

Oral Presentations (please note that presenting authors are **bold**)

Invited Talks

Plenary Talks

Monday

PHOTOSYMBIOSIS IN THE OCEAN

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Photosymbiosis is a symbiotic relationship between two or more organisms, one of which is capable of photosynthesis. Like other forms of symbiosis, photosymbioses can involve the full spectrum of trophic interactions from mutualism to parasitism. As in marine benthic environments (e.g. coral reef ecosystems), photosymbiotic associations are frequently encountered among plankton in marine pelagic environments and can involve various combinations of microalgae with bacteria, protists, or metazoans.

After a brief overview of current knowledge on the diversity of the organisms involved in mutualistic pelagic photosymbioses, I will focus on Radiolaria (Rhizaria) a poorly studied yet significant plankton group in oceanic ecosystems. Evolutionary relationships based on single cell morpho-molecular approaches for main radiolarian groups and their microalgal symbionts emphasize the necessity to re-evaluate traditional classification structures. Those molecular phylogenies using universal taxonomic marker genes (e.g. 18S rDNA) also provide references sequences that are critically required to appropriately annotate environmental meta-barcoding datasets and explore specific ecological patterns in relation to contextual parameters.

In order to investigate further the functioning of photosymbiotic relationships between protists, we develop a comparative genomic approach based on reference transcriptomes and dedicated bioinformatics tools such as similarity sequence networks. Identification of gene sets involved specifically in photosymbiosis will contribute to a better understanding of symbiotic relationships. Following this strategy, we believe that genomic functional traits can be characterized and help decipher the distribution of targeted biological functions in the environment but also be valuable to conduct evolutionary studies.

Tuesday

FROM FISHING TO FEASTING: THE CULTURAL IMPORTANCE OF MARINE ALGAE AND MARINE PLANTS FOR NORTHWEST COAST INDIGENOUS PEOPLES **Turner, N. J.**, University of Victoria, Canada, <u>nturner@uvic.ca</u>

Macrosopic marine algae and marine flowering plants have long had strong cultural associations, featuring as nutritious foods, medicines and a range of materials. Kelp forests in particular also serve as

key habitat for fish and countless other species of marine life. In this talk I highlight some of the key species of cultural importance on the Northwest Coast and describe their key uses, from the distant past to the present day. In particular I describe the various uses of bull kelp (*Nereocystis luetkeana*), giant kelp (*Macrocystis luetkeana*), red laver (*Pyropia abbottiae* and related species), rockweed (*Fucus* spp.), and eelgrass (*Zostera marina*). All of these are named in diverse Indigenous languages and in some cases are major items of trade. These species are an important component of biocultural diversity on the Northwest Coast.

Wednesday

THREE PERSPECTIVES ON GENE TRANSFER IN EUKARYOTES **Richards, T**., University of Exeter

Horizontal (or lateral) gene transfer (HGT) is a controversial factor in eukaryotic gene evolution. Objections to the idea that genes can be transferred across species boundaries into eukaryotic genomes focus on lack of evidence for a mechanism of transfer and/or lack of exploration of the functional consequences of gene transfer. My laboratory has sought to test these objections head on. Using a combination of genome sequencing (including single cell approaches), phylogenomics and functional cell biology, we present evidence that HGT can fundamentally change the biological capabilities of eukaryotic microbes including algae, saprotrophs, predators and pathogens. Our work also demonstrates a pattern of viral-mediated HGT into eukaryotes demonstrating a clear vector and mechanism for driving HGT. These data are consistent with the hypothesis that gene transfer augments eukaryotic gene repertories but plays a reduced role compared to prokaryote genomes.

Thursday

FLATTENING OF GLOBAL KELP FORESTS: TALES OF TURF, TEMPERATURE, AND TROPICAL HERBIVORES Wernberg, T., University of Western Australia, Australia

Kelp forests have, like so many other coastal ecosystems, undergone substantial declines over recent decades. This talk will expose global patterns of kelp forest transformation into flat seascapes dominated by turf algae and warm-water species, and discuss some of the key drivers and feedbacks that underpin these potentially persistent changes. Understanding the interactions between local to global perturbations such as increasing temperature, marine heatwaves and range-shifting herbivores is the foundation for solutions to confront these changes. We have a narrowing window of opportunity to do so. Kelp forests (Order Laminariales) form key biogenic habitat in coastal regions of temperate and polar seas worldwide, providing ecosystem services valued in the range of billions of dollars annually. A global analysis of kelp time series reveals a high degree of geographic variation in trends of kelp change through time, with regional variability in the direction and magnitude of change far exceeding a small global average decline. This analysis identified declines in 38% of ecoregions for which there are data, increases in 27% of ecoregions, and no detectable change in 35% of ecoregions, painting a complex picture of the fate of kelp forests over the past half-century. We use qualitative modelling to explore the role of climate change and local stressors (e.g. fishing) in driving these patterns of kelp forest change. Results reveal that climate change, leading to increases in temperature, storm frequency and intensity, and pCO₂, is likely to cause declines in kelp in nearly all regions examined. The relative importance of climate change vs. local stressors varied by region, however, with local stressors in some cases overwhelming the effects of climate change. We conclude that while global drivers may be affecting kelp forests at multiple scales,

local stressors and regional variation in the effects of these drivers dominate kelp forest dynamics, contrasting many other marine and terrestrial foundation species.

ISOP Past President's Address

PROTISTS OF THE MESOPELAGIC AND A BIT ON THE LONG PATH TO THE DEEP SEA **Dolan, J. R**., CNRS, France, <u>dolan@obs-vlfr.fr</u> Ciobanu, M. C., Université PIerre Marie Curie Paris VI, France Marro, S., CNRS, France Coppola, L., CNRS, France

Through weekly sampling we examined the dynamics of the mesopelagic protist fauna in the N.W. Mediterranean Sea. We focused on three distinct groups in which species identification is relatively unambiguous using light microscopy: tintinnid ciliates, phaeodarian radiolarians, and amphisolenid dinoflagellates. These groups of protists showed remarkable seasonal changes corresponding with changes in water column structure. The deep-water community of tintinnid ciliates is comprised of forms apparently restricted to deep waters (deep water natives) and species also found in the surface layer (invasives from the surface layer). This latter group of invasive species dominated during the winter mixis period when tintinnid concentrations were highest and subsequently declined with water column stratification. The community structures of the tintinnid assemblages, deep water natives and invasives, showed distinct population structures. Phaeodarian radiolarians and the amphisolenid dinoflagellates, largely absent from surface water samples, were regularly found in deep samples and showed distinct seasonal patterns. Phaeodarian radiolarians declined with water column mixing and then increased in concentration with water column stratification whilst amphisolenid dinoflagellates concentrations showed no large shifts in abundance but a distinct change in species composition. We conclude that for all three groups of protists, there are distinct mesopelagic forms and they show seasonal dynamics much like surface layer assemblages.

THE UNSUSPECTED ROLE OF LARGE PROTISTS (RHIZARIA) IN THE SILICA AND CARBON CYCLES

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There is an emerging recognition of protists as key players in biogeochemical processes. Among them, Rhizaria are diverse protists, some of which build silicified skeletons (e.g., Radiolaria and Phaeodaria). Using a combination of sediment trap coupled with simultaneous in situ imagery, we quantified the abundances, biogenic silica (bSiO₂) export and carbon flux-attenuation of two abundant families of large Phaeodaria (Aulosphaeridae and Castanellidae) in the California Current Ecosystem. These two families alone contributed on average 10% (range 0-80%) to total bSiO2 export from the euphotic. Their proportional contributions increased markedly towards regions of low bSiO₂ fluxes. Their contribution was also elevated in the upper mesopelagic (0-500 m) where they show peak abundances. With estimated turnover times of Aulosphaeridae ranging from 2.1 to 19.4 d, we estimated their minimum in situ growth rates at 0.05-0.5 d-1. We further found that the Aulosphaeridae alone could intercept >20% of sinking particle-related organic carbon produced in the euphotic, before these particles reach 300 m, thus attenuating carbon fluxes. These two examples suggest that these large protists could play a major role in oceanic ecosystems, in both the silica cycle and the biological carbon pump. However, beyond the ecological significance of specific families, rhizarians also encompass a large diversity of species that span a broad size range and inhabit multiple ecological niches, from the euphotic down to the mesopelagic. Therefore, additional studies are required to better appreciate the full extent of the contribution of Rhizaria to oceanic ecosystems and biogeochemical processes.

PUSHING THE LIMITS OF 18S RDNA VARIANTS: A TEMPORAL AND SPATIAL STUDY ON MARINE HETEROTROPHIC FLAGELLATES **Massana, R.**, Institut de Ciències del Mar, CSIC, Spain, <u>ramonm@icm.csic.es</u> Obiol, A., Institut de Ciències del Mar, CSIC, Spain, <u>obiol@icm.csic.es</u>

Studies on microbial diversity have greatly advanced by high throughput sequencing of phylogenetic gene markers retrieved from natural assemblages. Now, it is feasible to simultaneously obtain thousands of sequences from hundreds of samples, and one of the main challenges is to discern very-similar real variants from the numerous low-frequency generated errors. Our main aim here is to identify the relevant variability within marine heterotrophic flagellates, a functional group of small bacterivorous protists including many uncultured species, and survey these variants along extensive temporal and spatial datasets. We use metabarcoding data from the V4 region of the 18S rDNA targeting marine picoeukaryotes, which were sampled at the global Malaspina expedition and the Blanes Bay Microbial Observatory (monthly during 10 years). By comparing different clustering methodologies, we identify single-nucleotide variants with contrasted temporal and spatial distributions. The combination of extensive sampling efforts and powerful sequencing and analytic tools enables us, for the first time, to conduct a global study to identify the dominant heterotrophic flagellates and better define their prevalence and relevance in the oceans.

PSA Presidential Symposium: The Macroalgal Legacy of the Pacific Northwest

ONE STUDENT-OF-SEAWEED'S JOURNEY WITH FREQUENT DEPARTURES FROM RELEVANCE FROM WEST BEACH, WHIDBEY ISLAND TO BAMFIELD AND OCTOGENARIAN-SHIP: SCAGELEAN ADVENTURES AND A MARINE STATION **Druehl**, L., Simon Fraser University

An exploration of Robert F. Scagel from Assistant Manager, Sylvia Hotel, Vancouver, through his seaweed contributions at Botany, UBC, to his FRSC and now, his exploration of abstract visual art. Coincidental with this journey, is the establishment of the Bamfield Marine Station. Aspects of this talk are embellished with reminiscences of Louis Druehl.

THE REMARKABLE JOSEPHINE TILDEN AND THE MINNESOTA SEASIDE STATION **Hansen, G. I.,** Oregon State University, United States, <u>hansengi@outlook.com</u>

In 1895 when natural history surveys were especially important to science, Josephine Tilden was hired as a faculty member by the University of Minnesota. At 26 and the only phycologist in the Department of Botany, she was given the task of maintaining the library and of collecting and identifying freshwater and marine algae, particularly from the Pacific, for later distribution in exsiccatae. From 1896 to 1898, she made several trips with her mother to the west coast to collect algae in Puget Sound and the San Juan Islands. Working with local fishermen, she gathered a collection of 50 sets of >150 macroalgal species from the area and distributed it in 1898 as a part of her Centuries of American Algae, an exsiccatae that eventually reached 37,000 specimens. While in Victoria, she heard rumors of a richly diverse and beautiful "pothole beach" that occurred on the outer coast of Vancouver Island near Port Renfrew. Traveling to the area by steamboat to obtain additional collections, she was so noticeably impressed by the area that a local settler gave her a 4-acre plot of land that she named "Botanical Beach". In

Minnesota, her description of the area was so enticing that the head of the Department joined her in establishing a summer seaside station there where up to 80 students could study and do research. The Minnesota Seaside Station was immensely popular and lasted from 1901 to 1906 (1907). The activities and contributions of the station will be examined as will its demise. After this, Tilden's interest turned to the South Pacific, and her adventures and algal exsiccatae produced from this part of the world filled up much of the rest of her professional life.

RAMBLINGS OF AN ITINERANT PHYCOLOGIST IN A POST-GLACIAL LANDSCAPE Lindstrom, S. C., University of British Columbia, Canada, <u>Sandra.Lindstrom@botany.ubc.ca</u>

The Pacific coast of North America is a topologically rich environment with a fascinating geological history and significant oceanographic and meteorological clines. Geographically, it represents a bridge between North America and Asia as well as a link between the North Pacific and the North Atlantic through the Arctic Ocean. Collections of seaweeds began in the eighteenth century, mostly by Russian naturalists, followed by Americans in the late nineteenth Century, and Canadians in the mid twentieth Century. When I returned to Alaska after obtaining a M.Sc. degree at UBC, my working hypothesis was that Alaska's seaweed flora represented a depauperate extension of the BC flora, which in turn was a diminished version of the California flora. This idea was based on these northern regions having been covered by glaciers during the Pleistocene. The idea was quickly shattered when I looked at seaweeds around my hometown of Juneau, an area under at least 1 kilometer of ice during the last glaciation. I eventually described two new genera and nine new species of seaweeds from this formerly glaciated area. During my work on environmental assessments in Alaska, I have sampled seaweeds from much of the State. Use of molecular tools has allowed development of additional biogeographic hypotheses, most notably the extension of western Pacific species into Alaskan waters, Alaska as a source for Arctic and north Atlantic species, and recognition of additional endemics both within and outside the glacial boundary.

HEROES IN THE SEAWEED AND OTHER EVENTS IN THE PHYCOLOGICAL EXPLORATION OF VANCOUVER'S ISLAND AND BEYOND **Hawkes, M. W.**, University of British Columbia, Canada, mhawkes@mail.ubc.ca

This overview of marine phycological exploration in the Pacific NW covers a 200 year period starting with voyages in the late 1700's. Collections by the ship's surgeon-naturalist often included seaweeds. Archibald Menzies, on both the Colnett (1786–1789) and Vancouver (1791–1795) Expeditions, was the first 'Hero in the Seaweed' to make noteworthy collections in what became British Columbia. Lt. C. Wilkes commanded the US Exploring Expedition (1838–1842), which collected seaweeds in Puget Sound; 4 were new. Following establishment of the Colony of Vancouver's I. (1849), a coastal survey was conducted (1857-1862) by Capt. G. Richards, Royal Navy. David Lyall and C. Wood, both surgeonnaturalists, participated; their seaweeds being described by W. Harvey (Ireland), J. Gray (England), and J. Agardh (Sweden). Frank Collins (USA) included collections by J. Macoun, Dominion Botanist, in his 1913 paper on marine algae of Vancouver I. Establishment of marine labs at Botanical Beach (1901), Friday Harbor (1904), and Bamfield (1972) made the Pacific NW a destination for seaweed research. Nathaniel Gardner began his phycological career on Whidbey I., WA and in collaboration with W. Setchell described many Pacific NW seaweeds. Harald Kylin (Sweden) visited Friday Harbor twice in the 1920's, describing 3 new genera and 31 new species. In the 1960's and 70's, R. F. Scagel at UBC sent out several seaweed survey expeditions; the CSS Parizeau (1976) being the last. Collections from this trip were the basis for a paper reporting all seaweeds known from northern British Columbia to that date.

Host-Associated Microbial Communities and their Evolution

A TUNICATE, FOUR HUNDRED APICOMPLEXANS AND THREE BACTERIA: AN INTERDEPENDENT MUTUALISTIC COMMUNITY Lane, C.E. University of Connecticut, <u>clane@uri.edu</u>

Apicomplexa is an enormous phylum of parasitic species that infect every animal that has been investigated for infection. Parasitism is often considered an irreversible evolutionary dead end, but members of the genus Nephromyces are apicomplexans with an apparent mutualistic relationship with their hosts, molgulid tunicates. Additionally, three types of bacteria have become endosymbionts within various species of Nephromyces. Multi-species infections are common among species of apicomplexans; however, unlike other apicomplexans where co-infection is coincidental, co-infection appears to be mandatory for Nephromyces. No molgulid host has been found with only a single Nephromyces species, and transcriptomic data indicates that a mix of bacterial symbionts in the system is also critical for community functioning. A combination of cloning, multi-gene amplicon sequencing, genomic and transcriptomic sequencing, laboratory culturing, and single cell isolation have uncovered a highly complex, interdependent, endosymbiont community comprised of many species within a single genus of apicomplexan. This community has evolved in relative isolation from competition because of the unusual tunicate organ they live in, and has developed into a complex mosaic of inter-dependent closely related species. The community structure, diversity, relationships, and genomic consequences of community dependence will be discussed.

METABOLIC FUNCTIONING OF A CILIATE-METHANOGEN SYMBIOSIS FROM ANOXIC HABITATS

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Symbioses between anaerobic protists and methanogenic archaea are common in oxygen depleted habitats ranging from aquatic sediments to gastrointestinal tracts, yet little is known about the mechanisms and dynamics of metabolic interaction between the partners. In these putatively syntrophic associations, it has been hypothesized that host fermentation is facilitated by symbiont consumption of fermentative end-products (e.g., H2) during symbiont methanogenesis. However, at present there is very little information about the metabolic pathways employed by both host and symbionts. Here, we report the gene content of a 93% complete genome from the Methanobacterium symbiont of a common anaerobic ciliate from the genus *Heterometopus*. In addition, we also investigated coupled host-symbiont metabolism during different stages of host growth, as well as when exposed to micro-oxic conditions, with experimental measurement of symbiont CH4 production and assessment of host and symbiont gene expression via transcriptomic sequencing. Given that, in some habitats, protist-associated methanogens can account for a significant portion of CH4 production, data regarding host and symbiont metabolic processes is not only foundational to our knowledge of the physiology and ecology of protist-methanogen symbioses, but may also critical to our understanding of biogeochemical processes in the ecosystems they inhabit.

EVOLUTION OF TERMITE HINDGUT PROTISTS: NEW PERSPECTIVES FROM 18S AMPLICON SEQUENCING

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Lower termites harbor symbiotic wood-eating Parabasalia and Oxymonadida in the distended paunch of their hindguts. These obligate symbionts are transmitted from parents to offspring via proctodeal traphallaxis, or anal feeding, which has led to a certain degree of coevolution between the protists and

their hosts. Proctodeal trophallaxis also serves to replenish the hindgut fauna of worker termites after each molt, and is therefore expected to maintain a consistent hindgut community or "social stomach" throughout each termite colony and across each termite species. However, recent work in our lab has hinted at higher levels of both inter- and intra-colony variation than this model suggests. We have therefore investigated the hindgut protist community of *Heterotermes* and *Coptotermes* species (Rhinotermitidae) using both single cell isolation for description of live protists and high-throughput 18S rRNA amplicon sequencing of total hindgut DNA. In the case of *Coptotermes formosanus* and *Coptotermes gestroi*, hybrid termites exhibited variable hindgut communities due to stochastic maintenance or loss of parental protist symbiont species. In the case of *Heterotermes tenuis*, specimens collected across its geographical range revealed significant inter-colony and inter-individual variability in the number of protist species present. In *H. tenuis*, the protist community is best described as a pansymbiome: the total microbiota of the species is not seen in any one individual or colony.

PHYLOGENY AND EVOLUTION *PIRIDIUM SOCIABILE* A NEW PARASITIC LINEAGE CLOSELY RELATED TO APICOMPLEXA

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Apicomplexa are obligatory intracellular parasites of animals. They possess several adaptations for their intracellular lifestyle (for example the apical complex) and a highly reduced non-photosynthetic plastid called the apicoplast. The apicoplast lost many plastid functions, but still contains a small plastid genome and remains an essential organelle as it harbors several important metabolic pathways (i.e. heme and isoprenoid synthesis). Several new free-living lineages, collectively called chrompodellids, were recently discovered and shown to be closely related to Apicomplexa. The best studied of these new lineages are two photosynthetic algae called *Chromera velia* and *Vitrella brassicaformis*, whose genomes have been fully sequenced. *Piridium sociabile* is a parasite of a marine gastropod *Buccinum undatum*) described as a member of apicomplexa in 1936. Here, we present transcriptomic and genomic data from *Piridium*. Phylogenomic analyses show, with highest support, that it is not a member of Apicomplexa, but, in fact, a sister lineage to the photosynthetic *V. brassicarformis*. It therefore most likely represents a lineage that is closely related to Apicomplexa but that acquired a parasitic lifestyle independently. Analyses of the plastid genome and plastid targeted proteins have shown that its non-photosynthetic plastid is very similar to the apicoplast of Apicomplexa. Where possible, we have also explored the transcriptomic data to compare genomic adaptations to parasitic lifestyle in *Piridium* and Apicomplexa.

Kelp Forests in Flux

DECADAL CHANGES IN LONG-LIVED KELP FORESTS REFLECT ENVIRONMENTAL AND HISTORICAL FACTORS

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Fluxes in kelp forests are a product of environmental and biological drivers. However, the ecological processes shaping a community are difficult to disentangle as they often occur on long time scales. We examined decadal-scale changes in forests of the long-lived kelp, Pterygophora californica, at 11 sites off the west coast of Vancouver Is., Canada. We used shallow sublittoral *Pterygophora* forests (where waves exclude grazing sea urchins) to control for site-specific factors and compared the age structure, density, biomass, and growth of the shallow stands to adjacent deeper Pterygophora forests that recruited after sea urchins were removed by sea otters. The deep *Ptervgophora* stands exhibited narrow age ranges (pulsed recruitment) compared to shallow forests at the same sites. As the modal age of deep stands passed 10 yrs, stipe density declined. By a modal age of 17 yrs most deep *Pterygophora* forests had senesced but slowly recovered via recruitment. Over 25 years, the net mean biomass of stipitate kelps (Ptervgophora and Laminaria spp.) in the shallow forests declined significantly by 50%, whereas the change in net mean kelp biomass in the deeper stands was not significant perhaps reflecting the patchwork of successional states at the 11 sites. Although growth rates, based on age-at-size, changed slightly on a decadal scale, growth rates in the shallow and deep *Pterygophora* stands did not differ, suggesting factors that affect growth were not the cause of the biomass change in the shallow stands. These results emphasize the importance of considering ecological history and environmental factors when evaluating long-term changes in kelp communities.

CHANGES IN BENTHIC DIVERSITY AND ECOSYSTEM PRODUCTION FOLLOWING WIDESPREAD KELP LOSS IN THE ALEUTIAN ARCHIPELAGO **Edwards, M. S.**, San Diego State University, United States, <u>medwards@mail.sdsu.edu</u> Konar, B. H., University of Alaska Fairbanks, United States, <u>bkonar@alaska.edu</u> Kim, J. H., Kunsan National University, South Korea, <u>juhyoung@kunsan.ac.kr</u> Gabara, S., San Diego State University, United States, <u>scottgabara@gmail.com</u> Sullaway, G., San Diego State University, United States, <u>genoahs@gmail.com</u> McHugh, T., San Diego State University, United States, <u>tristin.mchugh@gmail.com</u> Spector, M., San Diego State University, United States, <u>mspector.ecology@gmail.com</u> Small, S., San Diego State University, United States, <u>subtidal.sadie@gmail.com</u>

Changes in the distribution and/or abundance of species that are ecosystem engineers can have dramatic impacts on primary production, biodiversity and nutrient cycling across a range of habitats. Nowhere may this be as dramatic as in the coastal zones of the Aleutian Archipelago where large declines in forestforming kelps have occurred in recent decades due to a dramatic increase in sea urchin abundances. We studied patterns of biodiversity and Net Ecosystem Production (NEP) in small isolated kelp forests, urchin barren grounds, and in areas that were in the transition between the two states at 10 islands during 2016 and 2017. Specifically, we deployed replicate benthic respiration chambers in each habitat to measure oxygen production/consumption over 24-hour periods, and conducted shipboard respiration measurements of the dominant organisms in each habitat to estimate their contribution to NEP. Our results show that that the loss of kelp forests resulted in decreased biodiversity and an overall reduction in NEP across the Archipelago. The transition areas exhibited intermediate levels of both metrics. Nighttime respiration rates were greater in the kelp forests due to higher abundances of organisms indicating that the effects of kelp loss on NEP varied over the diurnal cycle. Further, these patterns varied among islands and regions of the Archipelago, presumably due to differences in light (cloud cover) and ocean conditions (salinity and temperature), such as observed on the opposing sides of biogeographic breaks.

KELP FORESTS IN THE NORTHEAST PACIFIC: THEIR DYNAMICS AND FUNCTION IN A CHANGING CLIMATE

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Kelp forests are known to be key habitats for species diversity and coastal productivity. What is less known is how these biogenic habitats interact with seawater chemistry and phytoplankton productivity, thus affecting the potential productivity of coastal habitats. With concerns about their dynamic trends globally, as well as their broad water column effects, we examined kelp forest function in Washington state. We found that kelp forests in Washington greatly affected the carbonate system locally, increasing pH and aragonite and calcite saturation states for calcifying species, a pattern that was persistent over several sites. While canopy kelp decreased light and nitrate levels, water column productivity declined relatively little within a kelp bed, suggesting that nutrient regeneration and recycling in proximity to kelp may provide additional opportunities for primary production and carbon fixation. We further document century- and decadal-scale persistence of outer coast and western kelp forests in Washington state, while those in proximity to population centers have declined. Thus, the known function of foundational species as biodiversity hotspots and their important functional role in carbon and nitrogen chemistry makes their persistence a key priority for ocean conservation efforts.

GLOBAL PATTERNS AND DRIVERS OF KELP FOREST CHANGE OVER THE PAST HALF CENTURY

Krumhansl, K., Fundy Ocean research Center for Energy

Kelp forests (Order Laminariales) form key biogenic habitat in coastal regions of temperate and polar seas worldwide, providing ecosystem services valued in the range of billions of dollars annually. A global analysis of kelp time series reveals a high degree of geographic variation in trends of kelp change through time, with regional variability in the direction and magnitude of change far exceeding a small global average decline. This analysis identified declines in 38% of ecoregions for which there are data, increases in 27% of ecoregions, and no detectable change in 35% of ecoregions, painting a complex picture of the fate of kelp forests over the past half-century. We use qualitative modelling to explore the role of climate change and local stressors (e.g. fishing) in driving these patterns of kelp forest change. Results reveal that climate change, leading to increases in temperature, storm frequency and intensity, and pCO2, is likely to cause declines in kelp in nearly all regions examined. The relative importance of climate change vs. local stressors varied by region, however, with local stressors in some cases overwhelming the effects of climate change. We conclude that while global drivers may be affecting kelp forests at multiple scales, local stressors and regional variation in the effects of these drivers dominate kelp forest dynamics, contrasting many other marine and terrestrial foundation species.

From Pharm to Table: Frontiers in Applied Phycology (Prof. Milton Sommerfeld Memorial Symposium)

NRC'S ALGAL CARBON CONVERSION PROGRAM- CONVERTING CO₂ AND OTHER INDUSTRIAL WASTE STREAMS INTO VALUABLE BIOPRODUCTS **O'Leary, S.**, National Research Council Canada <u>stephen.oleary@nrc-cnrc.gc.ca</u>

The Algal Carbon Conversion (ACC) Flagship Program focused NRC's algal research platforms on the technical and economic barriers to using microalgae cultivation to intercept industrial carbon dioxide emissions in a Canadian context. A biorefinery approach was explored to derive the maximum economic benefit from the biomass produced while utilizing local waste streams as inputs. The primary testbed for the program was a 25,000 litre photobioreactor operating within a pilot plant jointly deployed by NRC and industry partners Pond Technologies and St Marys Cement Canada. At the pilot plant, algae is

cultivated on raw emissions from a cement kiln. In addition to activities at the pilot plant, the ACC program has hosted more than a dozen large, multi-year, internal research projects and collaborations to address bottlenecks at key points in the pathway to commercially scaled microalgae production. These projects are grouped into four main areas: (1) algae strain selection and characterization; (2) photobioreactor technologies; (3) microalgae harvesting, dewatering and extraction technologies; and (4) biomass utilization. The range of projects include three different approaches to harvesting and dewatering dilute microalgae cultures, advanced biomass characterization, genomic analysis of strains and the development of algae-derived products including biofuels and other bioproducts such as pigments, animal feed ingredients, and bioplastics. This presentation will provide an overview of the activities and achievements of the Algal Carbon Conversion Flagship Program and discuss the current capabilities and future opportunities for NRC's algal research teams and facilities.

TURNING THE TIDE ON PROTEIN MISFOLDING DISEASES: CAN SEAWEEDS HELP? **Ewart, K. V.**, Dalhousie University, Canada, <u>vewart@dal.ca</u>

A number of proteins share an inordinate tendency to misfold and some have roles in human diseases. For example, Parkinson's disease involves the misfolding of a protein, alpha-synuclein, into an amyloid form. Although drugs are available to address the symptoms of protein misfolding diseases, none of them target the underlying misfolding events. However, preventing the formation and proliferation of amyloid aggregates could offer effective means of inhibiting the progression of these diseases. An approach to this problem is to identify naturally occurring substances that impede the misfolding of proteins. Protein misfolding is expected to be a particular challenge in organisms inhabiting coastal marine temperate waters, where salinity and temperature can vary greatly. Therefore, intertidal and subtidal seaweeds may have effects on human proteins. Extract fractions from a subtidal brown algal species were shown to impede the conversion of alpha-synuclein to an amyloid form. In addition, an extract from an intertidal brown alga was found to favour the native helical fold of alpha-synuclein, consistent with the prevention of amyloid formation. Identification of the algal extract components responsible for these activities may lead to new means of addressing protein misfolding diseases. Furthermore, investigation of these solutes may offer insight into the adaptations of coastal seaweed species to their environment.

SEAWEEDS AS PLANT BIOSTIMULANTS: CHEMICAL COMPONENTS AND MECHANISMS OF ACTION

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Plant biostimulant is a new class of agricultural input that improve crop vigor, yield, quality and tolerance to abiotic stress. Seaweed extracts are a major class of plant biostimulants. Among the seaweed extracts, *Ascophyllum nodosum* extract is the most used in Agricultural and horticultural crops. *A. nodosum* extract contains a complex mixture of bioactive compounds that includes simple sugars, polysaccharides, amino acids, fatty acids, polyphenols and trace elements. The chemical composition of extract largely depends on the geographical origin of the seaweed and the method of extraction. Seaweed extracts elicit a wide range of beneficial effects like early seed germination and seedling establishment, early flowering and fruit set, resistance against abiotic stress factors (low temperature, salinity, drought), biotic stresses (insects and disease), and longer shelf life and improved nutritional quality of the produce. Using the plant model; *Arabidopsis thaliana*, single gene mutants, and 'omic' techniques we investigated the molecular mechanism(s) of action of the *A. nodosum* extracts. This presentation will give an overview of the recent understanding of mechanism of biostimulant activity of *A. nodosum* extracts with special reference plant growth regulators and non-coding RNA.

OPPORTUNITIES, CHALLENGES AND FUTURE DIRECTIONS OF OPEN WATER SEAWEED AQUACULTURE IN NORTHEAST AMERICA FOR ECOSYSTEM SERVICES, FOOD, FEEDS AND BIOFUELS

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After more than 30 years of effort by scientists, industry, state and federal agencies, seaweed aquaculture is now considered an environmentally responsible practice and offers new opportunities for expansion in US coastal waters. With the nursery technologies developed at the University of Connecticut, Saccharina latissima and S. angustissima and Gracilaria tikvahiae have been successfully cultivated in open water farms in the Northeast. After out-planting juvenile kelp (< 1mm), we have found that our cultivated kelp grew as much as 7.0 m in length and yielded up to 24 kg FW m⁻¹ after 6 months in Long Island Sound and the Gulf of Maine. *Gracilaria* also grew rapidly, up to 16.5% d⁻¹ in the New York City's Bronx River Estuary during summer. Seaweed aquaculture provides ecosystem services by removing excess nutrients (carbon and nitrogen) from ecosystems and thereby improves water quality, potentially reduces ocean acidification. Seaweeds have significant value as foods for human consumption and agriculture as organic fertilizers, animal feeds, nutraceuticals, and cosmeceuticals. Seaweed aquaculture now offers other new opportunities with the planned expansion into the Exclusive Economic Zone with the ARPA-E (US DOE) MARINER Program. With improvements in productivity, kelp and other seaweeds could potentially be a viable feedstock for biofuels. There are now unique opportunities for phycologists to work with ocean engineers, plant breeders and others to develop and apply advanced breeding technologies that will increase growth and improve thermal tolerance for open water farm systems.

Origins and early evolution of eukaryotes

LECA CONCEPTS AND THEIR IMPLICATIONS FOR EVOLUTIONARY UNDERSTANDING **O'Malley**, **M**., University of Bordeaux/Sydney

By definition, LECA gave rise to all extant eukaryotes. LECA means more, however, than a node in an ancestral reconstruction. In addition to capturing an abstracted phylogenetic space, LECA refers to biological properties, capacities and activities. Based on work with Michelle Leger, Jeremy Wideman, and Iñaki Ruiz-Trillo, I analyze a continuum of different LECA conceptions and their implications for the evolution of early eukaryotes. This continuum runs from notions of LECA as a single cell, to LECA as an evolving population or species, and beyond any single population to LECA as a community. The talk focuses on a pangenomic notion of LECA, in which a diverse population exhibits genomic and phenotypic differences, despite being the same 'species'. I explore what this concept might mean for how LECA is understood evolutionarily and phylogenetically.

RECONSTRUCTING THE CELL BIOLOGY OF LECA

Wickstead, B., School of Life Sciences, University of Nottingham, Nottingham, United Kingdom All extant eukaryotes descended from a single ancestor (the last eukaryotic common ancestor, LECA) which existed ~1-2 billion years ago. Since then, they have diversified into the great variety of forms and lifestyles seen in modern lineages. To understand the biology of LECA and how this changed during the evolution of the major lineages, it is necessary to infer the gene content of LECA and to map content changes to the likely phylogeny. However, eukaryotic genome evolution has involved substantial innovation at the level of multi-gene families and defining orthology/paralogy (in addition to homology) remains a considerable challenge. We have developed a hybrid approach based on simple similarity searches and HMM-HMM comparisons to cluster the ~ 1 million proteins predicted to be encoded by 64 organisms spread across the eukarvotic tree. These clusters contain sequences with alignable sequence homology and were used to generate phylogenies that were then manually curated to reduce artefact, refine clusters and define non-lineage specific paralogs. There are ~6000 clusters, each approximating to a core eukaryotic protein type – around half of which are eukaryote-specific innovations. These clusters encompass ~ 12000 well-supported ancient paralogs. The majority of both protein types and paralogs were likely already present in LECA under most root hypotheses, but the specific LECA content differs substantially according to placement. With the exception of those associated with plastid acquisition, there is evidence for only low levels of protein gain during diversification.

EUKARYOGENESIS: PATTERNS AND PROCESSES

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The origin of the eukaryotic cell has remained enigmatic and controversial for a long time. Until recently, various kinds of models competed to explain the chimeric patterns observed in eukaryotes: informational systems (e.g. replication, translation, transcription) are archaeal-like, whereas operational systems (e.g. energy and carbon metabolism) but also the nature of membrane phospholipids are bacterial-like. The model that prevailed since the late 1970s invoked the existence of a proto-eukaryotic lineage in which most eukaryotic features evolved (cytoskeleton, endomembrane system, phagocytosis) in a hypothetical lineage sister to archaea prior to the acquisition of the alphaproteobacterial ancestor of mitochondria by endosymbiosis. Models proposing a symbiotic origin of eukaryotes directly from bacterial and archaeal ancestors were marginal. The situation has radically changed in recent times largely thanks to the exploration of microbial diversity in natural ecosystems. The discovery of the uncultured Asgard archaea, which share more and more similar genes with eukarvotes than other archaea, has lent new credit to symbiogenetic models for the origin of the eukaryotic cell. Nonetheless, the controversy is still there and different scenarios propose different evolutionary processes underlying the evolution of typical eukaryotic features. I will argue that existing models of eukaryogenesis are not satisfying and that establishing a plausible scenario of eukaryogenesis needs to fully explain the details of the evolutionary process. answering to crucial open questions, that resulted in the observed patterns in eukaryotic cells.

CONSERVATION AND REWIRING OF THE EUKARYOTIC CELL CYCLE FROM LECA TO BASAL FUNGI

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Cell division is an essential process that has been occurring in an uninterrupted chain for billions of years and, thus, one expects strong conservation. Here, we examine diverse genomes to show that the last eukaryotic common ancestor (LECA) already had a complex cell cycle, similar to that found in animals, and whose regulators have been maintained in nearly all eukaryotes. Surprisingly, the LECA cell cycle network in Fungi was rewired by an uncharacterized domain found in many DNA viruses, e.g. Megaviruses. We present evidence that this viral protein (known as SBF in yeasts) may have hijacked cell cycle control by activating genes targeted by the ancestral cell cycle regulator E2F and was subsequently entrained into the cell cycle regulatory network. Cell cycle evolution in the fungal ancestor proceeded through a hybrid network containing both E2F and SBF, which is still maintained in many basal Fungi. Such hybrid networks may represent a common mechanism through which core regulatory networks can dramatically evolve.

Protist responses to environmental stress: resilience to climate change?

RESILIENCE AND ADAPTATION TO EXTREME ENVIRONMENTS: THE SPECIAL CASE OF *EUPLOTES FOCARDII*, A STRICTLY PSYCHROPHILIC ANTARCTIC CILIATE **Pucciarelli**, S., University of Camerino, Italy, <u>sandra.pucciarelli@unicam.it</u> Ballarini, P., University of Camerino, Italy Priyan, K., University of Camerino, Italy Mozzicafreddo, M., University of Camerino, Italy Miceli, C., University of Camerino, Italy

Antarctica and the Sothern Ocean are the coldest and harshest places on the Planet. Antarctic marine organisms face a number of environmental challenges, in particular the adverse effects on key biological processes of cold and oxidative stress, the latter due to increased oxygen solubility in cold waters. Over the past 50 years, the west coast of the Antarctic Peninsula has been one of the most rapidly warming parts on the Earth. Antarctic species are dramatically impacted by thermal changes. As single cells directly exposed to environmental cues, marine Antarctic protozoa represent excellent models to unravel the full suite of cellular functions that allowed the ecological success of psychrofiles and the biological responses to fast environmental changes. To optimally address resilience and adaptation, it is necessary to access to large sample sizes of sequences in order to enable evaluation via statistical and computational approaches. Only genome or transcriptome sequencing can provide such data sets. In this context, we are studying the Antarctic hypotrichous marine ciliate Euplotes focardii by an "omic" approach, focussing principally on the genome and transcriptome, the latter obtained under both physiological and stress conditions. The analysis of these large data set allowed us to approch the study from three different points of view: the analysis of gene expression under stress, the biochemical characterization of "cold-adapted" enzymes and the study of a bacterial consortium associated to this ciliate. Our results strongly indicate that ciliates represent a good model to unravel the evolutionary mechanisms that determine environmental adaptation. The biology of *E. focardii* highlights the impact of environmental constrain on evolution and adaptation and indicates the strategy used by this microorganism to survive in fast evolving environmental conditions.

INSIGHTS TO TESTATE AMOEBAE RESPONSE TO ENVIRONMENTAL STRESS **Kosakyan, A.**, Institute of Parasitology, Czech Academy of Sciences, Czech Republic, <u>kosakyan@paru.cas.cz</u> Lara, E., Real Jardín Botánico de Madrid, CSIC, Spain, <u>enrique.lara@rjb.csic.es</u>

Testate amoebae are a polyphyletic group of amoebae characterized by a shell which can have different sizes and shapes that are used for species description. Species are mostly represented in Amoebozoa, Rhizaria (several clades) and also in Stramenopiles. The test being costly to produce, these organisms have in common slow growth rates and are characterized most often by narrow ecological tolerance. This designates them as excellent bioindicators for environmental perturbations, and they have been used in pollutant monitoring and even in forensic sciences. They have been also widely used in monitoring environmental quality of freshwater environments. However, their taxonomy is still sketchy, and recent works in molecular phylogenetics have shown that traditional systematics had to be revised. Having fulfilled this prerequisite at least partially will enable developing protocols to use entire communities of

testate amoebae to monitor environmental quality, based entirely on environmental DNA sequencing. Increasing drought events due to climate change may affect salinity levels in water reservoirs. We present here, as a possible application, a project aiming at monitoring water quality in Madrid's catchment lakes and the entire hydrographic basin that provides drinking water to the whole city and outskirts based on testate amoebae data and environmental DNA, in a fast and efficient way.

PROTISTS RESPONSES TO CLIMATIC CHANGES FOCUSING ON WATERBORNE PATHOGENIC PROTOZOA

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Global climate change is causing the average temperature increase and consequent intensifying of the hydrological cycles. Heavy precipitation events promote the sewage effluent provision in rivers, lakes and sea whilst drought leads to waterborne pathogen concentration. Both the extreme events affect the drinking water supply, indirectly, causing severe consequences to public health. Performing a scientific literature review on the basis of Medline database and appropriated key words, has been possible to identify waterborne outbreaks worldwide potentially linked to different types of extreme weather events. Unfortunately, it is still existing a gap in knowledge on relationship between extreme climatic events and diseases whereby research has to be encouraged to suggest actions for health prevention in this context. In our experience, one year monitoring of lake waters normally used for recreationally purposes, gave evidence of cysts/oocysts of pathogen protozoa belonging to different species, in presence of high rainfall. The analysis on the same sampling sites after two years of strong increase in drought and high decrease of water level has given evidence of a different environmental contamination pattern and a changed risk exposition for human health. This study provides useful suggestion for a health prevention approach in presence of extreme climatic events.

PROTISTS RESPONSES TO CLIMATIC CHANGES FOCUSING ON WATERBORNE PATHOGENIC PROTOZOA

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The consequences of global climate change are not just the creeping thawing glaciers, the steadily rising sea levels or extreme weather but also shifts of distribution areas of plants and animals. There is no doubt that such changes can have a major impact on parasites and on the epidemiology of the diseases they cause. Due to global warming and population growth, the emergence of new epidemics favored. The complex relationships between climate change, transmission of parasites and the occurrence of parasitic infectious diseases require the interdisciplinary cooperation of various specialists to find solutions to the most pressing problems. Pathogenic protists are particularly resistant to external environmental conditions due to their strong adaptation and survival outside of the host organism. Pathogenic protists are therefore suitable, as a model for changes in epidemiology of infectious diseases, the interrelationships between disease incidence and by potential impact of global climate change. The identification of research needs and the development of innovative research priorities are in focus. Furthermore, it will be discussed how the research results can be made accessible to a broad public and political decision-makers. Ideally, international collaboration against waterborne pathogenic protozoa in the form of standardized reporting system, especially in the face of adverse climatic events driven by climate change, which are currently on the rise globally and pose a threat of massive waterborne infectious disease outbreaks.

Contributed Talks

Monday, July 30

Bold Talks

EXPLORING ALGAL-FUNGAL SYMBIOSIS FROM *APOPHLAEA LYALLII*GENOME **Cho, C.,** Department of Biological Sciences, Sungkyunkwan University, South Korea, <u>endgus56@gmail.com</u> Nelson, W. A., National Institute of Water & Atmospheric Research; University of Auckland, New Zealand, <u>Wendy.Nelson@niwa.co.nz</u> Yoon, H., Department of Biological Sciences, Sungkyunkwan University, South Korea, hsyoon2011@skku.edu

In nature, symbiotic associations between red algae and fungus (i.e. mycosymbiosis) are common and constitute a strong evolutionary force, notably through coevolution. Particularly mycosymbioses are a great strategy to adapt to new environment, each member's gene repository complementing each other. The red alga genus *Apophlaea* forms an obligate mycosymbiosis with marine fungal species of the order Capnodiales. The genus is endemic to the intertidal zones of New Zealand and the two species are well known for their high resistance to ultraviolet radiation and desiccation. However, establishment of this symbiotic relationship and physiological interactions between the alga and the fungus still remain poorly understood. To investigate the symbiotic relationship and its impact on environmental adaptations, we sequenced the whole genome and transcriptome of *Apophlaea lyallii* using Pacbio RSII and Illumina HiSeq. From our genome sequencing data, we discovered new symbiotic fungal species and completed their mitogenomes. The 27.7 Mbp haploid genome of *Apophlaea lyallii* was assembled with ~800x coverage and 92% of eukaryotic BUSCO were discovered from annotated genes. Based on a comparative analysis with other red algal species, we identified *Apophlaea*-specific orthologous gene families and identified unique horizontal gene transfer of fungal origin. The sequencing of *Apophlaea lyallii* genome allowed us for the first time to address an algal-fungal symbiosis from a genomic point of view.

HOW A RED ALGA *HETEROSIPHONIA JAPONICA* ACQUIRES A SYSTEMIC RESISTANCE AGAINST OOMYCETE PATHOGEN

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Infection of pathogens causes cellular and physiological changes in host plants. Hosts are able to resist pathogens more effectively through these changes called as systemic acquired resistance, which enables long-term coexistence of host and pathogens. Filamentous red alga *Heterosiphonia japonica* underwent significant morphological and physiological changes when infected with the oomycete pathogen *Olpidiopsis heterosiphoniae*. Host filament can survive without fatal damage, and often coexist with pathogen in culture for more than several months. The infected filaments showed reduced growth compared to control even when several cells were actually infected with the oomycete. Infected filament showed much higher tolerance to osmotic stress compared to control. Transcriptome analysis showed significant changes in the regulation of the genes involved in cell wall biosynthesis from early infection stages. Salicylic acid treatment to the host induced a similar physiological change, and enhanced resistance to the pathogen. These results suggest that salicylic acid signals for the systemic acquired resistance in *H. japonica*.

A COMPREHENSIVE KELP PHYLOGENY SHEDS LIGHT ON THE EVOLUTION OF AN ECOSYSTEM Starko, S., University of British Columbia, Canada, <u>samuel.starko@gmail.com</u>

Ecosystems dominated by kelps (Laminariales, Phaeophyceae) are some of the most productive on the planet. However, the lack of a robust genus-level kelp phylogeny, with corresponding divergence time estimates, has substantially limited our understanding of their evolution. Here, we reconstruct the evolutionary diversification of habitat forming kelps by reconstructing a global phylogeny of genera using high-throughput sequence data. We then generate divergence time estimates using fossil and biogeographical calibrations to investigate the timing of this diversification. First, we resolve several important features of the kelp phylogeny, including relationships between early diverging lineages (putative ancestors) and the larger radiation of complex (putatively derived) kelps. Next, we demonstrate that the initial radiation of complex kelps resulted from an increase in speciation rate around the time of the Eocene-Oligocene boundary, supporting the hypothesis that kelps radiated during recent cool climates. Reconstruction of historical biogeography confirms the North Pacific origin of the kelps and demonstrates that repeated dispersal across the Pacific is a general feature of Laminarialean natural history. Our findings lend insight into the evolutionary history of a key habitat forming lineage with enormous ecological and economic importance around the world.

A MOLECULAR ASSESSMENT OF ULVA DIVERSITY IN CENTRAL SAN FRANCISCO BAY, CA, U.S.A.

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Several marine algae lack morphological characteristics that can reliably be used to differentiate species. The green tide forming genus, *Ulva*, exemplifies this challenge -its species taxonomy has been poorly resolved. While *Ulva* spp. are often combined into a single functional group with other ephemeral foliose algae, studies incorporating molecular species concepts have routinely revealed cryptic species with potential species-specific differences in physiology, recruitment mechanisms, and interactions with higher trophic levels. Given the increased frequency of ulvoid blooms globally, and the accompanying potential for ecological harm, this study set out to survey and identify the *Ulva* spp. found in central San Francisco Bay using the molecular barcode gene, *tufA*. This is the first study to use a molecular species concept in ulvoid identification within San Francisco Bay. Sixty-two specimens were collected from six central bay localities between 2013–15 and the *tufA* gene sequenced. These sequences were compared to 116 sequences available for the Ulvaceae in GenBank. Six OTUs were identified, two of which had not previously been reported in bay. This more complete diversity picture can guide future studies of *Ulva*, ulvoid blooms, and ulvoid introductions in San Francisco Bay and around the world.

MULTI-MARKER METABARCODING ASSESSMENT OF BIODIVERSITY WITHIN STREAM BIOFILM COMMUNITIES ALONG AN ACID MINE DRAINAGE RECOVERY GRADIENT **Wolf, D. I.,** Ohio University, Athens, Ohio, United States, <u>dw845316@ohio.edu</u> Vis, M. L., Ohio University, Athens, Ohio, United States, <u>vis-chia@ohio.edu</u>

In southeastern Ohio, active remediation of streams affected by Acid Mine Drainage (AMD) has proven to be successful for some streams, while others have not recovered based on macroinvertebrate assessment. In this study, biofilms were collected from three Not Recovered, three Recovered, and two Unimpaired streams. The biodiversity was characterized by metabarcoding using two universal barcode markers (16S & 18S) along with two algal specific markers (UPA & *rbc*L) and high-throughput amplicon sequencing. For each marker, the ordination of Bray-Curtis Index calculated from the total operational taxonomic units (OTUs) present in each stream showed the Unimpaired and Recovered streams clustered, while Not Recovered streams were more distant. Focusing on the algal OTUs only, the Shannon index for the *rbcL* and UPA markers showed significantly lower alpha diversity in Not Recovered streams compared to Unimpaired streams, but the Recovered streams were not significantly different from the other two stream types. Further examination of the UPA marker revealed a decrease in relative abundance of diatoms in Not Recovered streams compared to Recovered and Unimpaired streams. The same 10 algal phyla were identified with the 18S and UPA marker, but the UPA included Cyanobacteria as well. A total of 21 diatom genera were identified from the *rbcL* marker data and these were investigated for taxa that have been reported to be indicators of high water quality. Overall, results from this study corroborate previous findings using individual groups of organisms to assess stream impairment from AMD.

SPATIOTEMPORAL VARIATIONS OF MICRO-ORGANISMAL ASSEMBLAGES WITHIN BIOLOGICAL SOIL CRUSTS AT THE JORNADA RANGELAND, NM **Omari, H.,** New Mexico State University, United States, <u>hanomari@nmsu.edu</u> Pietrasiak, N., New Mexico State University, United States, <u>ferrenbe@nmsu.edu</u> Ferrenberg, S., New Mexico State University, United States, <u>ferrenbe@nmsu.edu</u> De Ley, P., University of California, Riverside, United States, <u>pdeley@ucr.edu</u> Nishiguchi, M. K., New Mexico State University, <u>nish@nmsu.edu</u>

Biological soil crusts (BSCs) are prominent features of dryland regions with essential ecosystem functions. They are especially sensitive to dryland degradation processes, and what structures their microbial composition remains poorly understood. BSCs can be composed of cyanobacteria, bacteria, fungi, archaea, bryophytes, lichens and various associated microfaunal components. Few studies have explicitly evaluated how season, surrounding vegetation type, and BSC type impact BSC communities. Using three types of BSCs: light algal, dark algal, and cyanolichen crusts, black grama grass and woody shrub sites at the Jornada LTER, patterns in micro-organismal abundance and diversity were elucidated across differing spatial and temporal scales. We used a modified extraction method for nematodes, tardigrades, rotifers, amoebas, ciliates, and flagellates to obtain direct counts for three time points throughout the year; Phospholipid Fatty Acid Analysis (PLFA), soil texture, and soil chemistry analyses were applied for all BSC samples. Preliminary data analyses show that microfaunal biomass within BSCs correlates with PLFA based fungal and bacterial biomass values. Fall abundance counts are the greatest and have less variation than summer counts. Additionally, fall samples are structured by BSC type, while summer microfaunal abundances assemble by surrounding vegetation type. These data provide first insights into microfaunal assemblage variations within different BSCs, plant community types, and seasons, thus enhancing knowledge on plant-soil feedbacks and microbial food webs in drylands.

THE EFFECTS OF DILUTED BITUMEN ON THE DEVELOPMENTAL STAGES OF THE INTERTIDAL BROWN ALGA, ROCKWEED (*FUCUS DISTICHUS*) **Chavoshi, R.**, Simon Fraser University, Canada, <u>ranahchavoshi@hotmail.com</u> Bisrove, S. R., Simon Fraser University, Canada, <u>sbisgrov@sfu.ca</u> Kennedy, C. J., Simon Fraser University, Canada, <u>ckennedy@sfu.ca</u>

Canada is the fourth largest exporter of oil in the world and major industrial projects have been proposed that will increase the transport of diluted bitumen to overseas markets. Scientific uncertainty exists in understanding of the risks associated with a petroleum spill in marine environments and in particular for habitat-forming algal species. The effects of diluted bitumen were examined on a keystone brown alga, *Fucus distichus*, a foundational species of many intertidal ecosystems. Acute toxicity tests using environmentally-relevant concentrations of diluted bitumen (as water accommodated fractions or WAFs) were completed on two populations of *Fucus*. One population was located in a Polycyclic Aromatic Hydrocarbon (PAH)-contaminated environment near an oil shipping terminal in Burrard Inlet, BC. The other population was located on Savary Island, BC that had no prior history of petroleum exposure. Reproductive fronds were sampled from both populations and stimulated to release zygotes which were

exposed to diluted bitumen in a controlled laboratory setting. Effects on early developmental stages were examined for differences in responses between these two different populations. Diluted bitumen altered algal development in both populations, with the Savary Island population (uncontaminated source of algae) being more sensitive than those collected from the contaminated site.

DISCOVERIES AND CHALLENGES: INTERPRETING DIVERSITY AND DISTRIBUTION OF CORALLINE ALGAE IN SOUTHERN NEW ZEALAND **Twist, B. A.**, University of Auckland, New Zealand, <u>brenton.twist@gmail.com</u> Sutherland, J. E., NIWA, New Zealand, <u>judy.sutherland@niwa.co.nz</u> Hepburn, C. D., University of Otago, New Zealand, <u>hepburnc@planta.otago.ac.nz</u> Nelson, W. A., University of Auckland and NIWA, New Zealand, <u>wendy.nelson@niwa.co.nz</u>

Coralline algae (Corallinophycideae) are calcifying red algae that form the foundation of many shallow marine ecosystems globally, providing settlement sites for a range of invertebrate larvae, stabilisation of reefs and habitat for grazing and infaunal species. Despite their ecological importance, little is known about their diversity, distribution and ecology, globally and within New Zealand. Phylogenetic analysis and species delimitation methods were used to examine species diversity from a range of habitats in southern New Zealand. A high level of diversity was found, with 74 species identified from three orders. Individual species are likely to respond differently to a range of local and global disturbances, such as ocean acidification and sedimentation. Disentangling these species-specific responses, and flow on effects for coastal ecosystems in response to environmental change, is likely to be difficult to assess for southern New Zealand coralline algae, given the high level of diversity discovered. High diversity was also found on small spatial scales, which creates unique challenges for future researchers sampling biodiversity. However, multivariate clustering techniques, grouping sites based on similarities in coralline algae community structure, identified a range of environmental variables correlated with these community groupings. These results could enable the prediction of coralline algae communities under specific environmental conditions and help to determine how changing conditions may shape these communities.

Diversity and Distribution of Marine Protists

METABARCODING OF BENTHIC DINOFLAGELLATES: COMMUNITY COMPOSITION AND DYNAMICS FROM TWO CONTRASTING COASTAL AREAS (NW MEDITERRANEAN SEA AND GERMAN WADDEN SEA)

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The diversity and distribution of benthic protists is still poorly known in comparison with those of their planktonic relatives, and many marine areas remain nearly unexplored. In this study, we characterized, by 18S rRNA gene metabarcoding, sand-dwelling dinoflagellates from sediment cores obtained monthly during six months (April to September 2017) at three beaches from the NW Mediterranean Sea and twice in two locations from the German Wadden Sea (June 2017). While Mediterranean communities are virtually unknown, those of the German Wadden Sea (North Sea) have been largely characterized using traditional methods. Light microscopy observations confirmed that many new, and sometimes dominant, species remain to be described, especially in the Mediterranean. To alleviate the scarcity of molecular information available for benthic dinoflagellates, we determined the 18S rRNA gene sequence for several individually picked dinoflagellate cells. This subsequently helped refining the taxonomic assignment of

amplicons from the18S rRNA V4 region generated for the same samples. Our metabarcoding results showed that dinoflagellate communities strongly differed between Mediterranean sublittoral and North Sea tidal flat samples. Mediterranean samples had higher richness and were more similar among them than samples from the two North Sea locations. In all cases, the communities were dominated by a few taxa, including both autotrophic and heterotrophic genera, and showed a quite stable composition. However, dominant taxa showed temporal trends from spring to summer.

THE MOST TRANSCRIPTIONALLY ACTIVE EUKARYOTIC PLANKTON IN THE SURFACE OCEANS: WHO IS DOING WHAT, AND WHERE?

