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PALATABILITY AND CHEMICAL DEFENSES OF MACROALGAE IN THE ANTARCTIC PENINSULA

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We examined palatability of 37 species of nonencrusting macroalgae from the Antarctic Peninsula. This represents approximately 30% of the entire antarctic macroalgal flora and 75% of the 49 nonencrusting species we collected. Organic extracts from most species were also prepared and mixed into artificial foods. We examined palatability using feeding bioassays with three common, macroalga-consuming animals (an omnivorous antarctic rockfish, *Notothenia coriiceps*; an omnivorous sea star, *Odontaster validus*; and a herbivorous amphipod, *Gondogenia antarctica*). Thallus pieces from 23 of 34 macroalgal species tested with the fish (68%) were rejected. Of the 23 species rejected as thallus, organic extracts of 16 were bioassayed using the fish with 9 (56%) unpalatable. Thallus pieces from 21 of 36 macroalgal species tested with the sea star (58%) were rejected. Of the 21 species rejected as thallus, organic extracts of 20 were bioassayed using the sea stars and 14 (70%) were unpalatable. Overall, 28 of the 37 species assayed as thallus (76%) were rejected by either or both the fish and sea stars. The amphipod assay was not suitable for use with thallus but was utilized with organic extracts of 23 macroalgal species that were rejected as thallus by either or both the fish and sea stars. Of these, 14 (61%) of the species' extracts were rejected by the amphipods. Unpalatability was highest among the brown algae examined with only an ephemeral, ectocarpoid species not rejected as thallus out of 10 species tested. Of the remaining nine brown algal species, six of seven tested were also unpalatable as extracts, including all the ecologically dominant, perennial species in the area. We conclude that unpalatability to herbivores is common in antarctic macroalgae and that chemical defenses may play an important role in the unpalatability of many algal species (NSF OPP9814538, OPP9901076).

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DINOFLAGELLATE GENOMICS: RESULTS FROM AN EST APPROACH

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Dinoflagellates are enigmatic protists with odd nuclear features, interesting plastid gene arrangements and a proclivity for endosymbiotic relationships. Relatively little molecular work has been done on dinoflagellates, and only a handful of genes have been characterized in these organisms. We have begun an Expressed Sequenced Tag (EST) project with the aim of collecting plastid targeted but nuclear encoded genes from peridinin-containing dinoflagellates. This provides an opportunity to understand the integration of endosymbiont genes into the host cell. Our sequencing effort has produced about 1000 unique ESTs from a *Gonyaulax polyedra* cDNA library obtained from David Morse. Of the 1535 total ESTs (72%, >400 bp), 466 sequences were identifiable by BLAST with a bit score above 50. The most abundant transcript is the peridinin-chlorophyll-binding protein, which constituted 3% of the clones. More recently, we began sequencing from an *Amphidinium carterae* cDNA library made in our laboratory. This provides insight into expression patterns and is a resource for other workers in the field. Several clearly identifiable plastid genes have been found and preliminary analysis confirms the chromophyte, and therefore, tertiary origin of the peridinin plastid.

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ALGAL STIMULATION OF THE BERKELEY PIT LAKE SYSTEM

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This study was conducted to evaluate four species of algae (*Chromulina freiburgensis* Dofl., *Chlorella ellipsoidea* Gerneck, *C. vulgaris* Beijerinck, and *Chlamydomonas acidophila* Negoro) isolated from an acid pit lake for their bioremediative potential. The Berkeley Pit Lake system, located in Butte, MT, USA is an aban-

doned open-pit mine. As a result of acid rock drainage the water in this pit is very acidic and contains high concentrations of dissolved metals. First, optimal nutrient levels for each species of algae were determined. Levels of nutrients tested include: 5, 10, 15 mg N/L, and 0, 3, 6 mg P/L. The experiment was conducted with sterile filtered Berkeley Pit water. Next, each species of algae were grown under optimal nutrient levels and dissolved metal removal potentials were examined, using Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). In addition, field sampling was performed to gain a better understanding of the microbial community within the pit. Finally, field parameters including, light, temperature, dissolved oxygen, specific conductivity, redox potential, and turbidity were examined to determine the actual conditions for microbial growth within the Berkeley pit.

