Protistology-UK's Autumn meeting 2022

Abstracts list

How does *Toxoplasma* manipulate its host cells via secreted parasite proteins?

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Toxoplasma gondii is an obligate intracellular parasite that can infect most endothermic animals. Key to the survival strategy of this ingenious protist is the controlled manipulation of its host cell. By secreting proteins into different host cellular compartments, the parasite is capable of elaborately re-engineering the host cells for its own purpose. The spatial location of proteins correlates with their functions. Therefore, the subcellular localisations of the parasite-secreted proteins in the host cells are of great significance in elucidating their functions in host manipulation. Many parasite-secreted proteins have been identified and their localisations in the hosts determined using methods such as immunofluorescence or proximity-dependent tagging via biotinylation. However, such methods generally have low throughput, and it is believed that multiple hundred different *Toxoplasma* proteins are secreted into their hosts. An alternative approach is localisation of organelle proteins by isotope tagging (LOPIT) that can determine the localisation of thousands of proteins simultaneously. LOPIT involves biochemical fractionation of lysed cells using density-gradient ultracentrifugation, followed by the assessment of distinct abundance-distribution profiles that correlate with their subcellular organelles. Our preliminary data indicate that the LOPIT approach has generated good resolution of the host cell spatial proteome, and we detect *Toxoplasma*-secreted proteins located to different host subcellular compartments. Future experiments will build on the resolution of these secreted *Toxoplasma* proteomes in their hosts, and validate the localisation of the proteins through tagging and microscopy followed by examining the protein functions.

Gregarine apicomplexan research: the old, the new, and the future

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According to the literature, the first gregarine was formally described by Dufour in 1828. For a long time, descriptions were based on light microscopy alone, accompanied by hand drawings. In addition to light micrographs, the development of the transmission and scanning electron microscopes in 1931 and 1938 respectively enabled the depiction of the cell's ultrastructure thereafter. Relationships were described using phylogenies since Darwin's tree of life, but it took quite a while until molecular data were used (late 1950s). Molecular data for gregarines were quite scarce for a long period of time but nonetheless, highlighted their importance in the apicomplexan evolution. It took a few more centuries until the first transcriptomes and genomes for gregarines appeared very recently. With a vast range of techniques now available the understanding of the evolution of the Apicomplexa has begun to unfold. To fully understand this evolution, it is important to study the adaptations gregarine's have undergone on the spectrum of symbiosis. What is still limiting here, is the inability of culturing gregarines. At this point I will enter the future of gregarine research with the immediate task to develop a gregarine culture system.

A complex cytoplasm enables inflation-based motility in a non-motile dinoflagellate

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It's long been understood that plankton diversity, distribution and migration can tip the balance of carbon sequestration in the ocean via a complex set of interactions encoded in the "biological pump". However, a cellular understanding of the migratory mechanisms in phytoplankton at the base of this process remains elusive. In this work we directly observe the migrating dinoflagellate Pyrocystis noctiluca, a major contributor to the largest daily movement of biomass on our planet, which it achieves without any ciliary swimming mechanism. We discovered that the cell undergoes a coordinated “ballooning” (a cell inflating its volume 10-fold in less than 10 minutes) event to remain afloat. Although such a process would in principle dilute cellular contents to the point of catastrophic disruption of biochemical activity, we find that a complex reticulated topology of the cytoplasm enables this inflation without dilution. Furthermore, by performing perturbation assays and transcriptomic analysis, we underpin the role of membrane channels in this inflation-driven propulsion. We merged the field ecology and cell biology with sedimentation physics to create a generalizable model for non-mechanical migration. This incorporated a wide range of length scales (cell radius approx. 50 microns to ocean stratification depth approx. 100 meters) and time scale (ballooning approx. 10 mins to cell cycle approx. 1 week), in a single differential equation; thus presenting a framework in which to understand the vertical plankton migrations. Over the past 4.5 billion years gravity has shaped the evolution of all life-forms in the oceans, with gravitational pull and vertical ocean stratification setting bounds on the physiology of a cell. Predictions from our model propose a new explanation for why the cytoplasmic density of cells is around the current value. Our study enables the broader field to incorporate cellular physiology and cell cycle in planetary scale simulations.

From obscurity to centre stage; the role of system dynamics modelling in progressing marine protist plankton science

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Planktonic protists that combine phagotrophy and phototrophy have been known to protistologists for over a century. Widely viewed as curiosities, they were side-lined from mainstream science. In the early 21st century, applications of system dynamics modelling helped to focus interests that drew together disparate strands of protist plankton research, identified different protist functional groups, and provided a route to explore the contributions of these organisms to marine ecology and biogeochemical cycling. System dynamics modelling is an approach developed from economics where feedback mechanisms are recognised as important drivers, while the numeric data needed for traditional modelling methods, focussed on conceptual simplifications, are lacking. This approach provides a platform for describing protist physiology that exploits expert witness (empirical 'natural science') knowledge and thus engages protistologists directly with the modelling effort. Directly (via modelling) and indirectly (by providing a research focus), this effort has led to a sharp increase in research, leading to a reassessment of the role of these photo-phagotropic protists in marine ecology. In recognition of the importance of these protists, of their unique abilities to exploit both phototrophy and phagotrophy, a new term has been coined - 'mixoplankton'. The term 'mixoplankton' clearly delineates these organisms from the 'phytoplankton' that cannot engage in phagotrophy and the 'protozooplankton' that cannot engage in phototrophy. The subsequent explosion in current research interests has identified mixoplankton as global players in oceanic ecology, with regional significance due to the biodiversity exhibited across the range of functional types. Even exemplars of 'phytoplankton' such as *Emiliania huxleyi* and *Phaeocystis globosa* have been identified to be 'mixoplankton' through recent observations of their ability to eat bacteria. System dynamics modelling not only provides a focus for research, but also enables the development of teaching materials to train the next generation of protistologists in the ecophysiology of mixoplankton.