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The Tara Oceans expedition sailed the globe over a three-year period, collecting samples from over 150 individual stations. For eukaryotic plankton ranging from 0.8 µm-2 mm, we apply phylogenetic methods to Tara metatranscriptomic data to catalog transcriptional activity in the world's surface oceans. First, we map Tara Oceans metatranscriptomic sequences to a database of phylogenetic trees for 250 conserved genes to produce a eukaryotic tree of life with each branch weighted by its transcriptional activity. We find that, globally, metatranscriptomes show a similar representation of eukaryotic lineages to Tara metabarcodes (from the V9 region of the 18S locus), indicating that both sources likely reflect the biomass of active cells in the surface ocean. Within the pico- and nanoplankton (0.8-5 and 5-20 μ m), we find an unexpectedly high relative abundance of dinoflagellates and a substantial representation of transcriptionally active metazoans. We next apply our method to functional genes by studying the biogeographic expression patterns of the silicon transporter (SIT), which moves environmental Si across the cell membrane for use in biosilicified structures (such as diatom frustules). We find that diatoms dominate SIT expression in the Antarctic, where Si concentrations are highest, whereas choanoflagellates and copepods perform the bulk of SIT expression in the northern Indian Ocean and the Mediterranean Sea, where Si concentrations are much lower. We propose that our method can be used to study the ecology and biology of microbial eukaryotes in global pelagic ecosystems.

LATITUDINAL DIVERSITY GRADIENT OF PROTISTAN PLANKTON IN THE ATLANTIC OCEAN

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Latitude was long recognized as one of the main driver of species diversity globally and was first though to correlate with body-mass. Meanwhile, a latitudinal diversity gradient was also identified in marine microbes and uneven distribution of phytoplankton morphological species was also observed along latitudes. In this study, we aimed to address this latitudinal gradient for all marine protists. We sampled the DCM layer of a north-south transect in the northern Atlantic Ocean (17-49°N) in the late summer 2015. Genomic DNA was used to amplify the hypervariable V4 SSU rRNA region. PCR products were sequenced on an Illumina MiSeq platform at a depth of 1e^⁵ reads per station. The recovered communities were largely dominated by Dynophyta. Both OTU and phylogenetic based alpha diversity residuals (i.e. corrected for uneven sequencing depth) of all protists, only Dinophyta or only Radiolaria displayed a hump-shaped distribution along the gradient with a maximum between 30° to 35° latitude.

The other abundant protist groups showed contrasting patterns with significant changes as a function of latitude. For example, OTU diversity of Ciliophora was stable along the gradient while their phylogenetic diversity reached a maximum at 30° latitude. The decomposition of the beta diversity showed that both geographic and environmental parameters affect the change in community composition along the gradient but with different impact depending on the protist groups. These patterns may reflect the different functional role of these groups.

GLOBAL DIVERSITY AND DISTRIBUTION OF CLOSE RELATIVES OF APICOMPLEXAN PARASITES

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Apicomplexans are a group of obligate intracellular parasites, but their retention of a relict nonphotosynthetic plastid reveals that they evolved from free-living photosynthetic ancestors. The closest relatives of apicomplexans include photosynthetic chromerid algae (e.g., Chromera and Vitrella), nonphotosynthetic colpodellid predators (e.g., *Colpodella*), and several environmental clades collectively called Apicomplexan-Related Lineages (ARLs). The objective of this study was to investigate the global distribution and inferred ecology of the ARLs by expansively searching for apicomplexan-related plastid small ribosomal subunit (SSU) genes in large-scale high-throughput bacterial amplicon surveys. Searching more than 220 million sequences from 224 geographical sites worldwide revealed 94,324 ARL plastid SSU sequences. Meta-analyses confirm that all ARLs are coral reef associated and not to marine environments generally, but only a subset are actually associated with coral itself. Most unexpectedly, Chromera was found exclusively in coral biogenous sediments, and not within coral tissue, indicating that it is not a coral symbiont, as typically thought. In contrast, ARL-V is the most diverse, geographically widespread, and abundant of all ARL clades, and is strictly associated with coral tissue and mucus. ARL-V was found in 19 coral species in reefs, including azooxanthellate corals at depths greater than 500m. We suggest this is indicative of a parasitic or commensal relationship, and not of photosynthetic symbiosis, further underscoring the importance of isolating ARL-V and determining its relationship with the coral host.

MARINE DIPLONEMIDS OF CALIFORNIA

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Marine diplonemids are one of the most diverse heterotrophic protists group in the ocean based on environmental sequence surveys. Unlike their well-studied sister clade, the classic diplonemids (including *Diplonema* and *Rhynchopus*), the biology of marine diplonemid clades has yet to be elucidated. In 2016, we reported the first information on the morphology of marine diplonemids along with genomic information, derived from isolated single cells. Recently, we performed a field expedition in the eastern North Atlantic to isolate additional marine diplonemid cells. In this talk, we will share our latest observations on their morphology, including the first microvideography of live cells of these diverse, uncultured protists, as well as first glimpses into our single cell transcriptomic studies of marine diplonemids.

DIPLONEMIDS - EXTREMELY DIVERSE MARINE PROTISTS CAN BE GENETICALLY MODIFIED

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Diplonemids are heterotrophic protists recently found to be the most species-rich eukaryotic groups populating the world ocean, which is also unexpectedly abundant and omnipresent. So far, only a few diplonemid species have been described and we know close to nothing about their life style. However, the absence of reverse genetics in these protists has hampered elucidation of their molecular and cell biology. I will report first protocol for the transformation of *Diplonema papillatum* allowing effective electroporation of constructs, which become integrated into chromosomes and the genes they carry are transcribed, trans-spliced and translated. Moreover, I will present new data on the diversity, ultrastructure, endosymbions and cell cycles of marine diplonemids

LIPIDOMIC ANALYSIS OF *EUGLENA GRACILIS* CELLS, THE ORGANELLAR FRACTIONS AND THE BLEACHED MUTANTS

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Euglena gracilis is a photosynthetic flagellate with secondary endosymbiosis-derived plastids. Such plastids are usually bound by four membranes of various origin - two membranes of the primary plastid, the plasma membrane of the symbiont and the membrane of the host phagosome. E. gracilis plastids are surrounded by only three membranes, suggesting that one has been lost. We prepared plastid and mitochondrial fractions of *E. gracilis* and determined their lipid composition by HPLC/ESI-MS/MS. We also investigated the lipid composition of non-photosynthetic bleached mutants of E. gracilis, W10BSmL (W10) and WgmZOfl (OFL), which are believed to contain only the remnants of the plastids. The lipid composition of the whole *E. gracilis* cells was compared to cellular fractions as well as to bleached W10 and OFL mutant cells. E. gracilis cells contain seven classes of glycerolipids mainly represented by monogalactosyl diacylglycerols (MGDGs), digalactosyl diacylglycerols (DGDGs), phosphatidylglycerols (PGs) and sulfoquinovosyl diacylglycerols (SQDGs). The comparisons suggest that these four major classes are present in the chloroplast membranes, while the main mitochondrial lipids are phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and PGs. Finally, the major lipid class of membranes outside chloroplast and mitochondria are probably PEs, which compose 68% and 53% lipids of W10 and OFL mutant cells, respectively. Investigation of lipids in thylakoid and envelope chloroplast membranes will follow.

Evolution of Plastids and Algae

SELMA, THE PROTEIN TRANSLOCATION MACHINERY OF SECONDARY RED PLASTIDS HAS MULTIPLE EVOLUTIONARY ORIGINS **Moreira, D.**, CNRS - Universite Paris-Sud, France, <u>david.moreira@u-psud.fr</u> Ponce-Toledo, R. I., CNRS - Universite Paris-Sud, France Lopez-Garcia, P., CNRS - Universite Paris-Sud, France, <u>puri.lopez@u-psud.fr</u> Deschamps, P., CNRS - Universite Paris-Sud, France

Many eukaryotes acquired plastids by secondary endosymbioses of red and green algae. In contrast with the 2-membrane plastids of primary photosynthetic eukaryotes, these secondary plastids have additional membranes that require specific translocation systems to import proteins from the host's cytoplasm. In the 4-membrane red secondary plastids (found in cryptophytes, certain alveolates, stramenopiles, and haptophytes -the CASH lineages-), protein translocation through the second outermost membrane involves the complex multiprotein system SELMA (Symbiont-specific Erad-Like MAchinery). SELMA proteins are homologous to those of the ERAD system, involved in protein translocation across the endoplasmic reticulum. The presence of SELMA in the CASH lineages has been a major argument to support their monophyly. It was proposed that SELMA evolved from the red algal symbiont ERAD in a common ancestor of all CASH. If this hypothesis is true, the CASH lineages should form a monophyletic group in the phylogenies of all SELMA components. However, this test has been done for only a couple of SELMA proteins and very often using a reduced taxon sampling. We have analyzed the phylogeny of 18 SELMA proteins and found that only one, Cdc48 (and only under one substitution model), supports the CASH monophyly. The rest of phylogenies support different evolutionary scenarios, including an unequal contribution of host proteins in the different lineages. In particular, the SELMA system of cryptophytes appears to have been built with a set of proteins of different origin than in the other CASH. These results challenge the view that SELMA can support a single origin of all red secondary plastids.

ORGANELLAR PHYLOGENOMICS PERSPECTIVE ON THE EVOLUTION OF EUSTIGMATOPHYTE ALGAE

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Eustigmatophytes (shortly eustigs) are a small yet quickly growing group of unicellular algae living primarily in freshwater and terrestrial environments. Except for genomic resources built for the biotechnologically important genera *Nannochloropsis* and *Microchloropsis*, genomic explorations of eustigs have so far yielded only organellar genome sequences of three additional species. The most surprising aspect unveiled by these studies is a six-gene operon of unknown function, denoted *ebo*, present in the plastid genomes of two species and acquired by horizontal gene transfer from bacteria. To further improve our understanding of the eustig evolution we sequenced organellar genomes of seven additional members, covering thus essentially all main eustig lineages, including a novel one presumably sister to the order Eustigmatales. Analyses of the new data provided a much fuller picture of the evolutionary history of organellar genomes in eustigs. An example of the interesting new insights is the realization that the *rps4* gene in eustig mitochondrial genomes is split into two parts, one corresponding to

the previously identified unknown gene *orfX*. Most notably, none of the newly sequenced plastid genomes include the *ebo* operon, implying that is was acquired in a specific ancestor of Monodopsidaceae and the Eustigmataceae group and secondarily lost independently in the *Nanno/Microchloropsis* and *Characiopsis* lineages. We are presently exploiting the eustig organellar genomes to resolve the backbone phylogeny of the group.

INVESTIGATION OF NOVEL FREE-LIVING RELATIVES OF MARINE ALVEOLATES (MALVS) **Hehenberger, E.**, Monterey Bay Aquarium Research Institute, United States, <u>helisabe@mbari.org</u> Tikhonenkov, D. V., Penza State University, Russia, <u>tikho-denis@yandex.ru</u> Irwin, N. A., University of British Columbia, Canada, <u>nickatirwin@gmail.com</u> Keeling, P. J., University of British Columbia, Canada, <u>pkeeling@mail.ubc.ca</u>

The dinoflagellates, a major plastid-bearing protist lineage, can be grouped into the so- called core dinoflagellates and two non-photosynthetic deep-branching lineages, Marine Alveolates (or MALVs) and Oxvrrhis. The MALVs are generally considered to be parasitic and likely completely eliminated their plastids, while the free-living Oxyrrhis, although heterotrophic, seems to have retained a relic plastid. In this context, we are investigating two novel free-living and predatory lineages that were found to cluster with the deep-branching dinoflagellates in initial phylogenetic analyses, representing therefore putative models to better understand the early evolution of dinoflagellates in general and their plastids in particular. Using a combination of culture- and single cell-based approaches, we generated transcriptomic data to further explore these novel taxa. We performed a comprehensive phylogenomic analysis that resolves those novel free-living taxa with high support as a sister group to the parasitic MALVs, further expanding the complexity of the deep-branching dinoflagellates. To investigate whether these taxa have lost their plastid completely, like their closest relatives, the MALVs, or retained a reduced version, we are searching the transcriptomes for genes with a putative function in a non-photosynthetic plastid and N terminal sequences indicative of targeting to such an organelle. First results suggest the presence of several such plastid-associated genes, however further sequencing work is necessary to clearly identify the subcellular localization of the corresponding proteins.

PHYLOGENOMICS AND THE EVOLUTIONARY HISTORY OF THE FAMILY RHODOMELACEAE

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The Rhodomelaceae, with about 1000 species, is by far the most diverse family of the red algae. It is present worldwide and in all benthic habitats in the photic zone. Its high diversity and wide range of thallus architectures makes it a good model for testing evolutionary hypotheses. However, the lack of well resolved phylogenies has been a major barrier. We used a phylogenomic approach with the aim of resolving relationships among the major lineages of the family. On this basis, we constructed a time-calibrated phylogeny of >400 species to unravel the timing and tempo of lineage diversification and analyse the evolutionary history of morphological traits. The origin of the family was estimated at ca. 270 Ma and all major lineages emerged early in the evolution of the Rhodomelaceae. Both lineages and functional morphology exhibit an early rapid diversification followed by a slow-down, indicating that the Rhodomelaceae experienced an evolutionary radiation.

THE ORIGIN AND SPREAD OF RED ALGAL PLASTIDS **Strassert, J.**, Uppsala University, Sweden, <u>strassert@protist.eu</u> Irisarri, I., Uppsala University, Sweden, <u>iker.irisarri@ebc.uu.se</u> Burki, F., Uppsala University, Sweden, <u>fabien.burki@ebc.uu.se</u> Photosynthesis is widespread among various supergroups of eukaryotes. Whereas the ancestor of red algae acquired a cyanobacterium, other groups obtained their capability for photosynthesis by endosymbiosis events at higher levels, i.e., by the uptake of a red alga, or by the uptake of protists that already engulfed a red alga. To this day, the number and the complex order of these serial symbioses are highly debated. In this study, we combined phylogenomics with micropaleontology to constrain the timing of plastid transfers. About 320 genes were extracted from transcriptomic/genomic data from each of more than 730 taxa representing all eukaryotic supergroups. This so far largest taxon sampling enabled us to build an exceptional clean dataset by detecting contaminants, endosymbiotic gene transfers, and paralogs, which can easily be overlooked in smaller-scale analyses. Our phylogenetic analysis of this dataset resolved most of the deep evolutionary relationships among eukaryotes — a prerequisite for revealing the history of red algal plastid transmissions. Finally, we uncovered the chronology of the plastid acquisitions using molecular dating with numerous age constrains derived from a great taxonomic diversity of macro- and microfossils.

THE SHORT MOTILE PERIOD ADVANCED THE TERTIARY PLASTIDS FROM ENDOSYMBIONTS

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Dinoflagellates called 'dinotoms' are known to possess endosymbiotic diatoms (ESDs) as their tertiary plastids. We revealed last year that dinotoms utilise thirteen different species of ESDs respectively depending on host species. Furthermore, we found that the each species of dinotoms possess an ESD, which phylogenetically positioned in diatom groups living in a same habitat of the host. This observation implies that each species of dinotoms took up its ESD independently at their habitats. In contrast to the majority of dinotoms, five species of benthic dinotoms, which make a clade with bootstrap 100% in 18S rDNA molecular phylogeny, maintain the genetically concordant ESDs despite of their geographicallydiverse habitats. To clarify why they share identical diatoms, we microscopically observed three species of dinotoms in this clade: Galeidinium rugatum and two novel species. All of these dinotoms show an extremely short motile stage. Motile cells appear approximately once in three days after the cell division and the duration is from a few minutes to several hours, the rest of the time the cells are living non-motile cells. We hypothesize that the specific life style makes it less likely that the cells meet diatoms that they can take up. We also obtained an indication by CLSM that the nucleus of ESDs are extremely reduced the size compared to nuclei in ESDs of other dinotoms. This result implies that the ESD was acquired in the early history of dinotoms, and the ancestor of the five diatoms developed the non-motile life style after acquirement of the permanent ESD.

INTERPLAY AND POSSIBLE INTERACTION OF MITOCHONDRIA AND NUCLEI IN A BICOSOECID

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Stramenopiles comprise photosynthetic as well as non-photosynthetic groups. The plastids of the photosynthetic groups are complex plastids, which are derived from a red algal ancestor. There is quite some debate on the relationship between the different groups of algae in which such plastids are found (cryptophytes, haptophytes, plastid containing stramenopiles and most of the plastid containing alveolates), as well as on the number and sequence of plastid gains, losses or transfers between these

groups. Non-photosynthetic stramenopile groups like oomycetes or bicosoecids show no evidence of plastids. However, depending on the scenario of plastid evolution between the above-mentioned groups of algae, the photosynthetic stramenopile groups might either have lost plastids that were present in the last common ancestor of the stramenopiles, or might have never had plastids. Organelle evolution via endosymbiosis is accompanied by extensive re-targeting of proteins to intracellular compartments that are not always the same compartment as the one from which the gene originated. Therefore, one key to understanding plastid evolution in organisms with complex red algal derived plastids is knowledge of the organelle/cytosol/nucleus interactions in their plastid free relatives. In a yet to be described bicosoecid, we discovered close interaction of nucleus and mitochondria in structures that were to our knowledge never observed in plastid containing stramenopiles. Here we present results of our ultrastructural and molecular biological characterizations of the interplay of mitochondria, nuclei and cytosol in this bicosoecid.

Sex and Life History

CONSIDERING SEX IN THE EUKARYOTE LIFE CYCLE; IMPLICATIONS FOR IDEAS ABOUT EVOLUTION OF SEX Spiegel, F. W., University of Arkansas, United States, fspiegel@uark.edu

Sex is an idiosyncratic form of horizontal gene transfer that involves changes in nuclear ploidy and is unique to extant eukaryotes. The major events in a sexual life cycle are the alternation between karyogamy, where two haploid nuclei fuse to become a diploid zygote nucleus, and meiosis, a twodivision form of nuclear division where a diploid nucleus with replicated chromosomes undergoes haploidization in the first division and sister chromatids segregate in the second. Both karyogamy and meiosis result in the formation of novel genetic combinations. Meiosis limits sex to populations of closely related eukaryotes due to the accurate pairing of homologous chromosomes in Prophase I. No sexual organism is known where mitosis is not intercalated in some way between karyogamy and meiosis events. Most discussion of the consequences of sex have focused on organisms that have much or most of their mitosis occurring between karyogamy and meiosis. I suggest that the haplontic life cycle, where the zygote nucleus immediately undergoes meiosis and all mitosis is haploid is the primitive state in eukaryotes, and I will discuss the implications of this with respect to the origin and evolution of sex.

ONE DIPLOID TO RULE THEM ALL? PHASE-SPECIFIC RESPONSES TO ENVIRONMENTAL VARIATION IN A RED ALGA

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Genetic models suggest haplodiplontic life cycles are transitory states on the way to diploidy or haploidy. Yet, these life cycles, in which there are prolonged haploid and diploid stages, are found across the eukaryotic tree, particularly amongst the three lineages of macroalgae. The diversity of lineages maintaining haplodiplontic life cycles suggests they are not transitory states, but evolutionarily stable. It is only when ecological processes are considered that models predict stability. There are, however, few empirical tests of ecological niche differentiation between ploidies or sexes. Here, we used the

widespread, non-native red seaweed *Gracilaria vermiculophylla*, which has been shown to undergo dramatic demographic shifts to diploid dominance following the invasion of soft-sediment habitats. We tested the following hypotheses: (i) diploid tetrasporophytes exhibit greater plasticity than haploid gametophytes, and (ii) survival and growth varies by sex and ploidy. By comparing genotype-level responses to thermal and osmotic stress in thalli collected from the native and non-native ranges, we can assess the mechanisms that lead to diploid dominance in free-floating populations in the non-native range, as well as potential tradeoffs that may help explain the maintenance of haplodiplontic life cycles.

PLASTID GENOME EVOLUTION IN THE ISOGAMOUS BROWN ALGAE

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Among the major brown algal orders, only the Ectocarpales species are known to have isogamous fertilization in which male and female gametes are morphologically identical. The other being oogamous in which female gametes are much larger than the male gamete. Interestingly, the plastids of isogamous species are biparently inherited whereas the plastids of oogamous are maternally inherited. Furthermore in isogamous and oogamous species, the mitochondrial genome is always maternally inherited. To investigate how different modes of inheritance impact organelles genomes architecture, we sequenced the organelles genomes of three Ectocarpales species and compared them with previously sequenced organelles genomes of oogamous and isogamous species. We determined that the gene content and general architecture of the organelles genomes are not affected by the mode of inheritance. However, we show that the plastid genome of the isogamous species (i.e. biparentaly inherited) present numerous rearrangements when compared with each other whereas the plastid genome of oogamous species (i.e. maternally inherited) show no rearrangements with each other. We identified that the rearrangements are localized in one region of the plastid genome. This region is flanking one of the inverted repeat of the genome and we discuss the potential role of those inverted repeats in the rearrangements of the plastid genome. To further investigate the role of inheritance, we studied the highly variable region of the plastid genome in artificial hybrids of the Ectocarpales species.

SPATIAL PATTERNS OF GENETIC STRUCTURE IN *MASTOCARPUS STELLATUS* (RHODOPHYTA)

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Mastocarpus stellatus occurs in dense patches on many rocky shores on both eastern and western coastlines of the North Atlantic Ocean. The life cycle of *M. stellatus* consists of sexual generations alternating between upright, haploid gametophytes and diploid crustose tetrasporophytes, and in many northerly populations, a diploid asexual life cycle in which female fronds give rise directly to diploid females. Our research examines the spatial genetic structure of mapped individuals of *M. stellatus* within and among shores from seven sites in France and four sites in Maine using 15 DNA microsatellite markers. DNA was extracted from vegetative tissue of crusts and the stipes of fronds. Signatures of diploid genotypes (i.e., heterozygous at one or more microsatellite loci) indicated that almost all fronds collected in Maine were asexual at most sites. Reduced genetic diversity and high frequency of diploid

frond genotypes relative to Eastern Atlantic populations suggest that clonality is the primary mode of reproduction for *M. stellatus* lineages in the Northwest Atlantic. In France, high levels of genetic structure were observed between sexual (single allele at all loci) and asexual lineages within populations. There was no evidence of isolation by distance among pairwise combinations of individuals within sites (0.05–50 m distances). Our results suggest that short-distance dispersal does not hinder gene flow within populations of *M. stellatus*, but reproductive isolation between sexual and asexual lineages drives their divergence.

ISOLATION OF SEX-SPECIFIC IMPORTIN-ALPHA-LIKE GENES IN THE RED ALGA BOSTRYCHIA MORITZIANA

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The modes of sex determination in eukaryotes are diverse. The genetic pathways leading to sex determination are not known in red algae. We searched for sex-specific genes in the red alga Bostrychia moritziana by searching for genes that may be involved in sex differentiation. RNAseq results showed that two importin-alpha-like genes were specifically expressed in male and female gametophyte, respectively. Female specific homologues of importin-alpha (BmFS) was found only in female gametophytes, and plants bearing carposporophytes. The other was restricted to male gametophytes. The two sex-specific importin-alpha-like genes showed only 77 % sequence identity. The two sex-specific importin-alpha-like genes contain three conserved domains (Armadillo Repeats, Ankyrin Repeats, Structural Maintenance of Chromosomes). Genomic PCR showed that the genes are present only in respective gametophytes. Tetrasporophyte had both genes. These results suggest that sex may be determined by genes on two different sex chromosomes or regions. Differential expression of these two sex-specific genes was analyzed in the gametophytes, carposporophytes and tetrasporophyte, showing that they are expressed only in their respective stage, and both expressing in sporophytes. Shortly after fertilization expression levels of BmFS sharply increased suggesting that it might be involved in fertilization signaling. Importin-alpha is a type of karyopherin that transports protein molecules into the nucleus. Our results suggest that signal protein traffic to nucleus may be involved in sexual differentiation in red algae.

TRANSCRIPTOME DATA OF *COCHLIOPODIUM PENTATRIFURCATUM* (DISCOSEA, AMOEBOZOA) UNCOVER THE SEXUAL NATURE OF FUSED CELLS **Melton, J. T.**, Spelman College, United States, <u>imelton@spelman.edu</u> Wood, F. C., Spelman College, United States, <u>fwood@spelman.edu</u> Tekle, Y. I., Spelman College, United States, <u>ytekle@spelman.edu</u>

Amoeboid microbes were originally thought to be asexual. However, it has recently been shown that Amoebozoa is ancestrally sexual based on the detection of sex-related and meiosis genes in the genomes and transcriptomes of the majority of its clades. *Cochliopodium* spp. (Discosea) are known to go through a process of cellular fusion (plasmogamy) and nuclear fusion (karyogamy) to form large multinucleate or polyploid cells containing up to 30 nuclei. The cells then undergo fission resulting in single, uninucleate cells. A previous transcriptomic study of three *Cochliopodium* species identified an almost complete gene repertoire for recombination confirming that there is a sexual component to the life cycle. However, the exact mechanism of sexual development in *Cochliopodium* is poorly understood. Here, we sequenced the transcriptomes of single (unfused) and fused cells of *C. pentatrifurcatum*, a species that is known for its rapid growth and propensity to fuse, to help determine if the sexual part of the life cycle is during the fused state. A differential expression analysis of fused versus single cells revealed several upregulated genes in the fused state involved with chromosomal segregation and membrane fusion. Additionally, the meiosis specific gene SPO11, which encodes for a protein that makes double stranded breaks in the DNA

during meiosis, was only detected in the fused cells. This study provides evidence that the fused state represents a sexual part of the life cycle of *Cochliopodium* that likely allows for genetic exchange between or among cells.

THE LIFE CYCLE OF *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE) DISCOVERED BY IN SITU AND IN VITRO OBSERVATION

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Raphidophycean flagellate Heterosigma akashiwo (Hada) Hada is a bloom-forming algal species that has damaged many fish farms in coastal waters during recent decades. Consequently, many studies focused on the population dynamics of *H. akashiwo*, while its life cycle was not well studied. In this study, we investigated veiled life cycle of *H. akashiwo* through both field observation and culture based method. Firstly, in order to confirm life cycle stages of *H. akashiwo* in the natural environment, sea water and sediment samples were collected from the Youngsan River estuarine Bay (YRB) and Masan Bay in South Korea. The cysts found in the sediment of YRB were both single cell and clustered type. Cyst has a cell wall while cell wall is absent in vegetative cell, and the structure underneath the lid of germination pore remains characteristic. The cell clusters found in water of Masan Bay were thought to be precursor cells of the cyst, which is a stage of life cycle in which about 30 cells remain in clustered form. Since there is a limit to follow up the process of life cycle stage change in the natural environment, various types of H. akashiwo cells were confirmed during the incubation in the laboratory which were isolated and observed in detail. Results confirmed that H. akashiwo was observed to have sexual cycle and heterothallic. H. *akashiwo* was found to form large cells ($\approx 40 \, \mu m$) through sexual reproduction and large cells were observed to form small cells ($< 8 \mu m$) or multiple cells. These results improved the life cycle study of H. akashiwo and this fundamental investigation provide important new information and improve our understanding of H. akashiwo.

Taxonomy and Evolution of Algae I: A New Hope

MITOCHONDRIAL AND PLASTID GENOME EVOLUTION OF CORALLINE RED ALGAE Lee, J. M., Sungkyunkwan University, South Korea, <u>leejunmo331@yahoo.com</u> Nelson, W. A., University of Auckland, New Zealand Fredericq, S., University of Louisiana at Lafayette, United States Yoon, H. S., Sungkyunkwan University, South Korea, <u>hsyoon2011@skku.edu</u>

Because mitochondria and plastid genomes contain conserved gene contents and low mutation rates compared to nuclear genes due to maternal inheritance of organelles, these genes have been frequently used to phylogenetic studies. The phylogenetic approach using several useful molecular markers has been widely used to uncover inter-species relationships that were the basis for taxonomic conclusions. Recently, next-generation genome sequencing technique has enabled the construction of massive organelle genome databases from diverse eukaryotic organisms and used for species trees, based on concatenated gene matrices from genome data, without any concerns of bias between gene tree and species. Here we test conflicting phylogenetic signals from mitochondrial and plastid genomes in coralline red algae. We generated complete organelle genome data from five coralline red algal species (*Lithothamnion* sp., *Neogoniolithon* sp., *Renouxia* sp., *Rhodogorgon* sp., and *Synarthrophyton chejuensis*) for comparative analysis with published organelle genome data from two corallines (*Calliarthron tuberculosum* and *Sporolithon durum*). We found strong incongruent phylogenetic signals from both

organelle genomes that might derive from incomplete lineage sorting caused by rapid radiation, hybridization, or gene flow in the ancestry of coralline red algae.

PRASIOLALES OF NEW ZEALAND AND SURROUNDING REGIONS **Sutherland, J. E.**, National Institute of Water and Atmospheric Research (NIWA), New Zealand, judy.sutherland@niwa.co.nz Robinson, N. M., University of Auckland and National Institute of Water and Atmospheric Research, New Zealand, Nestor.RuizRobinson@niwa.co.nz

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The Prasiolales (Trebouxiophyceae) are an order of diminutive green algae that grow as blades, filaments and unicells. They are found from the high intertidal splash zone of marine environments to extensively human modified urban environments kilometres from the nearest shore, and in freshwater systems. Their tolerance for extremes of salinity, desiccation and temperature, and the association of some species with particular fungi, make them interesting objects of study. We have made collections of Prasiolales from the Three Kings Islands in the north of New Zealand to Campbell Island in the subantarctic. As a result of *rbcL* and *tufA* sequence data analyses we have recorded three species of *Rosenvingiella*, one species of *Prasiola novaezelandiae* is widespread throughout the North and South Island, in both supralittoral and terrestrial habitats. *Prasiola borealis* is found only in the south of the South Island, while *Prasiola snareana*, which is closely related to the Antarctic *P. crispa*, has been recorded only from the Snares Islands just South of the main New Zealand archipelago. The extraordinary genetic similarity of *Prasiola* species from different parts of the world implies some mechanism of gene flow, possibly through a mobile vector. Their association with guano deposits of bird colonies provides an *a priori* suspect, while their hardiness and tolerance to desiccation supports the feasibility of such transfers.

DNA SEQUENCING AND MORPHOLOGY SUPPORT SEGREGATION OF A NEW GENUS *PHYMATOLITHOPSIS* (HAPALIDIACEAE, RHODOPHYTA) FROM *PHYMATOLITHON* **Jeong, S.**, Chosun University, South Korea, <u>delesseriaceae@gmail.com</u> Won, B., Chosun University, South Korea, <u>giving_won@daum.net</u> Cho, T., Chosun University, South Korea, <u>tocho@chosun.ac.kr</u>

In the Hapalidiaceae, the genus *Phymatolithon* was established by Foslie (1898) for a single previously described species, *P. polymorphum* (L.) Foslie. The basionym of *P. polymorphum*, *Millepora polymorpha* L. is a superfluous substitute name for *Millepora calcarea* Pallas, and consequently the correct name of the type species of *Phymatolithon* is *P. calcareum* (Pallas) Adey & McKibbin. Since 1898, at least 47 specific and infraspecific taxa have been referred to the genus, and it has undergone several changes in circumscription. Currently, 17 species of *Phymatolithon* are recognized mostly based on morphological analyses. However, the genus *Phymatolithon* has been known as polyphyletic group during long time. A multigene phylogeny of the genus *Phymatolithon*, using COI-5P, *psbA*, and *rbcL*, was constructed to assess generic boundary. Based on our morphology and molecular analysis, we have concluded that some *Phymatolithon* species represent a distinct new genus *Phymatolithopsis*.

FLIP-FLOP ORGANIZATION IN CHLOROPLAST GENOME OF *CAPSOSIPHON FULVESCENS* (ULVOPHYCEAE, CHLOROPHYTA)

Kim, D. S., Sungkyunkwan University, South Korea, <u>dongseokkim6662@gmail.com</u> Lee, J. M., Sungkyunkwan University, South Korea, <u>leejunmo331@gmail.com</u> Yoon, H. S., Sungkyunkwan University, South Korea, <u>hsyoon2011@skku.edu</u> To better understand organelle genome evolution of *Capsosiphon fulvescens*, we sequenced and characterized its complete chloroplast genome (cpDNA). The cpDNA length is 111,561 bp with 31.3% GC content in a circular molecule that contains 108 genes including 77 protein-coding genes, two copies of rRNA operons and 27 tRNAs. We found that the special structure of cpDNA consists of two types of isoform called heteroplasmy by flip-flop. This is a unique chloroplast genome feature of *C. fulvescens* that reported in other green algal species. To determine the phylogenetic position within the class, we reconstruct phylogenetic tree using the cpDNA data including all available ulvophycean species. Individual gene phylogenies using 70 genes support, i) the monophyly with Ulotrichales (27 genes), ii) the monophyly with Ulvales (16 genes), and iii) the monophyly with Ulotrichales and Ulvales for *C. fulvescens* or *Pseudoneochloris marina*. Although 70-gene concatenated phylogeny supports the monophyly with Ulvales for both species, this phylogenomic analysis suggests that a taxonomic reinvestigation is needed using data-rich approach (i.e., organelle genomes) from broader taxon sampling.

INFERRING TRAIT EVOLUTION FROM PHYLOGENY TO BETTER UNDERSTAND SPECIES DIVERSIFICATION AND SUPPORT TAXONOMIC REVISION OF UDOTEACEAE (BRYOPSIDALES, CHLOROPHYTA)

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Udoteaceae is a family of marine green macroalgae belonging to the order Bryopsidales. Although they are characterized by a unicellular structure, Udoteaceae species are highly diverse morphologically both in their external habit and anatomical features. However, genetically distinct lineages can have similar gross morphology which raises questions about the evolution of morpho-anatomical characters among the family. In addition, several genera are polyphyletic which emphasizes the need for a deep taxonomic revision of the family. In order to better understand the diversification processes among lineages and through time, and to help with taxonomy decision-making, we inferred traits evolution based on a timecalibrated phylogeny of the family reconstructed from an extensive multilocus (tufA, rbcL and 18S rDNA) dataset. Both continuous and discrete morpho-anatomical traits were recorded. We applied phylogenetic comparative methods through statistical techniques (Brownian-motion and Ornstein-Uhlenbeck models) to infer patterns of trait evolution and test evolutionary hypotheses. Our results pointed out to differences in the informative power of the investigated characters with some standing out as key traits. Taxonomically, several synapomorphies support a number of lineages, including new ones, and they were identified as significant diagnostic characters for species descriptions. Homoplasies have also been found in distinct lineages and may indicate convergence or regression events in the evolution history of the Udoteaceae.

NEW INSIGHTS INTO THE PHYLOGENY OF SCYTOSIPHONACEAE (PHAEOPHYCEAE) IN THE SOUTHEASTERN PACIFIC COAST

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Due to the extremely simple morphology of members of the Scytosiphonaceae, defining generic and species boundaries is troublesome. Morphological characters used to define species within these genera, often influenced by environmental factors, vary within and among populations. On the other hand, phylogenetic studies highlight high diversity and the existence of a complex of cryptic species within *Scytosiphon lomentaria*. The aim of the study was to assess the taxonomic status of the family in the Southeastern Pacific coast, with special emphasis in the genus *Scytosiphon*, through morphological and

molecular approaches. Secondly, through the used of Bayesian phylogenetic approximations and the phylogenetic comparative method addressed the origin and diversification of both genera including samples representatives of most of their worldwide distribution. Our molecular results, based on nuclear and mitochondrial markers, demonstrated that the genera *Petalonia* and *Scytosiphon* are paraphyletic. Moreover, we detected a *Scytosiphon* complex which harbours a large cryptic diversity along the Chilean coast, including three lineages of *S. lomentaria* and a possible new species endemic to the region. The historical biogeographic analyses suggested that these lineages present different spactio-temporal origins based on their current geographic distribution and genetic diversity. It is suggested that the species with restricted distribution recently colonized the region, following human-induced introduction, while *S. lomentaria* would have come through an ancestral event of colonization. Financial support: CeBiB FB-0001.

PHYLOGENY OF CHARACEAE WITH PARTICULAR REFERENCE TO TRIBE CHAREAE (CHAROPHYCEAE, CHARALES, CHARACEAE) BASED ON THREE PLASTID GENE SEQUENCES

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Green algae in the Family Characeae (Order Charales), commonly called charophytes or stoneworts, have long been considered a distinct lineage within the green algae (Chlorophyta *sensu lato*) due to their large size and relatively complex morphology and reproduction. This study addresses phylogeny within the family and closely related groups, based on a broad taxon sampling within the family Characeae (53 taxa) and across the Charophycean green algae and embryophytes (249 taxa total). We report on a molecular phylogenetic analysis of three plastid genes (rbcL, atpB, and psbC) sampled across the family, with in depth focus on four genera in the tribe Chareae (*Chara, Lamprothamnium, Nitellopsis*, and *Lychnothamnus*). The tribe Nitelleae was also sampled (*Nitella, Tolypella*). We use the resulting 3-gene phylogeny to test hypotheses of the phylogenetic position of stoneworts within the Charophycean green algae, of monophyly of genera and subgnera of *Chara*, and monophyly of sections and subsections within the subgenus Chara, Tribe Chareae. The family, genera, subgenera and species of *Chara* are generally strongly supported as monophyletic, however, *Lamprothamnium* is nested within a paraphyletic *Chara*, and sections and subsections are only rarely supported as monophyletic groups. Most characters used to define sections and subsections are homoplastic, and a new classification of species within *Chara* is called for.

Tuesday, July 31

Nagoya Protocol and its influence on international research endeavours Chuck Delwiche

ISOP Symposium: UniEuk

UNIEUK: TIME TO SPEAK A COMMON LANGUAGE IN PROTISTOLOGY **Berney, C**., Station Biologique de Roscoff, France, <u>cedric.berney@sb-roscoff.fr</u> Quast, C., Max Planck Institute for Marine Microbiology, Germany, <u>cquast@mpi-bremen.de</u> Weber, M., Max Planck Institute for Marine Microbiology, Germany, <u>maweber@mpi-bremen.de</u> Rajan, J., EMBL-EBI/ENA, United Kingdom, <u>rajan@ebi.ac.uk</u> Mahé, F., CIRAD, France, frederic.mahe@cirad.fr Wegener Parfrey, L., University of British Columbia, Canada, <u>Iwparfrey@botany.ubc.ca</u> Kolisko, M., Biology Centre CAS, Czech Republic, <u>kolisko@paru.cas.cz</u> Brodie, J., The Natural History Museum, United Kingdom, j.brodie@nhm.ac.uk Edgcomb, V., Woods Hole Oceanographic Institution, United States, <u>vedgcomb@whoi.edu</u> Kim, E., American Museum of Natural History, United States, <u>ekim1@amnh.org</u> Adl, S., University of Saskatchewan, Canada, <u>sina.adl@usask.ca</u> Cochrane, G., EMBL-EBI/ENA, United Kingdom, <u>cochrane@ebi.ac.uk</u> del Campo, J., Institut de Ciències del Mar, Spain, <u>jdelcampo@icm.csic.es</u> Geisen, S., Netherlands Institute of Ecology, Netherlands, <u>s.geisen@nioo.knaw.nl</u> Glöckner, F. O., Max Planck Institute for Marine Microbiology, Germany, <u>fog@mpi-bremen.de</u> Simpson, A., Dalhousie University, Canada, <u>alastair.simpson@dal.ca</u> Yilmaz, P., Max Planck Institute for Marine Microbiology, Germany, <u>pyilmaz@mpi-bremen.de</u> de Vargas, C., Station Biologique de Roscoff, France, <u>vargas@sb-roscoff.fr</u>

UniEuk is an open, community-based and expert-driven international initiative to build a flexible, adaptive universal taxonomic framework for eukaryotes, focused primarily on protists, that will be implemented into the European Nucleotide Archive at EMBL-EBI. As a whole, the UniEuk system represents a 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 comprises three complementary modules allowing 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 V4 18S rDNA 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 contextual data. At the 2018 PSA/ISOP meeting we will report on the project's advances, advertise the next EukRef workshop (focusing on dinoflagellates) that will take place 5-9 Nov 2018 in Roscoff, France, and present the types of analyses made possible by the EukBank. Throughout the meeting, members of the UniEuk team will be available to answer your questions, and to provide live demonstrations of the EukMap.

Applied Phycology: Macroalgae

OPTIMAL GROWTH CONDITIONS FOR ERECT AND STOLON THALLI OF THE EDIBLE *CAULERPA OKAMURAE* IN KOREA

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To examine optimal cultivation conditions of the edible *Caulerpa okamurae* for erect fragments (3 cm long) and stolon fragments (5 cm long) were cultured in the combination of four temperatures (15, 20, 25, 30 °C) and three irradiance levels (20, 40, 80 μ mol photon m–2 s–1), and in four daylengths (8, 12, 16, 24h) and in seven salinity levels (15, 20, 25, 30, 35, 40, 45 psu). Relative growth rates (RGR) were between 0.93-4.72 % day-1 for erect thalli and between 0.85-5.57 % day-1 for stolon thalli in temperature and irradiance treatments. At 30 °C, erect thalli showed minus growth (-5.2 % day-1) but average RGR of stolon thalli was 1.96 % day-1. Maximal growth for the both thalli occurred in the combination of 25 °C and 40 μ mol photon m–2 s–1. Also, RGRs were maximal in the 16h daylengths for the erect thalli (6.46 % day-1) and for stolon thalli (6.31 % day-1), respectively. In the salinity experiment, maximal growth occurred at 30 psu and RGRs were 5.91 and 5.88 % day-1 for erect and stolon thalli, respectively. In this

study, stolon thalli were more tolerance than erect thalli in high temperature of 30 °C. Based on present results, we could cultivate *Caulerpa okamurae* in summer with long daylength (16h) and high temperature (25 °C), and in the water depth of 40 µmol photon m–2 s–1. Finally, *Caulerpa okamurae* could be cultivated in the wide range of salinity (20-45 psu) but maximal biomass can be obtained at 30 psu. These physiological data provide valuable information in relation to the successful large-scale tank or field cultivation of *Caulerpa okamurae*.

EFFECTS OF STOCKING DENSITY ON BIOREMEDIATION CAPACITY OF *GRACILARIA VERMICULOPHYLLA* IN BIOFLOC EFFLUENT

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Optimal stocking density of the marine red algae *Gracilaria vermiculophylla* was determined to maximize productivity and nitrogen removal in biofloc effluent. *G. vermiculophylla* was cultured at 0.2, 2, 4 and 8 g·L⁻¹ (FW) for 20 days at 140 µmol m⁻² s⁻¹ of light emitting diode and 12:12 L:D photoperiod. *G. vermiculophylla* was compared with effluent from a juvenile Paralichthys biofloc tank culture system and von Stosch enriched (VSE) at 20°C. The total nitrogen and phosphorus concentration of VSE medium was adjusted to the biofloc (1000µmol and 33µmol, respectively). Growth rates of both medium were inversely dependent on stocking density. However, the productivity was significantly higher at 8 than 0.2 g·L⁻¹ at both medium (VSE : 1.90 g·d⁻¹, biofloc : 1.82 g·d⁻¹). Tissue carbon contents were not significantly influenced by me medium at 8 g·L⁻¹ (34.9% in VSE and 34.0% in biofloc). However, tissue nitrogen contents were significantly higher VSE medium than biofloc medium at 8 g·L⁻¹ (3.7% in VSE and 3.4% in biofloc). The carbon removal rate was highest at the highest stocking density, 12.99 mgL·d⁻¹ (VSE) and 11.41 mgL·d⁻¹ (biofloc) respectively. Also the nitrogen removal rate was highest at the highest stocking density, 1.50 mgL·d⁻¹ (VSE) and 1.31 mgL·d⁻¹ (biofloc) respectively. The nutrient removal was not significantly influenced by me medium at 8 g·L⁻¹. The result show that *G. vermiculophylla* can be a perform productivity and nutrient removal capacity in biofloc effluent.

ENZYME-ASSISTED EXTRACTION AND CHARACTERIZATION OF PROTEIN FROM *PALMARIA PALMATA* (DULSE)

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In the coming decades, the global demand for protein is increasing mostly due to the growth of the population. The red seaweed *Palmaria palmata* is known to have high contents of protein, which makes it an interesting candidate in the search for new protein sources. The overall aim of this study was to develop a procedure to extract protein from this seaweed with high efficiency. It was an explicit goal that it should be possible to implement the process in industrial scale at a later stage. In this investigation, the optimal extraction method developed was enzyme-assisted extraction consisting of an aqueous/enzymatic extraction followed by an alkaline extraction. The protein content of the raw material and the fractions from the extraction were analyzed by Dumas method. The protein content in the raw material and in the different fractions were used to determine the protein recovery in the fractions and to determine the extraction efficiencies of the treatments. The results showed that the enzyme combinations containing Alcalase and either Celluclast or Shearzyme achieved the highest protein extraction efficiencies (> 90 %).

The amino acid content and composition in the raw material and in the fractions obtained from the treatment using the combination of Alcalase, Celluclast and Shearzyme were determined by LC-MS. The protein in the raw material and in the fractions had high quality and contained all the essential amino acids necessary for human nutrition.

OPTIMIZING SITE SELECTION FOR KELP-OYSTER CULTIVATION SYSTEMS IN RHODE ISLAND

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There is a growing interest among shellfish farmers to transition from monoculture to integrated multitrophic aquaculture (IMTA) systems that incorporate kelp. IMTA systems consist of the co-culture of multiple species from different trophic levels and attempt to reduce environmental impacts through water quality improvement. Industry interest is driving an expansion of kelp-shellfish IMTAs, however little information is available to help farmers determine where these systems will be successful. The primary goal of this research project is to develop a spatially explicit model that combines the biological requirements for kelp and oyster growth with the hydrodynamics of Narragansett Bay (NB). In order to determine conditions for optimal kelp growth, we established six kelp-oyster IMTAs in collaboration with industry partners across a biophysical and geochemical gradient; three sites were located in shallow coastal salt ponds and three in the deeper waters of NB. Two 60m longlines of nursery reared sugar kelp Saccharina latissima were planted at each site; the first in early November 2017 and the second 3-5 weeks later to test for optimal planting time. At harvest (late April 2018), the average blade length of kelp from the first planting was 0.68-1.33 m, while blades from the second planting ranged from 0.31-0.87 m long. Kelp blades reached over 1 m at sites in shallow salt ponds. Analysis of kelp production and tissue content from each site will inform a dynamic energy budget model for sugar kelp in southern New England.

SEAWEED MARICULTURE RESEARCH AND COMMERCIAL PROGRESS IN ALASKA Stekoll, M. S., University of Alaska Southeast, United States, <u>msstekoll@alaska.edu</u> Thomson, A. E., University of Alaska Fairbanks, United States, <u>aethomson2@alaska.edu</u>

Commercial seaweed production in Alaska has existed only recently. The principal species under commercial cultivation are Sugar Kelp (Saccharina latissima) Ribbon Kelp (Alaria marginata) and Dragon Kelp (Eualara fistulosa). All mariculture must be permitted through the Aquatic Farm Program operated by the state of Alaska. Permits are also required for the acquisition of parent stock, transport of live plants and the commercial operation of seaweed "hatcheries". The first successful commercial production of kelps was harvested in spring of 2017. About 5000 Kg of Saccharina and Alaria were harvested at three farms and sold to Blue Evolution. Procedures for the hatchery operations and certain aspects of the outplantings have been determined by applied research at the University of Alaska in Juneau. One bottleneck for S. latissima is locating beds for collecting parent stock. Unlike other kelps S. latissima occurs in semi-protected soft bottom locations attached to small rocks or shell fragments. Outplantings of mostly Saccharina latissima but also other kelp species on a monthly basis have laid the foundation for successful commercial outplantings. In general, outplantings of all kelps depend primarily on nutrients and secondarily on light. Growth of the kelps is optimal if baby sporophytes are set out in the fall near the time of nutrient upwelling, placed at 2-3 m depth and allowed to grow until the spring phytoplankton bloom. After the spring bloom nutrients decline and growth on the longlines slows to the point that fouling of the kelps by diatoms and bryozoans can become a problem.

EPIDERMAL SHEDDING OF BIOMASS FROM VEGETATIVE AXES AND RECEPTACLE SURFACES IN *ASCOPHYLLUM NODOSUM* (PHAEOPHYCEAE) **Garbary, D. J.,** St. Francis Xavier University, Canada, <u>dgarbary@gmail.com</u> Karcz, M., St. Francis Xavier University, Canada, <u>mkarcz@stfx.ca</u> Galway, M., St. Francis Xavier University, Canada, <u>mgalway@stfx.ca</u> Halat, M., University of British Columbia, Canada, <u>laryssa.halat@botany.ubc.ca</u>

Epidermal shedding of cell walls in *Ascophyllum nodosum* from Captains Pond in Nova Scotia was previously estimated to contribute about 1% of vegetative frond biomass per month or about 10% per year. We have now extended the quantification of shedding to include receptacle surfaces during their annual cycle. In summer, shedding from vegetative axes and from receptacles was highly correlated (r2 = 0.730, P = 0.006). Shedding was observed in all subsequent collections of receptacles carried out semimonthly during fall and winter. Based on biomass of 12 canopy fronds, divided between vegetative axes and receptacles, and morphometric analysis of receptacle surface area, we show that a single complete cycle of shedding from receptacles comprises over 1% of vegetative biomass. Extrapolating to an annual cycle brings the total production by shedding from receptacle surfaces is combined, shedding comprises 20% of total vegetative frond biomass. These results further emphasize the importance of epidermal shedding of *A. nodosum* as a contributor to coastal fertility and the impact of rockweed harvesting on biomass removal from the intertidal zone.

Cell Biology and Comparative Evolution

A NUCLEUS WITHOUT A NUCLEAR PORE COMPLEX? RECONSTRUCTING NUCLEAR MACHINERY IN NUCLEOMORPH-CONTAINING ALGAE Irwin, N., University of British Columbia, Canada, <u>nickatirwin@gmail.com</u> Keeling, P., University of British Columbia, Canada, <u>pkeeling@mail.ubc.ca</u>

Nucleomorphs are relict nuclei found in two lineages, the chlorarachniophytes and cryptophytes. These nuclei were derived following secondary endosymbioses with red and green algae and have undergone a remarkable degree of genome reduction. For example, the nuclear pore complex (NPC) is an essential nuclear feature that structures the pores of the nuclear envelope and facilitates nuclear transport, but previous studies failed to identify NPC components in nucleomorph genomes. This raises three nonexclusive possibilities: 1) the nuclear pore genes of the nucleomorph have been transferred to the host nucleus and are targeted back as proteins, 2) host nuclear pore proteins are dual targeted to both the host nucleus and nucleomorph, or 3) the nucleomorph has lost its NPC. To discern between these possibilities and investigate the composition of the NPC in nucleomorphs, we screened the transcriptomes and genomes of chlorarachniophytes, cryptophytes, red, and green algae for nuclear pore genes and characterized the hits phylogenetically. In both nucleomorph-containing groups we found little evidence of NPC gene transfer from the nucleomorph to host genome and no evidence of dual targeting. Two exceptions were algal-related NPC genes Nup98, found in chlorarachniophytes, and Rae1, found in chlorarachniophytes and cryptophytes. However, these proteins are also known to form a cell cycle regulating complex, suggesting they may function beyond the NPC. These data therefore suggest that nucleomorphs are the first eukaryotic nuclei identified to function without a canonical NPC.

HOW INOSITOL PHOSPHATES REGULATE PHOSPHATE HOMEOSTASIS IN UNICELLULAR EUKARYOTES

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Inorganic phosphate (Pi) is vital for all organisms from bacteria to humans because cells need it for synthesis of essential compounds as nucleotides and phospholipids. Deprivation or excess of Pi can be harmful, therefore normal metabolism requires a balance in cytosolic Pi. One form of buffering Pi levels is to store it as polyphosphate (polyP), a polymer of phosphates that can be degraded to release Pi to the cytosol. PolvP is stored mainly in acidic organelles that our laboratory discovered 20 years ago and named acidocalcisomes. We report here that production of polyP in acidocalcisomes and subsequent release of Pi are regulated by the signaling molecules called inositol phosphates. In this study, we characterized the vacuolar phosphate transporter Pho91, which has orthologs in protists, fungi and algae. Pho91 releases Pi from stores, so we studied Pho91 from T. brucei and Saccharomyces cerevisiae and discovered that it is a type III sodium/phosphate symporter. Pho91 knockout cells are more sensitive to DNA damage, revealing that an imbalance in cytosolic Pi may reduce nucleotide synthesis and consequently delay DNA repair. We also observed that the inositol phosphate 5-diphosphoinositol pentakisphosphate (IP₇) binds to the N-Terminal SPX domain of Pho91, known as phosphate sensor domain, and activates Pi/Na⁺ transport. The new mechanism of phosphate regulation we found is conserved between kinetoplastids and yeast, distant organisms with completely different evolutionary history and life strategies. Our findings highlight the significance of phosphate homeostasis regulation by inositol phosphates in unicellular eukaryotes.

THE DARK LECA RISES

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Reconstructing the cellular systems of the Last Eukaryote Common Ancestor (LECA) is an ongoing challenge. It is commonly accepted that LECA had all of the general features that extant eukaryotes share including a nucleus, mitochondria, flagella, a complex cytoskeletal and membrane trafficking systems, the capacity to engulf prey, and facultative sex. Although these general features of LECA are known, the degree to which LECA is a mystery remains mysterious. Ancestral reconstructions are limited by our cell biological knowledge of model organisms and the degree to which functional investigation in models might contribute to human health. This leaves a vast diversity of ancient genes neglected for which no functional information is available. In order to identify ancestral orthologue groups with little or no structural or functional information, we used sequence clustering approaches, tree building, and manual curation to identify two types of 'dark' genes in LECA. First, the dark proteome, which comprises genes encoding proteins for which domain information is available but their precise functions remain unknown. We suggest that between ~10-30% of genes in LECA lack functional or structural annotations leading to the conclusion that our knowledge of the cell biology of LECA is significantly lacking.

MEMBRANE TRAFFICKING SYSTEM SCULPTING IN HAPTOPHYTES AND ITS ROLE IN SCALE FORMATION AND CALCIFICATION **Herman, E. K.**, University of Alberta, Canada, <u>eherman@ualberta.ca</u> Zhang, X., California State University San Marcos, United States, <u>xiaoyu@csusm.edu</u> Read, B. A., California State University San Marcos, United States, <u>bread@csusm.edu</u> Dacks, J. B., University of Alberta, Canada, <u>dacks@ualberta.ca</u> The phylum Haptophyta (Diaphoretickes) includes marine algae that extrude large, distinctive calcium carbonate scales. These scales, or coccoliths, completely cover the cell, and play an important role in global carbon cycling. While the underlying cell biology of coccolith production is not fully understood, it relies on the cell's membrane trafficking system. To better understand the process of coccolith secretion, we performed a comparative genomic analysis of membrane trafficking machinery in the haptophytes Emiliania huxlevi and Gephyrocapsa oceanica, which produce coccoliths, and in the noncoccolith-producing haptophytes Isochrysis galbana and Chrysochromulina tobin. We then sought to determine how membrane trafficking genes are regulated under calcifying conditions. The calcifying E. huxleyi and G. oceanica and the non-calcifying I. galbana were grown in the presence or absence of calcium and/or bicarbonate, followed by transcriptomic sequencing. We show that haptophytes have a modified membrane trafficking system, with losses of key endolysosomal system components. In terms of membrane trafficking gene expression, components of the endolysosomal system show decreased expression under calcifying conditions in the calcifying haptophytes, suggesting an inverse relationship between calcification and the capacity for endolvsosomal degradation. We further note similar patterns of trafficking gene expression shared by the calcifying haptophytes in contrast to *I. galbana*. Overall, this work gives insight into the specific membrane trafficking pathways – and their regulation – that underlie scale formation; a biological process with global climatological consequences.

THE CRAZY AND MIXED-UP MOLECULAR EVOLUTION OF THE FORMALDEHYDE DEHYDROGENASE GENE FAMILY Littlefield, R., Clark University, United States Smith, G. J., Moss Landing Marine Laboratories, United States

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Formaldehyde is produced by all living cells as an intermediate in the breakdown of many organic compounds. The serial detoxification of formaldehyde is catalyzed by two well-conserved enzymes, formaldehyde dehydrogenase (FALDH) and s-formylglutathione hydrolase (FGH). FALDH has been shown to modulate intracellular nitric oxide levels and protein nitrosation in humans, plants, and yeast. Two independent fusions of the FALDH and FGH genes in two ciliates and two diatoms were reported previously. In this study, we provide a detailed examination of the molecular evolution FALDH with a specific focus on diatoms. FGH::FALDH fusions were observed in representative haptophytes, pelagophytes, and diatoms. Of note, two marine prasinophytes had copies of the FGH::FALDH. This gene fusion was not observed in other green algae and may represent a horizontal gene transfer. The majority of species with FGH::FALDH fusions had an additional gene encoding FALDH. Several of the key amino acids that are nitrosylated in humans, plants, and yeast are conserved in the diatom FALDHs (fused and unfused copies). The independent fusions of FGH and FALDH in ciliates and stramenopiles raises questions as to whether there is enhanced kinetic efficiency that might confer a selective advantage. In addition, it is unclear whether the fused and unfused genes serve redundant or divergent roles in cells that have multiple copies.

