these cases is the small amoebae from the order Vannellida. The taxonomic diversity of these amoebae may seem low due to their small size. This is refuted by molecular analysis showing at least six genus-level clades of marine and freshwater small vannellids. It is important that detailed morphological analysis of these taxa following discovery of their molecular diversity shows that light-microscopic differences between their locomotive forms may be significant, but require careful observations and analysis of large numbers of cells for detection. A similar situation occurs in the order Himatismenida where negligence to the electron microscopic characters may lead to a significant underestimation of the taxonomic diversity. Some examples of the recent studies also show, that a lack of attention to the previously published morphological works may lead to a significant bias in our understanding of the relationships among different taxa. In all, collection of a full set of data not limited to molecular characters only is in itself feasible and needed in most cases to really understand and classify the diversity of naked lobose amoebae.

Original data shown were obtained using the resource centers of St. Petersburg University Research Park; partially supported by the budgetary theme AAAA-A19-119031390116-9.

Website: www.ecop2019.org

Chair of the Congress- Maria Cristina Angelici
Istituto Superiore di Sanità_ Rome, Italy
President SIP onlus (www.siponlus.it)
mariacristina.angelici@iss.it

Congress venue
Congress Center – Hotel Crowne Plaza Rome-St Peter's
Via Aurelia Antica, 415
00165 Roma (Italy)
Tel: +39 066642114

Congress Secretariat: info@ecop2019.org

Congress and events organization:

M.T.B Management of Tourism and Biodiversity
Elly Travel srl - Roma
cristina.compagno@ellytravel.com