Establishing an *in-vitro* culturing system for apicomplexan gregarines

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Gregarines are a group of apicomplexans that exclusively infect invertebrates, and therefore have little to no clinical significance to humans. As such, minimal research has been dedicated to them, despite the possibility of elucidating the evolution of parasitism amongst Apicomplexa through studying these organisms. To stimulate further research of gregarines, an *in-vitro* culturing system should first be devised in order to abate the lengthy procedure of manual extraction from hosts.

Within this project, attempts at creating primary cell cultures from isolated intestinal tissues of various freshwater invertebrates have been made, however thus far none have led to the establishment of a strong cell line that may be used as a culturing platform for gregarines. Contamination and poor cellular adhesion have been the major cause of these failures, congruent with issues faced by others attempting to create cell lines from invertebrates.

Nevertheless, a particularly promising group of hosts, the Trichopterans, has been identified for its consistent infection with gregarines and ease of sampling from Canterbury's local river. Isolation and sequencing of such gregarines from Trichopterans is currently being performed to expand the shockingly empty pool of molecular data for these apicomplexans.

A continuous cell line derived from *Trichopteran* intestines, or indeed intestines of any invertebrate parasitized by gregarines, is currently the main platform considered by this project, however the exploration of culturing and studying gregarines through utilisation of microfluidics devices as well as organoids is now being considered. These are both biotechnologies which have hardly been applied to invertebrate systems and will therefore require adjustments to accommodate gregarines. Yet, such methods would more accurately reflect the environment of an invertebrate gut and as such may prove to be tools powerful culturing gregarines.

Overall, culturing gregarines using any platform requires much more study to establish a robust and replicable protocol.

Determining the stage-specific thermal effects on the fungal pathogen, *Batrachochytrium dendrobatidis*

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A significant global conservation issue, the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is responsible for the decline and extinction of at least 501 amphibian species. Temperature is a key driver of *Bd* disease epidemiology, affecting the ability of the amphibian host to protect itself and both the intensity and prevalence of the disease chytridiomycosis. Many of *Bd*'s key processes are affected by temperature such as development and mortality rates, fecundity and size, the combination of which determines the overall thermal performance and therefore, fitness, of the organism. Most studies that examine these rates examine whole life cycles, rather than individual life stages. However, this is not always sufficient to summarise the full effect of thermal change on *Bd*. Through the application of traditional scientific methods such as cell culturing and microscopy and the development of a model to examine cohort progression, we assess stage-specific temperature effects that molecular methods would not be able to examine. Specifically, we address the influence of temperature on development rates, size, fecundity and mortality rates, and potential trade-offs between these factors.

The Soil Protist Communities of St Helena, South Atlantic Ocean

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St Helena is a small and remote island in the South Atlantic, characterised by a unique biota with high endemicity. Since the 17th century environmental degradation and spread of invasive species have lead to the reducing of most native species to relict populations and to several extinctions. The Peaks National Park has been established to protect the remnants of native cloud forest in the higher altitudes. Hosting a suite of specialised mist-catching plants, this territory is highly significant for St Helena's water security. While the native flora is well researched, only one study has looked at the associated fungi and most of the soil microbiome is unknown. My work aimed to characterise the soil microbiomes found near three endemics (*Dicksonia arborescens*, *Nesohedyotis arborea* and *Melanodendron integrifolium*) and two invasives (*Phormium tenax* and *Austroeupatorium inulifolium*) of the St Helena Peaks to set a baseline for the microbial diversity of the National Park. Metabarcoding was performed using 18S rRNA gene sequencing on 55 soil samples, followed by α- and β-diversity analyses in QIIME 2. Levels of diversity were similar across sites and host plants, revealing a rich protist community consisting of 30 phyla. Besides a high abundance of heterotrophs (Cercozoa, Ciliophora, Amoebozoa), obligate parasites were well represented by Gregarinasina, Ichthyosporea and Peronosporomycetes. As the only native land vertebrates of the island are birds, the detection of Ichthyosporea contributes to mounting evidence of a host range beyond fish and ubiquitous presence in soils. Labyrinthulomycetes, an almost entirely marine class, were also detected. This study serves as the first report of the protist diversity of St Helena and stands in contrast to previously reported depauperate soil microbial communities. While sequencing methods have allowed to survey a wide taxonomic and geographic scale, detected taxa of interest await direct observation and description.

The impact of heavy metals on multitrophic interactions in the soil microbiome: how soil pollution influences amoebic predation and bacterial interactions.

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The soil microbiome drives fundamental processes such as soil fertility, nutrient cycling, carbon sequestration and impacts plants and animals' health. Global change is often linked to the rapid increase in anthropogenic activities, which threatens the delicate equilibrium of the microbiome. Maintaining soil biodiversity is crucial for the ecosystem, and a diverse and interactive microbiome, as viruses, bacteria, fungi and protists all contribute significantly to soil health. Protists contribute significantly to the ecosystem to the maintenance, as they are bacterial predators.
The impact of heavy metal accumulation in soils and its microbiome is yet to be fully understood, however their presence in soils have been shown to promote antimicrobial resistance, causing a negative impact on human and animal health.
Our study seeks to investigate how the cosmopolitan protist *Acanthamoeba castellanii*, predates *Pseudomonas* in the presence of heavy metal pollutants. We will use strains of *Pseudomonas*, isolated from *Acanthamoebae* species retrieved from heavily polluted environments and tested for multidrug resistance (MDR).
Furthermore, we aim to simulate a microcosm by adding the free-living ciliate *Tetrahymena thermophila*, with the purpose to observe how the involved protists interact.
This research will contribute to the understanding of how heavy metal accumulation in soils impacts protists' predation of bacteria, how anthropogenic pollutants influence microbial interactions, and provide an insight upon the impact of climate change from the unexplored perspective of the microbiome, its dynamics, and consequences at different levels.