STUDY OF THE CONTRACTILE VACUOLE USING *DICTYOSTELIUM DISCOIDEUM* AS A MODEL

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The CV is an osmorregulatory organelle found in a wide variety of freshwater amoeba, algae, flagellates, and ciliates. It is composed of a central vacuole with associated set of membranous tubules that collect ions and water molecules feeding into the central compartment. The CV undergoes cyclical swelling and collapse, fusing with the plasma membrane and expelling Ca^{2+} and water. However, the function and the

evolution of the CV is poorly understood. In order to examine the genes involved in the CV, we have performed microscopic and transcriptomic analyses using of both wildtype and CV mutant strains *Dictyostelium* under inducing and non-inducing conditions. Analyses confirm that 1664 transcript were differentially expressed in the wild type strain while 2394 transcript were differentially expressed in the CV mutant. Results confirm the differential regulation of known membrane-trafficking markers associated with CV function (including Rab11A, sec1, Rho GTPase-activating protein gacBB) confirming our protocol's induction of CV activity. Saliently, our results also showed modulation of the transcriptional program at the systems level and specifically in the membrane-trafficking machinery involved in CV action in *Dictyostelium* and are potential targets for functional characterization. Our RNASeq results will be compared with ongoing equivalent experiments in ciliates (*Tetrahymena thermopila*) and other organisms possessing CVs, in order to assess whether CVs across eukaryotes are homologous and to gain insight into the mechanistic underpinning of CV function.

SINGLE-DOMAIN NA⁺ AND CA²⁺ CHANNELS UNDERLIE MEMBRANE EXCITABILITY IN PHYTOPLANKTON

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Four-domain voltage-gated Na⁺ and Ca²⁺ channels mediate rapid sensory responses in metazoans through fast action potentials. The ability to generate action potentials evolved in unicellular organisms, although the ion channels responsible have not been elucidated. A family of single-domain homologs of bacterial Na⁺ channels was discovered recently in phytoplankton, suggesting a unique signaling role for these proteins in unicellular eukaryotes. We expressed two homologs, one from the diatom *Odontella sinsensis* and one from the coccolithophore *Scyphosphaera apsteinii*, in the mammalian cell line HEK293. Whole cell patch clamp analysis revealed both proteins mediate fast activating and rapidly inactivating inward currents in response to membrane depolarization. Mammalian four-domain channels have similar kinetics, suggesting that the single-domain channels form complexes of homotetramers. However, the kinetics and permeability of the diatom and coccolithophore single domain channel were distinct. The coccolithophore channel exhibits fast kinetics and Na⁺ selectivity, whereas the diatom channel mediated slower currents mediated by Na⁺ and Ca²⁺ influx. Substitution of aspartic acid for glutamic acid in the pore sequence of the channel may explain the Ca²⁺ permeability of the diatom channel suggest unique roles in each organism, with the diatom channel specifically playing a role in Ca²⁺ signaling.

Ecology: Community Interactions

LOCAL HABITAT HETEROGENEITY CONTRIBUTED TO THE PERSISTENCE OF *SARGASSUM SILIQUASTRUM* POPULATIONS AGAINST SEA URCHIN GRAZING Yeung, C. W., The Chinese University of Hong Kong, China, <u>bugyeung@gmail.com</u> **Ang, P.**, The Chinese University of Hong Kong, China, <u>put-ang@cuhk.edu.hk</u>

Barren ground due to sea urchin overgrazing is common in rocky shores in Hong Kong, southern China. However, in some sites, populations of *Sargassum siliquastrum* persist. We hypothesized that

heterogeneous habitat with isolated rocky outcrops surrounded by sand patches served as refugia for *Sargassum* as the main grazer *Anthocidaris crassispina* cannot cross the sand patches. We tested this hypothesis by setting up artificial habitats using granite rocks seeded with *Sargassum* thalli and fixed number of urchins. In the heterogenous habitat set-up, isolated rocks with *Sargassum* were separated from rocks with urchins by sand patches; whereas in the homogenous habitat, these rocks were bridged with additional rocks in between. Cage covered isolated rock with *Sargassum* served as the control. Four replicated sets were set up in three different sites, including a mesocosm site in the Laboratory. Results indicated that within the first 10 days, up to 95% of urchins moved into the bridged rocks but only <5-15% of them moved into the isolated rocks. *Sargassum* thalli on bridged rock were significantly grazed down within 10-30 days between sites but not in isolated rocks. All surviving thalli, including those on control rocks, eventually declined naturally. The experimental results supported the hypothesis and suggest that *Sargassum* populations in these rock outcrops could persist and serve as the source of *Sargassum* propagules to repopulate the barren ground, helping to maintain the algal population against sea urchin grazing.

FROM SINGLE SPECIES TO ECOSYSTEM RESPONSE: IMPACT OF CLIMATE CHANGE ON PRODUCTION AND TROPHIC TRANSFER OF ESSENTIAL FATTY ACIDS IN THE COASTAL FOOD WEB

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Single species responses to future ocean conditions are becoming increasingly better understood. There is now a need to investigate from a more complex perspective including interactions of numerous environmental stressors and responses on a whole ecosystem level. Marine food webs, which include many economically and ecologically important species, ultimately depend on energy generated by primary producers such as seaweeds. Seaweeds are particularly unique as they metabolize omega-3 and omega-6 polyunsaturated fatty acids (PUFA), in particular the long-chain (≥C20) PUFA (LC-PUFA), which play essential physiological roles both in the algae themselves and in animals that consume them. The environmental conditions strongly impact the concentrations and composition of fatty acids in seaweeds. The expected climatic changes including increased temperature and levels of dissolved CO2 in the future ocean are projected to lead to a decline in essential LC-PUFA in seaweeds with flow-on effects to higher trophic levels. The presented project aims to investigate the impacts of ocean warming and ocean acidification on production and trophic transfer of the key LC-PUFA in a laboratory based coastal model community. Results will provide an improved understanding of lipid turnovers and foster a better understanding of energy flow in the current and future coastal food web.

ECOLOGICAL APPROACHES FOR COMMUNITY SHIFT FROM BARRENS TO MARINE FOREST AND THE OPTIMAL DENSITY OF SEA URCHINS FOR SUSTAINABLE MAINTENANCE

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Ecological approaches have been conducting in two coastal areas, the east coast and Jeju coast in Korea, to recover kelp beds from existing urchin barrens. Korean east coast has been suffering marine deforestation last decades, comprising about 60% of all rocky shallow subtidal area, with the average density of urchins 7.8 individuals/m² in the completely barren sites. Experimental manipulation of sea

urchin density was conducted on the nine natural bed rocks of barren condition, each separated by sandy barriers in between. Three experimental conditions were applied: all urchin removal, "half"-removal, control for no-removal. At all removal sites, macroalgal community was recovered in good shape for three consecutive spring seasons (2015-2017), whereas barren state continued at the no-removal control sites. For the "half"-removal condition, we started with 4.0 individuals/m², and then switched to 2.5 individuals/m² to achieve an optimal density level, which may be necessary for the maintenance of kelp forest. A larger scaled approach is on-going this year. In the case of Jeju coast, where sea urchin is not common, barren condition is presumably caused by multiple stress factors. We applied the metapopulation concept to artificial reefs (with different substrate types) to improve the chance of natural recruitment of seaweeds as the function of distance from nearby existing source kelp beds. On-going results will be introduced in the presentation.

PHOTOSYNTHETIC PHYSIOLOGY OF CORALLINE ALGAE ACROSS DEPTH/LIGHT GRADIENTS AND BETWEEN CRUSTOSE AND ARTICULATED GROUPS IN A KELP FOREST IN SOUTHERN NEW ZEALAND

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Coralline algae are a dominant component of rocky reefs in temperate climates. A comparison of the photosynthetic physiology of the articulated coralline algae, *Arthrocardia* sp., and a group of unidentified crustose species in the order Hapalidiales was made in a kelp forest in Southern New Zealand. Comparisons were also made between the crustose coralline algal community at 2 m and 10 m depth. Photosynthetic performance of coralline algae was determined at 24 irradiance levels ranging from 0 to 1800 µmol photon m₋₂ s₋₁ at 12°C. Photosynthesis vs. Irradiance (PE) curves were used to provide key photosynthetic parameters including Pmax (maximum O2 evolution rate at saturating irradiance), alpha (slope of the PE curve at limiting irradiances), Rd (dark respiration rate), Ec (compensation irradiance) and Ek (saturation irradiance). Coralline algae were shown to photosynthesis across a broad range of light intensities. Pmax of the articulated coralline algae at 2m depth was significantly higher (20.38 ± 2.34 µmol O₂ gdw⁻¹ h⁻¹) than crustose corallines at 2 and 10 m depth (6.11 ± 2.46 and 2.79 ± 0.74 µmol O₂ gdw⁻¹ h⁻¹, respectively). There was no significant effect of depth on photosynthetic performance but variability in these parameters was greater at the shallower depth. This was likely driven by greater variability in the light environment in shallower sections of the reef as a result of habitat complexity.

ASSESSING BOTTOM-UP AND TOP-DOWN CONTROLS USING MICROCOSM AND 'OMICS. **Grattepanche, J.,** Smith College, United States, jgrattepanche@smith.edu McManus, G. B., University of Connecticut, United States, george.mcmanus@uconn.edu Katz, L. A., Smith College, United States, <u>lkatz@smith.edu</u>

Marine microbial eukaryotes play critical roles in planktonic food webs, but their interactions and controls are not yet well understood. We used a microcosm experiment and high-throughput sequencing to analyze taxonomic diversity of SAR (Stramenopila, Alveolata, and Rhizaria by amplicon) and functional diversity (metatranscriptome) related to trophic pressure. We carried out microcosm experiment for which we incubated in situ community with in increasing abundance of phytoplankton (a diatom, a haptophyte, and a chlorophyte from none to $10e^4/mL$) and copepod (from none to 20/L). We observed that (1) copepods tends to decrease SAR diversity and the contribution of Alveolata, in particular Spirotrichea, while Stramenopila increase in particular from the Phaeophyceae and Xanthophyceae; (2) Ciliate contribution increase with increasing phytoplankton. Looking at the community function, we observe that the main function that are upregulated in our microcosm are related to translation (EF1 alpha, ribosomal proteins), energy production and conversion (photosynthesis), and

cytoskeleton (Actin, tubulin). Using our phylogenomic pipeline, we observed that these upregulated transcripts are mainly from phytoplankton, but not the same that the phytoplankton we added. This suggest that, while taxonomy showed an increase of heterotroph, (1) phytoplankton are more active than heterotroph, and (2) that adding phytoplankton have a positive impact on phytoplankton function. Together, these data show the great potential of combining experiment and 'omics, and reinforce that we still have much to learn about microbial diversity in marine ecosystems to understand the biogeochemical cycles.

WITH A LITTLE KELP FROM MY FRIENDS: BEASTS THAT UTILISE IRISH KELP FORESTS AND THE MULTIFACETED WAY THAT KELPS STRUCTURE THESE EMERALD SEAS. Schoenrock, K. M., NUI Galway, Ireland, <u>kathryn.schoenrock@nuigalway.ie</u> Stengel, D. B., NUI Galway, Ireland, <u>dagmar.stengel@nuigalway.ie</u>

Until recently ecological processes within kelp forests along the Irish coastlines have not been studied intensively. In this current study we examine the productivity of kelp forests dominated by *Laminaria hyperborea*, and kelp-associated biodiversity at multiple scales. We evaluate seasonal kelp productivity through growth, kelp surveys, total standing stock of biomass, and we investigate patterns storage compounds across age groups. Assessment of age distribution within kelp forests allows the evaluation of population dynamics and a prediction of sustainability of the kelp forests at different sites. The biodiversity of kelp forest macro-fauna is assessed through kelp forest surveys seasonally, and then compared to other habitats structured by bedrock or calcifying organisms. Kelp as a habitat, the biodiversity associated with kelp stipes and holdfasts, is assessed through in lab dissections of kelp collections. The diversity of organisms that select to settle within kelp forests is assessed through recruitment habitats in the water column and benthic habitats seasonally. Such data provide information on seasonal patterns in kelp forest populations, kelp productivity, and can foster future management strategies for conservation or harvesting of these natural resources.

DETERMINANTS OF COMMUNITY STRUCTURE ON A HIGH-LATITUDE ROCKY SHORE **Klinger, T**., University of Washington, United States, <u>tklinger@uw.edu</u>

We used a 19-year time series of observations to identify physical and biological forces that shape *Fucus* communities on semi-protected rocky shores in the Gulf of Alaska. We conducted annual censuses of permanent plots in Kasitsna Bay, Alaska. Initial analysis using non-metric multidimensional scaling (NMDS) revealed that community composition shifted significantly between positive and negative phases of the Pacific Decadal Oscillation (PDO). Because temperature is a major component of the PDO, we explored the influence of annual air temperature and June water temperature, plus annual freshwater discharge (FWD) and biological interactions, on the abundance of dominant taxonomic groups. Using structural equation modeling (SEM), we found that the abundance of *Mytilus* was directly positively influenced by the abundance of Pterosiphonia, June water temperature, and FWD. Notably, the effect of Pterosiphonia on Mytilus constituted the strongest interaction in the model. The abundance of barnacles other than spat was positively influenced by Fucus abundance, FWD, and the abundance of littorines, and was negatively influenced by the abundance of *Pterosiphonia*. The abundance of adult *Fucus* was negatively associated with annual air temperature and with the abundance of lottids. In contrast, the abundance of *Fucus* germlings was positively influenced by the abundance of barnacles and negatively influenced by the abundance of *Mytilus* and *Pterosiphonia*. The strong signal of the PDO and the clear importance of FWD to the structure of this community indicate the importance of physical factors in this downwelling-dominated high latitude system.

Insights from Novel Genomic Tools

A GENOMIC APPROACH TO THE THERMOACIDOPHILIC ENVIRONMENTAL ADAPTATION OF CYANIDIALES (RHODOPHYTA)

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Cyanidiales is the only one eukaryotic lineage that thrives in highly acidic, high temperature, and heavymetal rich extreme environment found around volcanic hot springs around the world. Although some Bacteria and Archaea are reported from extreme environments, there are any eukaryotes, except Cyanidiales, reported from these harsh conditions. In addition, they show trophic differences being either autotroph or mixotroph. From previous researches, the genomes of Cyanidiales are highly reduced, but horizontally acquired the bacterial gene set to adopt in extreme habitats. To better understand the genome evolution of the Cyanidiales, we produced two high quality nuclear genome data from *Galdieria maxima* 8.1.23 and *Galdieria sulphuraria* SAG 108.79. We conducted comparative genome analysis with three available genomes of *Cyanidioschyzon merolae*, *Galdieria sulphuraria*, and *Galdieria phlegrea*. In this presentation, we will discuss about genomic differences in five Cyanidiales genomes in terms of gene contents, mutation rates, and repeat regions. Furthermore, we found unique horizontally the transferred gene set that related to heavy metal reduction (mercuric reductase, arsenite methyltransferase) that likely related to the adaptation in this harsh condition for *Galdieira maxima* 8.1.23.

DEVELOPMENT OF AN EXPERIMENTAL MODEL FOR STUDYING LATERAL GENE TRANSFER IN EUKARYOTES

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Lateral gene transfer (LGT) is well recognized as an important evolutionary force in prokaryotes. LGT has been proposed to play a role in eukaryotic evolution as well, based on evidence generated during whole genome sequencing projects. However, there is currently little or no experimental evidence to support the occurrence, frequency, and mode(s) of eukaryotic LGT. To this end, an experimental system is being developed with which to test the possibility of LGT into a eukaryotic organism, *Acanthamoeba castellanii*, through feeding with transgenic bacterial prey. Nanopore sequencing has been used to generate a new reference genome sequence for *A. castellanii* strain Neff, and, combined with molecular biological methods, is helping to elucidate the process of transgene integration when the same transgenes are transfected artificially into *A. castellanii*. Feeding experiments are underway in an attempt to observe gene transfer events in laboratory cultures, and characterize the frequency and mechanism of these events

AN EXPLORATION OF LONG TERMINAL REPEAT RETROTRANSPOSON ACTIVITY IN THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM* USING FINE-SCALE GENOMIC INVESTIGATION

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The marine pennate diatom *Phaeodactylum tricornutum* has emerged as a model system for exploring genetic factors underlying the unparalleled diversity and success of diatoms in the world's oceans. Previous genomic analyses have indicated that transposable elements (TEs) are abundant in *P. tricornutum*, comprising ~6% of the of the 27.4 Mb genome. The majority of TEs present in *P. tricornutum* are long terminal repeat retrotransposons (LTR-RTs), which are characterized by long terminal repeat sequences flanking the central coding domain and replication via reverse transcription of

an mRNA intermediate. Recent reinvestigation of the *P. tricornutum* genome using long-read sequencing technology has provided additional insight into the unusually active nature of LTR-RTs in this species. Our sequencing efforts have detected copia-type LTR-RTs that are both chromosomally integrated as well as non-integrated, suggesting that at least some of the elements are undergoing continuous reverse transcription. The potential impact of LTR-RTs on the structure, regulation and diversity of diatom genomes will be discussed.

DEVELOPING A GENE TRANSFER SYSTEM FOR DINOFLAGELLATES **Sprecher, B. N**., University of Connecticut, United States, <u>brittany.sprecher@uconn.edu</u> Zhang, H., University of Connecticut, United States Lin, S., University of Connecticut, United States

Dinoflagellates are an important class of phytoplankton encompassing species responsible for harmful algal blooms, species that symbiotically live with cnidarians and are critical for healthy coral reefs, and others that are important primary producers or consumers within their ecosystems; however, despite their importance, current molecular understanding of basic dinoflagellate biology remains limited. To circumvent this shortcoming, much effort has focused on dinoflagellate transcriptomics and genomics. Approximately 50% of genes found through these methods remain uncharacterized or novel. We are currently developing a genetic manipulation system for dinoflagellates, allowing for the study and understanding of important gene regulation pathways that underlie dinoflagellate biology. Using various selection agents and a green fluorescence reporter, we show our preliminary results for two dinoflagellate species, *Oxyrrhis marina* and *Karlodinium veneficum*. If these methods prove to be reproducible, researchers from various fields can begin to develop a deeper molecular understanding of dinoflagellates.

SINGLE-CELL RNA SEQUENCING OF ENVIRONMENTAL PROTISTS TO EXPAND THE EUKARYOTIC TREE OF LIFE

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Genome sequencing efforts of eukaryotic species have a strong bias towards more complex, multicellular organisms such as animals, plants and fungi. Yet, the majority of eukaryotic diversity is dominated by microbial eukaryotes (protists). Recent technological advances are now allowing for the generation of genomic data from environmental protists without establishing a culture, opening a window to explore the diversity of microbial eukaryotes at the genomic level. In the current study, we have applied RNA sequencing at the single-cell level to sample the diversity of environmental protists, focusing on protists in the $5 - 25 \,\mu$ m size range. Our results show that our method is robust and can be applied on a wide diversity of protist lineages with varying cell sizes. By collecting samples from 12 different sites and generating 120 cDNA libraries we could generate transcriptomic data of new species from every major clade in the eukaryotic tree. Among these new taxa we could find several Katablepharid species, which branch in a poorly characterized part of the tree. Other new species we could find were excavates from the group Skinetoplastids, Euglenids and Diplomonads, amoeba from the class Discosea, Rhizaria from the group Cercozoa and green algae from the order Pyramimonadales. On average we retrieved 55 markers per cell from a 159-marker dataset that was used to build phylogenies, indicating the suitability for this method to perform phylogenomic analysis.

DEVELOPING FUNCTIONAL GENETIC TOOLS FOR DINOFLAGELLATES: PROGRESS AND LESSONS

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Dinoflagellates are an ecologically important group of protists with many cytological peculiarities and evolutionary distinction. Their generally huge and varied genomes harbor numerous unique features. The

molecular bases of all these outstanding characteristics are poorly understood because the large number of genes these organisms possess remain to be functionally characterized and yet there is no effective functional genetic tool available. To address the gap, my laboratory has launched efforts to develop a tool through several different venues, ranging from expressing dinoflagellate genes in model organisms (mammalian cells and diatoms), using small interfering RNA (siRNA) to depress expression of genes in dinoflagellates, and gene transformation and CRISPR/Cas9 in dinoflagellates. Challenges have been encountered in most of these methodologies, but encouraging progress has been achieved in some of these. Some of the technical challenges include codon incompatibility, lack of effective selection marker, autofluorescence interference in reporter signal, and poor reproducibility of transformation. Initial results and lessons learned will be presented.

INSPIRATION FROM RED ALGAL LECTINS FOR THE ACTIVE EXPRESSION OF RECOMBINANT LECTINS

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Red algal lectins have a remarkable structure, consisting of small tandem repeat peptides. The tandem structure is predicted to be a factor contributing to their stability and active conformation. Since many marine algal lectins cannot be expressed in their active forms in a bacterial expression system, it has been a hurdle to commercialization or application in research. In this study, we performed a trial experiment to create an active recombinant green algal lectin, BPL3, which cannot otherwise be expressed in bacterial cells. Inspired by the tandem repeat structure of red algal lectin, artificial dimerization of BPL3 sequence was performed, and the dimeric form (rD2BPL3) contributed to the expression of active protein. The expression level was influenced more than 50-fold compared to that of monomeric form (rD1BPL3) by the treatment of 1 mM IPTG at 20 °C. The expressed protein showed hemagglutination properties similar to those seen in native BPL3. Both proteins could agglutinate human erythrocytes and were inhibited by N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-galactosamine (GalNAc). Despite their similar properties in the agglutination test, its activity was not notably higher than that of the native protein. Glycan array results showed that recombinant and native BPL3 exhibited different binding properties; both showed weak binding to α-Mannose (α-Man-Sp). While native BPL3 showed strong binding specificity to the alpha conformation of amino sugars, rD2BPL3 had binding specificity for the beta conformation. The suitability of a process, developed in this study, for the quality-controlled production of recombinant lectins is discussed.

Genomics opportunity session and discussion

OPPORTUNITIES FOR PROTIST RESEARCH FUNDING FROM THE NATIONAL SCIENCE FOUNDATION.

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Over the past ten years, the National Science Foundation (NSF) has devoted significant resources toward projects attempting to clarify under-sampled areas of the tree of life. Starting with the Assembling the Tree of Life (AToL) program, and the subsequent GoLife program, the Division of Environmental Biology has placed an emphasis on systematics and evolutionary research in protist lineages. Recently the GoLife program was ended and a new track in the core Systematics and Biodiversity Sciences cluster was opened for proposals on Poorly Sampled and Unknown Taxa (*PurSUit*). Opportunities to collaborate and connect laboratories within and outside the US will be discussed, as well as a workshop and planned

Research Coordination Network proposal to stimulate larger coordinated proposals to advance protist research using NSF funding.

10KP: A PHYLODIVERSE GENOME SEQUENCING PLAN

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Much of the tree of eukaryotic life remains unexplored at the level of genome sequencing. The recently announced 10KP (10,000 Plants) Genome Sequencing Project (<u>https://db.cngb.org/10kp/</u>) aims to sequence genomes from ~9,000 embryophytes and ~4,000 algae and protists within the next five years. Supported by the Beijing Genomics Institute (BGI) in Shenzhen, China, 10KP is structured as an international consortium and is open to the global scientific community. The resulting data will be freely available. Here I will provide a brief overview of the project with special emphasis on the genomes and transcriptomes of ~1,000 heterotrophic protists to be sequenced.

ALGAL GENOMICS FOR ENERGY AND ENVIRONMENT

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Algae account for about a half of Earth's photosynthesis, play a critical role in the carbon cycle, and are being explored as sources for bioproducts and biofuels. To fully exploit the vast diversity of algae, US Department of Energy Joint Genome Institute (JGI) is sequencing the genomes of representatives from the major clades, often including the 1st or only genome of that clade to be sequenced. Over forty algal genomes are available through the JGI Genome Portal (<u>https://genome.jgi.doe.gov/Algae/</u>) equipped with interactive analysis tools to enable comparative genomics. Multi-omics data production empowers metabolic modeling and strain optimization. The JGI Community Science Program (CSP) enables the scientific community to accelerate genomics exploration of algae by providing access to high-throughput sequencing, synthesis, and functional genomics resources JGI for projects of relevance to DOE missions.

Protist Behavior and Ecological Strategies

IDENTIFICATION AND FEEDING CHARACTERISTICS OF THE MIXOTROPHIC FLAGELLATE *POTERIOOCHROMONAS MALHAMENSIS*

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In this study, a mixotrophic flagellate chrysophyte was observed in samples from outdoor mass *Chlorella* culture. Data from field studies demonstrated that once the predatory flagellate was observed in the algal culture, there was a dramatic reduction in *Chlorella* cell concentration. The grazer was identified as *Poterioochromonas malhamensis*, based on both morphology and molecular barcoding employing 18S rDNA gene sequences. Transmission electron microscopy revealed that *P. malhamensis* digested the ingested *Chlorella sorokiniana* cells within a food vacuole, with residual cell wall from the prey being observed in the final phase. It was noted that during the process of ingesting and digesting the *C. sorokiniana* cells, the chloroplast of *P. malhamensis* reduced in size, whereas the number and volume of mitochondria increased. After the prey had been completely digested, the chloroplast and mitochondria of *P. malhamensis* returned to their pre-feeding status. Feeding experiments in the laboratory demonstrated that cell concentrations of *C. sorokiniana* and *P. malhamensis* were negatively correlated. Furthermore, once *P. malhamensis* reached 5×105 cells mL–1 and the ratio of C. sorokiniana to *P. malhamensis* was in the range 15:1 to 30:1, the cell concentration of the *C. sorokiniana* culture decreased dramatically. The

optimal temperature for *P. malhamensis* grazing on *C. sorokiniana* was 25 °C; the grazing ability of *P. malhamensis* in light conditions was higher than that in dark conditions; and *P. malhamensis* preferred weakly acidic conditions. In addition, it was demonstrated that this flagellate could graze on other common microalgae of commercial value.

BACTERIA INFLUENCE THE MULTICELLULAR DEVELOPMENT IN THE AMOEBA COPROMYXA PROTEA

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Copromyxa protea is a dung inhabiting sorocarpic amoeba that forms macroscopic multicellular fruiting bodies through the cooperation of thousands of individuals. Its life cycle is similar to the model dictyostelids, but they are not specifically related, occupying different branches of the deepest bifurcations of Amoebozoa. In wild multicellular fruiting bodies isolated from dung, bacteria are dispersed within a slime matrix of Copromyxa spores. Strains cultured onto sterile dung, with E. coli as food, are able to maintain the multicellular phenotype indefinitely. Interestingly, when *Copromyxa* is cultured on artificial agar media with E. coli, although the cells grow, the multicellular phenotype is lost and not recoverable when inoculated back onto dung. Through 16S bacterial identification on multicellular and non-multicellular strains, we find that a bacterial community is much more diverse in in multicellular strains with 44 unique bacterial OTUs. Notably, when the strain was moved from dung to artificial media, the multicellular capability was lost along with a large subset (40 bacterial OTUs). Reintroduction of a dung maintained bacterial community recovers multicellularity in non-multicellular strains. These data imply that the bacterial community are directly influencing the multicellular phenotype. This provides a testable and manipulatable system for examining the interplay between the bacterial community and the capability of multicellularity in this amoeba. Interestingly, this same pattern of behavior is observed in the dung inhabiting sorocarpic members of 3 other supergroups suggesting coevolution with bacteria may have led to these convergent behaviors.

METATRANSCRIPTOMICS AND THE ECOLOGY OF *NEMATODINIUM ARMATUM* (DINOFLAGELLATA, WARNOWIACEAE)

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Warnowiids are perhaps the most enigmatic dinoflagellates, and are characterized by elaborate, eye-like ocelloids. *Nematodinium armatum* is the most abundant warnowiid in temperate waters, and--bearing complex "nematocysts" in addition to its ocelloid--is a paragon of cellular complexity. Yet both the ecology and evolution of warnowiids and their remarkable features are poorly understood. To clarify this, we sequenced the first warnowiid metatranscriptome, obtaining 232,025 transcripts from approximately 200 cells of *Nematodinium* (and their prey), as they underwent a rare bloom event in Saanich Inlet, BC. We used these data to investigate whether *Nematodinium* is mixotrophic or heterotrophic, to molecularly characterize its potential prey, and to test hypotheses regarding the origin of its nematocysts. Through live observation of net-caught cells, we also recorded its diurnal migratory behavior as well as novel interactions with prey and parasitoids.

PARASITES OF HETEROTROPHIC PROTISTS: GIANT VIRUSES AND TINY BACTERIA AT THE EDGE OF LIFE

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Heterotrophic protists are abundant in aquatic ecosystems. Their phagotrophic lifestyle makes them vulnerable to highly unusual pathogens, ranging from the largest known viruses to the smallest bacterial

parasites, the former often surpassing the latter in complexity. This relationship is exemplified by two parasites infecting the abundant heterotrophic fresh water nanoflagellates, *Bodo saltans* and *Spumella elongata*. With a genome of 1.4 Mbp, Bodo saltans virus (BsV) is one of the largest viruses ever isolated and sequenced. The BsV genome reveals surprising adaptations to the unusual biology of its kinetoplastid host, such as a turnover in its translation machinery. Genomic expansions, forced by an escalating host-virus arms race, provide insights into the evolution and origin of giant viruses. BsV's features are contrasted by *Chromulinavorax destructans*, a member of the candidate phylum TM6/Dependentiae that infects the Chrysophyte *Spumella elongata*. With a genome of 1.2Mbp, smaller than that of BsV, this obligate intracellular parasitic bacterium replicates in viral-like replication bodies that are surrounded by the host mitochondrion. Extensive genomic reduction resulted in the complete absence of detectable metabolic pathways, instead, metabolites, as well as ATP, are imported by an extensive array of transporters. Together, these pathogens show a remarkable case of convergent evolution across the boundary of life. At the same time, their unusual genome content, 50% of which shows no recognizable homologies to known genes, suggests that these parasites represent one of the last frontiers of unexplored genomic diversity.

SLIDING BEHAVIOR OF *TETRAHYMENA PYRIFORMIS* NEAR A WALL **Nishigami, Y**., Kyoto University, Japan, <u>nishigami@chem.scphys.kyoto-u.ac.jp</u> Ohmura, T., Kyoto University, Japan, <u>ohmura@chem.scphys.kyoto-u.ac.jp</u> Ichikawa, M., Kyoto University, Japan, <u>ichi@scphys.kyoto-u.ac.jp</u>

To survive in nature, protists exhibit intelligent behaviors. Though it's well known that ciliates swim freely in bulk water, they also accumulate on interfaces of structure (bottom of a pond, surfaces of stone, waterweed, and biofilm, etc.), which is important to obtain sufficient nutrients and stable environment. The object of this study is elucidating the mechanism of the accumulation of ciliates near the liquid-solid interface. Observation of behavior of *Tetrahymena pyriformis* revealed that they slid on the glass surface and beating of cilia which attached to the glass substrate was stopped. To verify whether this mechanosensing phenomenon of each cilium induced sliding behavior of *T. pyriformis*, we performed hydrodynamic simulations of self-propelled swimmers. When the swimmer was considered as spherical with the mechanosensing system, the numerical swimmer didn't slide on the surface. On the other hand, when the shape of the swimmer was ellipsoid with the mechanosensing system, the numerical swimmer slid on the surface. These experiments and simulation results indicated that the shape of cell and the mechanosensing system of cilia induce sliding motion and the accumulation of *T. pyriformis* on the liquid-solid interfaces.

PHAGOCYTOSIS IMPLIES DYNAMIC NUTRITIONAL STRATEGIES IN DIPLOID CALCIFYING COCCOLITHOPHORES

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Mixotrophy is widespread in unicellular marine algae with smaller size groups obtaining up to 25% of their biomass via bactivory (Zubkov, M.V. et al. 2008 *Nature* 455, 224-248). Photosynthetic dinoflagellates in particular exhibit a variety of nutritional strategies, often combining phagotrophy with autotrophy. The heterotropic and mixotrophic origins of the haptophytes are also well recognized, and genomic studies of haploid and diploid (calcifying) stages of coccolithophores suggest they possess the molecular machinery and capability to phagocytose and enzymatically degrade potential prev particles (Houdan, A., et al. 2006 *Aquat. Microb. Ecol.* 44, 291-301, Rokitta, S.D., et al. 2011 *J. Phycol.* 47, 829-838). Using fluorescently labeled pH sensitive prev and confocal microscopy, we demonstrate phagocytosis and development of acidic phagosomes in the 2N non-motile calcifying stages of

Scyphosphaera apsteinii. Treatments with pharmacological compounds suggested novel H⁺ transport mechanisms may be involved phagosome development. Experiments with fluorescently labeled beads revealed *S. apsteinii* ingested and accumulated beads up to 2 microns in diameter. This implies larger coccolithophores may not be limited to bactivory, and could potentially consume larger picoeukaryote prey. Overall, our data supports mixotrophy through phagocytosis in calcifying coccolithophores that has important implications for understanding nutritional strategies in these globally important marine protists.

THE *MESODINIUM* GENUS AS A MODEL FOR SECONDARY ENDOSYMBIOSIS? **Moeller, H. V.**, University of California, Santa Barbara, United States, <u>holly.moeller@lifesci.ucsb.edu</u> Neubert, M. G., Woods Hole Oceanographic Institution, United States, <u>mneubert@whoi.edu</u> Peltomaa, E., University of Helsinki, Finland, <u>elina.peltomaa@helsinki.fi</u> Johnson, M. D., Woods Hole Oceanographic Institution, United States, <u>mattjohnson@whoi.edu</u>

Kleptoplastidic microeukaryotes transiently obtain photosynthetic abilities by retaining stolen chloroplasts from their algal prey. These acquisitions provide insight into the endosymbiosis pathway that led to the permanent incorporation of plastids into diverse extant eukaryotic phytoplankton lineages. However, understanding the steps along this pathway is hindered by our ability to effectively contrast the ecology and physiology of different kleptoplastidic lineages. Therefore, we are developing the *Mesodinium* genus of kleptoplastidic ciliates as a model system for secondary endosymbiosis. This genus includes species that range from purely heterotrophic (the basal lineage *M. pulex*) to almost exclusively phototrophic (*M. rubrum*, which obtains >95% of its carbon from photosynthesis conducted using stolen organelles). Using a series of experiments quantifying grazing rates, plastid retention, and photophysiology, we find that as *Mesodinium* lineages evolved toward greater reliance on photosynthesis, they also evolved toward greater prey specificity. In part, this is likely driven by increasingly elaborate plastid control mechanisms. By combining our empirical findings with an eco-evolutionary model, we demonstrate that specialization—on prey and on metabolism—can enable coexistence of multiple lineages. Our findings therefore have implications for the evolutionary diversification of modern eukaryotic phytoplankton.

Biogeography of Seaweeds

MESOPHOTIC MACROALGAE REVEALED: NEW DISCOVERIES IN THE NEW ZEALAND ARCHIPELAGO Nelson, W. A., NIWA / University of Auckland, New Zealand, <u>wendy.nelson@niwa.co.nz</u> D'Archino, R., NIWA, New Zealand, <u>roberta.darchino@niwa.co.nz</u> Neill, K. F., NIWA, New Zealand, <u>kate.neill@niwa.co.nz</u> Sutherland, J. E., NIWA, New Zealand, <u>judy.sutherland@niwa.co.nz</u> Twist, B. A., University of Auckland, New Zealand, <u>brenton.twist@niwa.co.nz</u>

Over the past decade, important data on the presence and distribution of mesophotic algae (ca. 30-150+m depth) have been collected as part of programmes conducted to characterise the coastal and shelf region of the New Zealand archipelago [which extends from 29°S (Rangitāhua/Kermadec Islands) through to 53.5°S (Campbell Island)]. Using video and still imagery, mesophotic macroalgae have been revealed at depths up to 160m, with specimens collected via epibenthic sleds and dredges. Molecular sequence data are critical for investigating the identity and relationships of these deepwater algae. Examples of the mesophotic flora will be presented including previously undocumented beds of macroalgae (including kelps), new species' distribution data, and investigations of specific deepwater assemblages (non-geniculate coralline algae in biogenic habitats, rhodolith populations), in the context of current understanding of the evolution and biogeography of the New Zealand flora.

SUBTIDAL SEAWEEDS AND SEAGRASSES IN 3 OREGON MARINE RESERVES Hansen, G. I., Oregon State University, United States, <u>hansengi@outlook.com</u>

To protect its marine flora and fauna, the State of Oregon has established along its outer coast 5 subtidal no-take marine reserves, off-limits to all harvest except as necessary for monitoring and research. Before closure, baseline studies of sub-canopy seaweed and seagrass species richness and biomass were completed at 3 of these reserves (Redfish Rocks, Otter Rock, and Cascade Head). A depth-stratified (5-15 m) random sampling method was used to investigate the "reserve areas" which consisted of the reserve and a nearby comparison area. $30-36 (0.25 \text{ m}^2)$ quadrats were placed within each reserve area and extracted by divers. The quadrat material was then examined for macrophytic species richness and biomass. Together, the reserve areas yielded a total of 142 species: 30% were common to all 3 areas, but 50% were found in only 1 area. Red algae comprised 80% of the species, but the highest biomass occurred among the brown algae: Laminaria longipes dominated Redfish Rocks and Cascade Head and Desmarestia herbaceae dominated Otter Rock. Otter Rock had the greatest overall diversity with an average of 32 macrophytic species/quadrat. It also had the greatest species uniformity with 19% of the species occurring in >50% of the quadrats. Redfish Rocks was the most depauperate and patchy averaging 9 species/quadrat with only 6% that extended into >50% of the quadrats. The most prevalent species was the red alga *Callophyllis flabellulata*, which occurred in 92% of the sampled quadrats. Bray-Curtis-MDS plots will illustrate the overall and within-area quadrat similarities.

ON THE ORIGINS OF NORTH AMERICAN ARCTIC MARINE MACROALGAE SINCE THE LAST GLACIAL MAXIMUM AND EVIDENCE FOR ARCTIC PERIGLACIAL REFUGIA **Bringloe, T. T.**, University of New Brunswick, Canada, <u>Trevor.Bringloe@unb.ca</u> Saunders, G. W., University of New Brunswick, Canada, <u>gws@unb.ca</u>

Marine macroalgae are expected to move northwards into the Arctic as isotherms retreat and perennial ice-cover diminishes. Understanding Arctic recolonization since the Last Glacial Maximum may aid in biomonitoring efforts by identifying key pathways for marine flora entering the North. We sought to improve understanding of Arctic recolonization in marine macroalgae by supplementing existing DNA barcode records with sampling from Baffin Island, through Northern Labrador (Canada), the Beaufort Sea and Nome (Alaska, USA), and Bergen (Norway). The origins of North American Arctic populations were inferred based on haplotype patterns. We accumulated sequence data for 4231 specimens, which represented 42 species of red, 46 brown, and 12 green algae. Of these species with Arctic populations, 38 had uncertain origins. 47 had origins in the North Atlantic, five had origins in the Pacific and the Atlantic. while the remaining 10 had origins in the North Pacific. Pacific contributions to Arctic recolonization, however, were inferred in 9/12 of the best sampled species (e.g. broad coverage across all three basins and >20 Arctic records). Our results indicated that the Pacific contribution to Arctic recolonization has been historically underestimated. Surprisingly, 18 genetic groups and some Arctic haplotypes in 28 species were not assignable to Atlantic or Pacific populations, suggesting marine macroalgae may have survived in periglacial refugia along seasonally ice-free coastal areas of Siberia or Greenland. The Arctic may therefore represent an independent evolutionary basin, with input from both the Atlantic and the Pacific following glaciation events, as well as Arctic refugia.

THE KELP CONVEYOR HYPOTHESIS: EXPLORING LONG-DISTANCE GENETIC CONNECTIVITY OF RED ALGAE IN THE NORTHEAST PACIFIC **Brooks, C. M.**, University of New Brunswick, Fredericton, Canada, <u>cbrooks3@unb.ca</u> Saunders, G. W., University of New Brunswick, Fredericton, Canada, <u>gws@unb.ca</u>

Roughly 10% of the seaweed flora in Haida Gwaii is also found in California, but not in nearby southern British Columbia presenting an unexpected disjunct distribution pattern. The kelp conveyor hypothesis posits that winter storms in California dislodge massive quantities of kelp which subsequently raft on the Winter Davidson current – a northbound current dominant from November to February. These kelp rafts are expected to transport non-buoyant algal species from California to Haida Gwaii, thus causing the observed disjunct distribution. If the hypothesis is valid, this pattern initially observed at the species level should also be reflected at the population level in species with continuous distributions along the west coast of North America. As the kelp conveyor is expected to have greater impact on subtidal species, we selected the predominantly low intertidal to subtidal species *Cryptopleura ruprechtiana* and the predominantly mid intertidal *Mastocarpus intermedius* for this study. Samples were collected from California, Haida Gwaii and southern British Columbia and sequenced using the mitochondrial cytochrome *c* oxidase subunit I and the nuclear internal transcribed spacer. Although our results are preliminary, the observed patterns of haplotype diversity and genetic differentiation are consistent with the kelp conveyor hypothesis. While kelp rafting has been reported elsewhere, this study marks the first test of this particular pattern at the population level.

EFFECTS OF WARM OCEAN TEMPERATURES ON BULL KELP FORESTS IN THE SALISH SEA Schiltroth, B., Simon Fraser University, Canada, <u>bschiltr@sfu.ca</u>

Kelp beds are marine sanctuaries, providing some of the most productive ecosystems on the planet and serving as critical habitat and refuge for many marine species. Rising ocean temperatures associated with climate change is a major stressor contributing to declines of kelp forests worldwide. In the Salish Sea, we identified bull kelp (*Nereocystis leutkeana*) populations growing under two different temperature regimes. Since 2011, kelp growing in the central Strait of Georgia has been exposed to sea surface temperatures (SSTs) of 15-20 °C in the summer months, which is 5-6 °C warmer than temperatures in the Strait of Juan De Fuca. To assess the effects of high summer SSTs, populations growing at a warm and a cold site (Stanley Park and French Beach respectively) were sampled in the summer and fall of 2017. Warmer summer SSTs at Stanley Park correlated with significant declines in spore production, indicating negative effects on the reproductive capacity of that population. Growth and development of healthy spores from either population was reduced when incubated in the lab at temperatures above ~ 17 °C and temperatures of ~ 20 °C resulted in mortality of nearly all spores. Analyses of stress levels in spores exposed to different temperatures, based on the production of reactive oxygen species, is now underway.

SEAWEED DIVERSITY OF THE SOUTH ATLANTIC ISLANDS: DISCOVERY OF NOVEL FLORAS?

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Vast areas of ocean between island archipelagos in the South Atlantic raises three key questions in relation to seaweed diversity: i) how much is there, ii) what is it and iii) how does it relate to floras in other parts of the world? The Falkland Islands, an archipelago of over 770 islands, is a case in point. Historical collections have been made of the seaweeds from the Falklands since the 1840s, but the taxonomy of the flora has barely been studied. During a seaweed survey undertaken in January-February 2018, over 1090 specimens were collected from 30 sites from a wide range of shores, including some which were studied for the first time. Preliminary results using molecular approaches indicate that there are potentially many undescribed taxa, particularly amongst the red algae. This raises the question as to whether the Falklands seaweeds represent a novel flora or whether the results reflect a paucity of data from other related islands, including South Georgia and Tristan da Cunha, and land masses such as Chile, South Africa, New Zealand and Antarctica. Comparison of the coralline algae, for example, indicates a link with those in Chile. In this talk, we will explore these results and discuss them in relation to the

recent geological history of the Falklands which appears to have been a refuge during the last glacial maximum and a reservoir of founder populations.

AN UPDATE ON THE PHYCOGEOGRAPHICAL RECORDS OF HONG KONG **Cheang, C.**, Education University of Hong Kong, Hong Kong, <u>cccheang@eduhk.hk</u> Yiu, W., Education University of Hong Kong, Hong Kong, <u>whyiu@eduhk.hk</u> Wong, T., Education University of Hong Kong, Hong Kong, <u>s1112874@s.eduhk.hk</u> Ang, P., Chinese University of Hong Kong, Hong Kong, <u>put-ang@cuhk.edu.hk</u>

Hong Kong is located in the northern part of the South China Sea and has a long history of marine macroalgal taxonomic and phytogeographical studies. The first seaweed record in Hong Kong can be dated back to 1836-1837 when the French Botanist C. Gaudichaud documented seaweed species from Hong Kong in a Pacific expedition. W.A. Setchell and C.K. Tseng are two important phycologists in the early to mid 1900's who contributed much to our understanding of brown and red macroalgae in the region. This long history of phycogeographical works renders Hong Kong as an algal biodiversity hotspot for this region with more than 300 species recorded. Of these, Hong Kong is the type localities of at least nine *Sargassum* and 20 red algal species or subspecies. New species continued to be discovered as recently as 2018. This presentation is to report an on-going 1.5-year project in revisiting the sites around Hong Kong to update its phycogeographical records. To date, 68 species were recorded from Nov 2017 to Mar 2018, including 23 brown, 20 green and 25 red algal species to be further verified. One green species was suspected to be *Derbesia marina* (Lyngbye) Solier, which could be a new phycogeographical record for Hong Kong. Finding all the previously recorded species in Hong Kong would be a great challenge as economic development in the last 70 years has disturbed many of the coastal sites, including some of the type localities.

POPULATION GENETICS OF THE BULL KELP *NEREOCYSTIS LEUTKEANA* Gierke, L., University of Wisconsin-Milwaukee, USA, <u>lggierke@uwm.edu</u> Coelho, N., University of Pittsburgh, USA, <u>castilho@pitt.edu</u> **Alberto, F.**, University of Wisconsin-Milwaukee, USA, <u>albertof@uwm.edu</u>

We present the first study on the population genetics of the Bull Kelp *Nereocystis luetkeana*. We developed 10 polymorphic microsatellite markers with 454 sequencing and used them to genotype a total of over 1,000 samples distributed from over 47 sites along the species distribution range, with particular focus in the Salish Sea region. Within the Salish Sea and adjacent North East Pacific coast, we used a landscape genetics approach to study which factors drive genetic differentiation in Bull Kelp. Accordingly, we tested the relative importance of spatial, oceanographic and environmental distances between sample sites. Four clusters of genetic co-ancestry resulted from our analysis, the extent of some of these suggest long distance dispersal is possible. Genetic diversity was the highest at the Southern limits of species range in Central California, although the levels at the northernmost samples in Alaska were also high. The lowest levels of genetic diversity were observed inside the Salish sea in agreement with increasing isolation of kelp beds. Oceanographic distance explained the best the patterns of genetic differentiation, although there was some support for isolation by environment associated with Summer sea surface temperature and light penetration in the water (kd490).

Disturbance and Climate Change

POTENTIAL FOR KELP FORESTS TO ATTENUATE COASTAL WAVE STRESS **Elsmore, K. E.**, Bodega Marine Laboratory, University of California, Davis, United States, <u>keelsmore@ucdavis.edu</u>

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Coastal erosion is a major threat to coastal communities, and may increase as global sea levels rise. Such erosion is induced by wind-generated waves as they interact with the shore. Although some research has suggested that kelp forests may dissipate energy from waves, other studies are equivocal, and few empirical efforts have been positioned well to directly test this possibility. Here we exploit a unique opportunity to definitively examine the capacity of kelp forests to damp nearshore waves, by utilizing an ongoing kelp forest restoration project off the coast of Palos Verdes, California. Areas that were once devoid of kelp, termed 'urchin barrens', have returned to thriving kelp forests after managed culling of unnaturally high densities of sea urchins. We are now comparing ratios of wave energy flux across the kelp forest site for conditions both prior to, and subsequent to, the establishment of the kelp, to quantify the overall percent reduction in wave energy flux as a function of kelp density. Preliminary results suggest limited benefits of kelp forests for shoreline protection.

THE LEGACY OF THE BLOB: ONGOING EFFECTS OF THE PACIFIC WARM WATER ANOMALY ON THE TRADITIONALLY HARVESTED ALGA, *PYROPIA ABBOTTIAE* **Clark, J. S.**, University of British Columbia, Canada, <u>Jennifer.Clark@botany.ubc.ca</u> Lindstrom, S. C., University of British Columbia, Canada, <u>Sandra.Lindstrom@botany.ubc.ca</u> Liggan, L. M., Hakai Institute, Canada, <u>Lauran.Liggan@hakai.org</u> Hessing-Lewis, M., Hakai Institute, Canada, <u>Margot@hakai.org</u> Martone, P. T., University of British Columbia, Canada, <u>Patrick.Martone@botany.ubc.ca</u>

The warm water anomaly (nicknamed 'the Blob') that developed in the Gulf of Alaska and circulated the Pacific Ocean in 2014-2015 caused cascading impacts on the biological structure and composition of many coastal ecosystems of the Northeast Pacific. In 2016, the coastal First Nations of British Columbia reported coastwide declines in the quality and quantity of the traditionally harvested red alga *Pyropia abbottiae*, with concerns that the Blob may have been the cause. The microscopic sporophyte stage of *P. abbottiae*, the conchocelis, has specific environmental cues such as changes in temperature, photoperiod and light intensity required to progress to different life stages. It is hypothesized that the alga may have missed these cues due to warmer temperatures, stratification (restricted nutrients) or lack of bright sunlight during the Blob anomaly years, which may have affected the gametophyte stage. To test these hypotheses, relationships between different *P. abbottiae* life stages and hypothesized environmental data and satellite imagery from Calvert Island, BC and vicinity are presented to test hypotheses whether environmental anomalies from the Blob affected *P. abbottiae* populations coastwide and whether these effects are ongoing.

ASSESSING THE IMPORTANCE OF MORPHOLOGICAL VARIABILITY ON THE PRODUCTIVITY OF *MACROCYSTIS PYRIFERA*: LABORATORY AND FIELD EXPERIMENTS **Buschmann, A. H.**, Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, <u>abuschma@ulagos.cl</u> Pereda, S. V., Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, <u>spereda@ulagos.cl</u> Kappes, J. L., Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, <u>ilkappes@gmail.com</u> Villegas, K., Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, <u>villegaska@gmail.com</u> Altamirano, R., Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, robinson.altamirano@ulagos.cl Camus, C., Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, <u>carolina.camus@ulagos.cl</u> Labbé, B., Centro i-mar & CeBiB, Universidad de Los Lagos, Chile, <u>b.labbe.bima@gmail.com</u> Hernandez-Gonzalez, M. C., Centro i-mar, Universidad de Los Lagos, Chile, <u>mhernand@ulagos.cl</u> Celis-Plá, P., Universidad de Playa Ancha, Chile, <u>paulacelispla@upla.cl</u> Figueroa, F. L., Universidad de Málaga, Spain, <u>felix_lopez@uma.es</u>

One relevant feature of giant kelp forests is their morphological variability; it can be a mean to compensate environmental changes or can allow a better performance for farming. This study summarized 3 years of research, seeking to relate kelp productivity to morphology by describing some morphological trends along the Chilean coast, and to assess whether productivity of Macrocystis pyrifera sporophyte is enhanced by its initial morphology (few vs. multiple frond juveniles) carrying out laboratory experiments (under different temperatures and N-NO₃ concentration); mesocosm experiments (400-L outdoor tanks); and grow out in a pre-commercial farm were carried out. Our observations in natural populations showed that even when the populations can be denser in the north, sporophyte in austral areas, with more variable environmental conditions (e.g. light, temperature), have higher Canopy:Subcanopy ratio (C:SC). Morphological parameters that increase the photosynthetic area can improve productivity. Our results show that juvenile sporophytes with multiple fronds had a significantly higher growth and photosynthetic performance. The mesocosm showed that adult sporophytes with high C:SC ratio have a higher productivity when compared to the low C:SC ratio. Field grow out into the sea also confirmed that multiple frond *M. pyrifera* sporophytes have a significant higher biomass production after six months. These results indicate that besides the genetic management to improve productivity, it is also possible that the morphotype can be manipulated under controlled conditions to increment the biomass production. Financial support: FONDECYT 1150978 & FB-0001.

CARBON CONCENTRATAING MECHANISMS (CCMS) OF DRAGON KELP, *EUALARIA FISTULOSA*, (PHAEOPHYCEAE) ALONG THE DEPTH: IMPLICATION FOR A HIGH CO2 WORLD

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Recently, concerns about the impacts of climate change on sub-arctic marine ecosystems have increased because these waters are vulnerable to ocean acidification. Specifically, ecophysiological studies on sub-arctic macrophytes have increased our ability to predict distribution dynamics of submersed aquatic vegetation, many of which act as ecosystem engineers. In this study, photosynthetic performance and carbon concentration mechanisms (CCMs) of the dominant canopy forming alga, *Eualaria fistulosa*, was investigated along a depth gradient (benthos, mid-water, and surface) in the Aleutian Archipelago. Our results indicate that *E. fistulosa* photosynthetic capacity is not notably affected by depth, but productivity is significantly higher in the surface canopy due to higher irradiance. Apparent photosynthetic affinity of CO₂ was significantly higher than that of HCO₃⁻ in tissues near the benthos, while tissues near the canopy showed higher affinity for HCO₃⁻ than for CO₂, with increased reliance on CCMs. These results indicate that *E. fistulosa* may be acclimated to low light environments at the benthos, with down-regulation of CCMs, and that growth of these tissues might be stimulated under more acidic ocean. This suggests that climate change may support more dense kelp vegetation that reduces ecosystem engineer loss in the sub-arctic coastal ocean.

DOCUMENTING POPULATION DYNAMICS OF A TRADITIONALLY HARVESTED SEAWEED: DEVELOPMENT OF LONG-TERM MONITORING SURVEYS Liggan, L. M., Hakai Institute, Canada, lauran.liggan@hakai.org

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In Spring 2016, First Nation communities documented historically low seaweed (*Pyropia abbottiae*) abundance and quality in culturally important, intertidal foraging areas throughout coastal British Columbia (BC). Local communities are concerned about future seaweed harvests; they want to know (1) the cause of the seaweed decline, (2) whether future events can be predicted, and (3) how future declines can be mitigated and/or managed. In response, the Hakai Institute has developed monitoring methods aimed at documenting seasonal dynamics and drivers of the observed changes. Specifically, surveys focus on capturing P. abbottiae bloom dynamics, such as seasonal timing, abundance, biomass, and tissue quality (i.e. C:N and pigments) as well as examination of key stressors (temperature and nutrients). Bloom dynamics were documented at three sites monthly during spring and summer (2017/2018), around Calvert Island, BC. All three sites reached peak bloom in mid May, however overall abundance varied (5 -80%) in percent cover across sites. Blade biomass was extrapolated to reflect available harvest yields during the bloom. Additionally, we partnered with Coastal Guardian Watchmen, to create annual harvest field surveys that quantify the health of traditional harvesting sites, providing support for seaweed monitoring by local First Nation communities and complementing local indigenous knowledge of harvested P. abbottiae. This year, we are engaging community-based interest in data collection along the BC coast to determine if *P. abbottiae* has recovered following the observed decline.

BAY-SCALE ASSESSMENT OF MARINE MACROPHYTES WITH VIDEO, SIDESCAN AND ECHOSOUNDER

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The bay-scale assessment of marine macrophytes in turbid coastal waters is problematic. Remote sensing approaches can fail in waters as shallow as four meters. The author spent over a decade in Atlantic Canada developing and applying in-water methods to assess marine macrophytes at bay scales (10s of km). The results will be discussed, emphasizing two case studies; eelgrass in several northern New Brunswick bays, and seaweeds along the south shore of Cape Breton (Isle Madame, Nova Scotia).

GENOME SEQUENCING AND POPULATION GENOMIC ANALYSES OF UNDARIA PINNATIFIDA: INSIGHTS ON THE IMPACT OF DOMESTICATION ON INVASIVENESS **Graf, L**., Sungkyunkwan University, South Korea, <u>louis.graf@gmail.com</u> Shin, Y., Sungkyunkwan University, South Korea Viard, F., Station Biologique de Roscoff, France Yoon, H. S., Sungkyunkwan University, South Korea

Undaria pinnatifida (Laminariales, Phaeophyceae) is a popular multicellular kelp species playing important ecological roles in its natural habitat in Eastern Asia. It is extensively cultivated in Korea (~500.000 tons/year) for food consumption, abalone farming or for its extracts. *U. pinnatifida* is also well known worldwide for being an invasive species. Indeed, it was introduced to Europe in the 1970s initially to the Mediterranean coast and later to Brittany. In the late 1980s the species was also recorded in New Zealand. Introductions most likely occurred from cultivated individuals from the native range. Therefore *U. pinnatifida* represents an interesting model to investigate both the domestication (i.e. artificial selection) and the return to natural selection in new environments. To investigate these questions at the whole-genome level first we produced a high-quality draft genome of *U. pinnatifida* from a cultivated individuals from Korean natural populations and 10 individuals from Korean cultivated populations to investigate domestication. Finally,

we re-sequenced 21 individuals from introduced populations in France and in New Zealand. We describe the genome of *U. pinnatifida* and briefly compare it to previously sequenced brown algae genomes. We then discuss genome-wide variations between the different populations considered and identified putative selective sweeps at multiple loci. We notably discuss the importance of domestication on the capability of *U. pinnatifida* to invade new environments outside of its native range.