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PHYTOPLANKTON COMMUNITY COMPOSITION IN THE TRI-LAKES AREA OF CENTRAL WISCONSIN, USA

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Tri-Lakes (Upper and Lower Camelot, Sherwood, Arrowhead) in Adams County, WI, USA are man-made impoundments draining substantial agricultural lands and surrounded by considerable shoreline residential development. The planktonic algal community, as sampled from June to November 2000, was sparse-to-moderately dense, fairly diverse (69 genera from six divisions basin-wide), and unremarkable in taxonomic composition. All sites sampled displayed the general algal successional trends expected from northern-temperate, mildly eutrophic waters. These included sparse but taxonomically diverse communities in the spring; a late spring pulse of diatoms; a late summer pulse of green algae; and a steadily increasing component of Cyanobacteria leading to their community dominance by the end of the growing season. Upper Lake Camelot (55 genera) best represented this pattern. Lower Lake Camelot (53 genera) had a large green algal pulse but only a small diatom pulse. Lake Sherwood was the most taxonomically diverse body (63 genera) and had the most extreme pulses of diatoms and greens. Lake Arrowhead had the lowest taxonomic diversity (39 genera), was the most dominated by Cyanobacteria, and had only minor pulses of diatoms and greens. The algal communities indicate a mesotrophic to slightly eutrophic lake status. Con-

tinued agricultural and residential inputs of fertilizers and pesticides will likely exacerbate the cyanobacterial dominance leading to further reductions in aquatic health and aesthetic values. Previous chemical treatment and macrophyte removal have achieved limited success, and might have altered algal community dynamics. Remediation approaches that might improve water quality include: reducing upstream inputs via sediment traps or lagoons; reducing in-lake nutrients via sediment removal; reducing residential inputs via improved septic/sanitation systems; and shoreline vegetation filter strips.

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DESMIDS AND DINOFLAGELLATES OF ECUADOR

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The freshwater flora of Ecuador is poorly known. This report provides preliminary information about the freshwater Pyrrhophyta and two families in the Chlorophyta. There has been some work on diatoms but other groups remain unstudied. In June 2001, samples were collected as whole water or by using a 10-mm plankton net from 52 locations in the interior (Amazonian) and Andean regions and examined within hours using a Swift field microscope. Nine additional samples from Ecuador have been provided by Dr Miriam Kannan. Amazonian sites consisted of oxbow lakes along the Rio Shiripuno, and small ponds and lakes along the road from the river to Coca. Andean sites were principally in the Cajas National Park near Cuenca. Genera in the Closteriaceae include *Closterium*, *Gonatozygon*, *Spinoclosterium*, and *Penium*. Desmidiaceae include *Arthrodesmus*, *Bambusina*, *Cosmarium*, *Desmidium*, *Euastrum*, *Hyalotheca*, *Micrasterias*, *Pleurotaenium*, *Spondylosum*, *Staurastrum*, *Staurodesmus*, *Triploceras*, and *Xanthidium*. Pyrrhophyta include *Ceratium*, *Gymnodinium*, *Peridinium*, *Sphaerodinium*, and *Woloszynskia*.

6

PHORMIDIUM RETZII (OSCILLATORIALES): GENOTYPIC VARIATION AND PHENOTYPIC PLASTICITY

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Phormidium retzii is one of the most commonly encountered lotic taxa in North America and has been recorded from a wide range of habitats worldwide. The genetic variability of this cosmopolitan, freshwater cyanobacterium was assessed using gene sequences and random amplified polymorphic DNA (RAPD) markers. Strains consistent with the morphological species circumscription were utilized from British Columbia, Canada to Rio Claro, Costa Rica, and from Rhode Island to Washington State, USA. In addition, some strains were from geographically close (<25 miles apart) sites. The 16S rRNA gene was sequenced for eight of the 12 strains. Sequence homology ranged from 90.95 to 98.71. The nine RAPD primers utilized yielded a total of 133 distinct bands from the 12 strains. The strains were variously related and showed great variation in pairwise genetic distances (0.23–0.83). Given the lack of sequence similarity, *P. retzii* as presently circumscribed most likely represents several cryptic species not clearly distinguishable with light microscopy of morphological characters. This conclusion may explain the lack of correlation between geographic proximity and genetic similarity. To assess the level of environmentally induced phenotypic plasticity of this taxon, one strain was subjected to three different current velocity and nutrient regimes (low, medium, and high) employing six stream mesocosms. ANOVAs and Bonferroni multiple comparison tests were performed with cell volumes, ratios of cell length:width and percent cover data. Low nutrient treatments elicited significantly ($P < 0.05$) greater cell volumes and percent cover than did high treatments. Furthermore, current velocity did not show any significant effect on cell morphology. As such, it appears that a single strain is capable of wide morphological variability in response to some environmental conditions. The results of these two studies have great implications in the utilization of cyanobacterial morphospecies concepts.

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GREEN ALGAL CONQUEST OF THE LAND: MANY CONQUESTS, ONE VICTORY?