Novel nuclear proteins in dinoflagellates mediate the highly compacted liquid-like state of dinoflagellate chromosomes

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DNA in most eukaryotic nuclei is packaged using histone-based nucleosomes to enable DNA condensation and regulation. Dinoflagellates are an exception to this universal feature as they have apparently abandoned canonical nucleosomes. They re-engineered their chromatin biology coincident with the gain of novel nuclear proteins through multiple lateral gene transfer events. First dinoflagellates acquired genes for a family of DNA-binding proteins of putative viral origin called 'dinoflagellate/viral nucleoproteins' (DVNPs). DVNPs are found in all extant dinoflagellates including the early-diverging Syndiniales. Subsequently dinoflagellates gained bacterial proteins for 'histone-like proteins' (type-I and type-II HLPs) and these genes were acquired on multiple occasions independently but after Syndinales diverged. The outcome of these changes in the protein biochemistry of dinoflagellate nuclei is the enormous expansion of nuclear DNA content and the formation of liquid crystalline permanently condensed chromosomes, a highly unusual nuclear state. To understand the role of DVNPs and HLPs in dinoflagellate nuclei, we expressed and purified these proteins and examined their interaction with DNA. We observed both DVNP and HLPs rapidly bind to DNA; however, whereas DVNP forms phased separated coacervates with DNA, HLPs form solid DNA aggregates. Using optical tweezers to measure DVNP's effects on single DNA molecules, we observe changes to the physical properties of DNA and extremely rapid DNA contraction although through DNA tensions less than those produced by canonical nucleosomes. DVNP structural data and co-evolutionary analysis identifies a disordered C-terminal domain as playing an important role in DNA compaction. Together, our results suggest that DVNPs and HLPs interact differently with DNA implying different contributions of these two proteins to the highly unusual dinoflagellate nuclear state.

Coccolithophores as predators: integrating system dynamics with hydrodynamic-biogeochemical modelling to study impact of mixoplanktonic coccolithophores on ecosystem functioning

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Coccolithophores, an iconic 'signature' phytoplankton, have been recognised as an important functional group in microbial communities for decades. They are one of the most abundant oceanic calcifying primary producers, influencing air-sea CO_2 exchanges and, substantially contributing to the sinking of inorganic and organic carbon. Within ecosystem models, coccolithophores are typically included within the phytoplankton functional group. Various experimental studies have shown different life-stage phases of coccolithophorids to be capable of phagotrophy. More recently, the exemplar coccolithophore, *Emiliania huxleyi*, has been shown to be capable of bacterivory. This ability of the coccolithophores to engage in phototrophy plus phagotrophy for nutritional needs has led to a change in their ecological status from phytoplankton to "mixoplankton". Over the last decade, mixoplankton, protists that obtain nutrition through both phototrophy and phagotrophy synergistically, have been shown to be ubiquitous in the global ocean. This has led to the recognition of the new mixoplankton paradigm in marine ecology. The mixoplankton paradigm has been implemented in a few recent modelling studies showing their importance in oceanic biogeochemical cycling. However, none of these models have represented coccolithophores as mixoplankton. For the first time, we investigate the importance of coccolithophores as mixoplankton within a marine ecosystem model. Our methodology includes the implementation of the "Perfect Beast" system dynamics protist model within a biogeochemical coastal system model (the European Regional Seas ecosystem model, ERSEM), coupled to the one-dimensional physical oceanography based water column model GOTM (General Ocean Turbulence Model). We are currently optimizing our model (ERSEM-PB) to field data with the aim to deploy our ecosystem framework at a site of coccolithophore bloom initiation on the NW European shelf. We will present preliminary findings focusing on the impact of modelling coccolithophores as mixoplankton versus as phytoplankton on ecosystem dynamics and biogeochemical cycling.

Changes in diversity and function of protists in Arctic cyanobacterial microbial mat communities

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Global environmental studies of protists have demonstrated their ubiquity and contribution to nutrient cycling in freshwater environments. However, understanding of their diversity and function in the polar regions remains limited. Accelerated warming is altering the Arctic landscape in real time, raising concerns over the future integrity of Arctic freshwater systems and their unique biodiversity within. Microbial mats dominated by oscillatorian cyanobacteria are almost ubiquitous in Arctic freshwater systems. The phototrophic communities in these mats rely on internal nutrient scavenging systems to cope with the low allochthonous input of nutrients. Microscopy-based studies have long indicated that eukaryotes including metazoa are present in polar mats, but their microbial eukaryotic community remains understudied.