THE MICROBIOME OF *FUCUS* SPP., A SUITE OF ECOSYSTEM ENGINEERS **Quigley, C. T.**, University of Maine, United States, <u>charlotte.quigley@maine.edu</u> Morrison, H. G., Marine Biological Laboratory, United States Johnson, L., Université Laval, Canada Morozov, A., Marine Biological Laboratory, United States Capistrant-Fossa, K., University of Maine, United States Brawley, S. H., University of Maine, United States

Bacteria influence the development of macroalgae, but their specific identities remain poorly characterized. Stress gradients across the intertidal zone produce distinctive vertical distributions of Fucus spiralis, F. vesiculosus, and F. distichus that inhabit the upper, mid, and lower zones, respectively. We aim to characterize fucoid microbiomes and determine whether associations change across the environmental stress gradient. In order to determine whether microbial associations are species-specific or zone-specific, F. vesiculosus individuals were transplanted to the high zone (F. spiralis zone) and either watered with seawater, to simulate native mid-zone levels of desiccation stress, or not watered, to expose transplants to high zone stress. Additional individuals were back-transplanted to their native mid-zone (controls). Temperature sensors (iButtons) were deployed in the mid- and high zones. We amplified and sequenced the V4 hypervariable region of the 16S rDNA. Preliminary results show shifts in bacterial "species" (sequence variants) in the microbiomes of transplanted individuals (ANOVA, p=0.015); microbiomes of dry treatments differed significantly from back-transplants, with watered transplants having an intermediate microbial community. Mid-zone iButtons recorded a mean daytime air temperature of 15.7°C throughout deployment, whereas high-zone iButtons had a mean of 17.3±0.1°C. Experimental and natural comparisons of fucoid microbiomes will contribute understanding of how stress affects intertidal bacteria and whether bacteria play a role in differential stress tolerance.

Ecology of Protists

GROWTH AND CHLOROPLAST REPLACEMENT OF THE BENTHIC MIXOTROPHIC CILIATE *MESODINIUM COATSI*

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While the ecophysiology of planktonic *Mesodinium rubrum* species complex has been relatively well studied, very little is known about the ecophysiology of benthic *Mesodinium* species. Here we report the growth response of the benthic ciliate *M. coatsi* as functions of different cryptophyte prey using established culture. *M. coatsi* was able to ingest all offered cryptophytes prey, but not all cryptophytes supported its positive growth. While *M. coatsi* achieved sustained growth on offered all phycocyanin-containing cryptophytes, *Rhodomonas* spp., it showed different growth responses on phycoerythrin-containing cryptophytes, *Rhodomonas* spp., *Storeatula* sp., and *Teleaulax amphioxeia*. *M. coatsi* was able to easily replace the previous prey chloroplasts with newly ingested ones within 4 d, irrespective of prey type if there existed available cryptophyte prey. Once retained, the ingested prey chloroplasts seemed to be photosynthetically active. Fed *M. coatsi* was capable of growing heterotrophically in darkness, but its growth was enhanced significantly in the light (light:dark 14:10 cycle), suggesting that photosynthesis,

which is derived from ingested prey chloroplasts, leads a significant growth of *M. coatsi*. Our results expand the knowledge of autecology and ecophysiology of the benthic *M. coatsi*.

THE HIDDEN SECRETS OF CRYPTOPHYTE ALGAE III. GENE EXPRESSION OF *RHODOMONAS SALINA* IN RESPONSE TO VARYING SPECTRAL IRRADIANCE **Schomaker, R. A.**, University of South Carolina, United States, <u>schomakerrachel@gmail.com</u> Heidenreich, K. M., University of South Carolina, United States, <u>heidenrk@email.sc.edu</u> Richardson, T. L., University of South Carolina, United States, <u>tammirichardson@gmail.com</u> Dudycha, J. L., University of South Carolina, United States, <u>dudycha@biol.sc.edu</u>

Algal pigment diversity allows for variation in absorption across the spectrum of wavelengths of visible light. This variation in spectral absorption can play a role in the geographic distribution of algae in natural habitats because ecosystems can vary in spectral characteristics. Cryptophytes, a group of microalgae, contain a pigment complement that includes either of the phycobiliproteins (phycoerythrin or phycocyanin) that are distinctive in their absorption maxima, and there is not an obvious relationship between phycobiliprotein type and phylogeny. Despite this, they are ubiquitous across all types of aquatic habitats, ranging from CDOM-rich red-dominant environments to open-ocean blue-dominant environments. Because of this geographic and pigment diversity, we expect cryptophytes to acclimate to changes in spectral irradiance by adjusting their pigment concentrations per cell, which should be reflected by gene expression patterns. In this study, we investigated how Rhodomonas salina responds to changes in spectral quality by measuring phenotypic (pigment concentration and absorption spectra) responses and gene expression patterns in white-, green-, red-, and blue-dominant light environments. Pigment and RNA extractions were performed across five replicates in each environment, and differential gene expression analysis was performed using a *de novo* transcriptome pipeline. Preliminary data indicates that *R. salina* exhibits a plastic response in pigment concentration per cell so that the pigment complement reflects the available usable spectra.

THE HIDDEN SECRETS OF CRYPTOPHYTE ALGAE II: GROWTH RATES OF MARINE CRYPTOPHYTES UNDER VARYING SPECTRAL IRRADIANCE

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Cryptophytes are eukaryotic algae found in a variety of aquatic ecosystems. Water color in these ecosystems may vary from blue to red depending on CDOM concentration and/or trophic state. Thus, each will have varying wavelengths of light available for photosynthesis. Cryptophytes also display great diversity in their color, from "pink" species that contain cryptophyte phycoerythrins (Cr-PEs) to "green" species with cryptophyte phycocyanins (Cr-PCs). According to the theory of complementary chromatic adaptation, we would expect species to grow best in the spectral regime to which they are best suited. We asked: How do growth rates of differently-pigmented cryptophytes vary among light environments of differing spectral quality? And, how do observed variations in growth rates relate to cellular pigmentation? We grew 5 pink and 3 green species under red, green, blue, and full-spectrum irradiance each with an intensity of 30 μ mol m⁻² s⁻¹. All pink species grew fastest under blue light (0.4 to 0.6 d⁻¹ depending on species), indicating the efficient absorption of blue photons by their Cr-PEs and by non-PE pigments (chl-a, chl c_1 , alloxanthin, and α -carotene). Green cryptophytes grew fastest under red, white, or blue light depending on the species (0.5 to 0.8 d⁻¹), which Cr-PC they contained, and their complement of non-PC pigments. Not surprisingly, all green species grew slowest under green light (0.3 to 0.5 d⁻¹). Thus, while some trends were observed between pink and green cryptophyte groups, species-specific variation based on pigmentation was still common.

A COMPARATIVE ANALYSIS OF TWO TINTINNIDS: ADDRESSING THE SPECIES PROBLEM WITH "OMICS" Smith, S. A., University of Connecticut, United States, susan.smith@uconn.edu

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Currently, the best strategy for species delineation in unicellular eukaryotes involves an integration of DNA barcoding, morphology, and ecophysiology. However, even these methods fall short when it comes to identifying true reproductive isolation among ciliates, a group whose genomic DNA has been difficult to sequence because it goes through a unique rearrangement process prior to transcription. We used a single-celled "omics" approach to amplify the entire genomes and transcriptomes from single cells of two closely related species to investigate reproductive isolation. This new method was used in conjunction with traditional DNA sequencing (ribotyping), morphology, and physiological experiments to document species boundaries in two tintinnid isolates, one from the Atlantic and the other from the Pacific. Results revealed minor differences in DNA barcodes and morphology, yet significant differences in physiological responses to prey preference, toxic algae and environmental conditions. The single-cell "omics" approach showed broad differences in the genome architecture of the two ciliates, suggesting that these tintinnids cannot share essential genes, and are thus reproductively isolated. The future of this work may lead to the identification of genes that will clarify functional roles corresponding to the ecological niches and reproductive isolation of these tintinnids.

GENETIC AND MOLECULAR MECHANISMS TO COPE OXIDATIVE STRESS IN FRESHWATER CILIATES

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Heavy metals are the major toxic inorganic environmental pollutants present in both terrestrial and aquatic ecosystems. Increase in their concentration due to anthropogenic activities is a matter of concern. To control the increasing pollution, it is necessary to assess the heavy metal toxicity. In this present study, ciliates are used as cellular tool to assess the metal toxicity and to study the various mechanisms to overcome the heavy metal stress. The effect of Cu and Cd on the population growth of freshwater spirotrich ciliates (*Tetmemena* n. sp., *Notohymena australis* and *Euplotes aediculatus*) was observed. The 24-hr LC50 was measured where heavy metals negatively affected the growth of ciliates. Generation of reactive oxygen species (ROS) was detected in heavy metal treated ciliates by using fluorescence dye, dihydroethidium (DHE). Also, the activities of various antioxidant enzymes viz. superoxide dismutase, catalase and glutathione peroxidase (involved in SOD-catalase-glutathione cycle) were studied in the presence of Cu and Cd. The levels of antioxidant enzymes were found to increase significantly with

higher concentrations of heavy metals. In addition, transcriptional expressions of stress genes like hsp70 have been evaluated by qPCR. Significant increase in the expressions of these genes were observed with increase in heavy metal concentration. The results suggest that antioxidant enzymes and these stress genes may act as effective molecular biomarkers to evaluate heavy metal toxicity in ciliates. In addition to this, hsp70 and SOD genes have been sequenced and characterized in these ciliates.

SHATTERED GLASS; THE SPECIALIZED PREDATOR *PHRYGANELLA PARADOXA* (ARCELLINIDA, AMOEBOZOA) RUPTURES FRUSTULES OF DIATOMS BY FORCE **Kahlich, C.**, University of Cologne, Germany, <u>ckahlich@smail.uni-cologne.de</u> Dumack, K., University of Cologne, Germany, <u>kenneth.dumack@uni-koeln.de</u> Lahr, D., University of Sao Paulo, Brazil, <u>dlahr@ib.usp.br</u> Bonkowski, M., University of Cologne, Germany, <u>m.bonkowski@uni-koeln.de</u>

A major drawback in testate amoeba research is a general lack of scientific studies combining molecular approaches and classical laboratory experiments. We isolated five yet uncultured testate amoebae of the genus *Phryganella* Penard, 1902 from three different rivers and one pond in Germany. Based on established cultures we show their morphology, which we studied by light and electron microscopy, and present their unique feeding mode on abundant and common pennate diatoms like *Nitzschia* spp. and *Synedra* spp., whose frustules are bent and frequently, but not always, broken during the feeding process. We further obtained the first SSU rDNA sequences of strains of the family Phryganellidae, all of which contain introns. We used the sequences to confirm the taxonomic placement of the Phryganellidae in the Arcellinida (Amoebozoa), branching as a sister group to the Cryptodifflugiidae.

THE HIDDEN SECRETS OF CRYPTOPHYTE ALGAE I: AN EXPLORATION OF PHYLOGENETIC AND FUNCTIONAL DIVERSITY IN LIGHT CAPTURE ABILITY

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Our work explores relationships among the genetic, phylogenetic, and functional diversity of an understudied group of microalgae, the Cryptophytes. Here, we present results of a study focused on phenotypic traits related to light capture ability and molecular phylogenetic relationships for 33 cryptophytes from a range of aquatic environments. We assessed light capture by measuring cellular phycobiliprotein (PBP) and non-PBP pigments, whole cell absorption spectra and photosynthetically useable radiation. We constructed molecular phylogenies using nuclear SSU and partial LSU rDNA sequence data. Molecular and phenotypic data together supported the reclassification of *Cryptomonas irregularis* NIES 698 to the genus *Rhodomonas*, a revision of PBP diversity within the genus *Hemiselmis* to include cryptophyte phycocyanin (Cr-PC) 569, the inclusion of previously unidentified strain CCMP 2293 in the genus *Falcomonas*, even though it contains cryptophyte phycocrythrin 545 (Cr-PE 545), and the inclusion of previously unidentified strain CCMP 3175, a pink strain containing Cr-PE 545, in a clade containing green PC-containing *Chroomonas* species, making CCMP 3175 the first known Cr-PE-containing *Chroomonas*. Our results illustrate the remarkable genetic and phenotypic diversity even within this subset of 33 cryptophyte strains, and provide new information about links between phylogenetic and functional diversification.

AN INDIVIDUAL LOOK AT THE OXYMONAD *STREBLOMASTIX STRIX* AND ITS BACTERIAL SYMBIONTS USING SINGLE CELL GENOMICS

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Oxymonads are a group of protists which live as intestinal endosymbionts in the gut of insects and vertebrates. Since most oxymonads cannot be cultured in vitro, obtaining molecular information from them was limited to gene fishing techniques, thus the molecular data is scarce. This situation is even more evident in bigger oxymonad species which are found in the gut of wood feeding insects. Moreover, these oxymonads are often closely associated with prokaryotes located inside or on the surface of their cells. Our work focused on the oxymonad *Streblomastix strix* which lives in the gut of the termite *Zootermopsis angusticolis*. Using single cell techniques, we managed to amplify the DNA of *Streblomastix strix* and its bacterial symbionts and we sequenced it using next generation sequencing. Our preliminary results show the presence of 14 Bacteroidetes SSU sequences, one SSU from genus *Endomicrobium* and five other SSU sequences from other bacteria. Using FISH, we managed to confirm that all Bacteroidetes SSU sequences metagenome shows that these bacteria have enzymes involved in nitrogen fixation and cellulose degradation, pathways which are critical for the survival in the gut of termites.

Taxonomy and Evolution of Algae II: The Return

EVIDENCE FOR RECENT SPECIATION AND DISPERSAL IN THE LACUSTRINE DIATOM *STEPHANODISCUS* (EHRENB.)

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The diatom genus *Stephanodiscus* Ehrenb. shows repeated patterns of recent speciation events. Evidence from this comes from molecular, morphological and paleontological evidence in the S. niagarae Ehrenb. complex, and molecular and morphological evidence in other groups of Stephanodiscus species including S. minutulus and several close relatives (particularly S. cf. jamsranii, S. meyeri), and S. hantzschii and S. binderanus. In each case, molecular evolution in genes typically used in diatom systematics, including common bar-code genes, has been outpaced by morphological evolution and physiological evolution. We will illustrate that a common thread in each of these clusters of species is that there is one plesiomorphic nominal species (based on a phylogenetic interpretation of morphology), and at least one apomorphic species. In the case of the S. niagarae complex, this pattern has already been shown to be consistent with speciation by (ecological) peripheral isolation by examining the distribution of appmorphy in the context of environmental parameters. Corroborative evidence comes from a paleolimnological study of the evolution of S. yellowstonensis from S. niagarae in Yellowstone Lake sediments which revealed that morphological changed tracked climate-driven changes in the lake. Here we examine the correlation between apomorphy and the environment, and ecological distribution of S. minutulus, S. cf. jamsranii, S. meveri, S. hantzschii, and S. binderanus, to generate a hypothesis about speciation and later dispersal of these species.

A SEASCAPE GENETICS APPROACH TO STUDY GENETIC DIFFERENTIATION IN BULL KELP **Gierke, L. G.**, University of Wisconsin-Milwaukee, United States, <u>lggierke@uwm.edu</u> Alberto, F., University of Wisconsin-Milwaukee, United States Mumford, T., University of Wisconsin-Milwaukee, United States

Despite its vast geographic range and important role in coastal ecosystems in the Pacific Northwest, the bull kelp, *Nereocystis luetkeana*, has historically not been as widely studied as its globally distributed sister kelp, *Macrocystis pyrifera*. To date, there have been no published studies on the population genetic structure of *Nereocystis luetkeana*. We developed 10 polymorphic microsatellite markers with 454 sequencing and used them to genotype a total of over 1000 samples distributed from over 32 sites within the Salish Sea. Anthropogenic impact in the Salish Sea has been associated with decreasing kelp cover in areas close to large urban centers. The study of genetic differentiation can shed light on the demographic connectivity between disjunct populations in this system and identify areas of concern. We found that genetic diversity decreased with increasing isolation within the Salish Sea. In general, genetic differentiation was low in the Salish Sea suggesting high gene flow between sites. We tested different association models of pair-wise genetic differentiation within the Salish Sea. We will show how spatial distance, hydrodynamic transport and environmental distance between sampling sites are associated with the pattern of genetic differentiation found in bull kelp.

ELUCIDATING THE ENDOLITHIC ARCHITECTURE OF RHODOLITH-FORMING CORALLINE ALGAE

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Endolithic single-celled microalgal stages have been found in the interior of cells of biogenic rhodoliths, such as *Lithothamnion* spp. (Hapalidiales). This work explores the interior, endolithic environment of rhodoliths from the NW Gulf of Mexico, Caribbean Panama, and the Florida Keys by making casts of the lumina of the interior cells and the connections between them using Spurr's resin. We used scanning electron microscopy to view the casts, and ImageJ to evaluate the area and volume of the micrographs taken of the casts to determine the percentage of resin-filled spaces versus the percentage of calcium carbonate walls within a rhodolith. Several patterns have been produced and recorded for calcified and decalcified resin-embedded rhodoliths. The architecture of the intercellular spaces within rhodoliths that were filled with Spurr's resin clearly show the variety of the internal anatomy within different rhodolith species. The results imply that pattern of internal architecture may be a newly-found character to separate species of coralline algae and this new approach will allow us to further explore the micro-environment of rhodoliths. The research may determine whether there are suitable interconnections that are part the endolithic rhodolith cell architecture for microalgal cells to move through. This research may also have important implications for assessing the changes in cell wall deposition vis-à-vis the effects of increased ocean acidification on calcified organisms.

ON THE SHOULDERS OF GIANTS: WHAT EPIZOIC DIATOMS ARE TEACHING US ABOUT DIATOM EVOLUTION

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A recent increase in the sampling of epizoic diatoms growing on marine vertebrates, such as sea turtles and manatees, has not only increased our knowledge of diatom diversity, but also our understanding of diatom diversification and evolution. One family in particular, the Rhoicospheniaceae, has yielded several interesting epizoic raphe-bearing diatoms from manatees and sea turtles in the southeastern US. In one example, we have found non-photosynthetic species of the genus *Tursiocola* Holmes, Nagasawa & Takano. While non-photosynthetic diatoms have been identified previously, they have all been in the order Bacillariales. The morphology of *Tursiocola* suggested no affinity to the Bacillariales, and DNA evidence, collected by single-cell DNA amplification techniques, confirmed that the loss of photosynthesis in *Tursiocola* was novel and unrelated to the losses in the Bacillariales. DNA data also question the placement of *Tursiocola* and the epizoic diatom *Poulinea* Majewska, De Stefano & Van de Vijver in the Rhoicospheniaceae, and suggest that *Poulinea* is quite distant genetically from other asymmetrical marine "gomphonemoid" raphid diatoms. We also present DNA data from cultures of the monoraphid diatom genus *Achnanthes* isolated from manatees and sea turtles, suggesting that this genus may be an ideal model to study not only the diversification of diatoms to the epizoic habit, but also across hosts.

ORGANELLAR GENOMES ARCHITECTURE AND EVOLUTION IN GRACILARIACEAE. Iha, C. Department of Botany, Institute of Biosciences, University of São Paulo, R Matão 277, São Paulo, SP, 05508-090, Brazil

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Organellar genomes (mitochondria and chloroplast) were sequenced for ten species of Gracilariaceae (Rhodophyta), combined with published genomes, to infer phylogenies and compare genome architecture among species. Similar and highly supported topologies were obtained for both chloroplast and mitochondrial genomes phylogenies. *Melanthalia intermedia* is sister to a monophyletic clade including *Gracilaria* and *Gracilariopsis*, which were both resolved as monophyletic genera. Mitochondrial and chloroplast genomes were highly conserved in gene synteny, and variation mainly occurred in regions where insertions of plasmid-derived sequences (PDS) were found. In mitochondrial genomes, PDS insertions were observed in two regions where the transcription direction changes: between the genes *cob* and *trn*L, and *trn*A and *trn*N. In chloroplast genomes, PDS insertions were located in different positions, but generally found between *psd*D and *rrs* genes. Gracilariaceae is a good model system to study the impact of PDS in genome evolution due to the frequent presence of these insertions in organellar genomes. Furthermore, organellar genomics are very useful tool to establish highly supported phylogenies.

A CONSENSUS SECONDARY STRUCTURE OF ITS2 FOR THE ORDER CYMATOSIRALES (BACILLARIOPHYTA) AND REAPPRAISAL OF THE ORDER BASED ON DNA, MORPHOLOGY, AND REPRODUCTION

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In 1983, Hasle and colleagues removed cymatosiroid diatoms from the pennates, and erected a new centric diatom family, the Cymatosiraceae, mainly to accommodate for their newly discovered mode of sexual reproduction. This family consisted of two subfamilies differing in frustule structure. We revisited intra-ordinal relationships within Cymatosirales using combined genetic (DNA sequences), morphological (valve and frustule structure), and reproductive (auxospore development) characters. In total, 36 cymatosiroid strains from 19 species representing 13 genera (80% of the total number of extant genera) were used in this study. A consensus secondary structure model of the Internal Transcribed Spacer 2 (ITS2) constructed for this order aided in sequence alignments for ITS2 and multi-gene phylogenies, separately and combined. An extensive search for compensatory base changes (CBCs) in ITS2 secondary structures was undertaken and mapped on the multi-gene (18S rRNA + ITS2 + rbcL) phylogenetic tree topology. In these trees, all taxa grouped into two morphologically and genetically distinct clades. Each clade was supported by the CBCs, as did all the clades representing genera. However, these clades did not correspond to the previously erected subfamilies. Consequently, we emend the Order Cymatosirales and family Cyamtosiraceae, and propose a new family, the Leyanellaceae. Overall, we demonstrate a novel approach to study diatom systematics across a broader taxonomic range using ITS2 secondary structural information. Our results suggest that this approach might be useful in establishing higher taxonomic relationships in other groups of diatoms.

EVOLUTIONARY DYNAMICS OF THE CHROMATOPHORE GENOME IN THREE *PAULINELLA* SPECIES

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The thecate amoeba *Paulinella* is a valuable model for understanding plastid organellogenesis because this lineage has independently gained plastids (termed chromatophores) of alpha-cyanobacterial provenance. Plastid primary endosymbiosis in Paulinella occurred relatively recently (90-140 million years ago, Mya), whereas the origin of the canonical Archaeplastida plastid occurred >1 500 Mya. Therefore, these two events provide different perspectives on plastid formation. Here we generated the complete chromatophore genome sequence from *P. longichromatophora* (979 356 bp, GC-content = 38.8%, 915 predicted genes) and P. micropora NZ27 (977 190 bp, GC-content = 39.9%, 911 predicted genes) and compared these data to existing genome from P. chromatophora. Orthologous gene family (OGF) analysis shows that chromatophore genome reduction is, as expected, relatively limited when compared to Archaeplastida plastids. Our analysis suggests that when a basal split occurred among photosynthetic *Paulinella* species ca. 60 Mya, only 38% of the ancestral OGFs from the cyanobacterial endosymbiont remained in the chromatophore genome. Following major gene losses during the early stages of endosymbiosis, this process slowed down significantly. Comparison of nucleotide substitution and insertion/deletion events among different P. micropora strains demonstrated an elevated AT-content. These data also provided evidence that chromatophore genome reduction is an ongoing process in this lineage.

BACK TO THE FUTURE: USING THE RDNA OPERON FROM ENVIRONMENTAL SAMPLES TO RESOLVE THE EUKARYOTE TREE OF LIFE AND DISCOVER NOVEL LINEAGES Jamy, M., Uppsala University, Sweden, <u>mahwash.jamy@ebc.uu.se</u> Bass, D., Natural History Museum, United Kingdom, <u>d.bass@nhm.ac.uk</u>

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In the quest to reconstruct the eukaryotic tree of life, the 18S rDNA was instrumental to transition from morphology-based to molecular-based phylogenetics. Today, phylogenomics has progressively replaced the use of one or few genes to reconstruct the backbone of the tree, but the 18S rDNA is still the most widely-used marker for environmental sequencing. This approach typically generates millions of short reads, which has resulted in an unprecedented wealth of data but with poor phylogenetic resolution. This problem may be overcome by the advent of relatively high-throughput, long-read sequencing platforms such as PacBio. Here, we test the power of long-read environmental sequencing to improve our understanding of the eukaryotic tree and reliably assign taxonomy to environmental sequences. Soil samples were targeted by long-range PCR to amplify a ~4500 bp region of the rRNA operon, spanning 18S, ITS1, 5.8S, ITS2, and 28S. Amplicons were sequenced on the new PacBio Sequel system, generating a total of ~130,000 long-reads. I will present our pipeline for filtering and analysing this data and show the potential of longer environmental reads to assess eukaryotic diversity using stronger phylogenetic signal. Notably, we adapted the Evolutionary Placement Algorithm (EPA) to take advantage of the improved phylogenetic information of long environmental query reads, which allows building reference trees including the queries and to infer the taxonomic affiliation of these queries at the same time. This approach also populates databases of ribosomal genes other than the 18S rDNA, significantly increasing taxon sampling and phylogenetic coverage of ITS, 5.8S, and 28S regions.

Wednesday, August 1

Algal Toxins and Defense

DIFFERENTIAL GENE EXPRESSION ASSOCIATED WITH COLONY FORMATION IN THE BLOOM-FORMING HAPTOPHYTE *PHAEOCYSTIS GLOBOSA* (PRYMNESIOPHYCEAE) **Mars Brisbin, M**., Okinawa Institute of Science and Technology, Japan, <u>margaret.marsbrisbin@oist.jp</u> Mitarai, S., Okinawa Institute of Science and Technology, Japan, <u>satoshi@oist.jp</u>

The haptophyte genus *Phaeocystis* includes three colony-forming species, and each blooms at different latitudes. Colonies are believed to protect *Phaeocystis* from small grazers and viral lysis, but excess organic matter from large colonies that are typical in blooms can negatively affect marine ecosystems. The triggers for colony formation, however, are enigmatic. Experiments indicate that light, temperature, nutrient availability, and grazing can increase or decrease rate of colony formation, but a clear link between these factors and the initiation of colony formation remains elusive. In this study, we applied RNA-seq to investigate the molecular pathways associated with colony formation. We compared gene expression in *P. globosa* CCMP1528 cultures (n=4) producing colonies to expression in cultures of the same strain without colonies (n=4). Biological replicates of colonial and non-colonial cultures were grown in identical culture conditions and sampled in late exponential growth phase. With the MMETSP *Phaeocystis sp.* CCMP2710 transcriptome as the reference, 284 genes were significantly differentially expressed between colonial and non-colonial replicates and sample types clustered separately in a

principal component analysis. Despite a low annotation rate for differentially expressed genes, ammonium transport and meiosis genes were upregulated in colonial replicates. A more appropriate reference transcriptome with more complete annotation is necessary to better describe molecular pathways associated with colony formation, and a *de novo* transcriptome assembly for *P. globosa* CCMP1528 will be used in future analyses.

LAGOSINEMA TENUIS GEN. ET SP. NOV. (PROCHLOROTRICHACEAE, CYANOBACTERIA): A NEW BRACKISH WATER GENUS FROM TROPICAL AFRICA Akagha, S. C., University of Lagos, Nigeria, <u>sandraakagha@gmail.com</u> Johansen, J. R., John Carroll University, United States, <u>johansen@jcu.edu</u> Nwankwo, D. I., University of Lagos, Nigeria, <u>dikenwankwo2@yahoo.com</u> Yin, K., Sun Yat-Sen University, Nigeria, <u>yinkd@mail.sysu.edu.cn</u>

A novel filamentous, non heterocystous cyanobacterium was isolated from the brackish Lagos Lagoon, Nigeria which is organically polluted. The isolate was <3.0 µm wide, untapered, with small rounded polar aerotopes visible at the crosswalls, and consequently fit the morphological description of *Limnothrix planctonica*. The 16S rRNA gene and the 16S-23S ITS region was amplified and sequenced. Although morphologically inseparable from that species, it was molecularly distant from that taxon, with genetic identities between the two taxa ranging 90.73-92.49%, a degree of separation typical of different genera. Phylogenetic analyses reveled that *Lagosinema* was sister to a large clade of strains assigned to *Limnothrix* sp. and *L. planctonica*. The ITS structures in *Lagosinema* were very different from those in the Order Prochlorotrichaceae. *Lagosinema tenuis, L. planctonica*as well as *Limnothrix rosea* are phylogenetically in the Prochlorotrichaceae, distant from the type species of *Limnothrix, L. redekei* which is in the Pseudanabaenaceae. The isolate is herein described as *Lagosinema tenuis* gen. et sp. nov. This species is ecologically significant because of its harmful bloom tendencies and role in aquatic ecosystem food web as one of the dominant phototroph in coastal waters around south-west Nigeria during the dry season.

TRANSCRIPTIONAL REGULATION OF PHOTOSYNTHETIC GENES LHCF2 AND LHCF15 IN RESPONSE TO HERBIVORY INDUCED ALDEHYDE IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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During herbivory, various diatom species release toxic polyunsaturated aldehydes (PUAs) as a defense response to impair reproduction of grazers. Unwounded diatom cells exposed to PUAs may exhibit reduced growth, viability, photosynthetic efficiency and altered photosynthesis-related gene expression. However, mechanisms of PUA-induced photosynthetic gene regulation are understudied. In this study, photosynthetic light harvesting complex (LHC) gene expression was explored in the marine diatom Phaeodactylum tricornatum in response to a model PUA, 2E,4E-decadienal (DD). At 10 µM, DD showed no detectable effects on cell viability and chlorophyll a content compared to the solvent control up to 6 hr. At this concentration of DD, RNA-seq showed an overall suppression of LHC mRNA levels at 3 and 6 hr. Analysis by qPCR showed Lhcf2 mRNA was significantly down-regulated (0.64 fold) while Lhcf15 mRNA was the most up-regulated (2.21 fold) at 6 hr compared to the solvent control. To determine whether rates of transcription of these genes changed, metabolic pulse labeling with an analog of uracil, 4-thiouracil, was developed. The rate of synthesis of Lhcf2 mRNA was strongly suppressed (0.01 fold) at 1 hr. In contrast, Lhcf15 mRNA rate of synthesis was 1.82 fold at 1 hr. Thus, DD induced rapid differential effects on the transcription of Lhcf2 and Lhcf15 genes. Experiments to determine the effects of DD on Lhcf2 and Lhcf15 mRNA turnover rates are in progress. Future studies to assess functional effects of the gene expression changes may elucidate adaptive benefits under biotic stress conditions.

CIGUATOXICITY AND MAITOTOXICITY OF REPRESENTATIVE *GAMBIERDISCUS* AND *FUKUYOA* SPECIES

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Dinoflagellate species belonging to the genera *Gambierdiscus* and *Fukuyoa* produce ciguatoxins (CTXs), potent neurotoxins that concentrate in fish causing ciguatera fish poisoning (CFP). They also produce maitoxins (MTXs) that may contribute to CFP when gut or liver tissues are consumed. Currently, few data exist on the intra- and interspecific variations in toxicity among these *Gambierdiscus* and *Fukuyoa* species. Quantifying these variations is critical for understanding how CFP events develop. An initial study analyzed the ciguatoxicity of 34 strains representing seven *Gambierdiscus* and one *Fukuyoa* species using the cell based Neuro-2a cytotoxicity assay. The average toxicity was inversely proportional to growth rate, suggesting an evolutionary trade-off between investments in growth versus production of defensive compounds. *Gambierdiscus belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, *Gambierdiscus* ribotype 2, *G. silvae* and *F. ruetzleri* exhibited low toxicities (0 to 24 fg CTX3C equivalents per cell), compared to *G. excentricus*, (469 - 1426 fg CTX3C eq. per cell). These findings indicate *G. excentricus* may be a primary source of Atlantic ciguatoxins. *G. excentricus* also exhibited the highest maitotoxicity of the eight species tested using a human erythrocyte lysis assay.

DIVISION TIME (TD) OF THE HAB DINOFLAGELLATE *KARLODINIUM VENEFICUM* FOR *IN SITU* GROWTH ESTIMATION IN BLOOMS

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Blooms of Karlodinium veneficum vary widely in toxicity for unknown reasons. One hypothesis is that stationary phase blooms are more toxic than growing ones. Testing this requires measurement of *in situ* or "gross" growth of K. veneficum in blooms, independent of "net" population changes due to convection or mortality. In situ growth is based solely on the proportion of cells that are dividing, but requires knowledge of the duration of cell division (td). To determine td in K. veneficum, we induced asynchrony in cultures via continuous illumination, and the fraction of dividing cells (f) was measured during log growth every h for 27 h. Under standard conditions, f remained constant at $\sim 2\%$ and td was calculated to be 1.1 h. This value was confirmed in cultures growing synchronously on a light:dark cycle, where in situ growth was identical to net growth (0.3-0.4 div/day). Potential for td to vary was examined in three geographically distinct strains at temperatures 15, 20, and 25 °C. Calculated td values ranged from 1.1 h -4.9 h, with the shortest and longest durations corresponding to the warmest and coldest temperatures, respectively. A strain effect was observed wherein td values for a putative warm-adapted strain varied significantly (td = 1.1 h at 25 °C, td = 4.9 h at 15 °C), whereas a eurythermal strain did not (td = 1.5 h at 20 °C, td = 1.4 h at 15 °C). These differences may indicate "ecotype species" in K. veneficum. Overall, mean td with upper and lower bounds were established for the first time to allow *in situ* growth rate estimation in K. veneficum blooms.

HOST-CELL ENTRY OF AN OOMYCETE *OLPIDIOPSIS PYROPIAE* IS MEDIATED BY A LIPID-RAFT ENDOCYTOSIS

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Olpidiopsis pyropiae is a causative agent of the *Olpidiopsis* blight which causes million dollars of economic loss in *Pyropia* farms every year. Host cell entry is the most crucial step in the infection of this obligate parasite. Often several parasite cells enter the host and develop zoosporangia while the host cell is still alive. How can the parasite cells get into the host without killing the cell? Oomycete and fungal effectors with RXLR and RXLR-like motifs can bind phospholipids, specifically phosphatidylinositol-3-phosphate (PtIns-3-P), and mediate cell entry via lipid raft-mediated endocytosis. The entry of *Olpidiopsis pyropiae* cell into the host, *Pyropia yezoensis*, was blocked with the treatment of PtIns-3-kinase inhibitors. PtdIns-3-P binding proteins and inositides that competitively inhibit effector binding to cell membrane also inhibited infection. Fluorescent labeling showed changes of PtdIns-3-P distribution along the cell surface during the infection. Homologues of PtIns-kinase and phosphatidylinositol-binding clathrin assembly protein (PICALM) were isolated from *P. yezoensis* transcriptome, and their expression profiles during the infection process were observed using qPCR. Our results show that the oomycete pathogen, *Olpidiopsis pyropiae*, may use a lipid raft-mediated endocytosis for its host cell entry.

Cryptic Species of Seaweeds

CRYPTIC SPECIES DIVERSITY OF OCHTODENES-PRODUCING *PORTIERIA* SPECIES (GIGARTINALES, RHODOPHYTA) FROM THE NORTHWEST PACIFIC **Yang, M. Y.**, Jeju National University, South Korea, <u>agape0307@naver.com</u> Kim, M. S., Jeju National University, South Korea, <u>myungskim@jejunu.ac.kr</u>

The red alga *Portieria* has been known to produce secondary metabolites apakaochtodenes A and B which are effective deterrents against herbivores. *P. hornemannii* samples from various sites exhibit the different concentrations of these metabolites suggesting the existence of probable genetic diversity and cryptic species. To evaluate genetic diversity and haplotype distribution of the genus in northwest Pacific, we analyzed *rbcL* sequences of *Portieria* in Korea, Japan, and Taiwan. Our analysis revealed five distinct lineages as species level in this region. One of them was recognized as *Portieria japonica* lineage and others as cryptic species of *P. hornemannii*. However, the *rbcL* haplotypes of *P. japonica* lineage were genetically fragmented and the traces of two subgroups of geographical origin, Korean and Japanese groups, were clearly discerned. Four cryptic species within *P. hornemannii* were also geographically structured but at much finer scale. This study suggests that different genetic populations of *Portieria* have been evolved through microhabitat differentiation, which might have influenced the significant concentration variation in secondary metabolites. Further studies focusing on the relationship between genetic and secondary metabolite variations of *Portieria* are required to resolve this.

A MOLECULAR SURVEY OF *RALFSIA* SENSU STRICTO (RALFSIALES, PHAEOPHYCEAE) IN BRITISH COLUMBIA UNCOVERS THREE NEW SPECIES

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Currently there are two species assigned to the genus *Ralfsia* (excluding 'species' now known to apply to alternate stages in the life histories of non-ralfsialean species) in British Columbia including *R*.

fungiformis (Gunnerus) Setchell & N.L. Gardner and *R. pacifica* Hollenberg However, a DNA barcode survey of Ralfsiales in British Columbia using the markers COI-5P and rbcL revealed five genetic groups assignable to *Ralfsia* sensu stricto. Further complicating matters, of those listed previously only the type species *R. fungiformis* is assignable to *Ralfsia* sensu stricto. The four other genetic groups in British Columbia assignable to *Ralfsia* sensu stricto are new species of which three will be presented: *R. roberti* sp. prov., *R. tenebris* sp. prov., and *R. unimaculata* sp. prov. The fourth consisted of a single sterile specimen from Haida Gwaii and was not characterized. *Ralfsia pacifica*, previously reported in British Columbia, and *R. verrucosa*, added to that flora through work here, both failed to join the generitype and belong to separate lineages in the Ralfsiales.

VARYING METHODS MUST BE EMPLOYED TO EFFECTIVELY EXPLORE ENDOLITHIC MICROHABITATS OF RHODOLITHS

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Cryo-electron microscopy (Cryo-EM), scanning electron microscopy (SEM), novel uses of Spurr's resin and decalcification, and confocal microscopy were used to investigate the internal microhabitat of rhodolith-forming coralline algae. Cryo-EM or the use of liquid nitrogen to fix and dehydrate rhodolith samples proved to be more informative than traditional fixation methods utilizing glutaraldehyde, paraformaldehyde and osmium. Embedding rhodolith samples in Spurr's resin filled the empty endolithic space. We then decalcified the rhodolith removing the cell wall and leaving a cast of the endolithic space that is extensive and forms intricate patterns. The internal architecture of the microhabitat may be a newly-found character to separate species of coralline red algae or it may shed light on the habitat of the endolithic microcommunity. This research may also have important implications in assessing changes in cell wall deposition. The microscopy work was conducted at the University of Louisiana at Lafayette Microscopy Center.

IMPLICATIONS OF *PLAKOBRANCHUS* CF. *IANTHOBAPSUS* (GASTROPODA) FOR HERBIVORE ECOLOGY, ALGAL COMMUNITY STRUCTURE, AND INVASIVE SPECIES DETECTION **Wade, R. M**., University of Hawai'i at Manoa, United States, <u>rmwade@hawaii.edu</u> Sherwood, A. R., University of Hawai'i at Manoa, United States, <u>asherwoo@hawaii.edu</u>

Siphonous green algae are important members of the marine community. Many siphonous genera are morphologically cryptic, particularly those that are diminutive, resulting in their limited descriptions and modest presence in the literature. To address the difficulty of their detection and identification, we used the sea slug *Plakobranchus* cf. *ianthobapsus* as a tool to explore green algal diversity. *Plakobranchus* sequesters chloroplasts from its algal hosts, a phenomenon known as kleptoplasty, which can be exploited to explore the diversity of algae they originate from. This sampling technique has resulted in the discovery of several new algal records for the Hawaiian Islands, and the first confirmed herbivory of the invasive alga *Avrainvillea amadelpha*. However, kleptoplast metabarcode data suggest that kleptoplast diversity is not representative of community siphonous algal diversity. To overcome this discrepancy and more thoroughly examine *Avrainvillea*'s distribution, this study used metabarcoding of live rock communities to assess total siphonous algal diversity from across the Main Hawaiian Archipelago. *Plakobranchus* was found to sequester chloroplasts from a narrow representation of the total community diversity, suggesting its host selection is more specialized than previously suggested. Additionally, *Avrainvillea* was confirmed to have spread beyond its known distribution. We conclude that the

application of metabarcoding to explore algal diversity can be a powerful tool to both better understand algal community composition and herbivore host selection, as well as early alien species detection.

MOLECULAR PHYLOGENY AND FEMALE REPRODUCTIVE STRUCTURE OF THE GENUS *PACHYMENIOPSIS* (HALYMENIACEAE, RHODOPHYTA) FROM THE NORTHWESTERN PACIFIC

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Recent taxonomic surveys have revealed that *Grateloupia* is divided into several taxonomic groups in generic level. The genus *Pachymeniopsis* separated from *Grateloupia* has been reported including just three species, *Pachymeniopsis lanceolata, Pa. gargiuloi* and *Pa. volvita*. We examined *Pachymeniopsis* with the relatives from the northwestern Pacific using *rbc*L gene and female reproductive structure to define the generic characteristics. In the *rbc*L molecular phylogeny, three *Pachymeniopsis* species and five *Grateloupia* species (*G. angusta, G. chiangii, G. elliptica, G. imbricata* and *G. kurogii*) were monophyletic with the strong support value, which are clearly distinguished from *Grateloupia filicina*, the generitype of *Grateloupia*. In the observation of female reproductive structure of *Pachymeniopsis*, the fine differences were recognized in the ampullae development. As a result of our study defining the phylogenetic relationships of *Grateloupia* from the northwestern Pacific, we suggest that five *Grateloupia* species should be transferred to the genus *Pachymeniopsis*: *Pa. angusta*, *Pa. chiangii*, *Pa. elliptica*, *Pa. imbricata* and *Pa. kurogii*.

COMPARATIVE PHYLOGEOGRAPHY OF TWO COMMERCIAL RED ALGAE, *PYROPIA* SUBORBICULATA AND PY. YEZOENSIS (BANGIALES, RHODOPHYTA) FROM KOREA **Koh, Y**., Department of Biology, Jeju National University, South Korea, <u>grandcross1999@nate.com</u> Kim, M., Department of Biology, Jeju National University, South Korea, <u>myungskim@jejunu.ac.kr</u>

Some species of foliose Banigales are commercially important in Asian countries. Their taxonomy and ecophysiology have received much attention because of the potential for improving aquaculture techniques. In this study, we investigated patterns of genetic diversity in *Pyropia suborbiculata* and *Py. yezoensis* based on COI-5P and *rbc*L analyses because they have been traditionally used for food materials in Korea. The results revealed that the populations of *Py. suborbiculata* and *Py. yezoensis* from Korea showed high levels of genetic diversity. In the both of COI-5P and *rbc*L analyses, *Py. suborbiculata* and *Py. yezoensis* separated into three major groups showing closely related population between east-coast of Korea and Japan. These results indicate that the north-west Pacific region has a possibility of genetic hotspot of *Py. suborbiculata* and *Py. yezoensis*. This is the preliminary data for the phylogeographic study of Korean foliose Bangiales to provide the information of distributional patterns based on the genetic diversity.

ASSESSMENT BY DNA SEQUENCING OF NONGENICULATE CORALLINE TYPE SPECIMENS (RHODOPHYTA) NAMED BY F. R. KJELLMAN DNA

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Sequencing of type specimens has been shown to be the only reliable method to unequivocally apply the correct names to coralline algae. Kjellman, between 1877 and 1889, named 12 Arctic and Subarctic nongeniculate coralline species that all predate Foslie names by at least one year. To determine the identity of the earlier Kjellman names we analyzed DNA from his type specimens. *Lithophyllum arcticum*, currently known as *Leptophytum arcticum*, is conspecific with *Neopolyporolithon loculosum*,

whose basionym, *Lithothamnion loculosum* Kjellman it predates by 12 years. The holotype of the currently recognized *Lithothamnion glaciale* is a single rhodolith that appears not to have been recognized for the past 135 years, but is an exact match to the specimen illustrated by Kjellman in 1889. *Lithothamnion soriferum*, currently and incorrectly considered a synonym of *Lithothamnion tophiforme*, is an earlier available name for the recently published *Lithothamnion erinaceum*. The holotype of *Lithothamnion intermedium* is comprised of two individuals, one identical to *L. glaciale* and the other to *L. soriferum*. *Lithothamnion foecundum* is correctly applied, but belongs in either *Mesophyllum* or a new genus, but not in *Leptophytum*. *Lithothamnion durum* is correctly classified as a heterotypic synonym of *Clathromorphum circumscriptum*. *Lithothamnion compactum*, basionym of *Clathromorphum compactum*, is distinct but two species are passing under this name. The distributions, habits and ecology of all of these species will be updated.

Eukaryotic Genomes: Transcription and Introns

RECURRENT UNCOUPLING OF THE MEANING OF THE UAG AND UAA CODONS IN EUKARYOTES

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Translation of mRNA molecules critically depends on termination (stop) codons recognized by specific release factors. Eukaryotes employ a single release factor, eRF1, to recognize all three termination codons. However, a number of eukaryotic lineages have reassigned one, two, or even all three termination codons to encode an amino acid, implying specific changes in their eRF1. Until recently, the meaning of the UAG and UAA codons seemed to be inseparable, as both codons have been reassigned to encode the same amino acid at least 13 times independently, whereas no UAG-only or UAA-only reassignment was encountered. However, last year we reported on two lineages that have managed to reassign UAG while keeping UAA as a termination codon: the fornicate *Iotanema spirale* and a novel uncultured rhizarian with UAG encoding glutamine or leucine, respectively. More recently we discovered three additional organisms from three different taxonomic groups (Ciliophora, Stramenopiles, Heterolobosea) with the same genetic code as we described in *I. spirale*. Genomic data from one of them, the ciliate *Trochilia petrani*, enabled identification of the newly evolved UAG-cognate Gln-tRNA gene. Our results thus indicate that the eukaryotic translation system can be modified to discriminate between UAG and UAA codons, and that this capability evolved at least five times independently. We are presently analysing

eRF1 proteins from the five lineages to illuminate how they lost the ability to recognize UAG while still binding UAA.

UNIQUE FEATURES OF PRE-MRNA SPLICING IN THE FREE-LIVING UNICELLULAR RED ALGA *PORPHYRIDIUM PURPUREUM* **Wong, D. K.**, University of British Columbia, Canada, donald.wong@alumni.ubc.ca

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Pre-mRNA splicing is a key eukaryotic feature, although our understanding of this process is based largely on well-studied organisms such as humans and yeast. Indeed, there is considerable diversity in mechanisms and components of pre-mRNA splicing, including examples from lineages that have evolved under the pressures of genome reduction. Red algae are thought to have undergone an ancient genome reduction prior to the lineage's radiation, resulting in overall gene and intron loss in extant groups. Previous transcriptomic studies on the extremophilic red alga Cyanidioschyzon merolae revealed a highly reduced spliceosome and unusually high levels of intron retention in its transcripts. To determine if these trends applied to other red algae, we investigated multiple aspects of pre-mRNA splicing in the free-living mesophilic red alga Porphyridium purpureum. Through strand-specific RNA-Seq, we observed high levels of intron retention across many of its 235 introns, and nearly half of the transcripts are not spliced. To assess links between intron retention and splicing machinery, we bioinformatically assembled the P. *purpureum* spliceosome, and assessed potential links to intronic sequence features. Our results suggest highly divergent or missing U1 snRNP components, despite the presence of a potential U1 snRNA. These unusual aspects highlight the diverse nature of pre-mRNA splicing in lesser-studied eukaryotes, and our transcriptomic data and comparative analyses provide further insight into red algal genome evolution, while raising the importance of investigating fundamental eukaryotic features in non-model organisms.

MICROSPORIDIAN INTRONS RETAINED AGAINST A BACKGROUND OF GENOME REDUCTION

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Spliceosomal introns are ubiquitous features of eukaryotic genomes but the mechanisms responsible for their gain and loss are difficult to identify. Microsporidia are obligate intracellular parasites that have among the smallest eukaryotic genomes known and have lost many, if not all, of their introns. Indeed, parallel reductions are also observed in their spliceosomal machinery. In the microsporidian *Encephalitozoon cuniculi*, one intron is an outlier in that it is three times longer than the average microsporidian intron and is spliced a higher level. In general, intron sequences are divergent in nature; however, we found that the long intron is part of an intron family that shows high levels of sequence conservation in two unrelated genes across diverse microsporidia. We identify an unusual extended interaction between these long introns and the U2 snRNA, which we predict plays a role in increased splicing levels. These long introns also share an additional internal conserved region, which includes a potential stem-loop structure, and infers a function for these introns. This makes their loss in intron-lacking taxa all the more intriguing. The retention of the stem-loop structure, the maintenance of strict spliceosomal motifs, and the unusual relative size of the long microsporidian introns offer unique insights into intron evolution that could be obscured in larger, more complex eukaryotic genomes.

BLASTOCRITHIDIA, THE TRYPANOSOMATID WITH ALL THREE STOP CODONS REASSIGNED

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According to the textbook definition, stop codon does not encode any amino acid but instead defines the end of translation. However, this view has been challenged by the discovery of eukarvotes that use all the three stop codons to encode amino acids. One of such organisms is a monoxenous trypanosomatid Blastocrithidia, the only known euglenozoan with a modified nuclear genetic code. Here, we have sequenced and analyzed genomes and transcriptomes of two cultivable Blastocrithidia species and another trypanosomatid Fi-14 ("jaculum" group), a close relative of Blastocrithidia with the canonical genetic code. We have created a new software for annotation of *Blastocrithidia* genome, as existing annotation programs are not able to deal with ambiguous stop codons. This allowed us to look at the reassigned stop codons from a wider perspective to see the general trends in their features and distribution. Besides, we have performed mass-spectrometry analysis of *Blastocrithidia* proteins to check experimentally the predicted meaning of the in-frame stop codons. Also, we have predicted and experimentally analyzed tRNA species that are responsible for stop codon decoding. The ultimate goal of our study is to address the following intriguing questions: How does translation termination function without defined stop codons? How do numerous in-frame stop codons influence translation? What are prerequisites of *Blastocrithidia* lineage that made the reassignment possible? What could be the intermediate steps between a system with a standard genetic code and one with all three stop codons reassigned?

LEADERLESS MRNAS ARE CIRCULARIZED IN *CHLAMYDOMONAS REINHARDTII* MITOCHONDRIA

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The mitochondrial genome of Chlamydomonas reinhardtii encodes eight protein coding genes transcribed on two polycistronic primary transcripts. The mRNAs are endonucleolytically cleaved from these transcripts directly upstream of their AUG start codons, creating leaderless mRNAs with 3' untranslated regions (UTR) comprised of most or all of their downstream intergenic regions. After cleavage, three untemplated oligonucleotide additions can occur on the 3' termini of these mRNAs - adenvlation, uridvlilation, or cytidvlation. The oligoA is very likely a degradation signal, U additions may also be a degradation signal but its role is unknown, and oligoC is hypothesized to play a part in the maturation process. In this study, we demonstrate that a portion of the processed linear mRNAs is circularized. This demonstrates that some of the Chlamydomonas mitochondrial mRNAs have leader sequences created by circularization and suggests that these leader sequences may negate the need for an alternative translation initiation mechanism. Sequencing of the 3'-5' junctions of the circularized mRNAs found the intramolecular ligation occurred between fully processed 5' ends (the start AUG) and a variable 3' terminus. For five genes (cob, cox, nd2, nd4, and nd6) some of the 3'ends were oligocytidylated and/or oligouridylated prior to ligation, and for two of them, cob and nd6, these 3' termini were the most commonly recovered sequence. This suggests these additions may play a role in the maturation of the 3' ends and may play a protective role to facilitate ligation.

TRANSCRIPTOMIC ANALYSIS OF STRESS RESPONSES IN *C. MEROLAE* Stark, M. R., UNBC, Canada, <u>stark@unbc.ca</u>

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Cyanidioschyzon merolae is a unicellular red alga found with a global distribution in acidic hot springs. Its surprisingly small complement of introns (27 in ~5500 genes) raises the question of what process has led to the elimination of introns (and the associated splicing machinery; see Stark poster), and whether the remaining splicing events have an important biological role. To address these questions, we have carried out a transcriptomic analysis of gene expression and splicing under two stress conditions, nitrogen starvation and phosphorus starvation. The results are startlingly different. Whereas phosphorus deprivation results in dramatic changes in gene expression, there is very little effect on splicing at short time points, and sequencing fragment profiles across genes are relatively stable. In contrast, nitrogen deprivation leads within 30' to massive RNA degradation across the genome, with most genes showing regions that are completely absent, while other regions are resistant to the degradation. Dramatic up- and down-regulation of splicing also occurs within 30'. We are currently analyzing these data to look for networks of genes that may be coordinately regulated, and to seek further evidence that splicing is in fact regulated, as there are very few proteins in *C. merolae* known to be involved in the regulation of splicing.

HOW TO LOSE A MITOCHONDRION: COMPARATIVE GENOMICS OF PREAXOSTYLA

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Preaxostyla is a lineage of anaerobic flagellates consisting of paraphyletic free-living trimastigids endowed with mitochondria-like organelles, and a crown group of endobiotic amitochondriate oxymonads. We have sequenced and assembled draft genomes of a trimastigid *Paratrimastix pyriformis* and two oxymonads *Monocercomonoides exilis* and *Blattamonas nauphoetae*. Annotation of multiple cellular systems of these protists provides insights into the dramatic evolutionary changes they underwent. An updated picture of *P. pyriformis* mitochondrial organelle is presented together with comparisons of selected metabolic pathways between *P. pyriformis* and the oxymonads, which were putatively affected by the transition to amitochondriality and symbiotic lifestyle. The distribution and evolutionary history of iron-sulfur clusters assembly pathways in Preaxostyla is also discussed. We hypothesize that it is the lateral gain of SUF system for iron-sulfur clusters assembly from bacteria, which served as a preadaptation for the complete loss of mitochondrion in oxymonads.

Symbiosis: From Parasites to Beneficial

DUAL RNASEQ TRANSCRIPTOMIC PROFILING OF THE FISH PATHOGEN SPIRONUCLEUS SALMONICIDA DURING OXIDATIVE STRESS AND INFECTION Stairs, C. W., Uppsala University, Sweden, <u>courtney.stairs@icm.uu.se</u> Svärd, S. G., Uppsala University, Sweden, <u>Staffan.Svard@icm.uu.se</u> Ettema, T. J., Uppsala University, Sweden, <u>thijs.ettema@icm.uu.se</u> The 'salmon killer' Spironucleus salmonicida is an anaerobic protistan parasite of salmonids. Unlike its close relative Giardia intestinalis that infects the gastrointestinal tract of humans, S. salmonicida is able to cause systemic infections. After colonization of the anoxic intestines, S. salmonicida can invade the blood and infect tissues throughout the host – including the oxygen-rich skin and gills. Since many aspects of S. salmonicida biology and metabolism are sensitive to oxygen, particularly within the anoxiaadapted mitochondria, we are interested in exploring how these parasites cope with oxygen-stress during infection. To investigate the parasite's biology in vivo, we developed an experimental infection system in juvenile salmon. We have compared the gene expression profiles of both host and parasites during infection as well as oxygen-stress using RNAseq. We observed that over 20% of the Spironucleus transcriptome is differentially expressed during both infection and oxygen stress. Parasites actively infecting salmon tissues upregulate genes encoding proteins involved in the oxygenstress response, cysteine metabolism, differentiation, as well as hypothetical proteins with no detectable homology to other proteins. Here, I present our working model for Spironucleus infections in terms of oxygen tolerance and host interaction. Altogether, this study has provided new insights into the intriguing biology of S. salmonicida. Understanding how the parasite is able to occupy different host environments will aid in the development of novel approaches to comba

ORGANELLOGENESIS LESSONS FROM STUDYING EUKARYOTE-BACTERIA ENDOSYMBIOSES

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For almost two billion years, eukaryotes have carried beneficial bacterial symbionts inside their cells that we now call mitochondria. The exact origin of this mitochondrial organelle and evolutionary steps in the transition from an endosymbiont to the organelle (organellogenesis) are still hotly debated. What is certain is that the organelle now relies heavily on protein import from the host cell employing genes of both bacterial and archaeal/eukaryotic origin. Even with their ongoing genome reduction and functional loss, mitochondria are still essential for majority of eukaryotes. This antiquity, widespread retention, and massive cellular, metabolic and genetic integration of mitochondria with their hosts is often viewed as an incredibly rare and stable example of a symbiosis that was essential to spark eukaryogenesis. Here, we inspect the symbiont-organelle transition using a broad sampling of endosymbioses that either seem to be on the trajectory to becoming organelles or not. We include not only model endosymbioses of multicellular eukaryotes such as insects, but also numerous endosymbioses from protists, including our novel data on diplonemids, ciliates, and parabasalids. We pay particular attention to the timing and stability (i.e. independent origins, losses, and replacements) of these symbioses, their intimate interactions with other microorganisms and organelles, and the role horizontal and endosymbiotic gene transfer play in their evolution. We argue that these younger endosymbioses can give us at least a few lessons about what was possible during eukaryogenesis.

MALBAC AND CISH: THE MOLECULAR INTERACTIONS OF THE RED ALGAL HOST-PARASITE RELATIONSHIP

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Red algal parasites have independently evolved over one hundred times within the red algal class Florideophyceae. These organisms are morphologically reduced, lack pigmentation, and establish cellular connections with their host. Very few studies have looked at the development of these organisms, instead focusing on the morphological details of their reproductive structures (an important distinguishing feature) or their phylogenetic history and taxonomy. The parasitic red alga *Choreocolax polysiphoniae* infects an epiphytic red alga, *Vertebrata lanosa*. Previous studies of *Choreocolax* have focused on its reproductive structures and evolutionary history, discovering the first parasite maintained plastid. Using parasite pustule size as a proxy for developmental age, samples were collected from field material from Beavertail State Park in Jamestown, Rhode Island, USA. Utilizing the MALBAC single cell transcriptomics protocol, amplified mRNA was sequenced from *Choreocolax polysiphoniae* pustules, infected host, and uninfected host material. Samples were also collected for chromogenic *in situ* hybridization (CISH) microscopy tying together the morphological development and genetic expression of the host and parasite throughout infection. Combining molecular and morphological datasets has enabled a deeper understanding of the parasitic interactions in red algae.

TOWARDS AN INTEGRATIVE UNDERSTANDING OF CHYTRID PARASITISM AND ITS DRIVERS IN MASS ALGAL CULTURE

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Chytrids cause rapid declines in microalgal populations in both natural water bodies and industrial algal ponds, yet surprisingly little is understood about how and under what conditions chytrids infect microalgae. From extensive production pond monitoring, we determined that chytrid infection rates are correlated with water temperature and daily irradiance, though only when algal motility is low (cyst stage). Additionally, bacterial dynamics were tightly coupled to algal physiology and culture age. To further investigate drivers of chytrid infection, we isolated two chytrid strains from crashed ponds: Rhizophydium sp. and Paraphysoderma sp. Culture assays supported the dependence of chytrid infectivity on algal growth stage, with highest infectivity of both strains on cyst-stage algae. Unexpectedly, we observed that the chytrids can switch between infective (parasitizing algal cells) and non-infective (feeding on algal exudate) lifestyles. The chytrid's trophic strategy appears to be regulated by dissolved organic carbon availability, which in turn is determined by algal exudation and competition with heterotrophic bacteria. Ongoing work will address the molecular changes underlying the switch in trophic strategies and subsequent physiologies, using metatranscriptomics and metabolomics. While the full significance of multiple chytrid trophic strategies in algal-dominated ecosystems is still unclear, identifying the molecular mechanisms and ecological drivers controlling chytrid metabolism is critical for deciphering their complex environmental roles, and will ultimately benefit our understanding of aquatic carbon cycling and algal bioenergy production.

METABOLIC FATE OF UPTAKEN GLYCINE IN THE CHROMERID ALGA *CHROMERA VELIA* Schneedorferová, I., Biology Centre, Czech Republic, <u>Ivana.Schneedorferova@email.cz</u> Tomcala, A., Biology Centre, Czech Republic, <u>a.tomcala@centrum.cz</u> Karlínová, I., Biology Centre, Czech Republic, <u>opekarova@bclab.eu</u> Cihlár, J., Biology Centre, Czech Republic, <u>Jerremy1@seznam.cz</u> **Oborník, M.**, Biology Centre, Czech Republic, <u>obornik@paru.cas.cz</u>

Chromera velia is an alveolate alga recently shown to be able to infect larvae of the coral *Acropora digifera*, however, it is living more likely as a facultative parasite, rather than a mutualist or a commensal. This is not surprising when the close relationship of chromerids and apicomplexans is taken into account, however, mixotrophy as the combination of phototrophy and parasitism is unprecedented in protists. Mixotrophic life style was reported for *C. velia*, and uptake of external glycine was the most effective. We studied the catabolism of labeled glycine-1-13°C by means of liquid and gas chromatography and mass spectrometry. We developed methods to specify ratio and speed of 13°C incorporation to final metabolic products to trace the fate of 13°C from the glycine. The catabolic pathway was proposed based on Kegg database and genes encoding the involved enzymes were identified. The analysis was focused on 13°C incorporation into chlorophyll a, free amino acids, lipids and monosaccharides. We showed that the

majority of labeled glycine was catabolized in 15 hours after administration. Glycine related amino acids and lipids were primary targets of the glycine-bounded 13°C, which was detected in these compounds within 7 hours. Incorporation of 13°C to the chlorophyll a was slower and was recorded within 48 hours. We showed no presence of the artificial 13°C in monosaccharides. Only about 5% of the glycine was used as the substrate for aminolevulinate synthesis in the mitochondria and further for heme and chlorophyll syntheses.

A WIDESPREAD CORAL-ASSOCIATED APICOMPLEXAN WITH AN UNUSUAL PLASTID **Kwong, W. K.,** University of British Columbia, Canada, <u>waldankwong@gmail.com</u> del Campo, J., University of British Columbia, Canada, <u>jdelcampo@icm.csic.es</u> Mathur, V., University of British Columbia, Canada, <u>varshamathur1994@hotmail.com</u> Keeling, P. J., University of British Columbia, Canada, <u>pkeeling@mail.ubc.ca</u>

Corals' symbiotic relationship with the photosynthetic dinoflagellate *Symbiodinium* forms the basis of reef ecosystems. Identification of other key microbial symbionts of corals has proven elusive. Here, we used microbial community surveys, metagenomics, and microscopy to show that diverse corals harbor a single, predominant clade of Apicomplexan symbionts. The Apicomplexa are an important group of unicellular parasites that include the causative agents of human diseases like malaria and toxoplasmosis. They evolved from free-living, phototrophic ancestors to become completely dependent on animal hosts for propagation, but how this transition occurred remains an outstanding question. We found coral-associated Apicomplexa in all major groups of corals, with high prevalence in both wild (80%) and aquarium (50%) samples. This symbiont was located intracellularly, within the tissues of the coral gastric cavity. Sequencing of its plastid genome shows that it shares characteristics with both parasitic and free-living relatives, including genes for chlorophyll biosynthesis, implicating it as a potential evolutionary intermediate. Our results indicate that this coral-associated apicomplexan, like *Symbiodinium*, is a ubiquitous coral symbiont, but one which represents an unexplored component of coral biology.

MUTUALISM OR SERVITUDE? THE CILIATE *EUPLOTES* AND ITS CONSTANT SEARCH FOR OBLIGATE SYMBIONTS

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When two symbiotic partners depend on each other for survival they are usually assumed to be mutualists, co-evolved to benefit from their relationship. This may be true when the advantages for both lineages last over long evolutionary times – which is not always the case. All species in a freshwater clade within the ciliate genus *Euplotes* harbor essential endosymbiotic bacteria. The bacteria, mostly belonging to the genus *Polynucleobacter*, also do not seem able to grow outside their hosts, providing a typical case of mutually dependent symbiosis. Genomic data reveal, however, that different *Polynucleobacter* strains became symbionts many times independently, each triggering the process of genome erosion that is common for intracellular bacteria. Since the establishment of the obligate symbiosis happened only once in the evolution of *Euplotes*, it follows that new symbiotic bacteria are repeatedly recruited from a free-living pool. The rampant gene loss in symbiotic *Polynucleobacter* is most likely not leading to a streamlined and functional organellar-like genome, but proceeds instead until it stops serving the host, who then captures a new soon-to-be symbiotic strain. This scenario is confirmed and complicated by the rarer occurrence of other bacterial genera (*Devosia* and *Protistobacter*) as obligate symbionts in the same *Euplotes* species. Analyzing the genomes of these independently evolved symbiotic lineages, as well as

those of their free-living relatives, provides the opportunity to test hypotheses and perform comparisons more accurately than in any other known system.

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Algal Physiology and Biomechanics

ANOTHER RENAISSANCE FOR SWELLING WALLS? **Peters, W. S**., Washington State University, United States, <u>winfried.peters@wsu.edu</u> Knoblauch, M., Washington State University, United States, <u>knoblauch@wsu.edu</u>

In the early days of plant physiology, the swelling of cell walls was thought to pressurize the protoplast, thus generating turgor. This biomechanic principle fell into oblivion following the demonstration that cells are osmotic systems in the 1870s. After a first renaissance in the early 20th century, when wall swelling that prevented plasmolysis under hyperosmotic stress appeared an essential process in intertidal algae, swellable walls disappeared from the literature once again. In kelps (Laminariales), photoassimilates are translocated in sieve tubes that are evolutionarily unrelated but structurally similar to their counterparts in land plants. We found that in kelp sieve tubes, the cell walls may expand radially, reducing tube volume by up to 90%. Only the innermost wall layers swelled, which are rich in alginates, gelatinous polymers of the cell wall matrix. Wall swelling was rapid, reversible, and in balance with intracellular pressure, as demonstrated by pressure injection of silicon oil. As sieve tube transport is pressure-driven and controlled by tube diameter, a regulatory role of wall swelling in turgor maintenance and sieve tube transport is implied. The phenomenon has been overlooked for over a century, although state-of-the-art methodologies were applied to kelp anatomy and transport physiology, suggesting that similar unidentified cases may exist in other eukaryotes with walled cells. Osmotic stress-induced wall swelling is easily demonstrated in *Porphyra* spp. (Rhodophyta). The recently released genome of *P*. *umbilicalis* provides a model for studying the molecular basis of wall swelling, which we hope will drive another renaissance for swellable walls.

UNRAVELING THE MOTILITY OF THE TERRESTRIAL CYANOBACTERIUM *MICROCOLEUS* VAGINATUS

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Many ocean or lake dwelling microbes move from place to place by using cilia or flagella to create asymmetric movement, a method necessary in Stokes flow. However, the physics and necessary conditions of how the terrestrial cyanobacteria, *Microcoleus vaginatus*, move in a non-fluidic environment are less well understood. This cyanobacterium is indigenous to high elevation deserts, such as the Colorado Plateau and Mojave Desert in North America, and is essential to the health and preservation of biological soil crusts. It has been shown that these cyanobacteria will move to the surface of the soil from their deeper zones in the soil when sufficient moisture is present, but it is unclear if this movement is caused via fluid phenomena or self-propelled movement. It is also unclear if there are basic environmental drivers for motility other than moisture, such as heat and light. Here we present an

investigation of *M. vaginatus'* response to light and heat in order to determine if these basic stimuli affect motility. Preliminary data suggests that the cyanobacteria shows little to no response to light except in high intensity light conditions. Understanding the dependence of environmental factors on the bacteria movement is crucial to quantifying the speed and strength of restorative efforts and informing models for future restoration projects.

CHEMICAL COMPOSITION OF CORALLINE CELL WALLS Janot, K. G., University of British Columbia and Beaty Biodiversity Centre, Canada, kyra.janot@botany.ubc.ca Martone, P. T., University of British Columbia and Beaty Biodiversity Centre, Canada, patrick.martone@botany.ubc.ca

Genicula serve a similar function in all articulated corallines, allowing otherwise rigid thalli to retain flexibility. Differences in genicular development and tissue properties between articulated clades, however, suggest that there is more than one way to achieve this function. Chemical composition of the cell wall plays a role in both the decalcification process that is integral to genicular development, and the mechanical properties of the genicular tissue; we therefore investigated whether the content and identity of polysaccharides in genicular tissue would differ between articulated clades. We also analysed the chemical composition of intergenicular tissues and closely related crustose species, to see whether phylogenetic relationships could predict cell wall chemistry of calcified tissue across different coralline morphologies. Data presented here suggests that developmental and mechanical differences are underscored by differences in genicular cell wall chemistry; conversely, chemical similarity between calcified tissues across both articulated and crustose species suggests that little has changed in the cell wall of these tissues over the course of evolution.

THE ANTARCTIC RED SEAWEED *PLOCAMIUM "CARTILAGINEUM*" AND ITS WEALTH OF DEFENSIVE SECONDARY METABOLITES

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Inter- and intraspecific interactions between organisms can either be a form of communication, environmental sensing, or defence and are often mediated by chemicals such as secondary metabolites. Sessile marine organisms such as macroalgae commonly produce chemical defences against grazers, pathogens, as well as biofoulers. *Plocamium "cartilagineum* is a finely branched red understory alga that is common in Antarctic macroalgal forests. It supports a very high abundance of amphipods of which most are not able to feed on the heavily chemically defended *P. cartilagineum*, except for *Paradexamine fissicauda*. Different *P. "cartilagineum*" individuals produce differing mixtures of halogenated secondary metabolites which are referred to as chemogroups. Around Palmer Station in 2016 and 2017 a total of 13 different chemogroups were identified. The 13 chemogroups fit well into two distinct genetic groups (but not different enough to be considered different species), identified by the *cox1* and *rbcL* genes. These data suggest that chemogroups are to some extent site specific, they have some correlation with depth and that there is an underlying genetic differentiation.

THE EFFECT OF TENSILE STRESS ON SPATIAL GROWTH PATTERNS IN THE KELP NEREOCYSTIS LUETKEANA

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Many seaweeds exhibit morphological plasticity across gradients of hydrodynamic stress. The bull kelp, Nereocystis luetkeana, presents a relatively well-studied example of this. When sheltered from waves and currents, this alga develops blades that are broad and undulate in shape. However, when hydrodynamically stressed, it produces narrow, flattened blades. The functional significance of this is thought to be primarily to reduce the amount of drag the kelp experiences when ambient flow velocities are high in order to prevent dislodgement from the substratum. While it has been shown that changes in blade morphology can be brought about by tensile forces normally associated with water motion, little more is known about the physiological mechanisms that enable this kind of plasticity in algae. In this study. I seek to address the question of what is happening at the cell level to create whole-organism level changes in morphology when Nereocystis is subjected to tensile stress. I hypothesize that tension causes meristematic cells to divide primarily in the axis of the stress. To examine this, I took strips of blade tissue oriented longitudinally or transversely relative to the main blade axis and placed them in a growth tank. One group of strips of each orientation was subjected to constant longitudinal tension using a system of clamps, lines, and weights, while another group of each was left unweighted. All strips were left in place for a growth period of several days. The change in length, width, and thickness observed in all strips over the growth period, as well as the patterns of cell division, were compared between both orientation and stress level

PHYSIOLOGY OF *PICOCHLORUM OKLAHOMENSIS* IN CYCLOTURBIDOSTATS Franks, D. T., Oklahoma State University, United States, <u>dylan.franks@okstate.edu</u> Sabella, A. J., Oklahoma State University, United States, <u>asabell@okstate.edu</u> **Henley, W. J.**, Oklahoma State University, United States, <u>bill.henley@okstate.edu</u>

We grew *Picochlorum oklahomensis* in eight replicate cycloturbidostat cultures (14:10 L:D cycle) for over five weeks. We poised cultures at an influent nitrate concentration just above limitation to induce mild physiological stress. For the last two weeks we supplied two cultures at 300 μ M, and three cultures each at 250 and 275 µM nitrate, with a 5:1 molar N:P ratio. Turbidity tracks biomass density, with the signal decreasing at night due to respiration, and pumps cycle steadily only for the last 11-12 hours of the photoperiod. All cultures maintained a specific growth rate (μ) of about 1.2 per day and predawn Fv/Fm, a measure of maximum photosynthetic efficiency, between 0.3-0.6. These Fv/Fm values suggest mild physiological stress, despite the high µ. Rapid light curves (RLCs) demonstrated diurnal induction of photosynthesis, with increasing initial slope and light-saturated relative electron transport rate. Incipient nutrient limitation was also indicated by occasional mishaps with individual cultures. For example, interrupted medium supply for about one hour, even when immediately followed by makeup flow, was evident in reduced RLC parameters the next morning. Occasionally, a culture would rapidly decelerate growth for no apparent reason, and had to be rescued by spiking with N and P equivalent to a few pump cycles, which resulted in recovery of fluorescence parameters and growth within a few hours. Flow cytometry shows phased cell division, although at $\mu = 1.2$ per day (1.7 doublings per day), some cells must have divided more than once per day. We also intend to determine whether triglyceride content was elevated despite the high growth rate.

Protists as Bioindicators of Change

CLIMATOLOGICAL AND ANTHROPOCENE EFFECTS ON SANDFLAT MICROALGAL COMMUNITIES OF LAGUNA MADRE, TX **Shalygin, S.,** Texas A&M Corpus Christi, United States, <u>got.lifemusic@gmail.com</u> Zimba, P., Texas A&M Corpus Christi, United States, <u>Paul.Zimba@tamucc.edu</u> Huang,, I., Texas A&M Corpus Christi, United States, <u>got.lifemusic@gmail.com</u> Laguna Madre is one of the largest hypersaline lagoons in the world, with an overall length of ~ 185 km. This region is renowned for its fishing, seagrass beds, and extreme hypersalinity. Wind tidal-sandflats within the lagoon are critical habitat for overwintering shorebirds such as sanderlings, plovers etc. Little is known about algal composition of this ecosystem, other than recognition of brown tides in the late 1980-2000s, and two studies on sandflat mats. Historically, algal mats, which are the sole primary producers in these harsh environments, reached 3 cm thickness, and consisted of a few cyanobacteria and diatom taxa. Samples from 2014 showed noticeable differences in thickness and species composition by light microscopy and pigment analysis. Now cyanobacterial species richness is >32 species with 10 taxa identified to species level. Flora is unique, containing *Pleurocapsa minuta*, *Spirulina tennerima*, Oxvnema sp., and Bacularia sp. and others. Historically dominant cvanobacteria (such as Coleofasciculus chthonoplastes, Lyngbya sp.) have been displaced and mats currently contain 7-14 species, dependent on location (with maximum species richness detected in samples collected from Kenedy Ranch), all with a maximal thickness less than 1 cm. In the samples collected from submerged habitat within Kenedy Ranch we have found additional taxa (Leibleinia spp.) growing epiphytically on the dominant species, Lyngbya sp. Reasons for depletion of the mat thickness and increasing of the cyanobacterial species richness may include sea level rise, opening of passes to the Gulf of Mexico, and enrichment of coastal waters.

MULTI-YEAR ASSESSMENT OF MARINE EUKARYOTIC PLANKTON DIVERSITY FROM AMPLICON AND IMAGE DATA

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We have collected and evaluated multi-year high throughput sequencing and image data at the Martha's Vineyard Coastal Observatory (MVCO) to assess microplankton community structure variation with respect to seasonal and annual environmental conditions. Surface water samples for sequencing were collected monthly or bimonthly over the period 2013-2017, while plankton image data was collected every 20 minutes by the Imaging FlowCytobot at MVCO. Amplicon tag data showed seasonal patterns of diatoms each year, with summer being dominated by 'polar centric' species and the winter/spring by 'radial centric' species. Ciliate amplicons also revealed a seasonal pattern, with lowest levels in the summer, and choreotrichs contributing to late winter spikes in tag abundance. Dinoflagellate amplicons changed little in abundance between 2013-2015, after which they began showing monthly spikes usually dominated by Gyrodinium tags. Temperature was the environmental variable that best described the total variation in the data, with phosphorous also contributing. Slight differences in taxa contributed to the vearly patterns, reflecting the interannual environmental variability. Co-occurrence between parasite and putative host sequence tags was examined for Cryothecomonas and Guinardia delicatula amplicons, and Syndiniales and dinoflagellates, ciliates and cercozoa; suggesting possible new host interactions. Image abundance data shows seasonal patterns similar to the amplicon tag data, particularly for diatoms. Overall, we are finding the combination of both methods useful for developing a more complete picture of planktonic community composition and response to environmental variation.

CILIATE MICROZOOPLANKTON FROM THE NORTHEASTERN GULF OF MEXICO

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PROTIST COMMUNITIES IN VINEYARD SOILS: WHAT DO THEY TELL US ABOUT SOIL QUALITY AND HEALTH?

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The impact of management practices and pesticides on soil vineyard quality and health is a growing concern for consumers, vine-growers and land managers. To evaluate this impact, bioindicators such as protists have great potential, but their use has been limited since little is known about the response of protists to soil management practices and soil pesticides. Here we used high-throughput environmental sequencing to investigate the effects of several abiotic and biotic factors on protist species diversity and composition across a network of thirty-three vineyards in southwestern Switzerland. Our analyses show that copper and nitrogen contents were the most strongly associated factors with protist community composition. However, protist diversity was negligibly affected by these parameters. In addition, we used indicator species analyses as well as machine learning approaches to identify and select bioindicators (subsets of protists OTUs) from four groups of samples with low, medium, high, and very high levels of copper. Overall, our results suggest that protists are sensitive to management practices and confirm the suitability of high-throughput environmental sequencing approach as a potential tool for soil vineyard biomonitoring.

MICROBIAL COMMUNITIES ON HERON ISLAND REEF Langlois, G. A., Bryant University, United States, <u>langlois@bryant.edu</u>

Field and laboratory studies conducted at the Heron Island Research Station (HIRS) located in the Capricorn Region of the Great Barrier Reef 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 study, with comparisons to similar observations in 2006 and 2016, emphasized the diversity of ciliates and diatoms found in shallow reef sediments especially the relative abundance of different trophic groups. This study explored the interactive dynamics of opportunistic and/or potentially pathogenic species associated with damaged corals undergoing disease conditions such as Brown Band disease, including Porpostoma sp. These altered communities are characterized by rapid tissue breakdown in hard corals and release of high levels of dissolved organic matter. Benthic samples were taken by direct capture, observed with phase contrast and epifluorescence microscopy, recorded by video and photomicrography, and fixed for further identification and genomic assessment. The relative abundance of diatoms/flagellates/ciliates showed a higher proportion of microalgae in recent as compared to earlier observations. The presence or absence of epiphytic protista on macroalgae was correlated with extracted algal exudates. The Brown Band ciliate, Porpostoma sp., was not found to be associated with corals, sediments, algal surfaces, or plankton prior to bleaching in the 2016 or 2018 samples in late January, posing the question of their location when not acting as opportunistic pathogens. Future sampling will focus on characterizing the life cycle of *Porpostoma* sp. on the reef. Video and photo images will be submitted to an online library to be available for future researchers.

PALEOECOLOGY OF MARINE PROTISTS: COMMUNITY DYNAMICS AND POPULATION ADAPTATIONS REVEALED BY ANCIENT-DNA METABARCODING AND PHYSIOLOGY OF REVIVED DINOFLAGELLATES

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The effects of the environmental changes that occurred in coastal ecosystems during the last century on protist dynamics and physiology are barely understood due to the shortness of the plankton monitoring data series and the drift of original physiological traits in long time cultured strains. The analyses of sedimentary biological archives (ancient DNA and living resting stage banks) in cold and lacustrine ecosystems have proved to compensate this information gap. In coastal ecosystems, the paleoecological approach was considered to be challenging due to potential perturbations of the sediments. In the Bay of Brest (NW Atlantic, France) several sediment cores, collected from estuarine ecosystems, allowed reliable analysis of sedimentary archives up to 4800 BP (ca. 2900 BC). The metabarcoding of the ancient DNA revealed shifts in protist and dinoflagellate community dynamics during the last century. Using different culture conditions and germination biostimulants, up to one century-old dinoflagellate strains have been revived. Metabolomic analyses of strains of ca. 30-years difference, grown in depleted phosphate conditions, sustain the hypothesis of intraspecific variability of dinoflagellate populations of different ages and potential adaptation of modern strains to phosphate limitation. The limits of the exploitation of protist paleoecological archives in the coastal ecosystems have been analyzed and understood, nevertheless this approach proved to be both useful to fill in past information gaps and valuable to answer ecological and evolutionary questions on protists.

Species Delimitation in Protists

PLASTID VARIATION IN CLOSELY RELATED DIATOMS

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Stephanodiscus yellowstonensis, an endemic diatom from Yellowstone Lake (Yellowstone National Park, Wyoming), evolved from a Stephanodiscus niagarae-type progenitor during the Holocene. Morphological evolution is well-documented from lake core records, but no known genetic differences exist between these two species in barcode regions of the genome (rbcl and LSU). Here, we use next-generation sequencing to assemble the chloroplast genome of both *S. yellowstonensis* and *S. niagarae* to determine if there are any variable regions of the plastome between these two species. We found that there are 41 nucleotide polymorphisms between these two species in the chloroplast genome. Future work will include creating primers to amplify these regions in multiple individuals and populations of *S. yellowstonensis* and *S. niagarae* to determine which nucleotide polymorphisms are variable between and within species. These findings will be used to track the molecular evolution of *S. niagarae* to *S. yellowstonensis* and to determine population structing of *S. niagarae* populations across North America.

NEW MOLECULAR DATA FOR TERMITE-ASSOCIATED PARABASALIDS IN THE CLASS SPIROTRICHONYMPHEA

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Spirotrichonymphea is one of six classes of protists within the phylum Parabasalia. These morphologically complex protists reside exclusively in the hindguts of termites, where they contribute to the digestion of lignocellulose. Currently, most data available on the Spirotrichonymphea are morphological descriptions, while very little molecular data are available. We are currently obtaining molecular data to better classify the taxa within the Spirotrichonymphidea. We have determined the phylogenetic position of several genera that were previously lacking molecular data, including *Holomastigotes, Microjoenia*, and *Spironympha*. Our data support the monophyly of *Holomastigotes* and *Microjoenia*, while suggesting that *Spironympha* and *Spirotrichonympha* are polyphyletic. In addition, both Sanger sequencing of 18S rRNA genes from single cells and high-throughput 18S amplicon sequencing of whole termite guts indicate that there are numerous cryptic and undescribed species and genera within the Spirotrichonympha.

SYNONYMY BETWEEN TWO SPECIES OF *STYLONYCHIA*: *STYLONYCHIA NOTOPHORA* STOKES,1885 AND *STYLONYCHIA PUSTULATA* (MÜLLER,1786) EHRENBERG,1838 **Kaur, H**., Department of Zoology, University of Delhi, India, <u>hk2171@gmail.com</u> Prasad, S., Ciliate Biology Lab, SGTB Khalsa College, University of Delhi, Delhi, India, <u>shasbot.du@gmail.com</u> Negi, R. K., Department of Zoology, University of Delhi, Delhi, India, <u>negigurukul@gmail.com</u> Kamra, K., Ciliate Biology Lab, SGTB Khalsa College, University of Delhi, Delhi, India, <u>komalkamra@gmail.com</u>

The spirotrichean *Stylonychia notophora* has previously been reported in India and Germany although the descriptions are lacking in details. It has been suggested several times that *S. notophora* does not differ from *S. pustulata* but this has never been confirmed due to insufficient data for *S. notophora*. In the present study, we characterized six populations of *Stylonychia* from locations along the River Yamuna, India with detailed descriptions using classical and molecular approaches. On the basis of comparative studies among these populations and with previous descriptions of *S. pustulata* and *S. notophora* show only minor differences in morphometry (Body length, AZM length, number of marginal cirri and number of dorsal kinieties) and in 18S rDNA sequences (pairwise distances ranging from 0.005 to 0.026), but

none in morphogenesis. Five of these isolates were nominally identified as *S. notophora* based on the similarity of its 18S rDNA gene with reference sequences in NCBI database. The sixth was morphologically similar to the other five populations but the 18S rDNA gene was most similar to reference sequences of *S. pustulata*. These findings support the contention that *S. notophora* is synonymous with *S. pustulata*. The minor differences among the six populations are thought to be a consequence of deteriorating water quality (as determined by ten physicochemical parameters) along the river from where isolations were made. Whether these six isolates represent subspecies of *S. pustulata*, or if this is an example of cline gradient, will be discussed.

THE GENUS *PSEUDOPARAMOEBA* (AMOEBOZOA, DACTYLOPODIDA): MORPHOLOGY, MOLECULAR PHYLOGENY AND SPECIES DISTINCTION

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The genus *Pseudoparamoeba* in the modern system of Amoebozoa belongs to the family Paramoebidae (Discosea, Dactylopodida). This genus differs from closely related genera in presence of glycostyles with hexagonal base and dome-shaped apex. In contrast with Neoparamoeba and Paramoeba amoebae of the genus Pseudoparamoeba have no Perkinsela amoebae-like organism (PLO) in their cytoplasm. Pseudoparamoeba is a relatively rare genus. Since the description of a type species in 1975, only one more species was isolated from fresh water. It was described as P. microlepis in 2016. It was stated in paper that this species differs from P. pagei (type species) in details of a cell coat structure, but now we have reasons to suggest that differences in the observed structure of *P. microlepis* glycostyles are related to the different methods of observation. We found another marine species designated here as Pseudoparamoeba sp. with characteristic Pseudoparamoeba glycostyles. Molecular phylogeny shown that our isolate probably represents a separate species. 18S rDNA sequence of *Pseudoparamoeba* sp. never forms a clade with marine P. pagei, but branches separately at the base of Pseudoparamoeba clade. This tree topology could mean that *Pseudoparamoeba* is primarily marine genus, with some species secondary passed to freshwater habitats. All species have almost identical locomotive form shape and dimensions. Thus, they are morphologically indistinguishable. It seems to be that molecular data is a primary criterion allowing to discriminate Pseudoparamoeba species (COX1 also showed high genetic distances between them). Supported with the Russian Foundation for Basic Research 18-34-00595 grant.

TWO SPECIES OF EUGREGARINES PARASITES OF THE MEXICAN LUBBER GRASSHOPPER *TAENIOPODA CENTURIO*: NOTES ON ITS MORPHOLOGY AND PHYLOGENETIC POSITION **Medina Durán, J. H**., Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico, jorgemedinad@ciencias.unam.mx Mayén Estrada, R., Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional

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Eugregarines are common but understudied apicomplexan parasites of invertebrates from marine, freshwater and terrestrial environments. Most gregarines in terrestrial habitats parasitize insects, however gregarines have been described in less than 1% of total insect diversity. In orthopterans (katydids, grasshoppers and crickets), gregarines usually infect the digestive tract, but the knowledge of its diversity is still scarce. The objective of this study was to carry out a survey of the gregarines from the Mexican lubber grasshopper *Taeniopoda centurio* to provide data of their morphology and phylogeny. We isolated

the gregarines and observed their different life-cycle stages, and later we sequenced the SSU rDNA gene to conduct a phylogenetic analysis along with other known gregarine taxa. We identified two gregarine species from two superfamilies based on morphological characteristics, and on its phylogenetics position. Our results show that both species are phylogenetically distant from each other despite the fact that they parasitize in the same host. As a conclusion, gregarine-insect interactions are poorly understood, and a single host can harbor more than one gregarine species. Further research is necessary to unveil its high but unknown diversity, and its evolutionary significance. Acknowledgments. To Consejo Nacional de Ciencias y Tecnología (CONACYT) for the scholarship to conduct my masters' degree project, and to Posgrado en Ciencias Biológicas, UNAM. To Programa de Apoyo a los Estudios de Posgrado (PAEP) for the financial support for a short stay in Texas A&M University. To Biól. Margarita Reyes Santos for her technical support.

TAXONOMIC DESCRIPTION AND MOLECULAR PHYLOGENY OF THREE SPECIES OF GENUS *EUPLOTES*: FIRST REPORT FROM DELHI, INDIA

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Ciliates are considered as one of the most diverse protozoa and play major role in ecology as they form important part of the microbial loop. Among ciliates, the class Spirotrichea has been regarded as one of the most diverse group of ciliates and order Euplotida is considered to be a well-defined lineage which constitutes more than 70 morphospecies. Euplotids can be found in nearly every habitat, including freshwater and soil ecosystems. In order to explore the species diversity of euplotids, the morphology, infraciliature, silverline system and molecular analyses of the three different species of genus Euplotes, viz. E. aediculatus, E. woodruffi and Euplotes sp. were investigated. E. aediculatus and E. woodruffi was collected from freshwater lakes in Delhi and Euplotes n.sp. from soil of Delhi, India. The Indian isolate of *E. aediculatus* having body size about 97 x 66 µm with 8 dorsal kineties and double eurystomous type dorsal argyrome; E. woodruffi having body size about 95 x 66 um with 10 dorsal kineties and double eurystomous type dorsal argyrome; *Euplotes* n. sp. has size about 65 x 50 µm with 6 dorsal kineties and complex type of dorsal argyrome. The molecular analyses of the three species were done by isolating and sequencing the small subunit ribosomal rDNA (SSU rDNA) and internal transcribed spacer (ITS) gene to infer their phylogenetic relationships. In the SSU rDNA tree E. aediculatus is forming clade with E. aediculatus; E. woodruffi is forming clade with E. woodruffi and E. parawoodruffi with moderate bootstrap support; Euplotes n. sp. is clading together with E. novemcarinatus and E. encysticus with low bootstrap support suggesting it to be a new species.

RESOLVING CRYPTIC SPECIES PROBLEMS IN AMOEBOID MICROBES USING LARGE TRANSCRIPTOMIC DATA **Tekle, Y. I.**, Spelman College, United States, ytekle@spelman.edu

The discordance between molecular and morphological taxonomic studies has resulted in discovery of many cryptic species diversity in amoeboid microbes. Since microbes suffer from limited morphological

variability that can be used for taxonomic delineations, this number is expected to rise as more microbes are exposed to molecular studies. There are two major cases of discordances reported in members of Amoebozoa. In one case the same morphotypes are genetically distinct, while in other cases morphologically different species are genetically identical. Early studies using the commonly used DNA barcode markers (cytochrome oxidase I and ribosomal genes) in Amoebozoa reported mixed results indicating that these markers might be not ideal for species identification or delineation in all groups. Given the complexity of cryptic species diversity and the growing number of transcriptome data in Amoebozoa, we tested the utility of transcriptome data to resolve cryptic species diversity and to get a better understanding of the reported discordances. Using a custom developed pipeline, we analyzed up to 30,000 contigs per sample from diverse amoeboid lineages with known cryptic species problems and exemplified the reported discordances. Our transcriptomic comparative analyses reveal a consistent pattern of inter- and intra-strain divergences among known or suspected cryptic species indicating the appropriateness of transcriptome data for cryptic species diversity studies. We also identify several potential barcode markers from our transcriptome data for use in microbial amoeboids.

Evolution of Eukaryotic Lineages I: The Beginning

DIATOM DIVERSIFICATION THROUGH THE LENSE OF LARGE SEQUENCE, FOSSIL, TRAIT, AND DIVERSITY DATA: THE ROLES OF LIFE HISTORY STRATEGIES AND ENVIRONMENTAL GRADIENTS

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Variation of diversification rates across lineages, environments, and time is a core question in diatom evolutionary biology. The rich fossil record has and will teach us much about these patterns, however, it is incomplete and biased towards the marine plankton. Phylogenies, in turn, can consider the entire diversity and timespan of diatom evolution. We constructed a time-calibrated diatom phylogeny of 1151 species, the most-inclusive to date, and used it to test several hypotheses regarding species richness patterns across life history strategies (sexual reproduction, motility) and environments (plankton-benthos, marine-freshwater). Anisogamous diatoms diversified faster that oogamous diatoms, but this increase was restricted to the nested clade of actively motile raphid diatoms. Freshwater clades diversified faster than their marine counterparts, but there was substantial variation within each group, indicative of the influence of unobserved factors. Both the plankton-benthos and marine-freshwater divides were typified by strong niche conservatism: bidirectional transitions were possible, but transitions took much longer to occur than in situ speciation or extinction events. Overall, our results support active-motility and freshwaters are independently associated with increased species turnover. However, as the rate of colonization of freshwater was higher in the raphid clade, the observed effect of active motility could in part be due to the underlying dynamics of salinity switches. Our results provide the first phylogenetic examination of diatom diversification and set the stage for an integrated analysis of the fossil and phylogenetic data.

COMPARATIVE ULTRASTRUCTURE OF PROTOSTELOID SPOROCARPY AMONG AMOEBOZOA

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Protosteloid sporocarpy is a process found only among Amoebozoa where a single amoeboid cell either rounds up directly and develops into a fruiting body that consists of a microscopic stalk and one to a few

spores, or where a multinucleate amoeba cleaves into segments that follow the same developmental path. This type of development was originally considered to be a synapomorphy of Eumycetozoa, *sensu* L.S. Olive, but molecular systematics analyses have shown that protosteloid sporocarpy is present in both major lineages of Amoebozoa, and that it occurs in the taxa Variosea and Eumycetozoa, sensu Kang et al., in Tevosa and in the Centramoebia and Flabellinia in the Discosea. This distribution of protosteloid sporocarpy in Amoebozoa either indicates that the process was ancestral to all amoebozoans, has arisen more than once by convergence, or a combination of both. Light and electron microscopic examination of fruiting body development and mature sporocarps in protosteloid amoebozoans that has been carried out over the last five decades show remarkable phenotypic similarities among both tevosean and discosean examples. These features will be presented, and the possible explanations for the similarities will be discussed along with ideas for the resolution of these possibilities.

EVOLUTION OF ANAEROBIC LIFESTYLE IN HETEROLOBOSEA

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Heterolobosea (Discoba) is a group of ~150 described species of heterotrophic protists that shows an extraordinary ecological breadth and morphological diversity. Most heterolobosean species are marine or freshwater aerobes, but the group also includes many thermophiles, several clades of halophiles, and at least three unrelated lineages of anaerobes. This ecological breadth predestines Heterolobosea to be an important model group for comparative studies focused on adaptation of eukaryotes to unusual environments. However, lack of a transcriptomic and genomic data as well as unresolved phylogeny within the group limit further research. Here, we obtained substantial amount of transcriptomic data from all main clades of the group including all three lineages of obligate anaerobes ('Dactylomonas', *Creneis*, Psalteriomonadidae) and genomic data from *Neovahlkampfia damariscottae*. To robustly resolve the phylogeny of Heterolobosea, we carried out a phylogenomic analysis based on 305 protein-coding genes. Moreover, our comparative study shows that each of the three anaerobic heterolobosean clades possesses a different type of mitochondrion-related organelle (MROs). Surprisingly enough, our results suggest that the last common ancestor of Heterolobosea was most likely able to generate ATP anaerobically. Our findings also provide important new insights into the evolution of anaerobiosis in eukaryotes.

WHAT IS A MANTAMONAD?

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Mantamonads comprise a deeply-diverging (kingdom-scale) lineage of tiny gliding heterotrophs that were unknown to science until 2007. Transcriptomic data was sequenced from the original strain (Bass1), and a recent phylogenomic analysis including this data proposed a new 'supergroup', informally termed 'CRuMs', combining mantamonads with rigifilans and collodictyonids, two other 'orphan' lineages. Unfortunately, Bass1 died in culture shortly after being sequenced. We recently discovered a new strain of mantamonad (SRT-306), which has only ~94% identity to the original SSU rRNA sequence. We have sequenced and annotated both its transcriptome and its genome. We have also assembled a completely new phylogenomic dataset, to which we added SRT-306, and are currently analysing it. In addition to this, we have been assessing the physiology and life-history traits that can be determined from a complete genome. Surprisingly, SRT-306 appears to lack all known carbohydrate-storage pathways. We are currently exploring the implications of this finding.

NEW FINDINGS IN THE DIVERSITY, TAXONOMY AND LIFE CYCLES OF CENTROHELID HELIOZOANS

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The centrohelid heliozoans represent a sister group of haptophytes, together with them forming the phylum Haptista. During the last decade the molecular phylogenetics reshuffled the system of the group, indicating the presence of two subclades: Acanthocystida and Pterocystida. Those two groups are the prime example of parallel evolution. The external skeleton morphology, the main taxonomic character in centrohelids, has independently become similar. For example, genera *Raphidiophrys* and *Raphidocystis* are characterized with the same tangential plate scales with hollow inflected margin. Interestingly the parallelisms also can be observed in the structure of 18S rRNA molecule, which was considerably expanded with length increases independently in both clades. Being a perfect diagnostic criterion the external skeleton morphology sometimes demonstrates the confusing pattern of distribution with some heterogeneous riboclusters including the representatives with quite different structure of cell coverings. Recently this conundrum has received a good explanation. It was shown that at least some centrohelids are capable of changing the morphology of the skeleton in the course of their life cycle. The difficult question is the reconstruction of the phenotype for the common ancestor of centrohelids. Our last results favor the hypothesis of the complex ancestor with a double layer of siliceous scales present. Study support: RFBR grants 15-04-18101, 16-34-60102.

EVOLUTION OF MAJOR METABOLIC PATHWAYS OVER ALVEOLATA **Guillou, L.**, CNRS, France, <u>lguillou@sb-roscoff.fr</u>

Alveolata represent a major group of protists with a great diversity of lifestyles (phototrophy, mixotrophy, parasitism and mutualistic symbiosis). They include, among others, parasitic apicomplexes, heavily studied due to their importance in either medical or veterinary biology, and dinoflagellates, some of which are responsible for harmful algal blooms or have evolved as fish and shellfish killing parasites causing substantial impact to food security and economies. Our understanding of the evolution of

Alveolata is rapidly expanding, notably with the release of genomic and transcriptomic data providing us the unique opportunity to better understanding how trophic modes and lifestyle evolve in this complex lineage. Here we present the comparison of major metabolic pathways over various alveolate lineages, trying to understand the ecological success of this group.

NEWLY IDENTIFIED LINEAGE OF EUKARYOTES WITH FIVE DISCOVERED REPRESENTATIVES AND EARLY MITOCHONDRIAL GENOME REDUCTION **Tikhonenkov, D. V.**, Institute for Biology of Inland Waters, Russian Academy of Sciences, Russia, <u>tikho-denis@yandex.ru</u> Janouškovec, J., University College London, United Kingdom

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Predatory protists are understudied, but often represent important deep-branching evolutionary lineages with unique morphology, slowly evolving nuclear proteins, large and slowly-evolving mitochondrial gene sets. Here we characterize Ancoracysta twista, a new predatory flagellate that is not closely related to any known lineage of eukaryotes. It was found in a sample collected from the surface of a tropical aquarium brain coral. It actively feeds on bodonids, probably immobilizing its prey through discharging a previously unknown type of extrusome named an ancoracyst. Maximum Likelihood model supported a sister relationship of Ancoracysta to a grouping of haptophytes and centrohelids. The Bayesian tree placed Ancoracysta at even deeper position basal to SAR + Haptista. The Ancoracysta mitochondrion has a gene-rich genome with a coding capacity exceeding all other eukaryotes except the distantly related jakobids and Diphylleia, and uniquely possesses heterologous, nucleus- and mitochondrion-encoded cytochrome c maturase systems. Analyzing on mitochondrial genomes across the eukaryotic domain refines scenarios for rooting the tree of eukaryotes and suggests that gene transfer from mitochondria has been highly parallel and exponentially decreasing in nature. Surprisingly, Ancoracysta twista has a sister. Recently we discovered four more representatives of this lineage, related to Ancoracysta. They are all predatory biflagellate cells inhabiting marine waters of different geographical zones and not always associated with corals. This work was supported by the Russian Science Foundation (grant No 18-14-00239).

Applied Phycology: Microalgae and Biofuels

CHOLINESTERASE INHIBITORY AND DIS-AGGREGATION EFFECTS OF TWO *CHLORELLA* SPECIES: NEUROPROTECTIVE POTENTIALS FOR THE MANAGEMENT OF ALZHEIMER'S DISEASE

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The chemical composition, antioxidant, anticholinesterase and anti-amyloidogenic activities of hexane, dichloromethane and ethanol extracts of *Chlorella sorokiniana* and *Chlorella minutissima* recovered from a fresh water in Durban, Kwazulu-Natal, South Africa were investigated in this study. FTIR characterization of the algal biomass revealed the presence of polyphenols, polysaccharides, proteins and fatty acids. GC-MS analysis of the extracts also revealed the presence of some phenols, sterols, steroids, fatty acids and terpenes. Ethanol extract of *C. sorokiniana* and dichloromethane extract of *C. minutissima* had the highest total phenol and flavonoid contents respectively. All the extracts scavenged 2,2-diphenyl -

1- picrylhydrazyl (DPPH) and hydroxyl (OH) radicals. The highest metal chelating activity of the extracts was observed in the ethanol extracts of *C. minutissima* and *C. sorokiniana*. Furthermore, the cholinesterase inhibitory activities of the extracts showed that ethanol extract of *C. sorokiniana* exhibited highest acetylcholinesterase inhibitory activity while dichloromethane extract of *C. minutissima* showed the highest butyrylcholinesterase activity. Results of Thioflavin-T assay and electron microscopy study revealed that incubation of β -amyloid protein increased the aggregation of fibrils after 96 hours. However, ethanol extract of *C. sorokiniana* induced disaggregation of β -amyloid fibrils compared to the control. This study reveals the effective action of *C. sorokiniana* and *C. minutissima* extracts on some mediators of Alzheimer's disease and gives insights into their potential benefits as functional food or therapeutic agents in the management of this disease.

ALGONONICS: INTEGRATING NANODOTS TO ENHANCE ALGAL BIOFUELS DEVELOPMENT **Khalid, M**., Johns Hopkins University, United States, <u>mkhalid8@jhu.edu</u> Vij, A., Johns Hopkins University, United States Andleeb, S., Johns Hopkins University, United States Bhakti, M.F., Johns Hopkins University, United States Janjua, H.A., Johns Hopkins University, United States Betenbaugh, M.J., Johns Hopkins University, United States

Algal systems have the potential to provide viable next generation feedstock for biofuel production. The production of algae is directly linked to the absorbed sunlight and utilization of Carbon dioxide. However, not all the bandwidths of visible sunlight are used for photosynthesis. Blue and red light plays a key role in electron excitation for ATP generation for the process. In this study, the impact of enhancing light capture in microalgae was studied by supplementing *Chlamydomonas reinhardtii* cc503 with Green Graphene Quantum Dots (GGQD) to improve the absorption of blue bandwidth of light. Intake of GGQDs within the microalgae was determined by TEM. Biological compatibility was observed by nontoxic nature of GGQDs. There were not any morphological or chemical changes except for photo-quenching of GGQDs. Despite of photo-quenching cells showed growth and increase in lipid production upto 17%±1. Furthermore, investigation was done for lipid and Fatty Acid Methyl Ester (FAME) upsurge and we found that increase in concentration of H2O2 might have caused the enrichment of lipids and FAME. The results indicate specific relation between GGQDs intake and impact on electron gain or loss for florescence quenching activity and improving bio-compounds production by an external stimulus induced stress.

MICRO-ALGAL SWIMMING VELOCITIES SIGNAL GROWTH PHASE AND LIPID ACCUMULATION

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The use of microalgae in biofuel production has received much attention and research efforts have been put into overcoming challenges in commercialization of algal biofuel. Understanding the algal intracellular lipid accumulation in response to environmental conditions is essential to maximize algal biofuel production. Batch cultures of salt water green algae, *Dunaliella primolecta (D. primolecta)*, were cultivated in modified growth media with three different initial nitrogen concentrations. Samples and measurements were taken at four growth phases: lag phase, exponential phase, late exponential/stationary phase, and late stationary phase. Intracellular lipid accumulation was quantified by the changes in size and number of boron-dipyrromethene (BODIPY) fluorescent dye stained lipid droplets under the confocal spectral microscope. Swimming velocities of algal cells were measured in a two-dimensional micro-

particle imaging velocimetry system and their swimming trajectories were tracked and analyzed by a digital inline holographic microscopy system. The results demonstrate that changes in nitrogen concentration in the cultivation environment have impact on both swimming velocity and lipid accumulation of *D. primolecta*. The findings provide the potential exploration of algal swimming kinetics as indicators of associated growth phase and intracellular lipid accumulation.

GROWTH, LIPID CONTENT, AND BIODIESEL POTENTIAL OF *CHROMULINA FREIBURGENSIS*, AN ACIDOPHYLIC CHRYSOPHYTE ISOLATED FROM BERKELEY PIT LAKE **Mohler-Mitman, J. E.**, Montana Tech of The University of Montana, United States, <u>JMohler@mtech.edu</u>

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Microalgae are a promising source of lipids for biodiesel, however, achieving cost-competitive production is a challenge. One complication is culture contamination by microorganisms. An acidic medium offers a solution by excluding competitors/predators. Chromulina freiburgensis was isolated from Berkeley Pit, an abandoned mine filled with heavy metal-laden, acidified groundwater. This strain grows rapidly in pH 2.5 medium and amasses lipids as energy stores. Published research does not yet include evaluation of a chrysophyte for biodiesel, but previous studies demonstrate that microalgae increase lipid reserves with decreasing nitrogen availability, and that some respond favorably to CO₂ supplementation. This research seeks to ascertain which culture conditions improve quantity and composition of lipids produced by C. freiburgensis, toward maximizing the proportion of triglycerides suitable for producing biodiesel. Growth (cells/mL) was monitored for six treatments, during an eight-week cycle. Fluorescence spectrometry and light microscopy confirmed that C. freiburgensis increases lipid reserves with decreasing nitrogen, and tolerates CO₂ supplementation. After transesterification/extraction, fatty acid methyl ester (FAME) composition and relative quantities were determined by Gas Chromatography-Mass Spectrometry (GC-MS), indicating conditions that increased percentages of suitable lipids. With its ability to resist contamination by thriving in an acidic medium, while amassing lipids of a suitable composition, C. freiburgensis may hold an advantage as a cost-effective microalgal candidate for biodiesel production.

ALGAE FOUNDATION'S ALGAL-BASED STEM EDUCATION INITIATIVES FOR A SUSTAINABLE FUTURE AND THE DEVELOPMENT OF THE BIOECONOMY WORKFORCE Levine, I. A., University of Southern Maine, United States, <u>ilevine@maine.edu</u>

The Algae Foundation (AF) is a five-year old, non-profit organization dedicated to algal-based education. The AF formed the Algae Technology Educational Consortium (ATEC) to develop two novel degrees in Algal Cultivation and Biotechnology. The AF's K-12 STEM initiative, The Algae Academy, is gaining national attention through its rollout of its algal-based classroom curriculum kits. The AF's four-year DOE award for the development of algal-based bioeconomy workforce preparation programs is currently in its third year. The ATEC initiatives are providing applied algal curriculum to new tracks, minors, certificates, and degrees adopted by community colleges across the nation. The AF's Algae Academy distributed an algal-based STEM initiative reaching 300 and \sim 5000 students in 2016 and 2017, respectively. In 2018 the Algae Academy's intends to distribute kits in CA, ME, MI, NM, OH, and TX, ~ 20000 students. Accomplishments include: First graduating class, Santa Fe CC, May, 2018, initiation of algal biotechnology curriculum, Austin CC (September 2018), development of Summer Science Institute, University of Southern Maine, July 2018. ATEC curriculum has been adopted at five community colleges and in discussions with an additional 9 schools. Introduction to Algae Massive Open Online Course (Algal MOOC) was released on February 15, 2018, co-produced with the University of California, San Diego. The Algal MOOC is free for all participants (~ 1000 participants to-date) https://www.coursera.org/learn/algae

DESIGNING DIATOMS: USING OMICS AND PHYLOGENETICS TO CHARACTERIZE THE OPTICAL PROPERTIES OF DIATOM FRUSTULES

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Materials scientists are challenged with nanoscale assembly, while diatoms (Bacillariophyceae) have evolved thousands of micro- and nanoscale variations to their silica theca over hundreds of millions of vears. Anecdotes of color shifts and limited research have suggested diatom valves are capable of altering light, but the specific structures that influence light are still unknown. Seven distinct groups of diatoms were selected across a broad range of taxa to measure the optical properties of specific valve characters. The influence of hvaline areas will be tested by comparing the araphid pennate Serratifera (with a wide sternum) to the araphid pennates Hendeyella and Orizaformis, which share similar gross morphologies but lacking a wide sternum. The effects of valve symmetry on light will be evaluated using the araphid pennate diatom Astrosyne, which has lost the elongate outline/bilateral symmetry and builds a radiallysymmetrical valve with a circular outline, compared to its closest relatives, Lucanicum and Cyclophora, We also intend to look at closely-related pairs of radially-symmetric diatoms with distinctive apices; Triceratium dubium and Triceratium bicorne share identical valve ultrastructures but differ by the number of apices. Diatom phylogenies will guide genomic and transcriptomic analyses to identify differences in gene content and expression that leads to variations in valve characters. Other investigations include the identification of silica-related markers in silico, which should reveal key enzymes involved with frustule morphogenesis and the biosilicification process.

Diversity and Ecology

PHYLOGENETIC ESTIMATION OF NOVEL PROTIST DIVERSITY IN RECLAMATION SITES OF NORTHERN ALBERTA

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The advent of next-generation sequencing (NGS) technologies has exponentially increased the amount of ecological data available for characterising microbial communities. However, the classification of these DNA sequences is only as accurate as the reference data available, which can pose an issue in environments where substantial novel diversity is expected. One such environment is the oil sands reclamation sites of Northern Alberta; these communities are ideal observing adaptation of eukaryotic microorganisms to the unique ecological challenges present from substantial anthropogenic influence. In this study, we used 18S ribosomal DNA comparisons to reference databases to initially estimate diversity of protists in Base Mine Lake, a tailings pond reclamation site in Northern Alberta. The majority of the 752 eukaryotic OTUs had extremely tentative classifications from this methodology. We then used various comparative genomic and phylogenetic methods to determine which OTUs had a close relative in GenBank, and which appeared to be different to any other sequence which had previously been detected. We defined novel diversity as OTUs which grouped within strongly-supported clades to the exclusion of all other reference sequences. We found evidence for novel diversity across all eukaryotes, most substantially in the small heterotroph and parasitic clades of Cercozoa and Fungi. These ecological niches have been relatively understudied when it comes to their overall impact on the microbial community, and these data suggests they may have a more notable role in environmental reclamation that was previously thought.

EIGHT YEARS LATER: HAVE THE BENTHIC DEEPWATER ALGAE RETURNED TO THE NW GULF OF MEXICO? WHAT HAVE WE LEARNED?

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Prior to the April 2010 Macondo Well Blowout and resulting Deepwater Horizon Oil Spill (DWH) offshore Louisiana, rhodolith beds at 45-90m depth in the NW Gulf of Mexico harbored diversity-rich, lush assemblages of seaweeds. In contrast, after DWH, the macroalgae covering these rhodoliths disappeared and most rhodoliths themselves appeared bleached and fully or partially denuded of surface macroalgae, a situation that has persisted in the field as of September 2014, our last expedition to the NW Gulf. We will report on whether, and which, algae have returned to the NW Gulf of Mexico hard banks based on a recent 8-day exploratory and algal-collecting cruise conducted in May 2018. We will assess the current health of the deep bank ecosystem based on our invaluable algal reference collection of phototrophs from previous study sites in the NW Gulf dating back to 1997. Since rhodoliths and their associated macroalgae are ecologically important phototrophs at the base of the oxygen-based food chain, there may be important consequences for the health and recovery of the deep bank ecosystem in the NW Gulf if their drastic die-off in the field did persist. We will assess the important role of rhodoliths in the stability of eukaryotic algal communities in the region and their potential vital function in their regeneration and resilience in the face of anthropogenic disturbances. Our investigations may have major implications for the prediction of algal blooms, shifts in the benthic primary-producer community, and possible effects of global warming and ocean acidification on the calcifying rhodoliths and their microbiota.

EPIPHYTIC MICROBIAL COMMUNITIES AND NUTRIENT CYCLING ASSOCIATED WITH BLADES OF THE CANOPY-FORMING KELP, *NEREOCYSTIS LUETKEANA* **Weigel, B. L**., University of Chicago, United States, <u>brookeweigel@uchicago.edu</u> Pfister, C. A., University of Chicago, United States, <u>cpfister@uchicago.edu</u> Altabet, M. A., University of Massachusetts, Dartmouth, United States, <u>maltabet@umassd.edu</u>;

In addition to providing vital habitat for macroscopic organisms, kelps host an abundant microbial community in their surface mucus layer. We characterized epiphytic microbial communities associated with blades of the canopy-forming kelp *Nereocystis luetkeana* using next-generation Illumina sequencing of 16S rRNA genes. Microbes associated with blades of *N. luetkeana* were sampled along a spatial gradient in Washington, from the outer Olympic Coast to the southernmost kelp bed in Puget Sound. Across all sites, the *N. luetkeana* microbiome is distinct from the surrounding seawater. Epiphytic microbial communities on *N. luetkeana* displayed significant spatial variation, with outer coast sites enriched in Bacteroidetes (family Saprospiraceae) and southern Puget Sound sites enriched in Alphaproteobacteria (family Hyphomonadaceae). We examined microbial community development and succession on meristematic and apical *N. luetkeana* blade tissues throughout the summer growing season. Blade tissue microbial communities displayed significant temporal variation, with increasing phylogenetic diversity over time. In addition to characterizing the kelp microbiome, we examined carbon and nitrogen cycling functions associated with kelp and their microbial symbionts. We used a dual stable isotope tracer experiment (13C-bicarbonate and 15NO3) to examine carbon fixation and dissolved organic carbon production by *N. luetkeana*, as well as microbial nitrogen cycling on kelp blade surfaces.