In this study, environmental DNA (eDNA) was extracted from mat samples collected from freshwater systems across a diversity of Arctic ecozones, from the taiga to the ice shelves. The V4 16S and V9 18S rRNA gene DNA regions were sequenced from extracted eDNA samples by targeted amplicon sequencing to identify the prokaryote and microbial eukaryotic diversity, respectively. Amplicon sequence variants (ASVs) were assembled, and the eukaryotic sequences were taxonomically assigned using the PR^2 (Protist Ribosomal Reference) database. ASVs were assigned to three main functional modes, based on their taxonomic affiliation; phototrophic, consumers and symbionts. A consistent community of phototrophs and consumers was identified across all Arctic ecozones, however a notable restriction in richness was identified in the highest latitude samples along with a reduction in symbiotic organisms.

This comparative annotation was dependent on previous classification of nutrition and morphology through microscopy. Applying this annotation to eDNA approaches allows us to develop our understanding of the biogeography and function of protists in extreme and less studied environments.

A non-lethal method of gregarine apicomplexan identification in the freshwater amphipod *Gammarus pulex*

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The phylum Apicomplexa consists mainly of parasitic protists, some of which are the causative agents of potentially fatal infectious diseases in humans such as malaria (caused by *Plasmodium*) and cryptosporidiosis (caused by *Cryptosporidium* sp.). Despite the intensive study of medically important species, many aspects of apicomplexan biology and evolution remain unknown. In general, all Apicomplexa are referred to as being obligatory parasitic, but the large group of gregarine apicomplexans infecting a wide range of freshwater, marine, and terrestrial invertebrates have been shown to fall anywhere on the spectrum of symbiosis (mutualistic to parasitic). Studying the effects of these organisms on their hosts is currently challenging and requires killing the host to verify gregarine infection or isolation of specimens under a microscope. This technique comes with the caveat that early-stage infections can be difficult to determine using microscopy alone. We identified three separate gregarine species that infect the freshwater amphipod *Gammarus pulex* in the Water of Leith, Edinburgh, UK. Utilizing old school observational techniques and molecular biology methods, we present the development of a novel non-lethal protocol to determine the host's infection status, and if infected, which of the three gregarine species are present within the host. The use of this protocol may be extendable to other invertebrate-gregarine symbiotic systems.

Marine protist plankton parasites – insights from isolation and experiments in understanding their ecological impact.

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Protists thrive in the marine environment. Application of high-throughput sequencing approaches, such as metabarcoding of universal marker genes, to determine apparent marine protist diversity and infer ecology is now commonplace. These data dense DNA-based, and sometimes RNA-based, surveys have been used to propose ecological interactions between protists, including possible parasite-host interaction with negative host impacts. Marine diatoms are major players in the global carbon cycle and underpin marine food webs. Infection of marine diatoms by protists parasites has been known by microscope-based protistologists for some time. However, relative to other protist groups and activities, protist parasites of other marine protists remain poorly understood. We aimed to explore such interaction by isolating a protist parasite from the ecologically important marine diatom genus *Chaetoceros*. We identified the parasite as a novel Thraustochytrid (Labyrinthulomycetes) from a clade only previously known via DNA-based surveys. The Thraustochytrid appears widespread across several marine locations globally. Physiological experiments with both Thraustochytrid and *Chaetoceros* showed that the parasite targets senescent diatom cells over healthy cell. This physiology-selective targeting of 'unhealthy' cells appears to improve the overall health of the diatom population without impacting density. We provide support for 'healthy herd' dynamics in a protist–protist interaction, a phenomenon typically associated with animal predators and their prey. Such insights were possible through isolation and subsequent experiments, and would likely have been missed in high-throughput DNA sequencing diversity surveys. Furthermore, our study suggests caution against the assumption that protist–protist parasitism is always detrimental to the host population and highlights the complexity of marine protist interactions.

Evolving a non-photosynthetic dinoflagellate in the laboratory

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Dinoflagellates contain a wide variety of chloroplasts, acquired through numerous endosymbiosis events. The ancestral, peridinin-containing chloroplast is retained in some species, including the coral-reef associated *Symbiodinium* and the free-living *Amphidinium*. However, peridinin-containing chloroplast genomes are extremely reduced. Only genes for a small number of key photosynthetic proteins, as well as ribosomal rRNA genes, have been retained within the organelle. Furthermore, rather than residing on a large circularly mapping DNA circle, genes are found on much smaller plasmid-like DNA molecules termed minicircles. Typically, one gene is found per minicircle though some incidences of minicircles with a few genes have been discovered. We have been able to make use of this fragmented genome to establish a genetic modification system in *Amphidinium,* including the expression of heterologous genes.

Recently, by long-term growth of *Symbiodinium microadriaticum* on supplemented media, we have isolated strains which have lost the ability to carry out oxygenic photosynthesis. These strains have lost key minicircle genes, and are reliant on external glucose for growth. We are now investigating how minicircle loss may shape the evolution of this species and can lead to a transition to heterotrophy.

High-efficiency transfection of *Acanthamoeba castellanii* using a cationic polymer

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The free-living amoeba *Acanthamoeba castellanii* is an ecologically , clinically , and evolutionarily important microorganism. This amoeba is directly pathogenic to humans, and serves as a reservoir for bacterial pathogens (e.g., *Legionella pneumophila*), but also regulates the proliferation of other microorganisms in the soil. Despite its importance, no reliable genetic system has been developed, hampering the use of *A. castellanii* and related species as model organisms. Transfecting *A. castellanii* with plasmids is possible with commercial kits but is expensive, inefficient, and vulnerable to product discontinuation. We present a method for efficient transfection of *A. castellanii* with readily available and inexpensive polyethylenimines. We systematically explore the parameters of the method, obtaining as much as a twofold increase in transfection efficiency over the currently used reagents. The method presented here provides a robust step towards a full genetic toolbox for *A. castellanii*, hence expanding its use as a model organism.