Given the immense surface area of kelp forests, metabolic exchanges between kelp and their associated microbes have potentially far-reaching impacts on kelp forest productivity and coastal nutrient cycling.

AND YOU SEE YOUR GYPSY – A STUDY OF TERRESTRIAL CYANOBACTERIAL COMMUNITIES FROM GYPSUM SOILS

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Gypsum soils and substrates offer unique habitats for terrestrial cyanobacteria forming hypolithic. epilithic, endolithic, and biological soil crust communities (biocrusts). However, studies on the biodiversity and ecology of these communities are sparse for North America. Our objectives are to study terrestrial cyanobacteria from the largest gypsum dune field of the world and surrounding areas in White Sands National Monument, NM. We hypothesize that landscape structure, dune type, and age drive the composition and richness of the cyanobacterial flora. We also predict that soil moisture and stability will be important factors explaining community richness and diversity. Terrestrial cyanobacteria were surveyed along a dune front with dunes of varying types, mobility, and age. We used standard field biological methods such as quadrat and frequency methods to record varying types of cyanobacterial communities following established classification. Preliminary data showed a gradient of decreasing sand mobility, increasing dune stability and age that harbors cyanobacterial communities changing from incipient to stabilizing, to a stage with diverse ecosystem components including highly stable, nitrogen fixing biocrust communities, epi- and endolithic communities, and cyanobacterial symbiosis with fungi as lichens. First discoveries of cyanobacterial taxa from these substrates include morphotypes such as Trichormus sp., Myxosarcina sp., Pleurocapsa sp., Oculatella sp., and Nodosilinea sp. Our study brings first insights into enigmatic communities from a unique environment.

DISTINCT AND ABUNDANT MESOPHOTIC MACROALGAL ASSEMBLAGES IN THE HAWAIIAN ARCHIPELAGO

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Mesophotic coral ecosystems (MCEs) in tropical waters occur from 40 to beyond 150 m depths. In these low-light environments, macroalgae may be more abundant than coral-dominated reefs given their lower depth limits (> 200 m), greater diversity, faster growth rates, and ability to grow over soft and hard bottom habitats. The Hawaiian Archipelago extends 2,500 km across the north-central tropical Pacific Ocean, and consists of the inhabited Main Hawaiian Islands (MHI) and uninhabited Northwestern Hawaiian Islands (NWHI). Submersibles, remotely operated vehicles, and technical diving were used to survey benthic communities at 68 sites in the NWHI and MHI at depths ranging from 40 to 212 m. Across this survey range, we found 13 dominant mesophotic assemblages with distinct floras, with up to 100% algal cover over tens of kilometers squared. Some assemblages were distinct to specific regions or islands, such as large beds of *Microdictyon setchellianum* in the NWHI, expansive sand-dwelling meadows of *Halimeda kanaloana* in the MHI, and beds of an undescribed species of *Udotea* around west and south O'ahu. Beds of rhodoliths, *Halimeda distorta*, and *Distromium* spp. were common throughout the archipelago. The discovery of the invasive *Avrainvillea* sp. to 80 m depths off O'ahu highlights the

need to understand the dynamics of mesophotic communities if we are to understand the source and extent of invasive species, and their impacts on native communities. Mesophotic macroalgal assemblages in Hawai'i are abundant, diverse, and spatially heterogeneous with some assemblages unique to MCEs.

"PROTIST X" IS A PREDATORY ANAEROBE THAT REPRESENTS A NOVEL DEEP LINEAGE OF EUKARYOTES

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Anaerobic protists are widespread across the tree of eukaryotes but remain undersampled, especially in the free-living realm. "Protist X" is an enigmatic anaerobe with four flagella in an unusual cruciform arrangement which was reported once by Bernard et al. in 2000, from marine anoxic sediments. In the absence of molecular data, its phylogenetic position was entirely unclear. We cultivated two isolates of "Protist X" from marine and salt pond sediments from Nova Scotia and Curaçao, respectively. "Protist X" is an obligate predator of other flagellates, for example anaerobic jakobids and *Carpediemonas*-like organisms. It is capable of capturing prey with any part of the cell surface using morphologically-complex extrusomes that do not closely resemble those known from other protists. SSU rDNA phylogenies failed to place "Protist X" within any known major group of eukaryotes. We prepared transcriptomes from both strains, using full-culture and 'pooled' single-cell methods respectively. Phylogenomic analyses show that "Protist X" likely represent a novel lineage of anaerobes, and suggest that it does not branch within any recognised supergroup of eukaryotes.

Evolution of Eukaryotic Lineages II: The Revenge

THE FIRST DRAFT GENOME FOR THE KATABLEPHARID *ROOMBIA TRUNCATA* **Warring, S. D.**, American Museum of Natural History, United States, <u>swarring@amnh.org</u> Yang, A., American Museum of Natural History, United States Burnes, J. A., American Museum of Natural History, United States Kim, E., American Museum of Natural History, United States

Roombia truncata is a bi-flagellated unicellular heterotrophic generalist. It is a member of the nonphotosynthetic lineage Katablepharids, the sister group of the cryptomonads. Intriguingly, flow cytometry data indicate that this unassuming flagellate harbors a ~2 gigabase genome. Our ongoing efforts to assemble this genome support its large size and reveal that this expanded genome size is due mostly to repetitive sequence. The important phylogenetic placement of *R. truncata* means that these new genomic data present an opportunity to further resolve relationships amongst the corticate lineages and to address some of outstanding issues concerning the evolution of eukaryotic photosynthesis. Further, as free-living heterotrophic flagellates are underrepresented in genomic databanks, this new genome offers further insights into biological diversity in general.

LATERAL GENE TRANSFERS IN THE SYMBIOTIC *OPALINA - BLASTOCYSTIS* LINEAGE OF STRAMENOPILES FACILITATED THE ADAPTATION TO GUT ENVIRONMENT **Yubuki**, **N.**, Systematics and Evolution Unit. CNRS - Paris-Sud University, France, <u>naoji.yubuki@upsud.fr</u>

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Lateral gene transfer (LGT) plays an important role in adaptation to new environments in bacteria and archaea. In eukaryotes, the role of LGT remains highly debated, but recent research supports that gene transfer from bacteria to a wide diversity of eukaryotes may be much more common than previously appreciated. However, most of this research was focused on animals and there is room for improvement to better understand the true phylogenetic and functional impact of LGT in neglected protist groups. Eme et al. (Curr Biol 2017, 27:807-820) recently reported 74 cases of LGT from prokaryotes and eukaryotes to the human parasitic stramenopile Blastocystis sp. and suggested that the new functions acquired by LGT facilitated the adaptation to the human gut. Although several of those genes were inferred to have been transferred to a common ancestor of several Blastocystis lineages, the time of their acquisition remained unclear due to the poor taxon sampling available for non-photosynthetic stramenopile lineages. Ribosomal RNA phylogenetic analyses suggest that *Blastocystis* is related to the symbiotic Opalinata, represented by genera such as *Opalina*, found in intestines of frogs and toads. The Opalinata-Blastocystis assemblage is probably sister to Placidida, a lineage including small free-living marine flagellates. Here we report the first transcriptome sequences of an Opalinata species; *Opalina* sp. from the frog *Xenopus* tropicalis. Using phylogenomic approaches, we identified at least 28 genes transferred from either prokaryotes or eukaryotes to a common ancestor of *Opalina* and *Blastocystis*, including a SufCB gene functioning in anaerobic metabolism in mitochondria-related organelles. These data suggest that ancient LGT probably facilitated the adaptation to animal gut environments before the divergence of Opalinata and *Blastocystis* from a free-living ancestor.

EXPLORING THE PHYLOGENETICS AND DEEP EVOLUTIONARY HISTORY OF EUGLENIDS WITH SINGLE-CELL TRANSCRIPTOMICS OF PHAGOTROPHIC TAXA Lax, G., Dalhousie University, Canada, gordon.lax@dal.ca

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Euglenids are a ubiquitous group of flagellates that includes well-known phototrophic algae, but also osmotrophs and phagotrophs. Phagotrophic euglenids encompass far more phyletic diversity than the primary osmotrophs and phototrophs, which evolved independently from phagotrophic euglenid ancestors. The phylogenetics of phototrophic euglenids, and to lesser extent osmotrophs, is relatively well developed. In contrast, our basic understanding of deep euglenid evolution (for example including the exact origins of phototrophic euglenids), is severely hampered by limited knowledge of the phylogenetic relationships among phagotrophic forms. Almost all prior analyses of this group have used the SSU rRNA gene as the only phylogenetic marker. Early analyses suffered from poor taxon sampling, but despite continually generating SSU rRNA data for more phagotrophic taxa, recent analyses still do not robustly resolve many deep relationships amongst euglenids. To overcome the limitations of using a single gene, we used '-omics' methods, especially single-cell transcriptomics on isolated cells, to generate the first multigene datasets for a broad sampling of phagotrophic euglenid diversity. We demonstrate that single-cell transcriptomes from euglenids are more than adequate for subsequent phylogenomic analyses. We will present the first such multigene analyses carried out for euglenids as whole, and discuss evolutionary implications for this major group of unicellular eukaryotes.

ANURAN ORIGIN OF TRYPANOSOMES

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Amphibian trypanosomes were the first ever described trypanosomatids. Nevertheless, their taxonomy remains entangled because of the pleomorphism and high prevalence of mixed infections. Even though the first species in this group were described in Europe, virtually none of the trypanosomes from European anurans was analyzed using modern molecular methods. In all cases more than one morphotype per blood sample was observed, indicating mixed infections. One hundred thirty obtained 18S rRNA gene sequences were unambiguously subdivided into five groups, correspondent to the 3 previously recognized or 2 novel taxa of anuran trypanosomes. One of the species (Trypanosoma loricatum) represents a sister group to the clade of non-frog aquatic trypanosomes, thereby making anuran parasites paraphyletic, i.e. being ancestral to them. Apparently, such a transition may have occurred owing to a common vector of both groups, the leeches. Most anuran trypanosomes are transmitted by dipterans, but some of them (f.e. T. loricatum) utilize leeches as vectors. Thus, the origin of non-frog trypanosomes seems to be straightforward: an ancestral leech-transmitted anuran parasite has adapted to new host groups (fish and aquatic amniotes). However, this scenario poses several important questions. Firstly, in the current phylogenetic reconstruction this transition appears unique, although T. loricatum is not the only leechtransmitted trypanosome. Why in other lineages such switching did not occur? Secondly, why anurans could be the original trypanosome hosts? Thirdly, how are the terrestrial trypanosomes related to the aquatic ones? Further investigations will shed light on these questions.

TRANSCRIPTOMIC INSIGHTS INTO THE PHYLOGENY AND EVOLUTION OF DIATOMS

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Transcriptome data have provided valuable insights into the phylogeny and evolution of species-rich groups across the eukaryotic tree of life. The 100 or so sequenced diatom transcriptomes are restricted mostly to marine planktonic species and so do not fully capture major transitions in important functional, ecological, and life history traits. We sequenced high-depth transcriptomes for 180 diverse diatom taxa that, together with previously sequenced taxa, provide a broad representation of diatom diversity. Phylogenomic analyses highlighted the power of large nuclear datasets for resolving diatom relationships, though gene-tree conflict will likely be a persistent challenge in resolving some parts of the diatom species tree. Similarities in clade age, species richness, and primary productivity motivate comparisons between diatoms and flowering plants, whose genomes have been inordinately shaped by whole genome duplication (WGD). WGDs have been linked to speciation, increased rates of lineage diversification, and identified as a principal driver of angiosperm evolution. Phylogenomic analyses revealed an extensive history of WGD in diatoms as well, such that nearly all extant diatoms share deep polyploid ancestry. We also found strong evidence for allopolyploid events early in the evolution of the thalassiosirod and pennate diatom clades. The growing set of genomic resources across diatoms is making it possible to identify genomic changes that coincided with key transitions in diatom evolution.

FUNCTIONAL GENOMICS OF LABYRINTHULOMYCETES, AN ECOLOGICALLY AND BIOTECHNOLOGICALLY RELEVANT GROUP OF BASAL STRAMENOPILES **Collier, J. L.**, Stony Brook University, United States, jackie.collier@stonybrook.edu Rest, J. S., Stony Brook University, United States, joshua.rest@stonybrook.edu Rius, M., Stony Brook University, United States, mariana.rius@stonybrook.edu

Labyrinthulomycetes are ubiquitous and abundant protists thought to play important roles in the marine carbon cycle and food webs. Four phylogenetically and ecologically distinct groups of

labyrinthulomycetes have been cultivated, including thraustochytrids and aplanochytrids. First steps toward the routine genetic manipulation of these organisms have been taken with the thraustochytrid genus *Aurantiochytrium*, which has been the focus of biotechnological interest for production of essential polyunsaturated fatty acids, carotenoids, and squalene. In an effort to extend these methods to the labyrinthulomycete genera that appear to be of broader ecological relevance, we are taking a comparative genomic approach relying on three genomes sequenced by JGI plus transcriptomes for three additional strains produced by MMETSP. GC content (42 to 63%) and codon usage vary among the labyrinthulomycetes over ranges observed in other microbial eukaryotes. *Aurantiochytrium* and *Aplanochytrium* genomes reveal fairly typical Kozak-like translation initiation sequences, but *Schizochytrium* has different motifs. Gene structure is compact, with relatively few and short introns, though *Aplanochytrium* possesses 2-3X more introns than the thraustochytrids *Aurantiochytrium* and *Schizochytrium*. Genome annotations reveal a typical suite of transcription machinery and regulatory proteins. Together, these analyses suggest that constructs used for expression of exogenous marker and reporter genes in *Aurantiochytrium* may also be useful for the genetic manipulation of other labyrinthulomycetes.

Plastid Genomes

A GENOMIC INVESTIGATION OF THE PLASTID-LACKING CRYPTOMONAD *GONIOMONAS AVONLEA*: INSIGHTS INTO THE EVOLUTION OF COMPLEX PLASTIDS AND CRYPTISTA **Sibbald, S. J.**, Dalhousie University, Canada, <u>shannon.sibbald@dal.ca</u> Cenci, U., Dalhousie University, Canada Curtis, B. A., Dalhousie University, Canada Eme, L., Dalhousie University, Canada Roger, A. J., Dalhousie University, Canada Kim, E., Sackler Institute for Comparative Genomics, United States Archibald, J. M., Dalhousie University

Cryptomonads are single-celled eukaryotes comprised of both photosynthetic and non-photosynthetic species. While cryptomonads such as *Guillardia theta* harbor a plastid of secondary endosymbiotic origin, members of the Goniomonadea lack plastids. A long-standing question in the field of plastid evolution is whether the Goniomonadea, the closest relative of plastid-bearing cryptomonads, are ancestrally non-photosynthetic or whether they lost their plastid secondarily. To address this, we sequenced the nuclear genome and transcriptome of a heterotrophic Goniomonadea species, *Goniomonas avonlea*, and compared it to that of *Gu. theta* using phylogenetic evidence indicating red-algal endosymbiotic ancestry in *Go. avonlea*, but did find a close nuclear relationship of Cryptista and Archaeplastida in phylogenomic analyses. The *Go. avonlea* genome is a valuable tool for elucidating the physiology of heterotrophic cryptomonads, and the metabolic 'rewiring' that took place during secondary plastid integration.

EXPRESSION PROFILING OF *PAULINELLA MICROPORA* UNDER ENVIRONMENTAL ADAPTATION WITH THE EXPOSURE TO HIGH-LIGHT CONDITION **Ettahi, K**., SungKyunKwan University, South Korea, <u>ettahi.khaoula@gmail.com</u> Lhee, D., SungKyunKwan University, South Korea, <u>wanbyunori@gmail.com</u> Yoon, H. S., SungKyunKwan University, South Korea, <u>hwansu@gmail.com</u>

The non-photosynthetic filose thecamoeba *Paulinella micropora* engulfed photoautotrophic cyanobacterial and turned it into a steady photosynthetic organelle. *Paulinella* has been a typical model to assess the origin and the development of photosynthesis in eukaryotes with the view of independent primary plastid acquisition/endosymbiosis and the premature emergence of the host and the

photosynthetic organelle. For better understanding of origin and evolution of primary plastid within *P. micropora* under high-light induced environment, and regulation of genes in different scenario would give us insights of plastid evolution. In this study, *P. micropora* were cultivated under two environmental conditions: low light as a control and high-light with light/dark reversal by 12-h period transition for a complete 42 hours. Seven-time points were sampled for both conditions with each alteration of light/dark cycle. All the extracted samples were subjected to a high-throughput RNA sequencing analysis to investigate the molecular adaptation of high light-stress obtained in natural habitat along with the alteration of light/dark cycle in *P. micropora* photosynthetic process. Sequencing results provide a significant change in gene expression between control versus high-light condition from each time points. The genes which were affected due to light/dark cycle were considered critical for the current study. The reactive oxygen species (ROS) pathway of photosynthesis was subjected to further studies. Furthermore, the gene expression of *P. micropora* in the light-dark cycle has been analyzed using transcriptomic data.

THE TRANSCRIPTOME OF *EUGLENA LONGA* REVEALS PECULIARITIES OF THE PLASTID BIOLOGY IN EUGLENOPHYTES

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Euglenophytes are known to show various unusual features at the molecular and cellular level, but so far have been little studied on a genome-wide scale. Using our own transcriptomic data from the nonphotosynthetic Euglena longa and available transcriptomic resources from three photosynthetic euglenophytes we revealed new important characteristics of their plastid. First, the conventional eubacterial ribosomal protein L24 of the plastidial ribosome is missing and seems to have been replaced by a very different archaebacterial homolog. Second, no homolog of any key component of the TOC/TIC (translocon of the outer/inner chloroplast membrane) is discernible in euglenophytes, implying their extreme divergence or a use of an alternative import apparatus. The plastid division mechanism likewise remains elusive, as we have not identified homologs of even the highly conserved FtsZ protein. Third, the machinery for intraplastidial protein targeting has been simplified, owing to the lack of the cpSRP/cpFtsY system and the SEC2 translocon. Fourth, a number of plastid-targeted proteins are encoded as translational fusions, including newly identified cases where the two encoded proteins are functionally unrelated. Lastly, euglenophytes proved to encode a plastid-targeted homolog of the termination factor Rho horizontally acquired from a Lambdaproteobacteria-related donor, suggesting an unprecedented modification of the transcription mechanism in their plastid. The number of non-conventional features of the euglenophyte plastid make it an attractive target for future research.

COLOURLESS BUT NOT INVISIBLE: AN UNPRECEDENTED COMBINATION OF METABOLIC PATHWAYS IN THE CRYPTIC PLASTID OF *EUGLENA LONGA* **Füssy, Z.,** Institute of Parasitology, Biology Centre ASCR, Ceské Budejovice, Czech Republic, zoltan@paru.cas.cz Záhonová, K., Faculty of Science, University of Ostrava, Ostrava, Czech Republic, kika.zahonova@gmail.com

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Most secondarily non-photosynthetic eukaryotes retain a residual plastid due to localization of essential pathways into the organelle. One of the least characterized relic plastids is found in *Euglena longa*, a close relative of *Euglena gracilis*. To illuminate the function of this elusive organelle, we sequenced the *E. longa* transcriptome and reconstructed the plastidial metabolism *in silico*. Our analyses showed the absence of plastid-localized pathways for the synthesis of isoprenoid precursors and fatty acids, which are typical for other relic plastids. The plastidial tetrapyrrole pathway is only fragmentary and potentially redundant with a complete mitochondrial-cytosolic pathway. The most striking is the presence of a linearized Calvin-Benson (CB) pathway including RuBisCO yet lacking the gluconeogenetic part of the standard cycle, together with the ferredoxin/thioredoxin redox cascade that possibly mediates activation of the CB cycle and CO₂ fixation in response to the production of reduced ferredoxin. We hypothesize that in *E. longa*, reduced ferredoxin is generated by a plastid-localized ferredoxin. We hypothesize that in *E. longa*, reduced ferredoxin is generated by a plastid-localized ferredoxin of the cell. The pathway may thus function as a redox valve bypassing the glycolytic oxidation of glyceraldehyde-3-phosphate to 3-phosphoglycerate. The unprecedented combination of features makes the *E. longa* plastid unlike any of the previously studied relic plastids, including the apicoplast.

EVOLUTION OF CHLOROPLAST GENOMES IN SIPHONOUS GREEN ALGAE (BRYOPSIDALES, CHLOROPHYTA)

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Chloroplast genomes have undergone tremendous alterations through the evolutionary history of the green algae. Using a taxon-dense sampling of chloroplast genomes in the order Bryopsidales, we assess the evolutionary dynamics of the group and its chloroplast genomes. We investigated the evolutionary dynamics of genomic features using comparative phylogenetic methods, showing extensive variation in chloroplast genome architecture and intron content. Variation in genome size is primarily accounted for by intergenic space and freestanding open reading frames that do not show significant homology to standard plastid genes. We show the diversity of these non-standard genes based on their conserved protein domains, which are often associated with mobile functions (reverse transcriptase/intron maturase, integrases, phage- or plasmid-DNA primases, transposases, integrases, ligases. Investigation of the introns showed proliferation of group II introns in the early evolution of the order and their subsequent loss in the core Halimedineae, possibly through RT-mediated intron loss. The chloroplast genome-based phylogenies presented here provided good support for the major lineages (suborder and family level) in Bryopsidales.

WHY DO WE NEED MORE ALGAL PLASTID GENOMES?

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We investigated plastid genomes of two lineages of algae: Euglenophyta, with plastids, analyzed originated from endosymbiosis with green alga, and Dictyochophyceae, with plastid of the "red" origin. Euglenophytes genomes have been studied previously, but only the family Euglenaceae has been well represented. We sequenced cpGenomes of nine taxa representing families Phacaceae and Eutreptiaceae. We identified two inverted repeat regions containing rDNA operon in the majority of analyzed taxa, absent from other Euglenophyta. These results highlight the dynamic nature of the Euglenophyta chloroplast genome, in particular with regards to the IR sequence that underwent losses repeatedly. In contrast, no Dictyochophyceae plastid genomes have been known until now. We sequenced cpGenomes of two species, which turned out to be very similar. A thorough analysis revealed some interesting features such as a tic20-derived pseudogene or a 150 nt deletion in 16S rRNA gene. Some of the plastid genomes together with ones already available to search the TARA Oceans metagenomic data for plastid genomes. We gathered partial genomes and estimated their abundance in the environment using MetaPlastHunter, the new approach developed in our lab.

Contributed Posters (presenters are in bold)

P01. MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF MICROBIAL EUKARYOTE DIVERSITY AND COMMUNITY STRUCTURE FOR STREAM BIOMONITORING IN NEW JERSEY, USA

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Microbial eukaryotes, including diatoms and other algal groups possessing distinct morphological characters, have been traditionally used as water quality indicators. The limitations of morphology-based methods can lead to inconsistencies in taxa identification and an underestimation of cryptic species diversity. Phenotypic plasticity, poorly differentiated morphological characters, and an unknown degree of geographic intraspecific variation results in uncertainties in environmental inferences. Next Generation Sequencing provides an alternative, potentially more objective method of obtaining microbial community data. In this study, we used amplicon-based sequencing of the hypervariable v9 region of 18S rDNA to characterize epilithic microbial eukaryotic assemblages from fourteen New Jersey streams, representing a gradient of high to low water quality. We also characterized diatom assemblages from the same samples using standard morphological identification and enumeration methods. The molecular survey assessed within- and among-site variability of assemblage structure of all diatoms, all algal groups, and all eukaryotes. We examined response to environmental stimuli and reproducibility of both methods. Diatom and green algal sequences were among the most abundant in most samples, though there was a high degree of diversity among all algal groups. For our diatom data set, the molecular survey revealed a much higher level of diversity, but reproducibility of the results, expressed as relative abundance of OTUs, was stronger using the morphology-based analysis. We compare advantages and shortcomings of both approaches for bioassessment purposes.

P02. 'FLAGSHIP CILIATES' OF FRESHWATER PONDS AND SOILS FROM FLORIDA, USA **Hines, H. N.,** Bournemouth University, United Kingdom, hhines@bournemouth.ac.uk McCarthy, P. J., Harbor Branch Oceanographic Institute at Florida Atlantic University, United States, pmccart5@fau.edu

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The biodiversity and biogeography of flagship ciliates, even those with sizes over 1mm, remains an understudied area of microbial ecology. Many distinct flagship ciliates were originally documented in Africa and have remained unseen elsewhere. These ciliates are known from historic texts, with little ecological information and some lacking photographic record. We hypothesized that these species, thought to be endemic to Africa, could spread globally, and that Florida, USA, was a suitable habitat for tropical flagship taxa to thrive. We performed intensive sampling in freshwater habitats of Florida, USA, resulting in the discovery of several flagship ciliates that thrive in this region. Additionally, various soil samples were collected and observed for ciliates. Our findings include: the first record outside of Africa for several, poorly understood freshwater flagship ciliate species such as the 1.5mm *Bursaria caudata* the first records for the North America of soil species such as the blue ciliate *Condylostomides coeruleus* including photomicrographs and ecological reports of several flagships known previously only from drawings such as *Frontonia vesiculosa*. Our findings support previous research that showed that ciliates are able to disperse beyond a once hypothesized area of endemism, and likely are able to spread globally. Our discovery of previously unrecorded flagships from both freshwater ponds and soils from Florida further highlight issues with undersampling, and the potential ease of dispersal for these large single cells.

P03. CELL WALL MARKER ASSESSMENT OF NORTHERN EVERGLADES' DETRITAL FLOCCULENT LAYERS

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Wetland organic matter accretion rates are influenced by degradability of the resident macrophyte community after senescence. Decomposition rates differ between aquatic macrophyte species due to the abundance of labile or refractory structural cell wall polymers. In the Florida Everglades, the oligotrophic marsh dominated by sloughs of periphyton and submerged aquatic vegetation is being replaced by dense cattail stands due to eutrophication and sediment accretion rates are more rapid relative to the former marsh. In an effort to halt the loss of slough environments and dominance of cattail, a management strategy combining herbicide treatment with fire was used to create sloughs where *Chara* subsequently thrived. In this study, we sampled cattail, sawgrass, and Chara and detrital flocculent and applied plant cell wall immuno-cytochemical fluorescent markers to assess presence and persistence. In areas of the marsh dominated by cattail or sawgrass, we observed overlap in polymer labeling between plants and detrital floc. We found xylans and xyloglucans in *Chara* and floc from created sloughs; however, those polymers were also observed from emergent macrophytes. Polymers found in created sloughs, such as (1,4)-linked galactan, matched those from cattail or sawgrass, despite plant absence. Floc labeling patterns differed between the plots where emergent macrophytes continued to grow relative to created sloughs. Our results show that immuno-cytochemical fluorescence can be applied for rapid visualization of persistent cell wall components and that plant community composition and site physicochemical characteristics can influence the persistence of certain cell wall polymers.

P04. SUBSTANTIAL GENOME SIZE VARIATION IN THE CHRYSOPHYTE GENUS *SYNURA* AND ITS EVOLUTIONARY CONSEQUENCES

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The amount of nuclear DNA usually has broad consequences on cell functioning and its size. However, there is nearly no knowledge of genome size diversity in populations of freshwater microalgae when compared with higher plants or seaweeds. We present the first genome size measurements for Chrysophyceae lineage (Stramenopiles). We analysed DNA content of *Synura petersenii* strains from different populations across the Northern Hemisphere using flow cytometry, discovering a substantial among-population diversity in this trait. Despite their identical ITS rDNA sequences, the genome size varied more than two-fold (0.98 - 2.21 pg DNA) among 140 investigated strains. The source of this diversity is more likely a gradual increase/decrease in DNA content than genome doubling (polyploidization). Further, we tried to link the genome size diversity with its possible evolutionary drivers by investigating how it correlates with cell size, population growth rates and climatic conditions at sampled sites. However, no clear associations with the DNA amount were discovered. Interestingly, the genome size diversification indicates an independent evolution of lineages within *S. petersenii* and thus further level of cryptic diversity.

P05. TRUE DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF OXYMONAD PROTISTS IN THE GUT OF THE TERMITE *RETICULITERMES SPERATUS*Igai, K., Tokyo Institute of Technology, Japan, kigai@bio.titech.ac.jp Kitade, O., Ibaraki University, Japan, kitade@mx.ibaraki.ac.jp Ohkuma, M., RIKEN, Japan, mohkuma@riken.jp

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Oxymonads are obligately symbiotic flagellates in animal intestines. The hindgut of *Reticulitermes termites* is one of the best-studied habitats for oxymonads, where a large number of several oxymonad species coexist. Koidzumi (1921) reported the first comprehensive morphological study of the protist community in *R. speratus* and described eleven morphotypes of oxymonads belonging to genera *Dinenympha* and *Pyrsonympha*. In the present study, we revisited this complex oxymonad community with modern technologies including whole genome amplification (WGA) and high-throughput sequencing. We sampled single cells of distinct oxymonad morphotypes and determined near-full-length sequences of the 18S rRNA gene and its adjacent internal transcribed spacer (ITS) region, amplified by PCR after WGA. In addition, we performed sequencing of 18S rRNA genes amplified by PCR using newly designed oxymonad-specific primers from the entire hindgut community on an Illumina MiSeq platform. With these data combined, both the known and novel phylogenetic clades were catalogued with morphological characteristics and their phylogenetic relationships were inferred; revisions of the species and subspecies classification by Koidzumi are suggested. Our estimation of the true diversity and the phylogenetic relationships of the oxymonad protists in *R. speratus* guts provides the basis for studies of the evolutionary process of their speciation.

P06. PROTEORHODOPSIN IN DINOFLAGELLATES

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Proteorhodopsin (PR) is a photoactive proton-pump of bacterial origin found in both prokaryotic and eukaryotic marine microbes. While PR has been shown to drive ATP synthesis and other processes in

bacteria, its role in eukaryotic cells remains unknown. The basal dinoflagellate *Oxyrrhis marina* was the first eukaryote shown to express PR, and initial searches have revealed that other dinoflagellate genera do as well. The long-term objective of this study is to learn what cellular processes are driven by PR in dinoflagellates. The preliminary work presented here aims to look for patterns of retention or loss of PR between the diverse orders within this group. Transcriptome data were used to map proteorhodopsin and sensory rhodopsin onto a dinoflagellate character tree, as well as phytoene synthase (*crtB*), phytoene dehydrogenase (*crtI*), and retinal synthesizing enzyme (*diox1*), all proteins potentially involved in synthesizing retinal, the cofactor of PR. Transcriptome completeness was assessed to determine the confidence of whether absences were indeed "losses". The distribution of PR and associated proteins in dinoflagellates revealed its consistent presence across all established orders. Most species expressed both PR and sensory rhodopsin if they expressed any rhodopsin at all. These findings reveal that PR is a conserved trait across orders in dinoflagellates and that the production or function of PR and sensory rhodopsin may be linked. This will inform upcoming work to investigate the function of PR in dinoflagellates.

P07. NEW TOOLS FOR SELECTIVELY IMPROVING STRAINS OF SUGAR KELP FOR FOOD AND FUEL

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As a part of ARPA-E's MARINER program, we are pursuing a selective breeding program to improve the productivity and composition of sugar kelp, Saccharina latissima, which could serve as feedstock for biofuels. Our goal is to develop tools and a pathway toward low-cost (< \$100/DWT) feedstock that could supply 10% of US transportation fuels. Current markets are human and animal food, phycocolloids and ecosystem services. To facilitate high-throughput creation of family crosses, the NOAA Milford Lab and UCONN are developing cell-sorting methods to efficiently isolate and clone gametophytes. USDA/Cornell and HudsonAlpha will employ PacBio and Illumina sequencing to create a deepsequenced reference genome and establish a variant catalog for our founding populations and families. WHOI, UCONN and GreenWave will oversee field trials of 144 unique sporophytes resulting from crosses among 144 genotyped gametophytes sampled from across New England. These will be planted in triplicate plots on two farm sites (nearshore and offshore) over two growing seasons. The resulting family phenotypic data will be associated with genetic markers (GWAS), and we will identify variants significantly associated with primary productivity and composition traits. A goal is to develop methods to predict offspring (sporophytes) performance based upon genotype and breeding values of parents (gametophytes) as a short-cut around extensive and expensive field-testing. A separate ARPA-E project will test the potential laborsaving use of WHOI's autonomous underwater vehicles and sensors for conducting nutrient, acoustic, and optic measurements of macroalgae plots. These will be compared to conventional hands-on field measurements. Ultimately, our project goal is to select sugar kelp best suited genetically to offshore farm environments and possessing qualities of increased dry matter yield per unit area (up to 10% per generation) and improved composition for use as bioenergy feedstock.

P08. DETECTION OF ANCESTRAL POLYMORPHISM IN THE CILIATE GENUS *SPIROSTOMUM* (CILIOPHORA, POSTCILIODESMATOPHORA, HETEROTRICHEA) **Shazib, S.,** UNIVERSITY OF ULSAN, South Korea, shahed30ahmed@gmail.com Vdacný, P., Comenius University in Bratislava, Slovakia, vdacny@fns.uniba Shin, M., UNIVERSITY OF ULSAN, South Korea, mkshin@ulsan.ac.kr

We investigated genetic variation and evolutionary history of taxa belonging to the morphologically distinctive ciliate genus *Spirostomum*. Altogether 77 *Spirostomum* populations from seven morphospecies were analysed at intra- and interspecific levels using one mitochondrial (CO1 region) and five nuclear (18S rDNA, ITS region, 28S rDNA, alpha-tubulin) markers. Evolutionary relationships within this genus were shown to be very complex and incongruent between individual gene trees. Both nuclear and mitochondrial markers transcended species boundaries in *S. minus* and *S. teres* morphospecies. Phylogenetic gene trees and networks did not manage to unambiguously resolve relationships among *Spirostomum* species. To overcome this problem, we utilized Bayesian coalescent approach and PhyloNet analysis. Both techniques suggest that evolution of the genus *Spirostomum* was very likely shaped by ancestral polymorphism and incomplete lineage sorting. Individual gene trees were unable to overcome problems associated with ancestral polymorphism, while concatenation of multiple markers and coalescence methods can uncover the phylogenetic signal present in the data. Both protein coding genes (alpha-tubulin and CO1) were, however, revealed as phylogenetically uninformative and can be mostly useful in barcode analyses.

P09. TAXONOMIC STUDY OF THE GENUS *DESMODESMUS* (CHLOROPHYCEAE) **Anto, J. R.**, Chungnam National University, South Korea, jasmineruth@cnu.ac.kr Kim, J. I., Chungnam National University, South Korea, jongim@cnu.ac.kr Shin, W., Chungnam National University, South Korea, shinw@cnu.ac.kr

Desmodesmus species taxonomy is one of the most long standing issues in systematics due to problems associated with phenotypic plasticity. The species consist of four ellipsoidal cells, arranged in a single row which forms the coenobium and bears spiny projection at terminal cells. To understand the taxonomy of genus *Desmodesmus* we performed morphological and molecular analyses based on 250 culture materials, that were established from various ponds and lakes of Korea. The morphological studies for all isolates were examined using light microscopy. Molecular phylogenetic analyses based on a combined nuclear ITS and plastid *rbc*L sequence data were conducted by the Bayesian and RAxML programs. Our preliminary results suggest seven new lineages of *Desmodesmus* that were genetically different from the previously known taxa. Additional analysis of molecular data is necessary to resolve the phylogenetic relationships among seven lineages that are presently being determined. In this study, we will discuss about phylogenetic relationships coupled with morphological evaluation to better understand the taxonomy of these ubiquitous *Desmodesmus* species.

P10. PHYLOGENOMIC PLACEMENT OF A NEW ISOLATE OF *THECAMOEBIDA* (DISCOSEA, AMOEBOZOA) WITH A UNIQUE PERINUCLEAR MICROTUBULE ORGANIZING CENTER (MTOC)

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The family Thecamoebidae *sensu* Smirnov et al. (2011) has three known genera to date. Species in this family have been understudied and poorly characterized in molecular studies. Its members inhabit both terrestrial and aquatic environments as well as some medically important as parasite of animals. Here, we describe a new freshwater isolate of thecamoebid based on morphological (light microscope), cytological (immunocytochemistry, ICC), and transcriptome data. This isolate was discovered from a freshwater Amoeba proteus culture purchased from Ward's Science protist collections. Despite being morphologically similar to large, rugose Thecamoeba species, it is discernable by the following: 1) the existence of fibrillar cytoplasmic microtubules (MTs) that are arranged by a perinuclear microtubuleorganizing center (MTOC); 2) the ability to form lobose, digitate subpseudopodia projecting from anterior and lateral parts of the cell; 3) a distinct form of locomotion in a dragging motion. A phylogenomic analysis based on transcriptome data (511 genes) revealed a strongly supported sister relationship of the new isolate with Stenamoeba despite being more similar in light microscopy to Thecamoeba. Given its unique morphology and phylogenetic position within the Thecamoebida clade, we established a new genus and species named for its diagnostic features as revealed through light microscopy and ICC data.

P11. KIN RECOGNITION IN PROTISTS AND OTHER MICROBES: AN ANALYTICAL COMPILATION

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This is the first scientific compilation dedicated entirely to the genetics, evolution and behavior of cells capable of discriminating/recognizing taxa (other species), clones (other cell lines) or kin (as per gradual genetic proximity). It covers the advent of microbial models in the field of kin recognition; the polymorphisms of green-beard genes in social amoebas, yeast and soil bacteria; the potential that unicells have to learn phenotypic cues for recognition; the role of clonality and kinship in pathogenicity (dysentery, malaria, sleeping sickness and Chagas); the social/spatial structure of microbes and their biogeography; and the relevance of unicells' cooperation, sociality and cheating for our understanding of the origins of multicellularity. With 200+ figures, this work is conceptualized for a broad audience, including researchers in academia, post-doctoral fellows, graduate students and research undergraduates.

P12. TWO NEW EPIPHYTIC SPECIES OF CHAMPIA (CHAMPIACEAE, RHODYMENIALES) FROM BERMUDA AND THE WESTERN ATLANTIC Schneider, C. W., Trinity College, United States, cschneid@trincoll.edu Griffith, M. K., Trinity College, United States Lane, C. E., University of Rhode Island, United States Saunders, G. W., University of New Brunswick, Canada

Using molecular-assisted alpha taxonomy we have uncovered two new pseudocryptic species of *Champia* in Bermuda, as well as demonstrating that the European C. parvula, a name previously applied to historical Bermuda collections, and C. farlowii recently described from southern New England, USA are not part of the island flora. We present molecular, as well as morphological, evidence to describe these two endemic species for the islands, C. hasselbringii sp. nov. and C. insularis sp. nov. They are similar species with compressed but variable axes and a wealth of overlapping anatomical characteristics. However, the two species are distinguished not only by their genetics, but also reproductive features that are commonly found in the small prostrate, epiphytic species. It is possible that C. hasselbringii could be the correct name for species reported in warm waters of the western Atlantic as C. compressa or C. vieillardii species with type localities in South Africa and New Caledonia, respectively.

P13. IMPLICATIONS FOR ADAPTIVE RADIATION OF HALOPHILIC EUKARYOTES Park, J. S., Kyungpook National University, South Korea, jongsoopark@knu.ac.kr

Halophilic protozoa are independently scattered across the molecular phylogeny of eukaryotes; most of which are assigned to Heterolobosea. Here, a new biflagellate was isolated from a hypersaline water of 342‰ salinity. This isolate shared several morphological features with typical halophilic heterolobosean flagellates. Molecular phylogenetic trees of the 18S rRNA gene sequences clearly indicated flagellate is a heterolobosean species closely related to the halophilic Tulamoebidae. However, the flagellate was not closely related to any described genus. Cells were ovoid-shaped, and no amoebae were observed. The two unequal flagellates could grow at 100–200‰ salinity, suggesting an obligately halophilic species. Currently, it appears that the new halophilic *Aurem hypersalina* forms a strong clade with Tulamoebidae, and is sister to the Tulamoebidae, indicating that this clade is composed almost entirely of obligate halophilic taxa. Thus, *A. hypersalina* and the Tulamoebidae currently represent a unique adaptive radiation of halophilic eukaryotes.

P14. CHARACTERIZING VOLTAGE-GATED SODIUM CHANNELS IN *KARENIA BREVIS* AND *OXYRRHIS MARINA*

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Action potentials and electrochemical signaling are mediated in multicellular organisms by four-domain Voltage-Gated Sodium Channels (VGSC) and are important for evoking physiological responses. However, little is known about the functional roles of homologous single-domain VGSCs in unicellular organisms, particularly in marine phytoplankton. The dinoflagellate Karenia brevis has evolved to produce harmful brevotoxins that target eukaryotic VGSCs that result in physiological dysfunction. Many studies have characterized the mechanistic actions of algal toxins on mammalian sodium channels but there is a lack of knowledge about the molecular properties and electrical activity of VGSCs in the toxinproducing algae themselves. This study aims to characterize VGSCs from photosynthetic dinoflagellate K. brevis and basal, heterotrophic dinoflagellate Oxyrrhis marina utilizing molecular analyses and electrophysiology techniques. The objectives of this study are to 1) identify sodium channel genes in K. brevis and O. marina using BLAST sequence similarity searches with other known single-domain channel sequences and 2) heterologously express identified sodium channel genes in mammalian HEK293 cells and perform whole-cell patch clamp recordings to observe characteristic channel currents. Characterizing single-domain VGSCs from marine phytoplankton may better our understanding of sodium channel functionality relating to ion selectivity and toxin sensitivities as well as provide insight into the evolved functions of four-domain sodium channels in other eukaryotes.

P15. CALCEIN STAINING AS A TOOL TO INVESTIGATE COCCOLITHOPHORE CALCIFICATION

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Despite the oceanographic and geological significance of coccolithophores, the cellular mechanisms behind the intracellular production and secretion of their CaCO₃ coccoliths remain poorly understood. Novel tools for labelling coccoliths and coccospheres to track their production have not yet been described. We therefore evaluated the use of calcein, a derivative of fluorescein that fluoresces brightly when bound to Ca²⁺ as a method to stain CaCO₃ coccoliths. The calcein method readily labelled pre-existing coccospheres in a range of coccolithophore species, including diploid and haploid phases, without compromising coccolith structure or cell physiology. Calcite staining was verified though

epifluorescence and confocal microscopy, and both stained and unstained cell populations were readily distinguished using flow cytometry. The fluorescence of stained coccoliths was retained for more than three days allowing confirmation of the polar secretion of coccoliths by distinguishing pre-existing coccoliths from the accumulation and distribution of non-fluorescent coccoliths produced after staining. The calcein method represents a simple non-invasive, non-toxic optical technique to 'tag' calcite coccoliths and track their production in response to environmental manipulations or pharmacological treatments. This is the first description of the use of calcein to stain calcifying phytoplankton and this approach could be applied to detailed cytological investigations as well as high throughput analysis of cultured cells or field populations.

P16. METAGENOMIC EVIDENCE FOR NON-PHOTOSYNTHETIC PROTISTS IN ALGAL MICROBIOMES

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Comparative metagenomic analyses were performed for a phylogenetic spectrum of multicellular freshwater/terrestrial autotrophic algae to gain insight into the early evolution of autotroph microbiomes. 18S rDNA and 28S rDNA sequences filtered from the metagenomic data allowed the inference of eukaryotic associates, which included non-photosynthetic protists emphasized here. Pooled samples taken through a growing season of a globally-abundant, highly-branched freshwater chlorophyte revealed 11 species of Ciliophora inferred from 18S rDNA and 12 species from 28S rDNA; some of these ciliate species were also observed by means of light microscopy, providing ground truth. In addition, the chlorophyte microbiome was inferred from 18S rDNA to include 6 species of Amoebozoa and from 28S rDNA to include 3 Amoebozoa species. Kinetoplastid, centrohelid, and katablepharid representatives were also inferred for the chlorophyte microbiome. Sequences classified as endoglucanases included a type that clustered with an endoglucanase known from a dictyostelid. Nucleariid protists (closely linked with the ancestry of fungi) occurred in the microbiomes of freshwater streptophyte algae, and a rhizarian was a prominent component of the microbiome of a terrestrial lichenized cyanobacterium. These observations illustrate the types of non-photosynthetic protists that associate with modern multicellular freshwater/terrestrial algae of ecological or evolutionary significance and illuminate the evolutionary history of such associations.

P17. EXAMINATION OF THE SI REQUIREMENT FOR CALCIFICATION IN COCCOLITHOPHORES

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Coccolithophores are important contributors to global CaCO₃ through their species-specific production of calcite coccoliths. Although the overall biomineralization mechanism is well understood, the presence of silicon transporters (SITs) combined with a Ge sensitivity of coccolith production in species with SITs (*Calcidiscus leptoporus, Coccolithus braarudii*, and *Scyphospheara apsteinii*) suggests a mechanistic link between Si and calcification. The objective of this study was to further investigate the requirement of Si for calcification by monitoring dissolved Si (DSi) and Si incorporation into biogenic structures (BSi) for species with SITs. The silicifying haptophyte *Prymnesium neolepis* and a diatom were included as positive controls and two species of coccolithophores without SITs (*Emiliania huxleyi* and *Gephyrocapsa oceanica*) were also compared. All species were grown over 8 d in 5 μ M Si and sampled for DSi, BSi (through a modified alkaline digestion), growth rates, photosynthetic physiology (Fv/Fm), and biomineral structures (SEM) in a series of depletion experiments. Elemental analysis was also conducted using SEM with X-ray dispersive spectroscopy. Coccolithophore species with SITs did not drawdown (DSi) or incorporate (BSi) Si beyond levels of detection, confirming the Si quota is very low. However, significant Si was observed using EDS in coccoliths of SIT species. Higher resolution techniques are now required to further explore the functional role of Si in the biomineralization process.

P18. HETEROTROPHIC BACTERIAL PRODUCTIVITY OF MACROALGAL BIOFILMS **Hall, J. R**., University of Otago, New Zealand, <u>halja373@student.otago.ac.nz</u>

Secondary production by heterotrophic bacteria associated with macroalgal biofilms represents an important link between macroalgal primary production and consumers. Heterotrophic bacterial biomass production was determined on biofilms of representatives of different algal divisions that fulfill important roles in coastal ecosystems; 1) the giant kelp M. pyrifera, an ecosystem engineer 2) Ulva sp. often associated with eutrophication and algal blooms 3) the coralline A. corymbosa, a member of a group that is a dominant component on a cover basis of many reef systems. These species were collected from two wave-exposed habitats on the southeast coast of the South Island of New Zealand and the heterotrophic bacterial activity of biofilms on their surfaces determined using the [3H] leucine methodology. Results indicate that *M. pyrifera* biofilm was significantly more productive than both Ulva sp. and *A. corymbosa*. The Ulva sp. biofilm was also found to be significantly less productive than that of the other two species. Differences in biofilm productivity were consistent among species when compared between sites but rates of productivity were 40% higher in an extensive kelp forest habitat compared to a smaller fringing kelp bed site. These results are likely a consequence of physiological differences among species. Disparity in productivity between sampled sites suggests that abiotic factors also influence rates of heterotrophic production of biofilm microbes. Differences in productivity of the microbial community on macroalgal surfaces among key species suggests a further mechanism that changing macroalgal species composition can influence carbon flow and ecosystem functioning.

P19. TAXONOMY AND DESCRIPTION OF *CRYPTOMONAS* SP. (CRYPTOPHYCEAE, CRYPTOPHYTA), UNIQUE FRESHWATER CRYPTOMONAD **Nam, S. W**., Nakdonggang National Institute of Biological Resources, South Korea, seungwon10@nnibr.re.kr

Cryptomonas Ehrenberg is a photosynthetic freshwater cryptomonads. Here, we describe a new freshwater cryptophycean species *Cryptomonas* sp. and report putative cytostome and flagellar apparatus by transmission electron microscopy. A putative cytostome was located in the anterior region of the cell and supported by rim fiber and a mitochondrion. The major components of flagellar apparatus of *Cryptomonas* sp. consisted of long and keeled rhizostyle (Rhs), unique fibrous root (FR), FR-associated microtubular root (FRm), mitochondria-associated lamella (ML) and four type of microtubular roots (1r, 2r, 6r, and mr). The Rhs originated at the right side of the dorsal basal body and extended to the posteriad of the cell. The FR and FRm extended toward the left side of two basal bodies. The ML originated at the ventral basal body and connected to mitochondrion shortly. Four type of microtubular roots originated at the structure of flagellar apparatus was similar to those of previously reported *Cryptomonas* species such as *C. paramaecium, C. pyrenoidifera*, and *C. curvata*, However, *Cryptomonas* sp. was differentiated by cylindrical FR without striation, short ML and existence of the putative oral structure which showed the possibility of mixotrophy.

P20. TAXONOMY AND DESCRIPTION OF FRESHWATER MICROALGA *MYCHONASTES* SP. JANGDONG160806F, NEW SPECIES CANDIDATE

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Genus *Mychonastes* is one of the small coccoid algal genus belonging to Chlorophyceae. In this study, we described a new species candidate *Mychonastes* sp. Jangdong160806F. *Mychonastes* sp. was isolated from a freshwater sample in Jangdong reservoir and cultured in BBM medium at 20°C with 16:8 LD-cycle. *Mychonastes* sp. cells are a spherical shape and measured $2.37 \sim 4.20 \ \mu m$ ($2.99 \pm 0.49 \ \mu m$) diameter. A motile stage has not been observed by light microscopy. Ultrastructural data showed each cell had a

nucleus and a crescent-shaped chloroplast without pyrenoid. The chloroplast consists of thylakoid lamellae arranged in three almost parallel rows. The phylogenetic tree was inferred with combined gene sequences of partial small-subunit (SSU rDNA), internal transcribed spacer 1 (ITS1), 5.8s rDNA, internal transcribed spacer 2 (ITS2) and partial large-subunit (LSU rDNA) by maximum likelihood method. This phylogeny showed that *Mychonastes* sp. Jangdong160806F has formed a monophyletic group and had a sister group relationship to the *M. homosphaera* clade. Also, the predicting ITS2 secondary structures of *Mychonastes* sp. Jangdong160806F were significantly different from those of 10 species in genus *Mychonastes*.

P21. TAXONOMY AND DESCRIPTION OF *MESODINIUM ANNULATUM* SP. NOV., A NEW MEMBER OF THE *M. RUBRUM/M. MAJOR* COMPLEX **Nam, S. W.**, Nakdonggang National Institute of Biological Resources, South Korea, <u>seungwon10@nnibr.re.kr</u>

A red-tide bloom-forming ciliate, *Mesodinium rubrum*, have studied intensively on ecological roles in global marine ecosystems and on evolutionary aspects of stolen organelles (kleptoplastidy and karyoklepty), but their taxonomy is unclear. To understand taxonomy of Korean *Mesodinium* cf. *rubrum*, we isolated a new marine *Mesodinium* species from seawater samples at Gomso bay, Korea and established a culture strain under a mixotrophic condition by providing a red cryptomonad prey, *Teleaulax amphioxeia*. The cells consisted of two spherical hemispheres separated by two types of kinetids: dikinetid and polykinetid. The dikinety was approximately 38 in number and each dikinety consisted of up to 18 longitudinally arranged dikinetids. The polykinetids consisted of 16 kinetosomes organized into four longitudinal rows containing five, five, four and two kinetosomes. Two kinds of kinetids and its associated microtubules and fibers were similar to those of *M. rubrum* from Denmark. However, the Korean *Mesodinium* species was characterized by spherical aboral hemisphere, number of tentacles and presence of annulus. Molecular phylogeny based on ITS sequences showed that our *Mesodinium* grouped together with the Variant B of *Mesodinium rubrum/major* species complex, but not with the Variant F, the neotype of *M. rubrum*. Based on morphological and molecular data, we propose a new marine *Mesodinium* species, *M. annulatum*.

P22. STATUS OF BARREN GROUND AND COUNTERMEASURES IN KOREA WATERS **Oh, T. G.**, Korea Fisheries Resources Agency, South Korea, dharta@fira.or.kr

"Barren ground" that the coastal rocky area is covered with crustose coralline has been progressing 1,200ha every year since 1992 in Korea. For restoration barren ground, Korea government has progressed "Marine Forest Restoration Project (MFRP)" using macro brown algae since 2009, and has plan to enhance marine forest of 54,000ha until 2030. MFRP is divided by two methods called "Enhancement" and "Management". FIRA (Korea Fisheries Resources Agency) funded from government is trying to enhance marine forest with various methods such as artificial reefs for algae, long-line under water, spore bag, planting panel. Also for management of marine forest, FIRA has been enforcing control of grazing-pressure by exterminating grazers such as sea urchin, abalone at enhanced marine forest area. MFRP in Korea has been progressed at 129 places (15,200 ha) for 9 years (2009~2017) investing \$23 million. Ecological environment is significantly improving at enhanced marine forest (species increased 26% than last year did), and social effect showed 1.54 times by Benefit-Cost ratio analysis. Also, the world's first "Marine Gardening Day" was established by Korea government and FIRA in order to stimulate people's interest in MFRP, and 1st celebration was held on May, 2012.

P23. TEMPERATURE TOLERANCE OF THE SEA VEGETABLE CROP *ALARIA ESCULENTA* **Quigley, C. T.**, University of Maine, United States, charlotte.quigley@maine.edu Brawley, S. H., University of Maine, United States

Sea vegetable aquaculture will be increasingly dependent on a greater diversity of strains that are tolerant to coastal warming. Market demand offers increased opportunities for development of sea vegetable crops in Maine. This project aims to determine temperature tolerance of the crop Alaria esculenta (L.) Greville and to understand its potential in Maine's warming waters. We cultured gametophyte seedstocks from two source locations that span the Maine shore. Replicates were maintained at 12°C or slowly acclimated 1°C/12 h to 22°C. Heat treatment affected the health of cultured gametophytes, but the source location did not (MANOVA, treatment: p < 0.001, location: p = 0.364). Control and heated strains from each source location were crossed (within treatment) to produce sporophyte blades. Juvenile blades were grown in a common garden study in tanks at our aquaculture center, and then were transferred to a sea farm for grow-out. The largest juvenile blades produced at our aquaculture center were from northern strains that were previously acclimated to 22°C. Both gametophyte acclimation and source location affected the surface area of juvenile blades (Wilcoxon Signed Rank Test; Treatment: Z = 20.158, p < 0.01, Location: Z = 10.25, p < 0.01). Similar grow-out results are being found in the sea farm, based on observations and preliminary analyses. This research demonstrates that thermal tolerance of seedstock may vary by source location, but thermal acclimation protocols in seedstock nurseries may help boost crop yields universally, further supporting the sea vegetable aquaculture industry.

P24. INSIGHTS INTO THE CELL DEATH OF DINOFLAGELLATE *KARENIA BREVIS* TRIGGERED BY ENVIRONMENTAL STRESS

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Historically, marine phytoplankton were presumed to replicate indefinitely by binary fission. However, drastic changes in environmental conditions have been shown to induce algal cell death, and can greatly affect development of algal populations. The Gulf of Mexico (GoM), a vast region with extensive shellfish resources, is subject to frequent toxic blooms of the dinoflagellate *Karenia brevis*. Although the behavior and physiology of *K. brevis* have been extensively investigated in both laboratory and field contexts, the mechanisms of bloom decline and termination within the GoM are not well known, hampering our ability to forecast their impact on coastal waters and human health. In this research, oxidative stress, heat shock, cold stress, high irradiance and nitrogen starvation are used to induce cell death in *K. brevis*, and cellular response pathways are characterized by measuring stress indicators including photosynthetic efficiency, production of reactive oxygen species (ROS), caspase-like activity, and morphological changes. We expect to identify environmental stressors that can cause cell death in *K. brevis*, define stress-related death processes, and elucidate potential mechanisms of *K. brevis* bloom decline in the GoM, while adding to our ecological understanding of algal cell death in variable environments.