Morphological and phylogenetic observations of *Agogonia voluta* gen. et sp. nov. and *Ophirina chinija* sp. nov. shed light into the evolution of the deepest jakobid branch

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Jakobida (Discoba, Excavata) is a small group of flagellated protists with the most bacteria-like and gene-rich mitogenomes known for any eukaryotic group. Among them, Ophirinina is a recently described suborder with only one known species to date, *Ophirina amphinema*. Despite the acquisition and analysis of massive transcriptomic and mitogenomic sequence data from *O. amphinema*, its phylogenetic position among excavates remained inconclusive, branching either as sister group to all Jakobida or to all Discoba. From a morphological perspective, it has several typical jakobid features, but also unusual traits for this group, including discoid mitochondrial cristae and the presence of two vanes on the posterior flagellum. We have isolated, morphologically characterized, and sequenced genome and transcriptome data of two new Ophirinina species: *Ophirina chinija* sp. nov. and *Agogonia voluta* gen. et sp. nov. from marine and freshwater environments, respectively. *Ophirina chinija* differs from *O. amphinema* in having rounded cell ends, subapically emerging flagella and a posterior cell protrusion. The much more distantly related *A. voluta* has several unique ultrastructural characteristics, including saccular to flattened-curved mitochondrial cristae and a complex two-part 'B' fibre. Phylogenomic analyses with a large conserved-marker dataset supported the monophyly of *Ophirina* and *Agogonia* within the Ophirinina and resolved the conflicting position of ophirinids as the sister clade to all other jakobids. The characterization of the mitochondrial genomes showed that *Agogonia* differs from all known gene-rich jakobid mitogenomes by the presence of two maturase protein genes and four group II introns. A phylogenetic analysis of the diversity of known maturases confirmed that the *Agogonia* proteins are highly divergent from each other and define distant families among the prokaryotic and eukaryotic maturases. Therefore, Ophirinina appear to have retained additional mitochondrial markers as compared to other jakobids that may help to understand the early diversification of eukaryotes and the evolution of mitochondria.

Survival kit: the flexible lifestyle of marine diplonemids

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Recent revelation of diplonemids (Diplonemea, Euglenozoa) as one of the most abundant groups of planktonic microeukaryotes in the world ocean has revived interest in their biology. More than a dozen axenic cultures have been isolated and are being studied. The examination of diplonemid ultrastructure revealed the nutrition-dependent emergence of euglenozoan-specific features, such as tubular extrusomes and paraflagellar rod adjacent to the axoneme. This peculiar aspect indicates the adaptive flexibility of diplonemid cells to survive in a changing marine environment. In order to obtain better insights into the fascinating adaptations of diplonemids and their fundamental influence on the marine food web, we focused on nutritional modes of these heterotrophic protists. We demonstrated that cultured diplonemids, maintained in an organic-rich medium as osmotrophs, can gradually switch to phagotrophy under cultivation preconditioning. The studied diplonemids were shown to have species-specific feeding patterns, size-selective grazing preferences, and distinct feeding strategies. In addition, they were able to discriminate between low-quality food items and inedible particles (latex beads) even after ingestion. The unprocessed material was released from cells in the form of large waste vacuoles. Furthermore, we detected digestion-related endogenous autofluorescence emitted by lysosomes and production of melanin-like material. The documented feeding flexibility clearly serves as another adaptive advantage for the successful life strategy of diplonemids and provides strong clues to explain their ubiquity in different marine habitats.

Establishment of genetic modification tools in the dinoflagellate *Amphidinium carterae*

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Genetic modification of dinoflagellate algae has been a challenge for many years. After much trial and error, we have recently established a successful method for the genetic modification of the chloroplast of the dinoflagellate *Amphidinium carterae*. Dinoflagellates with a peridinin containing chloroplast have a fragmented chloroplast genome, made up of a collection of approximately 20 plasmid-like minicircles. By adapting these minicircles, we have been able to achieve heterologous gene expression in *A. carterae*. To date, we have created four different artificial minicircles, and have shown that, for all four, heterologous genes located in them can be expressed.

A long way of the genus *Thecochaos* (Amoebozoa, Thecamoebida) - from ancient stained preparation in NHM collection to molecular phylogeny and NGS sequencing.

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The problem of ancient amoebae species remains a key one in modern studies. Poorly described species, not documented with type material, usually have to be declared invalid. There is a widespread opinion that permanent stained preparations of amoebae are of limited use as a type material, because they do not retain many features visible in living cells. However, well-made preparations, done with special attention to amoebae adherence and fixation may be highly useful and sometimes are decisive for re-isolation of ancient species. The collection of slides of the British Museum of Natural History (London) harbors hundreds of amoebae preparations. Among them there are slides of two species made by E. Penard in 1900th and recognised by F. C. Page in 1981 as members of the genus *Thecochaos* – an organism resembling multinucleate thecamoebians. *Thecochaos* was never seen since Penard's time, and these slides remain the only material proof of this organism. Recently we re-isolated *Thecochaos fibrillosum* from the soil of West Siberia, and images and description of these slides were decisive for species identification. We had just a few cells of this organism. However, using single-cell techniques, we were able to obtain light- and electron-microscopic data, get SSU sequence and perform whole-genome amplification for NGS sequencing and multigene phylogeny. Taking into account this successful (yet rare) experience of reliable re-isolation and identification of an ancient amoeba species based on type slides, it is reasonable to suggest that key ancient slide collections should be re-investigated and documented using modern optical facilities. Properly made permanent preparation remains a preferable type material for amoebae, since living cultures show little reliability on a long-term time scale. Moreover, it is possible to consider the project of DNA isolation from old slides (techniques allow) to get genetic material from ancient species. Supported with RSF 20-14-00195 project.