P25. COMPARATIVE PLASTID GENOMICS OF SYNUROPHYCEAE: EVOLUTION VIA LATERAL GENE TRANSFER AND INVERTED REPEAT DYNAMICS **Kim, J.,** Chungnam National University, South Korea, <u>jongim@cnu.ac.kr</u> Shin, H., Chungnam National University, South Korea, <u>shmoon1107@naver.com</u>

The Synurophyceae is one of most important photosynthetic stramenopile algal lineages in freshwater ecosystems. They are characterized by siliceous scales covering the cell or colony surface and possess plastids of red-algal secondary endosymbiotic origin. Despite their ecological and evolutionary significance, the relationships amongst extant Synurophyceae are unclear, as is their relationship to most other stramenopiles. Here we report a comparative analysis of plastid genomes sequenced from five representative synurophycean algae. Most of these plastid genomes are highly conserved with respect to genome structure and coding capacity, with the exception of gene re-arrangements and partial

duplications at the boundary of the inverted repeat and single-copy regions. Several lineage-specific gene loss/gain events and intron insertions were detected (e.g., *cemA*, *dnaB*, *syfB*, and *trnL*). Unexpectedly, the *cemA* gene of Synurophyceae shows a strong relationship with the green-algal lineage, suggesting the occurrence of a lateral gene transfer event. Using a molecular clock approach based on silica fossil record data, we infer the timing of genome re-arrangement and gene gain/loss events in the plastid genomes of Synurophyceae.

P26. CHANGES IN ALGAL BIOMASS IN RELATION TO HARVESTING METHODS OF *GELIDIUM*

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Gelidium has traditional been the main source of income for fishermen in the island of Jeju. However, the population of *Gelidium* has been in a gradual decrease. Therefore, the objective of this study is to monitor the changes in the biomass (biomass recovery) following a furrow-method of harvesting Gelidium and proposing a self-management model for fishermen in order to increase the decreasing population of Gelidium and to sustain its usage. In the coast of Pyungdae, Jeju, where Gelidium predominates, experimental plots were installed with varying harvest ratios of Gelidiales (100%, 2/3, 1/2) utilizing the furrow-method in June of 2016. Then, emergent marine algae as well as their biomass within each experimental plot were monitored for 17 months until November of 2017. The biomass of Gelidiales per experimental plots all appeared in the order of 1/2-harvest-plots>2/3-harvest plots>100%-harvest-plots, while the biomass of marine algae excluding Gelidiales tended to appear in the order of 100%-harvest plots>2/3-harvest-plots>1/3-harvest-plots in 2016, although a difference between experimental plots was not observed in 2017. The proportion of Gelidiales in the wet weight of marine algae appeared in the order of 1/2-harvest-plots>2/3-harvest-plots>100%-harvest-plots during most of the experimental period. The results of this study demonstrate that overharvesting Gelidiales decelerates the regrowth of Gelidiales while accelerating the succession and growth of competing marine algae. This monitoring indicates a need to regulate the harvest with a fixed ratio in order to maintain the sustainable population of Gelidiales

P27. PROTOPLAST ISOLATION FROM SUSPENSION CULTURES OF *HECATONEMA TERMINALE* (ECTOCARPALES, PHAEOPHYCEAE) USING A SIMPLE MIX OF COMMERCIAL ENZYME

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Protoplast systems are essential for genome-editing and gene silencing technologies. In brown algae, protoplast isolation has been hampered by protocols that use non-commercial enzymes or crude extracts. This study reports for the first time the production of protoplasts from cell-filament suspension cultures of the brown alga *Hecatonema terminale* (Collins) Sauvageau using different mixtures of commercial enzymes and chelating pre-treatment. Cellulase Onozuka RS was more effective than cellulase R-10 in protoplast isolation, while macerozyme R-10 was not necessary for protoplast formation. Alginate lyase alone did not yield any protoplast and chelating pre-treatment had a positive effect on mixtures containing this lyase. Although there was an incomplete cell wall digestion, true and viable protoplasts were released through holes in the cell wall. The maximum protoplast yield was $3.87\pm0.91 \times 10^5$ cells g⁻¹ fresh weight with more than 98% of viability using 1% cellulase Onozuka RS and 3 U/ml alginate lyase from Sigma with chelating pre-treatment.

P28. ULTRA-MORPHOLOGY AND PHYLOGENY OF *PHYMATOLITHON PURPUREUM* AND *P. LAEVIGATUM* (HAPALIDIACEAE, RHODOPHYTA) BASED ON COMPARISON OF TYPE MATERIALS

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To elucidate the morphological boundary and phylogenetic relationship between *Phymatolithon purpureum* and *P. laevigatum*, we revised the taxonomy of the two species using anatomical observations and DNA sequencing from type materials. Although *P. purpureum* has been often confused with *P. laevigatum* because of their similar features (e.g., smooth surface and immersed tetra/bisporangial conceptacles), *P. purpureum* was distinguished by a thicker medullary system, deeply immersed tetra/bisporangial conceptacle pore plates, and buried tetra/bisporangial conceptacles. Our molecular analyses of the *psbA* and COI-5P genes indicated that sequence divergence between *P. purpureum* and *P. laevigatum* was 1.9% and 9.3–9.5%. We also found that the lectotype of *Phymatolithon polymorphum* f. *papillatum* was consistent with the features of *P. purpureum*.

P29. *PHYMATOLITHON DOSUNGII* SP. NOV. *P. PROCUMBENS* SP. NOV. (HAPALIDIACEAE, RHODOPHYTA), TWO NEW CRUSTOSE CORALLINE ALGAE FROM KOREA **Jeong, S.**, Chosun University, South Korea, <u>delesseriaceae@gmail.com</u> Won, B., Chosun University, South Korea, <u>giving_won@daum.net</u> Cho, T., Chosun University, South Korea, <u>tocho@chosun.ac.kr</u>

Worldwide, 17 species have been recognized in the genus *Phymatolithon*. We describe two new species of the genus from Korea, based on morphological and molecular data. *P. dosungii* sp. nov. is recognized by having orbicular thalli in initial shape, no protuberances, all reproductive conceptacles raised above the surrounding surface without raised rims, up to 7–15 pores in pore plate, and no buried tetra/bisporangial conceptacles. *P. procumbens* sp. nov. is recognized by having encrusting thalli, no protuberances, tetra/bisporangial conceptacles raised above the surrounding surface without raised rims, up to 11–20 pores in pore plate, and buried tetra/bisporangial conceptacles. *P. dosungii* sp. nov. and *P. procumbens* sp. nov. are showing differences in raised degree of tetra/bisporangial conceptacles above the thallus surface, thickness of tetra/bisporangial conceptacle roofs, and presence or absence of buried senescent tetra/bisporangial conceptacles. Phylogenetic analyses of *rbcL* and COI-5P genes also reveal that these species are placed in *Phymatolithon* and distinguished from congeners. The sequence divergence between them is 2.1–2.2% in *rbcL* gene and 5.6–5.8% in COI-5P gene.

P30. BIOCHEMICAL COMPOSITION AND TAXONOMIC DIVERSITY WITHIN CYANOBACTERIAL ORDER PLEUROCAPSALES Shalygin, S., TAMUCC, United States, <u>got.lifemusic@gmail.com</u> Huang, I., TAMUCC, United States, <u>wade76924@gmail.com</u> Zimba, P., TAMUCC, United States, <u>paul.zimba@tamucc.edu</u>

The recognition of benthic cyanobacteria as harmful algal taxa has increased in the past decade, filamentous forms typically being identified. Baeocyte producers from the order Pleurocapsales are one of the least studied groups in terms of taxonomy, ecology, and biochemistry yet have widespread distribution in benthic habitats. In various phylogenies, Pleurocapsales occupy a paraphyletic position to the Chroococcales and the proper name for the entire group is debated. Absence of sequence data for *Pleurocapsa fuliginosa* (the type species) is problematic for description of any new taxa within the order. Data on metabolite profiles of Pleurocapsales are lacking. Several pleurocapsalean strains, such as CalAg792, CCMP3459 and others, were analyzed for phylogenetic relationships using the multi-loci approach and using physiological/chemical measures (pigments, fatty acids, off-flavor, and toxin production). The freshwater strain CalAg792, isolated from a California aqueduct, belongs to an undescribed genus based on genetic analysis – is of interest since it was reported to produce off-flavor by other authors. Our preliminary experiments have identified several toxins in CalAg792 including

microcystin, aeruginosin, etc. Multiple toxins can be released from that cyanobacterium into the water column and affect aquatic biota. It is likely that impacts of benthic cyanobacterial communities will increase in the future, affecting aquatic organisms and human health. It is crucial for phycologist to completely describe taxa using both genomic and metabolomic approaches. Genomic and biochemical attributes of the rest of mentioned isolates will be presented on the poster.

P31. THE INVASIVE RED ALGA *GRATELOUPIA TURUTURU* (HALYMENIALSES, RHODOPHYTA) REACHES MID-COAST MAINE: ARE CURRENT BIOSECURITY MEASURES ENOUGH?

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The invasive red alga *Grateloupia turuturu* was discovered in the intertidal effluent drainage area of the University of Maine Darling Marine Center's flow-through seawater laboratory in Walpole, ME. This represents over 200 km of expansion in its range. Multiple life history stages were found: tetrasporophytes (September 2017), a carposporophyte (October 2017), and vegetative plants (November 2017/February 2018). Blades were removed when found, and none was left on the shore after February 2018. To investigate one of several potential sources of the invasion, we performed a chlorination experiment with an initial 50 ppm free chlorine treatment for 2 hr with blades of *Grateloupia* (February collection) and *Porphyra umbilicalis*. No differences were found between solutions made with commercial bleach versus pure sodium hypochlorite, and both *G. turuturu* and *P. umbilicalis* grew after treatment. *Grateloupia* showed little damage, but the *Porphyra* developed callus within dead areas of blade. The free chlorine concentration fell to less than 25 ppm after two hr. The DMC carefully follows State of Maine biosecurity regulations when housing quarantined organisms, which include a full 2 hr of chlorination maintained at 50 ppm free chlorine. Based on the results of this experiment, some algae appear more resistant to chlorination. Therefore, we are expanding our experimental work to consider whether existing State-mandated biosecurity rules are adequate.

P32. GROWTH AND INGESTION RATES OF HETEROTROPHIC PROTISTS ON THE TOXIC DINOFLAGELLATE *OSTREOPSIS CF. OVATA*

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Ostreopsis cf. ovata is a toxic dinoflagellate and has a wide distribution from the tropic to temperate waters. This species is a primary epiphytic benthic dinoflagellate, but lives in water column as well and sometimes forms harmful red tides. To investigate feeding by the heterotrophic dinoflagellates *Gyrodinium dominans, G. moestrupii, G. spirale, Oxyrrhis marina, Pfiesteria piscicida, Polykrikos kofoidii, Protoperidinium bipes,* and *Stoeckeria algicida* and the naked ciliate *Strobilidium* sp. are able to feed on *O. cf. ovata* was tested. The growth and ingestion rates of *G. moestrupii* and *P. kofoidii* on *O. cf. ovata* as a function of prey concentration were also measured because this prey supported positive growth of only these two predators. Furthermore, these growth and ingestion rates were compared with those on the other algal prey for exploring comparative nutritional value of this prey. *G. dominans, G. moestrupii, O. marina, P. piscicida,* and *P. kofoidii* were able to feed on *O. cf. ovata,* whereas *G. spirale, P. bipes, S. algicida,* and *Strobilidium* sp. were not. The maximum growth rates of *G. moestrupii* and *P. kofoidii* on *O. cf. ovata* were 0.86 and 0.73 /d, respectively, while the maximum ingestion rates were 6.2 and 33.3 ng C/predator/d, respectively. The maximum swimming speed of *O. cf. ovata* is lower than that of any other dinoflagellate prey except 1-2 species. The results of the present study suggest that slow swimming *O. cf. ovata* is an easily edible prey, but not nutritional prey for growth of *G. moestrupii* or *P. kofoidii*.

P33. COMPARATIVE PLASTID GENOMICS OF SYNUROPHYCEAE: EVOLUTION VIA LATERAL GENE TRANSFER AND INVERTED REPEAT DYNAMICS

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The Synurophyceae is one of most important photosynthetic stramenopile algal lineages in freshwater ecosystems. They are characterized by siliceous scales covering the cell or colony surface and possess plastids of red-algal secondary endosymbiotic origin. Despite their ecological and evolutionary significance, the relationships amongst extant Synurophyceae are unclear, as is their relationship to most other stramenopiles. Here we report a comparative analysis of plastid genomes sequenced from five representative synurophycean algae. Most of these plastid genomes are highly conserved with respect to genome structure and coding capacity, with the exception of gene re-arrangements and partial duplications at the boundary of the inverted repeat and single-copy regions. Several lineage-specific gene loss/gain events and intron insertions were detected (e.g., *cemA*, *dnaB*, *syfB*, and *trnL*). Unexpectedly, the *cemA* gene of Synurophyceae shows a strong relationship with the green-algal lineage, suggesting the occurrence of a lateral gene transfer event. Using a molecular clock approach based on silica fossil record data, we infer the timing of genome re-arrangement and gene gain/loss events in the plastid genomes of Synurophyceae.

P34. TAXONOMIC REVISION OF JAPANESE *SAUVAGEAUGLOIA* SPECIES (ECTOCARPALES S.L., PHAEOPHYCEAE)

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Molecular phylogeny using mitochondrial cox1 and cox3 and chloroplast *atpB*, *psaA*, *psbA* and *rbc*L gene sequences reveals that the Japanese endemic brown alga *Sauvageaugloia ikomae* (Ectocarpales s.l.) is phylogenetically distant from the genetitype *S. divaricata* described in Europe, and also suggests the occurrence of a cryptic species in the Seto Inland Sea and Pacific coast of Tohoku Region, while *S. ikomae* is distributed in The Sea of Japan. The new species is genetically distinct from *S. ikomae* and is morphologically distinguishable in having thicker erect thalli with a more developed subcortical layer. In the phylogeny the clade of Japanese *Sauvageaugloia* was sister to *Mesogloia* and nested in the clade of Cladosiphon and Myriogloea species. Because ectocarpalean taxa are considered to be over-split at the generic taxonomic rank compared to the genetic divergence within the order, we refrain from describing a new genus for *S. ikomae* and the new species, and instead suggest placing them in *Mesogloia*.

P35. THE COMPLETE GENOME OF A NON-PHOTOSYNTHETIC DIATOM AND ITS PLASTIDAL SULFOLIPID BIOSYNTHESIS

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Photosynthesis provides chemical energy converted from solar energy. Acquisition of photosynthetic plastids through endosymbiosis has, therefore, made a heterotrophic eukaryote autotrophic. Regardless of such benefits, some photosynthetic eukaryotes have lost photosynthesis secondarily and become parasites, phagotrophs, or osmotrophic heterotrophs. Genome and cellular evolution to achieve loss of photosynthesis and to adapt to a heterotrophic lifestyle remain to be fully understood. It is also interesting to see what are "NOT" changed after loss of photosynthesis. To address these issues, we sequenced the complete genome of the non-photosynthetic diatom Nitzschia putrida (Cohn) Benecke by HiseqX and PacBio RSII. The resultant reads were assembled with Falcon and Falcon unzip, followed by error correction with Pilon. The genome size is predicted to be 35 Mbp in length. Through the genome analyses, we found a plastidal glycolysis/gluconeogenesis pathway that leads to biosynthesis of sulfolipids, sulfoquinovosyl diacylglycerol (SODG), one of the main lipids for plastid membranes. We identified plastid targeting signals in proteins related to the pathway including SQD1 and SQD2, which function at the last two steps to synthesize SQDG. GFP-tagged N-terminal sequences from SQD1 and SQD2 were localized in plastids of the model diatom *Phaeodactylum tricornutum*, indicative of the plastid targeting potential. Indeed, SQDG was detected from the crude lipids extracted from N. putrida. Retention of the plastidal sulfolipid biosynthetic pathway in N. putrida would give insight into reductive evolution of non-photosynthetic plastid structures.

P36. ARGININE-CULTURE INDUCES NITROGEN-STARVATION RESPONSES DURING
PHOTOSYNTHETIC GROWTH IN CHLAMYDOMONAS REINHARDTII
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Growth and metabolism need sustainable supply of nitrogen (N) that requires monitoring of cellular Nstatus to search for additional N-sources (N-scavenging) and to mobilize internal N-rich molecules (Nsalvage). A key component of monitoring N-status is N-catabolite repression (NCR) whereby the presence of high-quality N-sources such as ammonia and glutamine repress N-limitation induced responses. Additionally, photosynthetic organisms reroute fixed carbons (C) into reserves such as lipids to ensure balance of the C:N ratio but the mechanisms coordinating N-starvation responses are poorly understood. Using gametogenesis as a phenotype repressed by high-quality N sources, we investigated Nstarvation responses in arginine-based media reported to induce gametogenesis in *Chlamydomonas* *reinhardtii.* Arginine cultures exhibit upregulation of N-starvation-induced genes, lipid accumulation, and decreased chlorophyll. In contrast, photosynthesis and proliferation were more robust in arginine versus ammonium cultures. We found increased photosynthesis rates in arginine and light-dependent growth if acetate is absent, indicating that arginine cannot serve as an organic C-source and likely releases CO₂ during assimilation. Similar lipid accumulation is observed in a diatom, *Phaeodactylum tricornutum*, grown with arginine as the sole N-source. These results suggest that supplying arginine as the sole N-source releases N-catabolite repression. Our results represent a study system where the N-limitation condition is readily controlled and will be instrumental in identifying how photosynthetic eukaryotes sense cellular nitrogen availability in growing cells.

P37. COMPARISONS OF TISSUE CARBON AND NITROGEN CONTENTS OF *SEAWEED* SPECIES GROWN IN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) AND ADJACENT SITES IN KOREA

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To determine the nutrient bioextraction capacity of seaweeds, we analyzed tissue carbon and nitrogen contents of seaweed species collected from two different locations in Tongyeong, Gyeongnam, Korea, 1) integrated multi-trophic aquaculture (IMTA) site, 2) Dara Harbor (>3 km north of the IMTA site). Four different seaweed species naturally grown in the IMTA and/or Dara were collected, including *Ulva pertusa* (June 2016 – May 2017), *Gracilaria* sp. (June – July, 2016), and *Undaria pinnatifida* and *Sargassum* sp. (January – May 2017). The tissue carbon contents of *U. pertusa* varied without seasonal or locational patterns, 25–32% at IMTA and 29–35% at Dara. The average tissue carbon contents of *Sargassum*, *Undaria* and *Gracilaria* were 32.76%, 30.56% and 31.44%, respectively, during the study periods. Tissue nitrogen contents of *U. pertusa* at IMTA were much higher than those from Dara Harbor during the study periods. At the IMTA site, the average tissue nitrogen content of *Ulva* (3.30%) was higher than that of *Undaria* (2.91%) or *Sargassum* (2.72%) during the period of January to May 2017. The high nitrogen contents in seaweed species grown in the IMTA site suggest that cultivation of these seaweed species in the IMTA system in Korea may be an efficient tool to remove nitrogen discharged from the fed–aquaculture (finfish) component.

P38. USING HERBARIUM SPECIMENS, INCLUDING MACROALGAE, TO ENHANCE LEARNING EXPERIENCES FOR FIRST-YEAR BIOLOGY STUDENTS **Clarkston, B.** and Jennings, L.

The Beaty Biodiversity Museum (BBM) is home to UBC's biological research collections and hosts over two million specimens, including 90,000+ macroalgal specimens—a wealth of potential for teaching and research projects. However, most UBC Biology Program courses do not use the Museum as a teaching resource or use only the public displays. This project focuses on first-year students and seeks to integrate research collections into large-enrollment (100+ students) courses. The questions discussed here are: 1) how are first-year students' knowledge, interests and perceptions of biological diversity, including macroalgal diversity, impacted by interacting with research collections? 2) does this impact differ if students interact with specimens online compared to in-person? Student responses to written questions in two first-year courses were collected via a survey given prior to and after students completed a 2-hour

activity using BBM specimens, either online or at the Museum. Preliminary results suggest that students benefited from interacting with authentic research collections, with both the online and in-person activities showing a similar impact for students.

P39. THE CHLOROPHYLL CYCLE AND ANTENNA PROTEINS IN SECONDARY CHLOROPLASTS OF *EUGLENA GRACILIS*

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The photosynthetic apparati functioning in the secondary chloroplasts of Euglenophyceae are thought to inherit those of the green plants (Chloroplastida) and hence contain chlorophyll a (Chl-a) and chlorophyll b (Chl-b) as photosynthetic pigments. In green plants, Chl-b synthesis is involved in a metabolic pathway called chlorophyll cycle. In the present study, we focused on a homologous gene of Chlorophyll a Oxygenase (CAO), here named Euglena gracilis CAO-like gene (EgCAOL), which, in the chlorophyll cycle of green plants, catalyzes the two-step oxidation of Chl-a to Chl-b. To examine its function of in vivo, we conducted an RNAi-based targeted gene knockdown experiment on EgCAOL in E. gracilis strain-z. In the knockdown cells, EgCAOL-kd, the average Chl-b content on total chlorophylls in cells decreased from 15% down to ca. 0.1%, suggesting that EgCAOL gene is requisite for synthesis of Chl-b in E. gracilis. EgCAOL-kd exhibited little morphological difference from the wild type under light microscopy but significant reduction of chlorophyll fluorescence intensity from the chloroplasts, implying some changes in the efficiency of light harvesting/energy transfer among the photosynthetic apparati. In fact, the photosynthetic activity of EgCAOL-kd was lowered about 30% compared to that of the wild type. Nonetheless, the cell growth rate of EgCAOL-kd in the exponential growth phase was only slightly affected (lowered about 10%), which is contradictory to the phenotype of ΔCAO mutants of plants (angiosperms) that exhibit impaired growth and developments.

P40. CHLOROPHYLL CATABOLISM BY PELAGIC NANOFLAGELLATES/AMOEBA FEEDING ON *PROCHLOROCOCCUS*

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Picocyanobacteria are known to dominate the primary production in the oligotrophic pelagic ocean. Those tiny picoplankton are likely to be predated by various protists through phagocytosis. Recent studies demonstrated that such protists feeding on microalgae commonly produce 132,173-cyclopheophorbide enols (CPEs), non-phototoxic catabolites generated after chlorophylls contained in their diets. On the other hand, Prochlorococcus produces unique chlorophylls called divinylchlorophylls. In this study, we tried to identify protists that potentially feed on picocyanobacteria by inoculating purely incubated cells of

Prochlorococcus sp. (NEIS-2882) into sample water obtained from oligotrophic surface layers of the Pacific Ocean in the south of the Kuroshio Current axis (27-30°N 138°E). A significant amount of divinyl-CPEs that presumably derive in divinylchlorophylls of Prochlorococcus were detected from extracts of such crude cultures, which are likely to have produced by protistan consumers of the picocyanobacteria. Protistan predators proliferated in the crude culture were then isolated using microcapillary under microscope, which established pure cultures of two nanoflagellates (*Paraphysomonas* sp. strain MZD003 and *Cafeteria* sp. strain MZD007) and two amoebae (*Neoparamoeba* spp.; strain MZD011 and MZD012). When these protists were cultured with *Prochlorococcus* as their diets, productions of divinyl-CPEs were confirmed in cultures of these two nanoflagellates (but not in those of the amoebae). Furthermore, divinyl-CPEs were detected from the untreated surface water samples, suggesting actual predation on *Prochlorococcus* by CPE-producing protists in the natural open ocean.

P41. PHYLOGENETIC POSITION OF THE ENIGMATIC SCUTICOCILIATE *CONCHOPHTHIRUS* (SCUTICOCILIATIA, CONCHOPHTHIRIDAE)

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Conchophthirus is an enigmatic scuticociliate that was redescribed by protargol staining and SEM, and whose division morphogenesis has been carefully described. Antipa & Small (1981) reviewed its stomatogenesis in relation to other scuticociliates, peniculines, and peritrichs, and concluded that it was difficult to unambiguously place this genus. We collected Conchophthirus spp. in California from two bivalve hosts: Pit River, 2 Anodonta californiensis; and Trinity River, 4 Margaritifera falcata. The ciliates were predominantly Conchophthirus curtus. The ciliates were preserved in 95% ethanol prior to DNA extraction. Standard methods were used to sequence the small subunit rRNA (SSUrRNA) and the cytochrome c oxidase subunit 1 (cox1) genes. Phylogenetic analyses used maximum likelihood (ML) for the SSUrRNA dataset and neighbor-joining (NJ) for the cox1 dataset. The cox1 sequences were obtained from four *M. falcata* populations, and these were all virtually identical. The NJ analysis placed these sequences with 96% bootstrap support as sister to Dexiotricha sp. The SSUrRNA sequences were obtained from five populations from both hosts. These sequences were almost identical. The ML analysis showed them to be sister with 99% support to two unpublished sequences from Chinese populations of Conchophthirus cucumis and C. lamellidens. The Conchophthirus spp. were sister to a Dexiotricha sp. with 100% bootstrap support. Conchophthirus is confirmed to be a scuticociliate, but consistent with the analyses of Antipa & Small (1981) is not closely related to either philasterine or pleuronematine scuticociliates.

P42. LIGHT EFFECTS GROWTH OF A HETEROTROPHIC DINOFLAGELLATE THROUGH AN UNKNOWN MECHANISM

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Crypthecodinium cohnii is a species complex of heterotrophic marine dinoflagellates that can be cultured on defined organic medium in complete darkness. Although not known to contain chlorophyll, some strains contain carotene pigments and a possible relict plastid, based on genetic (EST) analyses. In preliminary study of these characteristics, we observed an apparent light-induced difference in growth and pigmentation in *C. cohnii*. To examine this further, three geographically distinct strains of *C. cohnii* (CCMP 316, ATCC 30336 and ATCC 50050) were grown in triplicate over 15+ days extending through stationary phase under two treatments: continuous darkness versus a 12:12 h light:dark cycle. All replicates were grown under otherwise standard conditions in liquid A2E6 medium at 25°C. Cell abundance and biomass (biovolume) were measured daily using a Z2 Coulter Counter, and average

cellular ATP was measured using a luciferase luminescence assay. All strains exposed to light reached significantly higher cell abundance during log growth compared to dark treatments, and exhibited a darker pinkish-orange pigmentation. Strains CCMP 316 and ATCC 50050 also showed higher biomass production during log growth in light treatment compared to darkness. No differences were detected in cellular ATP between treatments. Results indicate that growth of *C. cohnii* is light-responsive in a way that suggests a form of facultative phototrophy. Further studies are required to determine mechanisms, which would be novel for this taxon whether due to relict plastids, carotenes, or other potential photoactive pigments such as proteorhodopsin.

P43. MORPHOLOGY AND PHYLOGENETIC POSITION OF NEW CENTROHELID HELIOZOANS FROM RUSSIA AND MONGOLIA

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Heliozoans or "sun-animalcules" is an assemblage of unrelated or distantly related protists with numerous radiating axopodia. The most of them belong to Centrohelida, a monophyletic group of the predatory organisms, which include both mobile and sessile forms, found in freshwater and marine environments. Two new species of centrohelid heliozoa from the treatment facilities in North Russia and river in Western Mongolia were isolated as clonal cultures and their SSU rRNA genes and transcriptomes are sequenced. The multigene phylogenetic analysis strongly supports the close relationship of centrohelid heliozoans to haptophytes. Electron microscopy was used to investigate the external morphology of scales. Clone HF-7 ("Choanocystis borokiensis") is characterized by slightly moving cells 14-20 µm in diameter. Thin needle-shaped radial spicules 7-10 µm in length form "halo" around the cell. Plate scales have been observed. Aggregations of cells have not been found. This species resembles Heterophrys myriopodia Archer, 1869 by the radial spicules structure and members of the genus Choanocystis Penard, 1904. Clone HF-20 ("Acanthocystis mongolica") is characterized by floating cells 15–20 µm in diameter. Plate scales of one type and radial scales of two sizes. Radial scales usually straight 2.0-2.8 µm and 4.5-12.0 um in length with the top furka which bears 2 or 3 teeth. This species resembles Acanthocystis myriospina Penard, 1890 and differs by two sizes of radial scales and two teeth in furca. This work is supported by the Russian Science Foundation (grant No 18-14-00239).

P44. BIODIVERSITY AND SYSTEMATIC POSITIONS OF SEVEN SCUTICOCILIATES FROM COASTAL WATERS IN SOUTH CHINA INCLUDING A NEW SPECIES: *URONEMA APOMARINUM* N. SP.

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The morphology and ciliature of seven scuticociliates isolated from mangrove wetland and intertidal zone of Shenzhen, China, including *Uronema apomarinum* n. sp. and a poorly known species *Homalogastra setosa* Kahl, 1926, were investigated using live observations and silver impregnation methods. The new species is characterized as: body size about $20-35 \times 10-15 \mu m$ in vivo; twelve or thirteen somatic kineties; M1 partly two-rowed, M2 two-rowed anteriorly and three-rowed posteriorly. Improved

diagnoses of *Homalogastra* Kahl, 1926 and its type species, *H. setosa*, are also provided. The genus *Homalogastra* is characterized as: non-thigmotactic Uronematidae with a pear-shaped body and a conspicuous subequatorial-positioned cytostome; apical plate not prominent; membranelle 1 two-rowed, shorter than membranelle 2, distinctly separated from the other two membranelles; paroral membrane extending anteriorly to about mid-level of membrane 2; one caudal cilium. Descriptions of other species, as well as comparisons with their congeners are also provided. Small subunit ribosomal RNA gene (SSU rDNA) of the six species are provided. Phylogenetic analyses indicate that *H. setosa* is separated from other sequences within the family Uronematidae, which is reflected by morphological arrangement of scutica. In addition, comparison of SSU rDNA sequences for populations or closely related species is performed and discussed.

P45. HOW CLIMATE CHANGE, CLONAL DIVERSITY AND PHENOTYPIC PLASTICITY OF *DAPHNIA MAGNA* AFFECTS THE COMMUNITY DYNAMICS IN FRESHWATER MICROBIAL FOOD WEBS

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Climate change caused by human activity will have a great impact on shallow freshwater ecosystems. It may cause evolutionary changes and an imbalance in microbial food web. To mitigate, or even effectively manage it, we must understand what limits the evolutionary potential of populations and what impact this has on biodiversity. To achieve this, researchers at the University of Liverpool are conducting an experiment that is assessing how levels of phenotypic plasticity of the keystone grazer *Daphnia magna* interact with warming to alter food web structure. Experiments are being conducted in 3000 L mesocosms containing natural freshwater species. Treatments vary the phenotypic plasticity of the *D. magna* population and the number of distinct clones in a mesocosm. On top of this, some mesocosms are warmed, to simulate temperature change. All treatments are replicated (n=3). As part of this large study, we are examining the impact of these treatments on the microbial food web. In general, we predict that: 1) *D. magna* populations with higher clonal diversity will have a wider food spectrum and stronger ability to resist the influence of climate change; 2) *D. magna* populations with higher plasticity will have a better adaption when global warming is simulated. To date, we have seen an expected strong top-down effect of *D. magna* on ciliates, but we have yet to recognize any effect of phenotypic plasticity, clone number, or warming on the structure of the food web.

P46. MORPHOLOGY AND PHYLOGENETIC SYSTEMATICS OF FOUR SAND-DWELLING BENTHIC DINOFLAGELLATES IN KOREAN COASTAL AREA Lee, J., Jeju National University, South Korea, <u>jblee@jejunu.ac.kr</u> Kang, S., Jeju National University, South Korea, <u>rosenmoon@jejunu.ac.kr</u>

As a primary producer of marine ecosystems, many studies of planktonic dinoflagellates have been conducted so far, but benthic dinoflagellates were not studied worldwide. In particular, sand-dwelling benthic dinoflagellates were not nearly reported in Korean waters. We examined the morphology of four sand-dwelling dinoflagellates in Korean coastal area, and analyzed 28S LSU rDNA genes for determining phylogenetic systematics. Sand samples were collected from intertidal zone at Gwakji Beach and Pyoseon Beach in Jeju coast, and Songpyeng Beach, Donggo-ri Beach, and Yulim Beach in the southern coast of Korea from January to April 2017. Nine strains from the samples were isolated and then incubated. The dinoflagellates were identified using light microscopes (LM) and scanning electron microscopes (SEM). In addition, DNA was extracted from each strains, and then PCR process was conducted. DNA sequences were determined, and then phylogenetic relations were analyzed using GTR+I model using MEGA v. 6.0 program. Four species were identified *Amphidinium thermaeum*, *Thecadinium kofoidii*, *Togula jolla* and *Heterocapsa* sp. nov. of benthic dinoflagellates. Three species of these were closely matched with the morphological shape and features compared to previous literatures. As a result of molecular phylogenetic

analysis of 28S LSU rDNA of each species, two species (*A. thermaeum* and *T. kofoidii*) matched with other strain. The strain SP02 of *T. jolla* was likely to be a cryptic species by systematic analysis, and *Heterocapsa* sp. nov. was considered a new species in terms of morphological and systematic analysis.

P47. SELF-CLEAVING PEPTIDE FOR GENE EXPRESSION IN *LEISHMANIA* **Podešvová, L**., Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic, Podesvoval@email.cz

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The genus Leishmania unites parasitic protists of the family Trypanosomatidae causing leishmaniases, closely related diseases that affect human and animal populations mainly in the tropical and subtropical regions. The clinical manifestations vary from spontaneously healing skin lesions to progressive and possibly fatal visceral infections. Leishmaniases represent a global health problem with over 500 million people at risk and an annual incidence rate of 5–10 million worldwide. Several molecular tools have been developed in recent years to study *Leishmania mexicana*, a causative parasite of cutaneous leishmaniasis. These methods have greatly extended knowledge concerning functions of numerous genes and their association to Leishmania virulence. One of such approaches relies on T7 polymerase-driven, Tetracycline-inducible gene expression. The main limitation of this system was its unsuitableness for developmental studies, due to the high impact the untranslated regions (UTRs), flanking both the gene of interest and T7 polymerase, have on the mRNA levels. Here, we report a novel system overcoming limitations of using exogenous UTRs. It is based on the 2A self-cleaving peptide, derived from the Porcine teschovirus-1. This approach enables simultaneous production of two separate proteins located upstream and downstream from its sequence. Importantly, protein expression is regulated by endogenous UTRs, thus allowing studying the protein function in cases which require its stable expression, e.g. during the life cycle of *Leishmania*.

P48. A NOVEL CRYPTIC SPECIES OF GREEN ALGAE FROM THE CALIFORNIA DESERT **Taylor**, **M**., Assumption College, United States, <u>melissa.taylor@assumption.edu</u> Pietrasiak, N., New Mexico State University, United States, <u>npietras@ad.nmsu.edu</u> Fucikova, K., Assumption College, United States, <u>k.fucikova@assumption.edu</u>

Green algae are a diverse group of photosynthetic life, yet are still vastly understudied compared to land plants. Traditionally, they were characterized using their morphology and life cycles. It is now clear that a better understanding of their diversity and evolution can be obtained through phylogenetic and genomic analyses. The desert green algal strain WJT24VFNP31 was isolated from Joshua Tree National Park (JTNP) as part of a larger biodiversity survey. The alga's organellar genomes were sequenced, annotated and used for phylogenetic analysis. A morphological assessment was conducted to determine its size, number of chloroplasts, flagellation, and number of nuclei. In addition, the secondary structure of the nuclear ribosomal 18S gene was compared to the structure typical for green algae. Although morphologically WJT24VFNP31 resembles the coccoid genera Bracteacoccus and Pseudomuriella, phylogenomic analyses demonstrate that it is not closely related to any other known species. The organellar gene content is consistent with other Chlorophyceae, but the architecture of the plastome is unusual, comprising two circular chromosomes, each with an IR-like region. The alga's GC-rich 18S sequence is highly divergent from other green algae, but its secondary structure appears preserved through numerous compensatory base changes. Given these unique characteristics and a distinct phylogenetic position, we propose that WJT24VFNP31 be placed into a new species and genus in Chlorophyceae. By studying cryptic green algae we expand our understanding of the desert biodiversity, but also of the evolutionary possibilities in gene and genome evolution.

P49. REVISION OF AMOEBOPHRYA CERATII KOEPPEN 1903 (DINOFLAGELLATA, SYNDINEA)
AS AN INTRACYTOPLASMIC PARASITE OF THE DINOFLAGELLATE CERATIUM TRIPOS
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The endoparasitic dinoflagellate Amoebophrya ceratii (= Hyalosaccus ceratii) Koeppen 1903 has been reported from numerous free-living dinoflagellates, but is now recognized as a species complex composed of more or less host-specific parasites. Here, we revise the diagnosis of A. ceratii from the dinoflagellate host Ceratium tripos as an intracytoplasmic parasite based on cytological stains and temporary cultures obtained from the Danish coast near Copenhagen in August 1999. We also use previously established cultures of A. akashiwo ex Akashiwo sanguinea to describe a new species of intranuclear parasite. Young trophont (before first nuclear division) of A. ceratii developed in the cytoplasm of the host C. tripos and contained a spherical or ovoid shaped nucleus ranging from 5.0X4.9 to 16.5X20.0 µm in size with a big nucleolus. As the parasite grew, parasite nucleus became elongated in a U shape with two nucleoli, presenting a synenergide development. When the trophont grew more than 35 μ m in size (> 200 nuclei), the trophont got rumpled inward, filling almost entire host cell, with the episome consistently oriented toward dorsal side of the host. In contrast, the intranuclear parasite A. akashiwo n. sp. developed inside the nucleus of host A. sanguinea. Young trophont of A. akashiwo had a small spherical nucleus ranging from 1.0X1.5 to 2.5X2.7 µm. Along with the growth of the trophont, this parasite exhibited immediate nuclear multiplication and formed a round or oval shape at maturity, with its episome orienting toward apical apex of the host. These features suggest that A. akashiwo n. sp. differs from previously described Amoebophrya species and is a new species.

P50. INVESTIGATIONS OF NUTRIENT REMOVAL EFFICIENCY FROM COASTAL MUDFLAT RESPONSE TO LIGHT INTENSITY: A CASE STUDY IN THE DIATOM-DOMINATED INTERTIDAL MUDFLAT

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Coastal mudflats, which are dominated by epipelic microphytobenthos, play important roles in regulating macronutrient (i.e., nitrate and phosphate) dynamics at the interface between overlaying seawater and sediments. To investigate the efficacy of nutrient removal from coastal mudflats, we collected sediment samples in the diatom-dominated intertidal mudflats of the Yellow Sea of Korea. The sediment samples were incubated via the continuous-flow microcosm system (seawater volume: 6 L; surface area: 60 cm²) for one week under four different light levels (45, 90, 145, and 220 µmol photons m⁻²s⁻¹). The results of the experiment showed that nitrate and phosphate concentrations decreased evidently with increasing light intensity, indicating a significant positive correlation between nutrient removal efficacy and light intensity. Based on our results, light intensity is a key factor controlling nutrient uptake in the study area, elevating the photosynthesis capacity of epipelic microphytobenthos at the seawater–sediment interface. Our study suggests the possibility that the coastal mudflat may act as a site of "blue carbon" storage.

P51. SYNTHESIS OF FRESHWATER RED ALGAL DIVERSITY (RHODOPHYTA) **Vis, M. L.**, Ohio University, United States, <u>vis-chia@ohio.edu</u>

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Since the advent of molecular systematics studies of freshwater red algae, the results have necessitated taxonomic changes. Within the Batrachospermales, rectifying the paraphyly of *Batrachospermum* has been a focus. New genera have been proposed to accommodate subgeneric sections as well as clades without previous section designation. These 'Batrachospermum-like' genera include Atrophycus, Lympha, Nocturama, Sheathia, Virescentia, and Volatus. Nothocladus has been expanded to include most species from Australasia. In the near future, it is likely that six more genera will be proposed to complete the task of rendering *Batrachospermum* monophyletic. Although this genus has been shrinking due to the description of these new genera, four new species have been added to *Batrachospermum* sensu stricto. Recently, the diversity in Thorea has expanded with at least nine species recognized from DNA sequence data and potentially 17 species based on the literature. Outside of these strictly freshwater orders, there have been two notable recent discoveries. In the Corallinales, *Pneophyllum cetinaensis* is a new crustose species discovered in a freshwater habitat even though all other species are marine. Within the Acrochaetiales, a new family and genus have been described for a red algal endophyte, Ottia meiospora from Australasia. The systematics of the freshwater red algae have been dynamic in recent years and this trend will continue in the near future. However, we have a new book project underway to synthesize these new developments and provide a comprehensive treatment of this charismatic group of organisms.

P52. SOME NEW MORPHOLOGICAL, GEOGRAPHIC AND PHYLOGENETIC DATA OF A MEXICAN POPULATION OF *EUPLOTOIDES OCTOCARINATUS* CARTER, 1972 **Mayén-Estrada, R.**, Universidad Nacional Autónoma de México, Mexico, <u>romaraf@gmail.com</u> Méndez-Sánchez, D., Universidad Nacional Autónoma de México, Mexico, <u>dms3v@hotmail.com</u> Hu, Z., Institute of Evolution and Marine Biodiversity, Ocean University of China, China Romero-Niembro, V. M., Universidad Nacional Autónoma de México, Mexico

The ciliate *Euplotoides octocarinatus* Carter, 1972 was isolated and described from the USA, and since their description no more morphological data have been updated, for which the goal of this work is to provide some new data, including its phylogenetic position and geographic distribution. We obtained individuals of one population in Oaxaca State, Mexico, and nonclonal cultures were maintained in laboratory. DNA extraction and PCR amplification were conducted, for SSU rRNA sequence. Maximum likelihood and Bayesian inference were carried out to construct the phylogeny. Our results include new data concerning the body length as well number of adoral membranelles, and also show the geographic distribution pattern includes at least, three major geographic regions. We obtained, after the phylogenetic results, the species is included in one clade with two more euplotids: *E. patella* and *E. daidaleos*. We concluded genus *Euplotoides* seems to be monophyletic.

P53. INDICATION OF A FOOD-CHAIN INTERACTION AMONG PROTISTS REPRESENTING FOUR TROPHIC LEVELS IN GWANGYANG BAY, MID-SOUTHERN COAST OF KOREA **Park, J. W.**, National Institute of Fisheries Science, South Korea, <u>cyanopark@korea.kr</u> Kim, H. S., Kunsan National University, South Korea, <u>mudskip@kunsan.ac.kr</u> Yoo, Y. D., Kunsan National University, South Korea, <u>ydyoo77@kunsan.ac.kr</u> Seong, K. A., Kunsan National University, South Korea, <u>scaway@kunsan.ac.kr</u> Oh, M. R., Kunsan National University, South Korea, <u>omr419@kunsan.ac.kr</u> Jeong, H. J., Seoul National University, South Korea, <u>hjjeong@snu.ac.kr</u> Yih, W., Kunsan National University, South Korea, <u>ywonho@kunsan.ac.kr</u>

Inter-annual variation in summer phytoplankton communities was explored in Gwangyang Bay (GYB) which is bridging the Seomjin River Estuary to the South Sea of Korea. The abundance of dinoflagellates are mostly less than 10% of diatoms that is predominated by the centric ones such as *Skeletonema*, *Chaetoceros*, and *Thalassiosira* throughout the whole monitoring period. Standing crops of the four protists, "cryptophytes(1)" as the prey for *Mesodinium rubrum*(2) which is predated by *Dinophysis* spp.(3), to be sometimes consumed by *Protoperidinium* spp., were measured by microscopic counting.

Distribution of the water samples on the four different scatter-diagram planes each with a set of preypredator axes or predator-(prey's prey) axes clearly indicated the food-chain interactions starting from (1) through (2) and (3) up to (4) in a row. Thus, prey-predator interaction among protist species could sometimes exert more impacts on the protistan community structures in semi-enclosed embayments like the GYB than previously thought.

P54. A METATRANSCRIPTOMIC APPROACH TO ASSESS DIATOM COMMUNITY COMPOSITION AND PHYSIOLOGY IN THE NORTH ATLANTIC

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Diatoms play a major role in global carbon cycling and are responsible for roughly 40% of the planktonic primary production in marine habitats. Nutrient availability is a key factor in shaping plankton community composition by influencing biodiversity, abundance, and growth rates throughout the world's oceans. Therefore, nutrient availability impacts primary production and the rate of carbon being sequestered to the deep sea. Much of our current understanding of diatom physiological ecology is derived from culture-based studies in lab settings. These studies provide a foundation for understanding the role of nutrient limitation on diatom physiology, however they also highlight distinct differences in how diatom species respond to nutrient availability. In this research we investigate the impact of nutrient availability on planktonic community composition and response across three biogeochemically unique provinces in the North Atlantic, the coastal shelf, the Gulf Stream, and the Sargasso Sea. From three stations in each of these oceanic provinces, the eukaryotic plankton community was collected from seawater on 5-micron filters. Both DNA and RNA were extracted from in situ samples collected at each station. Metabarcoding and metatranscriptomics were used to investigate community compositions and physiology of key species, across the three oceanic provinces. Analysis of gene expression patterns reveal that stations within each province cluster together, however no clear patterns emerged between nutrient availability and taxonomy. Diatom metabolic rearrangements are observed in the metatranscriptomic data as nutrient availability shifts across the biogeochemical provinces.

P55. GENOME DUPLICATION HISTORY WITHIN AMOEBOZOA SUPERGROUP **Apulu, N. J.**, University of Arkansas, Fayetteville, United States, <u>napulu@uark.edu</u> Brown, M. W., Mississippi State University, United States, <u>mbrown@biology.msstate.edu</u> Spiegel, F. W., University of Arkansas, Fayetteville, United States, <u>fspiegel@uark.edu</u>

Recent studies suggest complexity within a taxon is positively correlated with various genome duplication events. However, there is possible evidence to refute this trend. New publications show that aspects of complexity that occur in Amoebozoa are ancestral and the general trend is from complex to much simpler life cycles. We suggest that the Amoebozoa represents a group of organisms that do not follow this trend of complexity succeeding duplication events. We aim to demonstrate if genome duplications and losses could be considered drivers for increased complexity or if they show a pattern of genomic change independent of complexity. Perhaps duplication events are as likely to be associated with morphological and life cycle simplification as they are gains in complexity. The Amoebozoa has been interpreted to have many sub-lineages that have become simplified via loss of certain life cycle states. The added complexity seems to be the two origins of sorocarpy and two origins of external tests. Now, we have data that indicated that genome duplications have taken within Amoebozoa, and we will present data showing whether these duplication events are associated positively or not with increases in complexity. This

analysis will investigate an available transcriptome dataset of representative taxa within Amoebozoa by applying a variety of techniques such as gene tree reconciliation methods etc., previously employed in the analysis of genome duplication events in other eukaryotes. These techniques could reveal if genome duplications have occurred in Amoebozoa and if these duplication and loss events are consistent with the morphological patterns we observe in the group.

P56. DIATOM POPULATION GROWTH RESPONSES IN A DEFINED CULTURE MEDIUM CONSTITUTED FROM IONIC PROPORTIONS OF A LOCAL WATERSHED VERSUS TYPICAL ENRICHMENT MEDIUM

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Previously, Wee et al. (2016) used Skeletonema costatum strains isolated from the Lake Pontchartrain Basin (LPB) to investigate phytoplankton responses to petrochemical contamination in laboratory culture experiments designed to emulate a single watershed. In cultures exposed to light slop oil treatments, population growth was inhibited and relative abundances of cellular fatty acids and RuBisCO RNA transcript copy numbers were altered, and growth, food reserves, and photosynthetic potential were useful for assessing algal responses. However, the culture medium used LPB water enriched with f/2 nutrients and did not reflect the ionic proportions of LPB waters. The experiments described here utilized previous nutrient analyses to constitute Lake Pontchartrain Medium (LPM), a defined culture medium reflecting the ionic proportions of the LPB for targeting environmental impacts in a naturally-occurring system and constitute a paradigm-shift away from enrichment and defined culture media currently in general use. Population growth rates of S. costatum in f/2 were compared to LPM as well as a 1:1 mixture of the two media types. Population growth rates illustrated similar trends across the three treatments during the first two days of log growth, but the enrichment medium (f/2) exhibited significantly higher growth rates than either of the LPM treatments. The authors currently are monitoring a range of inorganic ions in media treatments during replicated growth experiments to assess whether experimental integrity increases when the algal culture medium reflects the ionic proportions of the investigated watershed over commonly used culture media.

P57. NEW DIVERSITY OF THE CORALLINE GENUS *SPOROLITHON* (SPOROLITHALES, CORALLINOPHYCIDAE) FROM THE GULF OF EILAT, ISRAEL (GULF OF AQABA), RED SEA **Kittle III, R. P.**, University of Louisiana at Lafayette, United States, <u>C00269538@louisiana.edu</u> Richards, J. L., University of Louisiana at Lafayette, United States, <u>joer207@gmail.com</u> Nguyen, A., University of Louisiana at Lafayette, United States, <u>a.nguyen210@gmail.com</u> Sauvage, T., Smithsonian Marine Station, United States, <u>tomsauv@gmail.com</u> Schmidt, W. E., University of Louisiana at Lafayette, United States, <u>wes4500@louisiana.edu</u> Fredericq, S., University of Louisiana at Lafayette, <u>slf9209@louisiana.edu</u>

Scanning electron microscopy and DNA sequence analyses (plastid rbcL and psbA, and nuclear LSUrDNA) of samples collected via snorkeling and SCUBA from the Gulf of Eilat, Red Sea (Israel), reveal the presence of at least two species of the coralline algal genus *Sporolithon* (Sporolithales, Rhodophyta) at these localities. All specimens possess a single layer of armored epithallial cells and both cell fusions and secondary pit connections. Two specimens show the presence of unshed and buried tetrasporangial compartments. A third specimen does not possess unshed and buried tetrasporangial compartments, and forms a clade with other species that slough off their tetrasporangial sori. These species will be compared with recently described *Sporolithon* species from the Gulf of Aqaba (Egypt) and the Gulf of Mexico.

P58. EFFECT OF EPIBIONT CILIATES (CILIOPHORA: PERITRICHIA) ON METABOLIC RATE OF THE CRAYFISH *CAMBARELLUS MONTEZUMAE* (CRUSTACEA: DECAPODA)

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Epibiosis is a facultative and interspecific association of two organisms, the epibiont and the basibiont. The ciliates are very common as epibionts of crustaceans, the body of the crustacean provides a suitable substrate for ciliate colonization, dispersion, refuge and food, with species of Epistylis and Cothurnia commonly attached to the gills of crustaceans. The aim of this work is to measure the oxygen consumption rate of decapod crustaceans, with and without epibiotic ciliates attached to the gills, to analyze the possible influence of the ciliates on the metabolic rate of the crayfish *Cambarellus* (Cambarellus) montezumae. Crayfish were collected at the Cantera Oriente pond (Mexico City), in rainy and dry seasons. The crayfish were individually placed in respirometry chambers. Oxygen concentration was measured before closing the chamber and one hour later, with a HANNA HI9143 oximeter; the measurements were made three times between 9 a 15 h. Oxygen consumption was calculated and compared between a group of crayfish with more than a 100 ciliate epibionts of the genus *Epistylis* on the gills and a control group without epibionts. A decrease in oxygen consumption rate was found in the crayfish with epibionts. We conclude that epibiont ciliates, at the studied density, decrease the metabolic rate of Cambarellus montezumae. Acknowledgements. To Posgrado en Ciencias Biológicas, UNAM and CONACyT, for the scholarship granted to MRB. To Biól. M. Reyes-Santos (Facultad de Ciencias, UNAM) for their technical support. Finally, our sincere thanks to REPSA (Reserva Ecológica del Pedregal de San Ángel) for allowing us to collect the crustaceans.

P59. DIVERSITY PATTERNS OF MICROBIAL EUKARYOTES FROM THE SEAGRASS EPIPHYTES OF *ZOSTERA MARINA* IN THE COAST OF BRITISH COLUMBIA (CANADA) **Segovia, B. T.**, University of British Columbia, Canada, <u>biatsegovia@gmail.com</u> Sanders, R. D., Hakai Institute, Canada, <u>rhea.smith@hakai.org</u> Parfrey, L. W., University of British Columbia, Canada, <u>lwparfrey@botany.ubc.ca</u>

Nearly 30% of seagrass has disappeared since 1980 and every year around 110 square kilometers of seagrass are lost globally. That is a dramatic scenario considering that seagrass meadows generate multiple high-value ecosystem services, by participating in carbon sequestration, protecting the coast from erosion, and improving water quality. Moreover, seagrass epiphyte provide food for invertebrates and juvenile fish, playing an important role in fisheries productivity. We investigated alpha and beta diversity patterns of microbial eukaryotes from seagrass epiphyte of Zostera marina and seawater in four regions (Calvert, Goose, McMullin and Triquet islands) along the coast of British Columbia (Canada) using 18S rRNA gene sequences. We found that microbial eukaryotes from the two sample types (seawater and seagrass) showed different taxonomic composition profiles and significantly different community structure. Taxonomic profiles were more distinct between sample types than between regions. When analyzing each sample type across regions, both seawater and seagrass microbiome samples showed differences in community structure; however, the proportion of shared taxa in seawater samples (51%) was much higher than in the seagrass epiphyte (15%). Furthermore, diversity among regions was more similar in seawater than in seagrass and this similarity decreased much faster with geographic distance for seagrass than for water samples. Altogether, our results show that microbial eukaryotes in seagrass leaves show distinct patterns from those present in the seawater and indicate provincialism of seagrass epiphytes in Zostera marina in the BC coast.

P60. ON THE COMPOSITION OF THE CRYSTALLINE BODY AND REDDISH GLOBULE IN THE EUSTIGMATOPHYTE – *VACUOLIVIRIDE CRYSTALLIFERUM* NIES-2860 **Pilátová, J.**, Faculty of Science, Charles University, Czech Republic, jana.pilatova@natur.cuni.cz Mojzeš, P., Faculty of Mathematics and Physics, Charles University, Czech Republic Bažantová, P., First Faculty of Medicine, Charles University, Czech Republic, <u>pavla.bazantova@lfl.cuni.cz</u> Hurková, K., Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague, Czech Republic, <u>kamila.hurkova@yscht.cz</u>

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The eustigmatophyte algae are widely used in biotechnologies while being also a fascinating object of basic research at the same time. Compared to other Stramenopiles they possess many distinct ultrastructural features – among others i.e. extraplastidial stigma in zoospores, reddish globule and lamellate vesicle in vegetative cells – originally described in 1972 and one more just emerged recently – the crystalline body in *Vacuoliviride crystalliferum* (Hibberd and Leedale, 1972; Nakayama et al. 2015). Until now not much is known about their composition, physiology or biogenesis.

In our work we contributed to more detailed knowledge of the composition of the lipophilic structures – the crystalline body and the reddish globule – by means of confocal Raman microspectroscopy *in vivo* and *in situ* followed by further confirmation via isolation by gradient ulracentrifugation and flow cytometry sorting (FACS) and analysis by GC MS. According to our results both organelles are lipophilic – the crystalline body is composed of elementary fatty acids showing simple Raman spectra, whereas reddish globule is way much more complex – possessing fatty acids, sitosterols, but contradictory to all predictions no carotenoids are present, however, some of the compound exhibits autofluorescence.

P61. *IN SITU* SUBMERSIBLE OBSERVATIONS OF WESTERN ANTARCTIC PENINSULA DEEP SEA MACROALGAL DRIFT

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The shallow subtidal zone along much of the Western Antarctic Peninsula (WAP) supports highly diverse macroalgal communities, which represent the highest floristic biomass of the continent. Several species of large, perennial brown macroalgae can dominate an area not only in biomass, but also in percent cover of the bottom. In some locations, several fleshy red algal species may co-dominate. As primary producers, macroalgae are carbon sinks and eventually with detachment from substrate, senescence or death contribute to the shallow subtidal nutrient cycle typically in the form of drift. There is little is known however, about the export of this vast carbon reservoir to deeper depths of the coastal WAP. In January and February 2017 a submersible-based cruise was conducted along the western Antarctic Peninsula. The focus of the cruise, organized by Japanese NHK TV, was to obtain high-definition video footage of scientists exploring the deep waters of Antarctica for a documentary (Deep Antarctica). The three-person 1000m-capable submersible carried a scientist (CDB, MOA, or KSRB) in addition to the pilot and a cameraman. Logging a cumulative 100 hours of dive time, survey sites included the Antarctic Sound, Fumarole Cove (Deception Island), Boyd Strait, Wilhelmina Bay, Buls Bay (Brabant Island), Fournier Bay (Anvers Island), and Palmer Deep. Macroalgal drift was observed at most sites, ranged between 150m and 1000m, and in many instances was identifiable to species.