The importance of long-term microbial ecosystem studies as a basis for mitigation strategies and environmental policymaking

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The study of microbial ecology in oiled marine ecosystems is complicated by multiple factors, including the type of petroleum-based pollutants, the duration and intensity of pollutant discharges, the rate of sediment deposition, the pattern of tidal variation and erosional forces, the level of toxicity, current shoreline influences, and the prevailing regulatory limits and thresholds. These variables are interactive and temporal changes can alter or disrupt initially observed patterns. Thus, short term observational studies may be misleading or provide insufficient information for modeling ideal mitigation procedures, particularly if researchers are attempting to characterize rapid assessment approaches or to devise successful intervention measures. Thus, longer term studies can capture more subtle clues about the rate of change in one or more of these parameters and may result in better regulations and policy formation based on multiple approaches for measuring the functional implications of microbial ecology in altered coastal ecosystems. An additional benefit is derived from integrating observational studies with new technologies for measuring microbial diversity and ecosystem change. A case study in Narragansett Bay, Rhode Island, USA, tracking the impacts of oiled environments on local microbial food chains in shallow coastal waters illustrates the value of long-term observational studies for framing future mitigation and other pollution prevention strategies.

Protist biocrystallization in the spotlight or Raman microspectroscopy

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In stark contrast to other well-studied cellular structures and organelles, crystalline cell inclusions are often of unknown composition, functioning, and biogenesis. Most of them have been observed since the beginning of microscopy. Some of them have been already mentioned by Charles Darwin and Ernst Haeckel in the mid-19th century. Obviously, these gentlemen did not possess appropriate analytical methods to study them, but we do. The employment of Raman microspectroscopy opens new horizons for studies of various cell compartments by getting the information on their chemical composition in vivo, in situ, almost real-time, and in a non-destructive way. I will present three examples of this state-of-the-art technique.

Revisiting crystalline inclusions in unicellular eukaryotes is a great example of how powerful the technique of Raman microspectroscopy is. Compared to well-known calcite scales, silicate frustules, and celestite skeletons, the intracellular crystals are oftentimes considered to be oxalates, sometimes proteins, and rarely purines. Herein we provide a great revision of the cellular microcrystals across the broad diversity of protists. The emergence of Raman microscopy enabled us to address various types of cell inclusions directly *in vivo* and *in situ*. We have found that the prevailing chemical nature of intracellular biocrystals corresponds with purines (guanine, uric acid, and xanthine). These are generally present across the vast eukaryotic diversity and based on our phylogenetic analysis we infer the parallel independent emergence in evolution. Further understanding of purine crystals' metabolism may bring important insights spanning from cellular biology to global ecology.

An evaluation of the phylogenetic and spatial distribution of vitamin B^{12}-dependent metabolism across the algal tree of life

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Vitamin B^{12}, or cobalamin, performs essential roles in algal functional ecology, and for human health (*c.f.*, pernicious anaemia), particularly as a cofactor for the essential enzyme methionine synthase (METH) (1). Vitamin B^{12} is highly complex, is synthesised uniquely by bacteria and is acquired by eukaryotes via phagocytosis, scavenging or symbiotic exchange (2, 3). Vitamin B^{12} scarcity may thus constrain global primary production (4) and many photosynthetic organisms retain a vitamin B^{12}-dependent methionine synthase (METE) either alongside METH (in algae) or in lieu of vitamin B^{12}-dependent metabolism (in plants). Here, we perform a detailed phylogenetic analysis of the distributions of METE, METH and five other vitamin B^{12}-associated enzymes (5) across over 1500 plant and algal genomes and transcriptomes (6). We reveal the striking retention of an ancestral copy of METH, alongside the vitamin B^{12} remodelling enzymes MTRR and CblB, in the bryophyte plant lineage hornworts, followed by at least two complete independent losses of this pathway in mosses and vascular plants. We show more limited distributions of other vitamin B^{12}-dependent enzymes involved in the propionate shunt (MCM, CblA), and the type-II ribonucleotide reductase (RNR) across eukaryotic algae; and the unique presence of an obligate vitamin B^{12} dependency throughout the major marine order haptophytes (2, 7). Finally, considering the bio-geographical distributions of vitamin B^{12}-related enzymes across photosynthetic eukaryotes, we propose freshwater-to-land transitions and symbiosis as the primary constraints of its biological function.

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Unveiling the diversity of Odontostomatea using 18S rRNA sequences and morphology

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Anaerobic ciliates, also referred to as sapropelic ciliates, thrive in sulfidic, microoxic habitats and have been observed for over a century. Because of the difficulty in culturing them and the limited methods available, at the time, most original descriptions were based only on in vivo observations from fresh samples, and the knowledge about their morphology was imprecise and species richness underestimated. In the last two decades, details of the morphology and phylogenetic positions of anaerobic ciliates from several different lineages have been revealed (e.g., Metopida, Clevelandellida, Caenomorphidae, Muranotrichea, Plagiopylea) showing the huge diversity of these groups. Nevertheless, the well-known class Odontostomatea has been largely overlooked, with only two species, *Saprodinium dentatum* and *Discomorphella pedroeneasi*, having been studied using modern morphologic and molecular methods. We sampled mud sediments from freshwater, brackish, and marine habitats, and partially unveiled the diversity of the class Odontostomatea through morphology based on in vivo and silver-impregnated specimens. Due, in large part, to the design of more taxon-specific primers for several odontostomatid groups we were able to successfully conduct phylogenetic analyses on this class based on partial 18S rRNA gene sequences and to improve their morphologic characterization.