P62. THE ROLE OF LIGHT IN SEASONAL GROWTH PATTERNS OF *NEOAGARUM FIMBRIATUM*

Borden, L. A., University of British Columbia, Canada, <u>lauraborden2@gmail.com</u> Martone, P. T., University of British Columbia, Canada, <u>patrick.martone@botany.ubc.ca</u> In Howe Sound, British Columbia *Neoagarum fimbriatum* is the dominant habitat-forming kelp on subtidal rocky reefs and is the preferential settlement habitat for juvenile pacific spot prawns, a commercially important species. Unfortunately, since 2013 this kelp habitat has declined locally due to a significant increase in urchin grazer abundance associated with the loss of a top-predator. Despite its local significance though, there is no existing information on basic seasonal growth patterns of *Neoagarum* in Howe Sound. In order to understand natural fluctuations of *Neoagarum* kelp beds I measured growth in the absence of grazer pressure over the course of two years to identify seasonal growth patterns and the relationship between growth and in situ temperature and light conditions. I found that *Neoagarum* grows throughout all seasons, with peak growth occurring in June/July at up to 6% d-1. Over the course of all seasons, growth was approximately 2-3% d-1. At the highest growth were significant during spring blooms in April when periods of zero light persisted for over a week on reefs. Ultimately, light proved the most significant driver of the seasonal patterns of growth observed. These data suggest that in the absence of significant grazer pressure, *Neoagarum fimbriatum* can form dense, multi-layered kelp beds in a relatively short time when light conditions are ideal.

P63. COST-EFFECTIVE AND EFFICIENT WAYS TO IDENTIFY ENDOLITHIC COMMUNITY MEMBERS INSIDE RHODOLITHS

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Rhodoliths are calcium carbonate nodules of non-attached, benthic crustose coralline algae. Corallines are marine red algae with hard skeletons, due to the precipitation of calcium carbonate (high-Mg biogenic calcite) within their organic cell walls. Following the 2010 Deepwater Horizon Oil spill offshore Louisiana in the NW Gulf of Mexico, the interior of individual rhodolith cells was shown by scanning electron microscopy (SEM) to contain endolithic stages of phototrophic microalgae (dinoflagellates, haptophytes, single-celled red algae), as well as microscopic stages of macroalgae (spores, filaments, propagules), observations subsequently confirmed by whole genome amplification of single cells and metabarcoding (environmental sequencing) of endolithic rhodolith DNAs. More cost-effective ways to quickly survey large numbers of rhodolith samples and identify their endolithic community members includes epifluorescence microscopy to quickly assess whether inclusions nested inside rhodolith cells are cellular in nature and, if so, to assess in which broad taxonomic division the inclusions belong. Once positive autofluorescence on a sample has concluded, one can then move onto the process of molecular identification of the single-celled inclusions using other cost-effective methods, such as molecular cloning.

P64. GENE EXPRESSION PROFILES OF SODIUM EXPORT MECHANISMS IN *CHARA LONGIFOLIA* C. B. ROB. AND *CHARA AUSTRALIS* R. BR **Phipps, S. R.**, University at Buffalo, United States, <u>shaunnak@buffalo.edu</u>

Species within the genus *Chara* have a range of tolerances when exposed to or cultured in varying salinities. We examine two potential genes (SOS1 and PpENA) related to sodium export that have been previously established in embryophtes to determine their potential role in the export of Na⁺ in salt-tolerant *C. longifolia* and salt-sensitive *C. australis*. Since *Chara* is more closely related to embryophytes than to other green algae, we hypothesize that similar genes are likely to be the mechanism behind sodium export in *Chara*. Illumina transcriptomes for both species assisted in identifying good candidate genes representing both a Na⁺-ATPase and a Na⁺/H⁺ antiport in *Chara*. These genes will be examined in expression profiles of both species with acclimation to salt. Creation of time-course Illumina

transcriptomes will be done using RNA from multiple time points (0hr, 6hr, 12hr, 24hr & 48hr) after salt stress for both species. The transcriptomes will provide sequence data to examine expression profiles genes involved in salt tolerance (presumably including *Chara* candidate genes representing SOS1 & PpENA). These expression profiles will give insight into the role of these sodium transport mechanisms in acclimation of salt for both *C. longifolia* and *C. australis*.

P65. ARE THERE WIDELY DISTRIBUTED RED ALGAE?

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Red algae are poor dispersers but paradoxically many species are widely reported. Records are commonly based on morphological identifications, but molecular data have revealed a range of issues with morphologically defined species boundaries. Few studies have analysed molecular data for widely distributed species over a broad sampling area. We analysed *rbcL* sequences of 12 widely distributed turf-forming Rhodomelaceae. *Ophidocladus simpliciusculus, Polysiphonia villum* and *Lophosiphonia obscura/P. hemisphaerica/P. boldii* show strong phylogeographic structure. Sequences from different regions (Europe, Atlantic America, Australia and South Africa) are distinibution of *Polysiphonia devoniensis/P. kapraunii* and *Symphyocladia dendroidea* is explained by human-mediated introduction events. *Pterosiphonia pennata/P. pinnulata/S. spinifera* and *Herposiphonia tenella* are species complexes involving three and eight different species, respectively, that were not resolved as monophyletic in our analyses. Widely distributed species are the exception in red algae, unless they have been spread by humans.

P66. CHARACTERIZATION OF PHOTOTROPINS INVOLVED IN BLUE LIGHT-INDUCED PHOTOMOVEMENT OF *SPIROGYRA VARIANS*

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Freshwater algae living in shallow waters have evolved various photomovement to stay in the optimum light condition for survival. The filamentous green alga *Spirogyra varians* shows photomovement in blue light. To decipher the genetic control of photomovement, two phototropin homologues were isolated from *S. varians*, and named SvPHOTA and SvPHOTB. Both phototropins have similar molecular structure consisted of two light–oxygen–voltage domains and a serine/threonine kinase domain. SvPHOTA and SvPHOTB had 48.7% sequence identity. Phylogenetic analysis showed SvPHOTA and SvPHOTB belong to different clades suggesting early divergence, possibly before the divergence of land plants from the Zygnematales. In quantitative PCR analysis, SvPHOTA was consistently expressed in the dark and in blue light, while SvPHOTB was expressed only when the plants were exposed to light. When SvPHOTs were heterologously expressed in the *Arabidopsis thaliana* phot mutant, SvphotA and SvphotB were localized on the plasma membrane. Phototropin responses of *A. thaliana* mutant were significantly rescued by the expression of SvPHOTA, while were not observed in SvPHOTB. These results suggest that the two phototropins may have different roles in the photomovement in *S. varians*.

P67. INTER-ANNUAL VARIATION IN THE RELATIVE DOMINANCE OF PLANKTONIC FLAGELLATES AND DINOFLAGELLATES AT TMNP WATERS ADJOINING THE HEBEI SPIRIT OIL SPILL SITE

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Summer phytoplankton communities were analyzed with water samples from surface and three meter depths at 15 stations around the Taeanhaean Marine National Park (TMNP) for 10 years (2008-2017) following the Hebei Spirit Oil Spill (HSOS) incident on 07 December 2007. The oil spill site was at some 10km away from the northern coastline of TMNP. During the first 5-year period (2008-2012) percent dominance by the phototrophic flagellate and dinoflagellate (PF-DF) species was gradually increasing since the outbreak of the HSOS. Moreover, dominant taxonomic group of the PF-DF was replace by new one each year: cryptophytes (30.6% in 2008), *Chattonella* and cryptophytes (24.8 and 11.5% in 2009; 12.2-14.0% in 2010), *Tripos fusus* (51.6% in 2011), and *Tripos furca* (47.7% in 2012). The relative dominance by the PF-DF, however, is somehow oscillating during the second 5-year period (2013-2017) with the dominance percentages at the trough in 2013 (12.3%) and 2016 (9.6%), at the peak in 2014 (24.9%) and 2017 (20.4%), and on the average of 2014 peak and 2016 trough in 2015 (17.3%). Rather than dinoflagellates the phototrophic flagellates such as *Chatonella* spp. and cryptophytes were the most dominant PF-DF groups during the second 5-year period. This kind of regime shift in the pattern of relative dominance by PF-DF groups might be driven by the ecosystem's responses to the abrupt oil spill stresses toward the final long-term resilient recovery.

P68. AGE DISTRIBUTIONS AND MORPHOLOGIC VARIABILITY IN SUBTIDAL *ECKLONIA ARBOREA* (SOUTHERN SEA PALM) AROUND ISLA NATIVIDAD, BAJA CALIFORNIA SUR, MEXICO

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The perennial stipitate kelp *Ecklonia arborea* persists along a wide latitudinal range in the Northeastern Pacific and exhibits distinct morphological features including stipe bifurcation and hollowing. Little is known about the persistence of E. arborea within Baja Californian kelp bed communities, and morphology to age relationships are poorly understood. Collections, swath surveys, and field stipe measured and compared in March 2018 to assess age distributions, morphology, and densities of E. arborea around Isla Natividad, Baja California Sur. Thalli were aged by quantifying dark growth rings. Age estimates ranged from 2 to 12 years (mean \pm SE = 4.2 \pm 2.2) and mean ages varied among sites, as did age distributions. Using stipe length as an age predictor, predictions were validated using thalli collected from a separate site around the island. Predicted age to growth rings comparisons at the validation site had an average deviation of 17.5%. Surveys and stipe lengths were measured at two additional sites within IN's two no-take reserves and were used to predict reserve age distributions. The reserves supported more persistent and denser E. arborea when compared to the other four sites distributed around the island. Thallus size correlated with age, but mean thallus size varied between sites, and relative thallus development with age varied between sites. These results reveal the variable morphology and persistence in *E. arborea* around IN and support increased persistence and density of *E. arborea* in reserves, suggesting more stable populations.

P69. CONTROL OF ALGAL GROWTH ON GREENHOUSE SURFACES USING COMMERCIAL ALGAECIDES

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P70. SOIL CRUST ALGAL COMMUNITIES OF WARREN PRAIRIE NATURAL AREA **Fawley, K. P.**, University of Arkansas at Monticello, United States, <u>fawley@uamont.edu</u> Baker, B., Arkansas Natural Heritage Commission, Little Rock, AR, United States, <u>Brent.Baker@arkansas.gov</u> Fawlev, M. W., University of Arkansas at Monticello, United States, <u>marvin_fawley@yahoo.com</u>

Warren Prairie Natural Area in Bradley and Drew Counties, Arkansas, is a strange mosaic of saline slicks that form flat, crusty depressions in a central area with a zone of lichens and a few rare angiosperms, and an outer zone of cyanobacterial mats. The edges of the saline slicks are home to the rare, diminutive vascular plant, *Geocarpon minimum* Mackenzie (Caryophyllaceae), which is a federally protected threatened species. The main objective of this project is to characterize the soil crust eukaryotic algal communities from two sites in Warren Prairie Natural Area using morphological and molecular techniques. We have characterized strains isolated from samples collected in February, 2016 and December, 2017. The eukaryotic algae of Warren Prairie slicks are highly diverse and include taxa from the Trebouxiophyceae and the Chlorophyceae (Chlorophyta), the Zygnematophyceae (Stramenopiles). Most of the genera are widely distributed in many types of soil, but a number of new species are present. Several strains represent new records for North America or the U.S. Our results show that the unusual chemistry of the barrens soil of Warren Prairie harbor unusual algae in addition to rare plants.

P71. A MOLECULAR ANALYSIS OF BACTERIAL COMMUNITIES ON THE EXTERNAL SURFACES OF MARINE SEAWEEDS

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The diversity of bacteria associated with algae form a complex dynamic ecosystem on the surfaces of seaweeds. There is little known about the existing relationships between macroalgae and bacteria, but potential symbiotic and commensal relationships have been hypothesized. The objective of this study was to determine and profile bacterial communities on the external surfaces of three seaweeds *Fucus* sp., *Bangia fuscopurpurea*, and *Cladophora* sp.) and also the surrounding water. Bacterial communities were sampled by swabbing the surfaces of the seaweeds, and the water sample was collected in 50 ml tubes. After DNA extraction, the V3-V4 regions of the 16S ribosomal RNA gene (16S rRNA gene) were sequenced via an Illumina MiSeq instrument. QIIME 2 and the demux tool were used to analyze the reads, and the DADA2 method clustered the reads into OTUs at 97% sequence identity. More than 12 bacterial phyla were detected in the four samples. Proteobacteria was the most dominant bacterial phylum followed by the phyla Bacteroidetes, Cyanobacteria, and Fusobacteria, which accounted for 81.2%, 8.7%, 6.1%, and 2.3% of the total sequence reads, respectively. However, the bacterial phyla Gracilibacteria,

Acidobacteria, Firmicutes, Spirochaetae, and Chlorobi were only observed in *Bangia fuscopurpurea* and not in the other seaweed samples. Interestingly, no sequences of Archaea were observed in the four samples despite using universal prokaryotic primers. Examining the bacterial composition using more algae samples and determining temporal bacterial shifts within the macroalgal species would provide a better understanding of algal-bacterial interactions.

P72. BAMBOO IS NOT JUST FOR PANDAS ANYMORE: AN EXAMINATION OF SOME NOVEL CYANOBACTERIAL EPIPHYTES FROM A BAMBOO GARDEN IN JACKSONVILLE, FL, USA **Tipley, D. J.**, University of North Florida, United States, <u>n00766786@ospreys.unf.edu</u> Garvey, A. D., University of North Florida, United States Rousse, J. D., University of North Florida, United States Casamatta, D. A., University of North Florida, United States, <u>dcasamat@unf.edu</u>

Epiphytic algae are common components of terrestrial environments in warm, humid climates. Preliminary research has shown that elevated temperatures and humidity commonly found in Florida are conducive to the growth of these epiphytes. Bamboo gardens were chosen for this study since they are both common in Florida and provide an excellent sampling site, given the ease of access and capacity for non-destructive removal of samples. Isolates were obtained by scraping bamboo culms and plated on Z8 medium, both with and without nitrogen. Strains were grown under ambient conditions in the lab for at least six months. Initial microscopic examination indicated morphological similarities to "common" aerophytic cyanobacterial taxa such as *Nostoc* and *Scytonema*, where Nostocalean lineages seem abundant. However, a total evidence analysis including morphology, 16S gene sequencing, and ITS folding patterns of recently isolated cultures has yielded a cornucopia of putatively novel taxa. Here we present three taxa putatively new to science: *Nostoc, Scytonema*, and *Oscillatoria* with ca. 95% sequence homology to established taxa. These strains exhibit some morphological incongruities with other, currently established species and possess unique 16S sequences, thus necessitating further study and elucidation. These types of aerophytic habitats may serve as excellent sources of future, heretofore unknown biodiversity.

P73. OMEGA-7 PRODUCING ALKALIPHILIC DIATOM *FISTULIFERA ALCALINUS* SP. NOV FROM LAKE OKEECHOBEE, FLORIDA

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Microalgae are an exemplary source of fatty acids since they can produce abundant biomass and oils in a short period of time. Algae derived oils are indispensable to human nutraceutical, pharmaceutical, and biofuel sectors. Achieving goals of producing algal-based products at competitive prices, however, involves further improvement of microalgae technology, especially the cultivation aspect. Manipulating cultivation conditions to promote growth and lipid storage while preventing contamination is desired. Contamination of algal cultures in open systems is a major impediment in algal biotechnology and can be avoided by choosing an extremophilic microalga. Our aim was to isolate alkaliphilic microalgae native to South Florida with characteristics suitable for production of omega fatty acids. For that purpose, water samples from Lake Okeechobee were inoculated into Zarrouk's medium (pH 9-12) and incubated for 35 days. This resulted in the isolation of three algal strains that were screened for biomass and lipid productivity. One of the isolates, *Fistulifera* sp. 154-3, was identified as a high-lipid accumulating, alkaliphilic diatom with biomass productivity of 0.285 g L⁻¹day⁻¹ and lipid content of 20% dry biomass at pH 10. Lipid analysis of *Fistulifera* sp. 154-3 indicated the most abundant fatty acid was palmitoleic acid

(or omega-7), followed by palmitic acid and eicosapentanoic acid. Based on morphological, ultrastructural, and 18S rRNA and *rbc*L gene results, we are also proposing this strain as a species new to science, *Fistulifera alcalinus* sp. nov.

P74. UNCOVERING CRYPTIC DIVERSITY OF PERIPHYTIC CYANOBACTERIA ISOLATED FROM ICHETUCKNEE SPRINGS, FL, USA **Garvey, A. D.**, University of North Florida, United States, <u>agarvey05@gmail.com</u> Gansert, E. A., University of North Florida, United States Casamatta, D. A., University of North Florida, United States, <u>dcasamat@unf.edu</u>

Springs provide a unique environment from which to study biodiversity given their relatively stable habitats. Springs are also economically and culturally significant. For example, over one million guests visit Florida springs every year, even as these springs are presenting evidence of anthropogenic impacts (e.g., alterations to water flow, nitrate contamination from surrounding land use, etc.). Further, algal blooms in Florida springs have recently been shown to contain toxic cyanobacteria. This study examined cyanobacterial species diversity in periphytic algae isolated from Ichetucknee Springs (Branford, FL, USA) to better understand the community structure. Field collections were plated on Z8 medium and samples were analyzed using morphology, genetic (the 16S rDNA gene), and molecular (secondary folded structures of the 16S-23S ITS region) methods. We recovered a number of "cosmopolitan" strains (e.g., *Oscillatoria, Phormidium, Leptolyngbya*, etc.), but also note the discovery of two potentially novel taxa thus far. One is a species of *Nodosilinea*, *N. fontis*. The other is an interesting strain that is morphologically similar to both *Westiellopsis* and *Fischerella*, yet falls into a highly supported clade (>95% bootstrap support) with "orphan" strains, sister to each genus, potentially necessitating the erection of a new genus. This study is the first to examine cryptic diversity among the cyanobacterial community isolated from Ichetucknee Springs.

P75. GROWTH AND BIOCHEMICAL RESPONSES OF TROPICAL AND SUBTROPICAL STRAINS OF *GRACILARIA DOMINGENSIS* (RHODOPHYTA) TO TEMPERATURE AND IRRADIANCE VARIATIONS

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Gracilaria domingensis (Kützing) Sonder ex Dickie is widely distributed in the Brazilian coast, and has a great potential as a resource for food industries. The objectives of the present work were to determine the limits of tolerance to temperature and irradiance variations, and to evaluate their effects on growth rates (GR), and contents of total soluble proteins (TSP) and pigments in female gametophytes of G. domingensis from Brazilian tropical region (ES strain) and from subtropical region (SC strain). Variations in temperature (15-35°C) and irradiance (20-250 μ mol photons m⁻²s⁻¹) were tested. Both strains tolerated temperatures from 15 to 30°C, but died at 35°C. The ES strain presented the highest GR (from 3.1% .d-1 to 6.1% .d-1) in relation to the SC strain (from 1.3% .d-1 to 4.1% .d-1). The ES strain presented higher TSP concentrations at low temperature while the SC strain showed higher concentrations at high temperatures. The irradiance variation influenced the GR of the two strains, and the lowest GR were observed at irradiance of 20 μ mol photons m⁻²s⁻¹ and the highest GR were observed at 40 and 60 μ mol photons m⁻²s⁻¹ for the SC and ES strains, respectively. The studied strains of G. domingensis showed higher concentrations of total soluble at low irradiances. The highest concentrations of phycoerythrin (0.42 mg/g FW) and phycocyanin (0.21 mg/g FW) were observed in the ES strain grown at low irradiance (40 μ mol photons m⁻²s⁻¹). Our results indicate that ES strain is more suitable than SC strain for maricultural purposes in tropical and subtropical waters, since ES strain showed higher growth rates in a wide range of temperature and irradiance variations.

P76. HABITAT HETEROGENEITY AND SCALE-DEPENDENT BIODIVERSITY LOSS IN BARKLEY SOUND KELP BEDS

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Biodiversity loss is driven by interacting factors operating at different spatial scales. Yet, there remains uncertainty as to how fine-scale environmental conditions mediate biological responses to broad-scale stressors. We surveyed mid-latitude kelp bed habitats to determine whether local habitat heterogeneity has mediated changes in community diversity after more than two decades of climate warming and extreme temperature events. Local wave exposure conditions were instrumental in determining responses, with some habitats remaining stable and others experiencing near complete diversity loss, leading to local declines without regional extinctions. Wave-sheltered shores, which saw the largest declines, are the most common habitat type in the Northeast Pacific and may be especially sensitive to climate-related losses in kelp diversity and abundance. Our findings highlight how local gradients can interact with global drivers to facilitate diversity loss and demonstrate how incorporating differences between habitat patches can be essential to capturing scale-dependent biodiversity loss across the landscape.

P77. ORGANELLAR PHYLOGENOMICS INFORM SYSTEMATICS IN THE GREEN ALGAL FAMILY HYDRODICTYACEAE (CHLOROPHYCEAE)

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Phylogenomic analyses are resolving relationships at the class, order and family levels, and highlighting unexpected patterns of organellar genome evolution in the green algae. We performed a phylogenomic study to resolve relationships within the green algal family Hydrodictyaceae (Chlorophyceae). Complete plastome sequences and mitochondrial protein-coding gene sequences were acquired from representatives of Hydrodictyaceae using Next-Generation sequencing methods. The resulting plastomes were examined for gene content and order and compared with plastomes spanning the Sphaeropleales. Single-gene and concatenated-gene phylogenetic analyses of plastid and mitochondrial genes were also performed. The Hydrodictyaceae contain the largest sphaeroplealean plastomes thus far fully sequenced, predominately due to infiltration of numerous genes of unknown function. Conservation of plastome gene order within Hydrodictyaceae is striking compared with more dynamic patterns reported across Sphaeropleales. Phylogenetic analyses resolved Hydrodictyon sister to a monophyletic Pediastrum, though the morphologically distinct P. angulosum and P. duplex remained polyphyletic. Analyses of plastid data alone supported the neochloridacean genus Chlorotetraëdron as sister to Hydrodictyaceae, while conflicting signal was found with the mitochondrial data. Results from this study resolved within-family relationships not obtainable with previous phylogenetic analyses, but additional data are necessary to resolve the sister lineage of Hydrodictyaceae.

P78. THE GENOME OF *COCHLIOPODIUM PENTATRIFURCATUM*: HIGHLIGHTING THE DIVERSITY OF UNDERSTUDIED AMOEBOZOAN GENOMES **Wood, F. C.**, Spelman College, United States, <u>fwood@spelman.edu</u> Melton, J. T., Spelman College, United States, <u>jmelton@spelman.edu</u> Tekle, Y. I., Spelman College, United States, <u>ytekle@spelman.edu</u>

Within the supergroup Amoebozoa, genomics is still in its infancy. Among the \sim 25 known orders of Amoebozoa, only 6 contain species with published genomes, and only 3 contain fully annotated genomes.

These genomes consist almost entirely of medically important species (e.g. *Entamoeba*, *Acanthamoeba*) or model organisms (e.g. *Dictyostelium*), and neglect the majority of the diversity of Amoebozoa. Amoebozoan genomes are highly diverse, with known or estimated genome sizes ranging from ~20 Mb (*Entamoeba*) to over 600 Gb (*Polychaos*). However, most sequenced genomes are less than 100 Mb in size, providing a limited view of genome evolution within the clade. In this study, we present the genome of *Cochliopodium pentatrifurcatum*. We chose this species for sequencing due to its phylogenetic position in a previously-unsequenced order of Amoebozoa (Himatismenida) and its unusual sexual life cycle. Using 10x Genomics Chromium sequencing and the Supernova genome assembler, we assembled 52.9 Mb of the *Cochliopodium* genome in 7133 scaffolds with an N50 of 9,888 bp and a GC content of 26.2%. Within these scaffolds, we used previously-sequenced de-novo-assembled transcriptome data to recover protein-coding genes using the MAKER annotation pipeline. A total of 16,203 genes were annotated using this method. In the future, we plan to conduct further sequencing using both short- and long-reads to better ensure the completeness of the new genome.

P79. EXPANDING THE REPRESENTATION OF PHOTOSYNTHEITC STRAMENOPILES IN A PHYLOGENOMIC FRAMEWORK

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Stramenopiles are a major eukaryotic clade consisting of macroscopic multicellular algae and microscopic unicellular organisms. This diverse group is classified into 17 different classes, of which 10 are photosynthetic. They are found in marine, freshwater, and terrestrial habitats and exhibit diverse life strategies such as parasitic, or free-living flagellates. Until recently, studies have looked at single gene or multigene phylogenies for this group. However, with transcriptome datasets expanding, this allows for a more reliable framework for phylogenetic studies. As part of a larger effort to construct a robust phylogeny for stramenopiles, novel transcriptomes were generated to interrogate relationships of photosynthetic stramenopiles (Ochrophytes). In this research, we cultured 20 photosynthetic stramenopiles from 6 different classes (Phaeothamniophyceae, Eustigmatophyceae, Chrysophyceae, Xanthophyceae, Dictyochophyceae, and Pinguiophyceae) and sequenced their transcriptomes. These data, coupled with existing data, provides robust support for the placement of these 20 organisms within the Ochrophytes and provide phylogenetic placement for Phaeothamniophyceae, Eustigmatophyceae, Dictyochophyceae, Dictyochophyceae. These data will also allow us investigate their evolution and diversification of this group.

P80. PREFERENTIAL GRAZING AND SECRETION OF HYDROLYTIC ENZYME BY MARINE HETEROTROPHIC NANOFLAGELLATES

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The functional and numerical significance of marine heterotrophic nanoflagellates (HNFs), a phylogenetically diverse protistan community of 2-20µm in size range, necessitates the study of their ecophysiology. HNFs have been identified as an essential component of the microbial loop and thereby regulate the marine biogeochemical cycling of carbon and nitrogen. Despite the crucial roles of HNFs in the marine microbial food web, their ecophysiological functions remain poorly understood. In this study, attempts have been made to evaluate the impact of preferential grazing on the physiology of marine HNFs using a model predator-prey system: one isolated HNF (*Jakoba* species) as the predator; various taxonomic groups of bacterial isolates (*Alteromonas, Bacillus, Flavobacterium, Pseudomonas, Micrococcus* and *Vibrio*) as the prey; and aminopeptidase as the enzyme. The results indicated that the isolated HNF had a strong numerical grazing response toward different taxonomic groups of bacteria. *Pseudomonas* was the most palatable food for this HNF, followed by *Flavobacterium* and *Vibrio*. The

taxonomic groups, *Alteromonas, Bacillus* and *Micrococcus* were found to be unpalatable foods. Furthermore, the isolated HNF had been observed to secrete a substantial amount of aminopeptidase, while preferentially feeding on *Pseudomonas, Flavobacterium* and *Vibrio*. There were no aminopeptidase activities detectable with other taxonomic groups of bacteria. The preferential feeding and production of the hydrolytic enzyme by this marine HNF have provided supportive evidences about the pivotal role of HNFs in shaping the bacterial taxonomic composition and in mediating the carbon and nitrogen cycles of the world's oceans.

P81. CARBOHYDRATES, PROTEINS AND PHOTOSYNTHETIC PIGMENTS IN DIFFERENT REPRODUCTIVE STAGES OF *GIGARTINA SKOTTSBERGII* FROM ANTARCTIC AND SUBANTARCTIC REGIONS

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The benthic marine macroalgae from Antarctic and sub-Antarctic regions require biochemical and physiological strategies to survive in the extreme environmental conditions. Therefore, the objective of this study was to analyze the contents of pigments, soluble proteins and carbohydrates of different life history stages of *Gigartina skottsbergii* Setchell & N.L. Gardner (Rhodophyta, Gigartinales) collected in a latitudinal gradient from Antarctica (7 collecting sites) to Chilean sub-Antarctic region (4 collecting sites). Cystocarpic and non-fertile specimens from both regions showed higher contents of phycocyanin, allophycocyanin, proteins, polysaccharides, and carrageenan with higher 3,6 anhydrogalactose content than tetrasporophytes. On the other hand, tetrasporophytes from both regions showed higher concentration of chlorophyll a, and carrageenan with high sulfation degree than cystocarpic and non-fertile specimens. The floridean starch content was higher in reproductive stages than non-fertile specimens. Considering the effect of latitudinal gradient, Sub-Antarctic samples presented higher concentrations of proteins, and pigments than Antarctic samples, while the Antarctic samples showed higher concentrations of low molecular weight carbohydrates, and floridean starch than sub-Antarctic samples. These results can be explained by the role played by low molecular weight carbohydrates and floridean starch in the protection against salinity variation and low temperatures.

P82. RECEPTACLE DEVELOPMENT IN ASCOPHYLLUM

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The dioecious brown seaweed *Ascophyllum nodosum* dominates intertidal zones of rocky shorelines around the North Atlantic, providing shelter and substrate for the growth of many macro- and microorganisms. *Ascophyllum* beds are a major source of biodegradable nutrients from the year-round shedding of epidermal cell wall layers and intermittent detachment of branches and plants in storm and waves, as well as the annual springtime release of gametes from conceptacles within reproductive receptacles. Mass receptacle dehiscence follows gamete release, unlike the more gradual loss of reproductive tissue from the model fucoid *Fucus vesiculosus*. Surprisingly little information is available about *Ascophyllum* receptacles development throughout the annual growth cycle. To remedy this, we began investigating receptacle and conceptacle development in July 2017. As expected, receptacle growth was greatest at the apices. A striking feature was the localized alignment of multiple conceptacles, identified by their ostioles (openings) visible on receptacle surfaces. Conceptacle distribution patterns

were unaffected by 2-4 weeks of frond growth under laboratory conditions, and no new conceptacle formation was detected. Fluorescence or confocal laser scanning microscopy of hand sections stained with fluorescent brightener (to view cell walls) and propidium iodide (for nuclei) provided a rapid and reliable way to track dynamic changes in conceptacle development, such as the appearance of elongated tip-growing cells on the inner surface of conceptacles in the early winter period.

P83. A MOLECULAR AND MORPHOLOGICAL IDENTIFICATION OF TWO NEW *COCHLIOPODIUM* SPECIES WITH MICROTUBULE ORGANIZING CENTERS FROM THE SOUTHEASTERN UNITED STATES

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Cochliopodium species are difficult to identify based on morphology alone due to cryptic diversity and plasticity of the often-used scale morphology to help delimit species. Here, we present morphological (light microscope, immunocytochemistry or ICC) and molecular data (COI) for two new Cochliopodium species, commonly named as "Crystal-like" and "Marr's Spring", from Arabia Lake, Lithonia, GA, and Marr's Spring on campus at The University of Alabama, Tuscaloosa, AL, respectively. The "Crystal-like" isolates were made from environmental samples in an attempt to re-isolate an undescribed species from Arabia Lake named as "Crystal". A phylogenetic analysis and pairwise comparison of all COI sequences of Cochliopodium species showed that both of our newly isolated species were genetically distinct from any described species. "Marr's Spring" clustered with C. actinophorum and C. arabianum, and was closest in pairwise sequence identity to C. arabianum (92.1%). "Marr's Spring" typically had oval to diamond shaped crystals and an average cell length and width of 44.1 µm and 47.0 µm, respectively. The closest COI sequence to "Crystal-like" was C. larifeili (91%). Additionally, "Crystal-like" was morphologically similar to "Crystal" but was molecularly distinct (88.2% identity). "Crystal-like" had square and spherical-shaped crystals, and an average cell length of 31.9 µm and width of 29.7 µm. Both of these species displayed a microtubule organizing center (MTOC). Our morphological and molecular data of Cochliopodium species will result in the description of two new species.

P84. MORPHOLOGY AND MORPHOGENESIS OF THE VEGETATIVE VALVE IN *BELLEROCHEA MALLEUS* (BRIGHTWELL) VAN HEURCK. **Porcher, E. M.**, Mount Allison University, Canada, <u>emporcher@mta.ca</u> Kaczmarska, I., Mount Allison University, Canada, <u>iehrman@mta.ca</u> Ehrman, J. M., Mount Allison University, Canada, <u>jehrman@mta.ca</u> Samanta, B., Mount Allison University, Canada, <u>bsamanta@mta.ca</u>

Bellerochea malleus is a polar centric diatom, and a member of a small genus belonging to the order Lithodesmiales. The order is also relatively small, but well circumscribed by a unique bilabiate process. *B. malleus* is planktonic, and found in tropical and warm temperate marine environments, typically close to shore. Like other members of the genus, *B. malleus* has variable morphology and very lightly silicified valves, and is thus difficult to examine using routine diatom-microscopy. The focus of this study is to investigate valve morphogenesis in *B. malleus* using scanning electron microscopy, as well as by tracing the incorporation of PDMPO during valve development. Silicification of the valve begins with an annulus, slightly off-set from the center of the valve. A bilabiate process is then formed inside of this structure. Silicification then begins to proceed outwards through radiating costae and the marginal ridge, which also bears costae. The silicification of marginal structures appears to occur prior to the silicification of the center of the valve face, showing a non-radial pattern of valve morphogenesis; additionally, silica structures are deposited within the annulus after its formation. While this is unusual, other forms of nonradial silicification patterns have been described in other bi-polar centric taxa. The stages of vegetative valve development found in *B. malleus* are compared to those seen in other taxa of the order, where valve genesis seems to proceed quite differently.

P85. THE FISH PARASITE DINOFLAGELLATE *HAIDADINIUM ICHTHYOPHILUM* WITH ITS UNUSUAL AND COMPLEX LIFE HISTORY, IS SISTER TAXON TO *PISCINOODINIUM* **Buckland-Nicks, J. A.**, St Francis Xavier University, Canada, <u>jbucklan@stfx.ca</u> Hehenberger, E., University of British Columbia, Canada, <u>helisabe@mail.ubc.ca</u> Keeling, P. J., University of British Columbia, Canada, <u>pkeeling@mail.ubc.ca</u> Reimchen, T. E., University of Victoria, Canada, <u>reim@uvic.ca</u>

An endemic population of unarmoured stickleback *Gasterosteus aculeatus* from a highly acidic bog pond on Haida Gwaii, becomes seasonally infected with dinospores of the dinoflagellate, *Haidadinium ichthyophilum* which induces epithelial hyperplasia that immobilizes the dinospores causing them to encyst. Even in cases of extensive infection the fish exhibit no obvious behavioural signs of stress, which is more suggestive of a symbiosis than a pathological association. Some traits of *H. ichthyophilum*, such as dinospores, autotrophic vegetative cysts, as well as a variety of resting cysts, pointed to an affiliation with autotrophic species in the Dinophyceae, while a temporary dinokaryon, a trophont stage and a variety of amoeboid stages suggested an affiliation with some heterotrophic parasites in the Blastodiniphyceae. Recent analysis of 492 base pairs of 18SrDNA has resolved an affiliation of *H. ichthyophilum* with *Piscinoodinium* sp, forming a clade of fish parasites, within the Suessiales (Dinophyceae). However, it reopens questions as to the origin of this dinoflagellate lineage, with its unusual haplotype, from an Asian-Pacific population and how long it has remained geographically isolated.

P86. SPLICING COMPLEXES IN C. MEROLAE

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Cvanidioschyzon merolae is a unicellular, thermoacidophilic red alga, found in globally in freshwater springs from Italy to Japan. It is notable for having only 27 introns in its entire genome, compared to ~300 in S. cerevisiae and tens of thousands in humans. The Rader Lab at UNBC, in collaboration with the Fast lab at UBC, demonstrated in 2015 that C. merolae's splicing machinery - the small, nuclear RNAs (snRNAs) and proteins that carry out the splicing reaction - is also highly reduced, with ~50 core proteins, compared to ~150 in humans. Strikingly, the U1 snRNP, responsible for the initial step of intron recognition in all other organisms studied, is completely absent. We have therefore sought to further characterize the complement of splicing proteins in this unusual organism as a first step towards characterizing its splicing pathways. Using a combination of bioinformatic analyses and proteomic experiments, we have identified five principal splicing complexes and a number of independent proteins. The U2 small, nuclear ribonucleoprotein (snRNP) is the most complex sub-particle, followed by U5, U4, and U6. In addition, C. merolae has a NineTeen Complex (NTC), the RNA-free particle that joins the splicing pathway just prior to the chemical steps of splicing. Unexpectedly, we find that the Pat1associated RNA degradation complex, normally localized to the cytoplasm, is physically associated with the splicing machinery, and appears to be abundant in the nucleus. We hypothesize that it may play a role there in degrading aberrant transcripts.

P87. CHEMICAL SIGNALING IN THE PHYCOSPHERE: GROWTH AND PIGMENTATION RESPONSES TO BACTERIAL SIGNALS AND PHYTOHORMONES **Hirsch, D.,** University of the Incarnate Word, United States, <u>hirsch@student.uiwtx.edu</u> Leverett, B. D., University of the Incarnate Word, United States, <u>leverett@uiwtx.edu</u>

Microalgae have been shown to respond to environmental and microbial dynamics through chemical signaling in the phycosphere, both by the algae and by other microorganisms. Of particular interest in mixed community dynamics is the response of microalgae to bacterial quorum signals, which are used by bacteria to coordinate population behaviors such as biofilm formation and toxin production. In these experiments, species from five classes of microalgae been grown in a multi-well plate format to study the differences in growth rate and pigmentation observed in the presence of select homoserine lactones, a class of bacterial quorum signals called autoinducers (AI-1). Microalgal response to plant hormones, including abscisic acid, salicylic acid, indole acetic acid, and methyl jasmonate have also been examined. Cell growth was determined using an automatic cell counter, cell density in microalgal cultures was monitored by refractometry, and pigmentation was estimated using chlorophyll fluorescence measurements. The freshwater microalgae, *Eustigmatos vischeri*, and the golden algae, *Tisochrysis lutea*, demonstrated small but detectable growth changes in the presence of each of the phytohormones tested. The marine picophytoplankton, *Nannochloropsis oculata*, showed decreased growth in the presence of higher concentrations of some, but not all AI-1 signals tested. This study demonstrates the utility of small scale microalgal growth modelling and has implications for mixed microbial signaling dynamics.

P88. THE IMPACT OF MICROALGAL EXTRACTS ON VIABILITY AND ANTIBIOTIC SENSITIVITY IN STENOTROPHOMONAS MALTOPHILIA BIOFILMS **Matulich, P. T.**, University of the Incarnate Word, United States, <u>matulich@student.uiwtx.edu</u> Leverett, B. D., University of the Incarnate Word, United States, <u>leverett@uiwtx.edu</u>

Biofilms are extracellular matrices produced by bacteria for adhesion and are a major factor in cell to cell gene transfer and the development of antimicrobial drug resistance (AMDR). AMDR is an increasingly prevalent and life-threatening feature of human infections, especially in hospital settings. Extracts from numerous species of marine and freshwater microalgae have been screened for bactericidal and antifungal activities, but fewer reports have directly examined anti-biofilm activity in microalgal extracts. This study compares the antimicrobial activity of two types of lipid extracts from select species of microalgae. Assays for bacterial biofilm viability and bacterial growth have been performed using established methods to assess the effect of microalgal extracts on film and planktonic growth in Stenotrophomonas maltophilia. Antibiotic sensitivity of S. maltophilia in both established biofilms and planktonic culture has also been assessed in the presence of microalgal extracts. Preliminary results demonstrate significant decreases in the viability of S. maltophilia biofilms treated with extracts from Botryococcus braunii, Nannochloropsis oculata, Eustigmatos vischeri, and Clorochromonas danica and Rhodomonas lens. A slightly enhanced sensitivity of S. maltophilia biofilms to both Azeotram and Cefatriaxone in the presence of microalgal extracts from Tisochrysis galbana and Mesostigma viride was also observed. The extracts did not exhibit a significant impact on antibiotic sensitivity in planktonic cultures of S. maltophilia, as demonstrated by the minimum inhibitory concentrations (MICs) of select antibiotics with and without extracts present.

P89. NOVEL PHYCODNAVIRUS SEQUENCES DETECTED IN *CYMBOMONAS TETRAMITIFORMIS* GENOME ASSEMBLY

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Cymbomonas tetramitiformis is a green alga with a large genome of greater than 1 billion base pairs. Nucleo-cytoplasmic large DNA viruses of green algae (phycodnaviridae) have been described for other species of green algae. The objective of this work was to identify phycodnavirus sequences from the C. tetramitiformis genome assembly. Initial BLAST analysis was performed against the Nucleo-Cytoplasmic Virus Orthologous Gene database. Results were confirmed against the non-redundant protein database available from NCBI. Further viral genome assembly was performed using the MIRA/MITObim pipeline. Epifluorescence microscopy to screen for viral particles in *C. tetramitiformis* culture was also performed. Greater than 400,000 base pairs from the *C. tetramitiformis* genome assembly were identified as possibly viral in origin. Results of epifluorescence microscopy suggest that *C. tetramitiformis* may be the host to a novel virus.

P90. REINSTATEMENT OF HOOK-FORMING *ACROSORIUM* SPECIES (DELESSERIACEAE, RHODOPHYTA) BASED ON MOLECULAR PHYLOGENY AND MORPHOLOGY **Kang, J. C.**, Manta's Marine Lab, South Korea, <u>mantachan@hanmail.net</u> Miller, K. A., University of California, United States, <u>kathyannmiller@berkeley.edu</u> Kim, M. S., Jeju National University, South Korea, <u>myungskim@jejunu.ac.kr</u>

Since the establishment of *Acrosorium* by Zanardini, there have been a lot of confusions on the identity of *Acrosorium* species with hook forming thalli from worldwide, such as *Acrosorium ciliolatum* (Harvey) Kylin, *A. uncinatum* (Turner) Kylin and *A. venulosum* (Zanardini) Kylin. By long and complicated nomenclatural history, *Acrosorium* species with hook-forming thalli world-widely distributed have been identified under the name *A. ciliolatum* in recent date. We re-examined these populations from eastern Atlantic, north-western Pacific, eastern Pacific and southern hemisphere including Australia, New Zealand and Chile using morphological and molecular analyses. We confirmed that these populations are separated into four distinct clades by *rbcL* gene phylogeny and the north-western population was conspecific ones with *A. flabellatum* Yamada. We discovered the fine differences on the position of tetrasporangial sori among these populations except the eastern Pacific population. Our results suggest that the distribution of *A. ciliolatum* is restricted in the southern hemisphere, *A. venulosum* and *A. flabellatum* are correct name for the populations from eastern Atlantic and north-western Pacific, respectively. We postponed the taxonomic conclusion for the population from eastern Pacific, because we could not observe any mature thallus.

P91. CONVERGENT GENOME EVOLUTION IN BACTERIAL ENDOSYMBIONTS OF MARINE DIPLONEMIDS

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Bacterial and archaeal endosymbionts are found throughout the eukaryotic tree of life. These endosymbiotic events have occurred multiple times independently, giving rise to reduced genomes and obligate symbiotic relationships. In most protist groups, however, bacterial symbioses are only poorly understood. Here, genome evolution of bacterial symbionts from two clades of newly described marine diplonemids was characterized. The symbiont genomes were sequenced, annotated, and analyzed from several diplonemid species and the symbiont metabolic pathways were reconstructed. In addition, transcriptomic data was generated from the host diplonemids to reveal possible host-symbiont interactions. The 16S rRNA gene and multi-gene phylogenies showed that these alphaproteobacterial endosymbionts belonged to Holosporaceae and Rickettsiaceae. The endosymbiont genomes were severely reduced with the smallest Holosporaceae endosymbiont genome containing only 505 proteincoding genes (616 kbp). Metabolic pathways involved in membrane synthesis including fatty acid and peptidoglycan biosynthesis were present, but genes involved in glycolysis and the tricarboxylic acid cycle were absent. ATP translocase genes were found suggesting that the endosymbionts import ATP from the host and genes for two bacterial secretion systems were also present. These results suggest that the endosymbionts rely on the host for numerous metabolites, but the question of what the endosymbionts provide to the protist host remains unknown.

P92. THE EVOLUTION OF MEIOTIC GENES IN THE INVASIVE RED ALGAL SPECIES, *BANGIA ATROPURPUREA* (RHODOPHYTA)

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Meiosis is a fundamental reproductive mechanism shared by sexually reproducing eukaryotic species and is a crucial event for the evolution of eukaryotes. However, there are numerous species that have been described to have unknown or an absence of sexual reproduction. The red algae and in particular, the order Bangiales, has an evolutionary history dating 1.6 BYA and some of these microfossils display life histories that are characteristic of meiotic processes. However, *Bangia atropurpurea*, an invasive species within the Laurentian Great Lakes, has been reported to only be asexual. This study has utilized a preliminary genome of *B. atropurprea* and has verified the presence of meiotic genes in this organism. This raises the possibility that this organism has the ability to undergo sexual reproduction but may not be observed in the natural environment. From the inventory of known genes related to meiosis in eukaryotes (27 genes in total), 16 are present in *B. atropurpurea* and are grouped with other red algal species such as *Porphyra umbilicalis*. These newly sequenced have enabled us to understand the evolution of meiotic genes in the red algae and their phylogeny among eukaryotes.

P93. ACCUMULATION OF TOTAL LIPIDS AND TRIACYLGLYCERIDES AS BIODIESEL PRECURSORS UPON CO-CULTURING A PHYCOSPHERIC BACTERIUM WITH INDIGENOUS *CHLORELLA VULGARIS*

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The cultivation of microalgae for the production of biomass and associated valuable compounds such as neutral lipids in the form of triacyglycerides (TAGs) has gained growing interest over the years. Currently, there has been a substantial attention given to Chlorella species as source of feedstock for biodiesel production due to its ability to grow rapidly, and accumulate high amounts of TAGs stored in the cytosolic lipid bodies. However, when grown under favorable conditions, Chlorella does not accumulate neutral lipids. This has become the major bottleneck in meeting standard industrial requirements for biofuels. To address this drawback, the study utilized a new approach by engineering a microalgal-bacterial community in the phycosphere. Hence, the main objective of this project is to determine the influence of a previously isolated phycospheric bacterium *Rhizobium* sp. to axenic and xenic freshwater C. vulgaris' total lipid content and TAG productivity determined by gravimetric quantification, TLC, and GC-MS. Results showed that xenic C. vulgaris inoculated with Rhizobium (CV+B) displayed more than two-fold increase on both total lipids (23.87 ± 1.23) and TAG productivity (8.21 ± 2.07) in comparison to the control and other variables. Meanwhile, the extracted TAGs from axenic C. vulgaris (CA) exhibited a variety in its FA composition (C16, C18, C18:1) amounting to 11.53%, 10.95% and 6.52%, respectively. Our study has demonstrated an efficient means of inducing microalgal biodiesel precursors by co-cultivation of algae and bacteria. To our knowledge, this is the first research undertaking in the Philippines that revealed its potential application for biofuel production.

P94. THE UNIQUE PHYCOBILIPROTEIN LIGHT-HARVESTING ANTENNA OF CRYPTOPHYTE ALGAE: STRUCTURAL INSIGHTS INTO ITS EVOLUTION **Green, B. R.**, University of British Columbia, Canada, <u>brgreen@mail.ubc.ca</u> Teng, C. Y., University of British Columbia, Canada

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Cryptophyte algae acquired their plastids from a red alga by secondary endosymbiosis, along with many nuclear-encoded genes for plastid proteins including the membrane-intrinsic LHC family. Unlike the other algal groups with secondary plastids, the cryptophytes retained a relict of the phycobilisome in the form of the phycoerythrin β subunit, acquired a novel subunit of unknown origin, and evolved a completely novel light-harvesting antenna system. The "new" α subunit was a small protein (8-10 kDa) with no homologs in any other organism, encoded by a nuclear rather than a plastid gene. The α subunit proteins are imported across four membranes into the plastid, where they assemble with β subunit proteins and phycobilin pigments to give a tetrameric protein made up of two $\alpha\beta$ units. Gene duplication/divergence resulted in diverse families of α subunit proteins but only minor changes to the β subunit. Three-dimensional structures from a number of species show that all the $\alpha\beta$ units share the same overall fold, and much of the sequence variation is found in surface loops and free N- and C-termini. This is true even in the genus *Hemiselmis*, where a single amino acid insertion in the α subunit has little effect on the conformation of the basic $\alpha\beta$ dimer but causes a large conformational change of the tetramer and loss of electronic coupling. The α subunits are natively unfolded in solution, and refold only on the template of a properly folded β subunit from the same or different species (Laos et al. Angew.Chem.56:8384-88, 2017). This promiscuity suggests that the small α subunit gene could have evolved by chance from a segment of non-coding nuclear DNA. The question remains whether it was involved in the loss of the phycobilisome or whether it simply rescued the β subunit from oblivion and in the process invented a novel antenna.

P95. THE ULTIMATE ENVIRONMENTAL GRADIENT: PATTERNS IN ALGAL COMMUNITY STRUCTURE ACROSS THE INTERTIDAL/SUBTIDAL INTERFACE

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Predicting and understanding community patterns (e.g. trends in biomass and biodiversity) is a core component in ecology. Despite this, studies exploring how algal community structure responds across the intertidal/subtidal interface, perhaps the ultimate environmental transition, are rare. Standing algal biomass and richness were measure across five strata from the high intertidal (1.5 m above MLW) down to depths 10 m below MLW on six representative rocky reefs in southern New Zealand. This is one of the first studies to describe a unimodal pattern between algal richness and biomass across the span of the intertidal/subtidal interface, where maximum species richness occurred at intermediate levels of biomass. These results are consistent with terrestrial plant studies across strong environmental gradients. Biomass and richness patterns varied significantly between depths examined and likely to be consequences of available Photosynthetically Active Radiation (PAR) and contrasting stability of environmental conditions. In addition, one species accounted for more than 60% of total biomass across all depth strata

examined. Although one species dominating biomass is common across a range of differing systems, very rarely is this proportion quantified. The strong environmental gradients over relatively small distances of coastal rock reefs provide excellent opportunities to further advance our understanding of the mechanisms controlling these important, but often poorly described, patterns in ecological systems.

P96. STRUCTURE OF TINTINNIDS COMMUNITIES IN THE EASTERN MEXICAN PACIFIC AND THE CALIFORNIA GULF

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The tintinnids, an important component of microzooplankton, have been poorly studied in Mexican waters, both coastal and oceanic. Until now, 182 species have been recorded, but this number can increase with greater sampling efforts. In this work, we focus on describe the structure of tintinnids communities in four regions: Todos Santos Bay (TSB) and the North, Center and South of the California Gulf (CG). For the latest region, a comparison between two seasons was realized using a Wilcoxon signed test and a Wilcoxon test with the Kruskal-Walis' correction. A total of 70 surface samples (< 110 m) were analyzed. The samples were taken in the cruises Marias 1603, Marias 1609, Exfinife 1609, BTS 1016 and Vaguitas 0217. A minimum of five and a maximum of 400 L were filtered through a 20 or 34 µm sieve. All the samples were fixed with 4 % formaldehyde neutralized with sodium borate. For the species identification and counting was used a phases contrast microscope at 40x. The counting was accomplished in a Sedgewick Rafter chamber. Fifty-eight species were reported, of which sixteen species constitutes new records for Mexico. In TSB the species richness and the total abundance was low (13 species, < 33 org * L⁻¹). For the CG these two variables were highest in the south area (55 species, > 500org $* L^{-1}$). A pattern of increase from the north to the south of the CG was evidenced. Nevertheless, some stations in the north of CG were identified as hotspots of species richness and abundance. The comparison between the two seasons in the South of CG indicated that despite share the 80 % of the species (Jaccard coefficient = 0.8) there was a significant difference for the species richness and specific abundance. The pattern exhibited by the variables is influenced by the water masses dynamic of the zone and for the TSB case, by the sampling effort.

P97. USING FLOW CYTOMETRY FOR KELP MEIOSPORE ISOLATION **Augyte, S.**, University of Connecticut, United States, <u>simona.augyte@uconn.edu</u> Yarish, C., University of Connecticut, United States, <u>charles.yarish@uconn.edu</u> Marty-Rivera, M., University of Connecticut, United States, <u>michael.marty-rivera@uconn.edu</u> Pitchford, S., NOAA NMFS Milford Laboratory, United States, <u>steven.pitchford@noaa.gov</u> Wikfors, G., NOAA NMFS Milford Laboratory, United States, <u>gary.wikfors@noaa.gov</u> Lindell, S., Woods Hole Oceanographic Institution, United States, <u>slindell@whoi.edu</u> Bailey, D., Woods Hole Oceanographic Institution, United States, <u>dbailey@whoi.edu</u>

Flow cytometry is a technique that measures light scattering and fluorescence features of individual particles suspended in fluid. These particles are detected and sorted based on optical signals such as reflecting cell size, structure, and pigmentation. The objective of this project was to isolate single sugar kelp, *Saccharina latissima*, meiospores using the Milford NOAA/NMFS Laboratory JSAN sorting flow cytometer for germplasm development. This rapid clonal strain automated isolation technique can sort up to 200-400 meiospores per hour, which is a faster method than the traditional manual isolation technique at 2-4 meiospores per hour. An initial trial study yielded successful isolation rates of up to 20% with single meiospores. Developing gametophytes can then be sexed and assessed for growth. Successful broodstock will be used for an ARPA-e MARINER breeding program. This technique has the potential to improve clonal meiospore isolation and will be instrumental for the development of a germplasm library for the emerging Northwest Atlantic aquaculture industry.

P98. GENOME WIDE ASSOCIATION STUDIES FOR BREEDING *MACROCYSTIS PYRIFERA* **Alberto, F.**, University of Wisconsin-Milwaukee, USA, albertof@uwm.edu Reed, D., University of California at Santa Barbara, USA, dan.reed@lifesci.ucsb.edu Miller, R., University of California at Santa Barbara, USA, miller@msi.ucsb.edu Nuzhdin, S., University of Southern California. USA, snuzhdin@usc.edu

We present our new genomics selection breeding program focused on *Macrocystis pyrifera*, the giant kelp. Using our previous results on spatial genetic structure, we identified a high diversity admixed population to start a large number of haploid clone cultures. A total of 600 individuals were sampled in Southern California from the admixed population and the three clusters of genetic differentiation that occur in this region (Santa Barbara Channel, Channel Islands and LA to San Diego). From these a total of 3,500 haploid cultures have been isolated to build our germ-plasm collection. We will sequence the species genome, estimate linkage disequilibrium, and cultivate diploid hybrid sporophytes (crossed from our haploid cultures) in the sea at Catalina Island for phenotyping. A genome wide association study between genetic variants and phenotypic traits will identify genetic markers associated with loci controlling biomass, growth rate, warm water tolerance and nutrient storage traits. These germplasm lines will constitute a 'seed stock' similar to that established for main agricultural crops that can be used by breeders to stage model-based, efficient, cost-effective and environmentally sound targeted genome-based selection.

P99. A SLIDING WINDOW OF MULTIPLE PATERNITY? MATING SYSTEM VARIATION ACROSS ECOTYPES IN AN INVASIVE SEAWEED **Krueger-Hadfield, S. A.,** University of Alabama at Birmingham, United States, <u>sakh@uab.edu</u>

Dioecy, or separate sexes, has often been used as a proxy for the mating system in macroalgae. Yet, dioecy does not prevent the fusion of gametes from male and female gametophytes originating from the same sporophyte. This is likely a common occurrence in red seaweeds in which there are no motile propagules. Here, paternity analyses were used to gain further insight into the role of mating system variation during the *G. vermiculophylla* invasion. There was an ecological shift from fixed to free-floating thalli, ostensibly necessitated by the invasion of soft-sediment habitats. This was correlated with shifts from sexual to asexual reproduction, resulting in the dominance of tetrasporophytes in many non-native populations. Yet, subsequent surveys have uncovered populations in which reproductive males and females are present in the non-native range. Thus, there may be a sliding window of multiple paternity correlated with standing genetic diversity in non-native populations. Further, native populations exhibited heterozygote deficiency, suggesting patterns of inbreeding, selfing, or both. To investigate the mating system across a range of *G. vermiculophylla* ecotypes in native and non-native populations, cystocarps were genotyped using 10 microsatellite loci. In addition to asexual reproduction, shifts in fertilization patterns may be a mechanism of reproductive assurance during range expansions, facilitated by a prolonged haploid stage.

P100: ENVIRONMENTAL-DNA METABARCODING TO ASSESS THE BIODIVERSITY OF AN INTERTIDAL SEAWEED COMMUNITY IN THE NORTH CENTRAL GULF OF MEXICO Sergei Bombin & Juan Lopez-Bautista, The University of Alabama, Department of Biological Sciences, Tuscaloosa, AL. USA.

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The sixth wave of plant and animal extinction is ongoing and it makes extremely important to find a faster and accurate way for a comprehensive evaluation of biodiversity. The traditional approach of biodiversity estimation relies on time intense morphological identification of taxonomic groups. The recent progress of molecular techniques made possible to develop a new approach for faster and more accurate identification of species richness. One of such methods is DNA barcoding, which set the taxonomic affiliation of the unknown sample through sequencing and identification of a short DNA marker. Our study is aiming to expand the biodiversity of photosynthetic organisms in an intertidal seaweed community at the Alabama Coast. An environmental metabarcoding of such intertidal algal community has not been studied yet and thus this research will be the first for the Gulf of Mexico. Using plastid and nuclear primers and HiSeq sequencing we have detected a preliminary database of photosynthetic organisms in this environment. A bioinformatic pipeline has been developed and our read results are representing all the major groups of algae (Cyanobacteria, Chlorophyta, Streptophyta, Euglenophyta, Rhodophyta, Dinophyta, Haptophyta, Brown Algae, Diatoms and others) and discovering non-native floristic elements for the Gulf of Mexico.