Exo-erythrocytic stages of avian *Parahaemoproteus* (Haemosporida, Apicomplexa) protists: how we study their diversity

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Parasitic protists of the subgenus *Parahaemoproteus* (Haemosporida, Haemoproteidae, *Haemoproteus*) are exclusive bird pathogens. For a long time, they have been neglected due to the assumption of being harmless, but recently gained interest as agents of severe and even lethal haemoproteosis in non-adapted avian hosts. The knowledge remains particularly scanty on exo-erythrocytic development of these parasites in natural hosts but is crucial to understand the patterns of infection development and mechanisms of virulence.

Microscopic examination of blood films determines naturally infected birds, and most *Haemoproteus* species were described based on morphological features of gametocytes, the intracellular stage developing in the blood of avian hosts. In parallel, molecular analysis (mainly DNA sequences of partial cytochrome *b* gene) are used to identify the lineages of the parasites. One parasite species often have more than one genetic lineage; one bird often is infected with more than one parasite species, which are common cases in wildlife. Combination of the microscopic and PCR-based tools is a gold standard in biodiversity studies of these protists. The diversity of *Haemoproteus* parasite is great, with 177 species described and more than 1800 lineages recognized. It is likely that bigger diversity occurs in nature. However, the exo-erythrocytic stages, which grow in the organs and proceed the development of gametocytes are known for less than 30 species. Diversity of haemoproteids on tissue stages remain insufficiently known.

Over the years, different techniques have been used to study the exo-erythrocytic stages of these parasites, including traditional microscopic examination of organ imprint preparations and histological sections. More recently, techniques such as chromogenic in situ hybridization, and laser microdissection provide new opportunities to approach the exo-erythrocytic stages. We will review these techniques as well as discuss and illustrate how they contribute to the investigation of biodiversity of this group of parasitic protists.

How Does Multi-Level Selection Impact Stable Genome Sizes in Endosymbionts

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The evolution of the eukaryotic cell is an evolutionary oddity. Central to the formation of the first eukaryotic common ancestor was the endosymbiosis of a prokaryote into another prokaryote. This endosymbiosis eventually gave rise to the mitochondria. This process of endosymbiont to organelle (organellogenesis) is central to eukaryogenesis, however there are gaps in our understanding of the genetic and metabolic transitions an organism must go through to live within another.

As endosymbionts are unable to recombine their DNA with free-living bacteria, an endosymbiont's genome degrades in quality over time with the accumulation of deleterious mutations. This puts endosymbiont genomes under the constraints of Muller's Ratchet, where deleterious mutations accumulate in a population which is unable to remove them and so once a mutation occurs, the population cannot regain the fittest genotype.

This is potentially one of the leading factors causing dramatically reduced genome sizes in endosymbiont populations, from the 1.5Mb genomes of *Wolbachia* to the 16kb genes of mitochondria. We have made a simple population genetics model which can simulate the genome degradation in an endosymbiont by also including how selection on the host may influence an endosymbiont's genetic traits. In doing so, we can study the effects of multi-level selection, mutation rate, and population size on stable genome size in endosymbionts.

This will provide novel insights into the theoretical study of endosymbionts as well as potentially highlight the factors which can motivate an organellogenesis-like event as seen at the origin of eukaryotes. In doing so we will be able to determine if all cases of endosymbiosis are the same and given enough time all endosymbionts will reduce their genomes to the point of becoming an organelle, or endosymbiosis is a fundamentally different process for each endosymbiont-host relationship, highlighting the oddity of the eukaryotic cell.

Challenges to decipher drivers of genetic differentiation in marine planktonic populations. Are we ready for protist population metagenomics?

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This past decade, high-throughput sequencing has transformed the study of planktonic communities and highlighted the extent of protist diversity in marine ecosystems. Still, little is known about their genetic diversity at the species-scale, and about their major speciation mechanisms. As data from large scale samplings are now available, we postulate that metagenomics could contribute to deciphering the processes shaping their genetic differentiation in the marine realm. As a proof of concept, we developed a findable, accessible, interoperable and reusable (FAIR) pipeline and focused on the Mediterranean Sea and chose three abundant protist species: *Bathycoccus prasinos*, *Pelagomonas calceolata* and *Phaeocystis cordata*. We compared the genetic differentiation of each species in light of geographical, environmental and oceanographic distances. We highlighted that isolation-by-environment shape the genetic differentiation of *Bathycoccus prasinos* whereas *Phaeocystis cordata* is impacted by isolation-by-distance. At present time, the use of metagenomics to accurately estimate the genetic differentiation of protists remains challenging as resulting coverages are lower compared to traditional genetic populations surveys. We performed similar analyses with Metagenome-Assembled Genomes, and the trend remains the same. Our approach sheds however light on ecological and evolutionary processes occurring within natural marine populations and paves the way for future protist population metagenomic studies.

Apical annuli are essential exocytic sites in the apicomplexan parasite *Toxoplasma gondii*

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Several unique cell features found throughout Myzozoa were re-employed by apicomplexan parasites for host cell invasion and exploitation. These include components of the apical complex that facilitate secretion of invasion factors, and a dedicated site for endocytosis called the micropore. These structures co-evolved with the elaborate multi-layered cell pellicle to facilitate material exchange across this barrier. This pellicle is common to all alveolates (apicomplexans, dinoflagellates and ciliates) and in Apicomplexa is called the inner membrane complex (IMC).

Following invasion of their hosts, apicomplexans release a suite of parasite-derived effector proteins involved in host cell manipulation from secretory organelles called dense granules. However, the sites of secretion of dense granules are yet to be identified. In *Toxoplasma,* small ring-like structures referred to as apical annuli have been found at the lower boundary of the apical cap cisterna of the IMC. The functions and significance of these enigmatic structures, however, have been unknown. Furthermore, all the previously identified annuli proteins are associated with the IMC, so it was uncertain if this structure has any interaction with or relevance to the plasma membrane and extracellular environment. We used hyperLOPIT spatial proteomics and proximity labelling to search for plasma membrane-associated proteins at the apical annuli and found four with this conspicuous location. One is a polytopic membrane protein containing LMBR1 domains of unknown function, and three are Q-SNAREs implicated in vesicle docking. Depletion of each of these proteins results in strong growth phenotypes. Specifically, these mutations manifest as defects in intracellular replication and the secretion of dense granules. Our data show that the apical annuli are essential structures to *Toxoplasma*, that their composition and activity span the plasma membrane, and that they represent additional exocytic sites to the apical complex in *Toxoplasma.* We speculate that the complexity and persistence of the IMC in Apicomplexa has driven the need for apical annuli as further specialised secretion sites in the cell periphery.

Diversity and phylogenetic partitioning of ciliated protists across 1,000 m in the Sargasso Sea

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The bulk of knowledge on the diversity of marine ciliates is from shallow and/or sunlit waters. The present work aims at studying ciliate diversity across the epi- and mesopelagic zones of a subtropical oceanic site using DNA metabarcoding and phylogeny-based metrics. We analyze sequences of the 18S rRNA gene (V4 region) from 369 samples collected at 12 depths (0-1000 m) in the Sargasso Sea (BATS station) monthly for 3 years. Preliminary analyses show three main findings. First, there is a gradual but significant decrease in alpha-diversity from surface to 1000-m waters (based on Faith's phylogenetic diversity index vs. depth; Pearson's $R = -0.52$, $p < 1 \times 10^{-5}$). The comprehensive depth and temporal resolutions analyzed allow us to settle contradictory relationships between ciliate alpha-diversity and depth reported in previous studies. Second, multivariate analyses of beta-diversity (using the phylogenetically-aware unweighted and weighted UniFrac distances) indicate that the taxonomic composition of the ciliate community changes significantly from photic to aphotic waters (ANOSIM, $p = 0.001$), with a switch in sequence prevalence from Oligotrichea to Oligohymenophorea. Third, phylogenetic placement of sequences and clade-level correlations (EPA-ng and GAPPA algorithms) show Oligotrichea, Hypotrichia, Litostomatea, Prostomatea and Phyllopharyngea as anti-correlated with depth, while Oligohymenophorea (except Scuticociliatia) and Protocruzia have a direct relationship with depth. Two enigmatic environmental clades with high relative abundances (OLIGO5 and NASSO1) include sequence variants distributed in photic, aphotic or both environments. Our results suggest functional changes in ciliate communities, from prevalence of algivory and mixotrophy in photic layers to bacterivory, detritivory and perhaps parasitism in aphotic waters. Integration of DNA sequences with organismal data (microscopy, functional analyses) and development of databases that link these sources of information remain as major tasks to better understand ciliate diversity and roles in the environment.

Transfer of protein translocation machinery from haptophyte endosymbionts to Kareniaceae hosts supports complex red plastid evolution by serial endosymbiosis

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Complex red plastids have a rhodophyte ancestry and are found among modern members of the groups Cryptophyta, Haptophyta, Ochrophyta and Myzozoa. Interpretations of the distribution of complex red plastids bifurcate between either strict vertical inheritance from a single common ancestor — the so-called chromalveolate hypothesis — or multiple horizontal transfers of red plastids between the polyphyletic eukaryote groups — the so-called rhodoplex hypothesis. Most complex red plastids are surrounded by four membranes and protein import across the second outermost membrane uses a nuclear-encoded translocation machinery called SELMA. SELMA is likely derived from the ER-associated protein degradation (ERAD) system of the rhodophyte endosymbiont common ancestor of complex red plastids. To explain the distribution of SELMA observed among modern eukaryotes, the rhodoplex hypothesis would require that genes for SELMA have repeatedly been transferred from endosymbiont to host genome, during each horizontal plastid acquisition event. Alternatively, under the chromalveolate hypothesis, only a single transfer of genes for SELMA is needed. Of these two scenarios, multiple transfers of SELMA is the least parsimonious, and this has been considered as an argument against the rhodoplex hypothesis. We therefore chose to investigate whether there is any evidence for multiple transfers of genes for SELMA. To do this we inferred the evolution of SELMA proteins from eukaryotes with complex red plastids using phylogenetics, and we investigated recent cases of complex red plastid acquisition in dinoflagellates to test for horizontal inheritance of SELMA. Among members of the Peridiniales with diatom endosymbionts, we found diatom-derived SELMA proteins that are most likely still encoded by the diatom endosymbiont genomes. Whereas among members of the Kareniaceae with plastids from haptophytes, we found host nuclear genome-encoded SELMA proteins that were likely acquired from haptophytes. These data provide strong evidence that horizontal transfer of SELMA genes has occurred in Kareniaceae dinoflagellates. This finding therefore supports a mechanism that could have similarly facilitated the horizontal spread of complex red plastids among other eukaryote groups, as is proposed by the rhodoplex hypothesis.