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Life on earth originated in a wet world and remained there for millions of years until the first terrestrial invaders made their initial forays into a new and unexploited terrestrial habitat. Although cyanobacteria, and perhaps fungi as well, were important participants in the conquest of the land, it was the evolution green algae into the initial land plants that started amazing evolution and diversification the terrestrial flora and fauna with which we are now familiar. Given the paradigm that life arose in the sea, it is perhaps surprising to find that freshwater green algae – not marine forms – were the successful conquerors, i.e. the algae that gave rise to the land plants. The first land colonizers were, of necessity, primary producers (i.e. food for organisms that arrived or evolved later) and were likely to have been cyanobacteria (blue-green algae), followed by green algae (at least four separate invasions) and fungi, which also made a successful assault on land together with algae in the form of lichens. The symposium will survey recent studies on these groups and their habitats, and describe how these colonizers changed and were changed by adaptations to the new, dry world. It will explore the question: ‘Why did only one group of terrestrial green algae give rise to the land plants?’ The success of these colonizers is relevant to all subsequent terrestrial life (including ourselves), as well as the search for extraterrestrial life, which, if it exists, is presumed to have begun in an aquatic environment.

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PHYLOGEOGRAPHY OF *BATRACHOSPERMUM HELMINTHOSUM* (RHODOPHYTA) IN NORTH AMERICA

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The freshwater red alga, *Batrachospermum helminthosum*, is distributed primarily in streams of eastern North America. Although it is often an abundant and conspicuous component of the algal flora for many streams, little is known about the genetic relationship among populations throughout its range. The purpose of our study was to elucidate geographic patterns among populations to gain insight into the biogeographic distribution of this species and possible modes of dispersal. Individuals were sampled in 13 stream segments as follows: four locations in Ohio, two locations in Michigan and one location each in Indiana, North Carolina, Tennessee, Louisiana, Rhode Island, Massachusetts and Connecticut. The mitochondrial spacer region (371 bp) between the

COX2 and *COX3* genes was chosen because it is variable among individuals. This region was sequenced for 15 individuals from each location. Thirteen haplotypes were identified among the 13 locations with most locations having one or two haplotypes. Two of the haplotypes were dominant being recorded from six of the streams. The Indiana, Louisiana and first Michigan population had a unique haplotype. The second Michigan and fourth Ohio populations shared a unique haplotype. The Tennessee population had four haplotypes, three of which were closely related but the fourth haplotype was not and linked to the haplotypes from Michigan and Ohio. The relationships among these populations are very complex but there may have been a recent dispersal event via birds among various streams.

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CHARACTERIZATION OF MACROALGAL EPIPHYTES ON *THALASSIA TESTUDINUM* IN TAMPA BAY, FLORIDA

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Blooms of seagrass epiphytes have potentially important economic and ecological consequences in Tampa Bay, one of the Gulf of Mexico's largest estuaries. As part of a Tampa Bay pilot study to monitor the impact of environmental stresses, precise characterization of epiphyte diversity is required for efficient management of affected resources, and thus may be used as a rational basis for assessment of ecosystem health. Fixed monitoring sites were selected north of Port Manatee, composed of dense and sparse *Thalassia testudinum* seagrass sites. A total of 13 epiphytic species encompassing green, brown and red macroalgae were manually collected in May 2001 from dense seagrass beds versus nine species from sparse beds. Epiphytes only collected in the dense beds were *Enteromorpha flexuosa*, *Sphacelaria rigidula*, *Ceramium byssoideum*, and *Herposiphonia tenella*; epiphytes only occurring in the sparse beds were *Griffithsia* and *Stylonema alsidii*. A correlation seems to emerge among attachment mode of epiphyte to host, presence of cortication and epiphyte length. A main goal of this ongoing study is the determination of indicator species for both healthy and stressed seagrass bed environments using both taxonomic and gene-sequencing techniques.

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NEW SYSTEMATIC INSIGHTS IN THE *CERAMIUM SINICOLACOMPLEX*: RESURRECTION OF *C. INTERRUPTUM* S. & G. (CERAMIACEAE, RHODOPHYTA)

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Setchell & Gardner (1924) provided a taxonomic treatment for the genus *Ceramium* from lower California and the Gulf of California, Mexico, in which they described several new species, including *C. sinicola* S. & G. described from Ensenada, BCN and *C. interruptum* S. & G. described from the vicinity of La Paz, BCS. The latter was later reduced to variety rank in *C. sinicolabey* Dawson (1950), a taxonomic decision that has subsequently been widely adopted. Phylogenetic analyses inferred from three molecular markers (chloroplast-encoded *rbcL*, the RUBISCO spacer, and nuclear encoded SSU rDNA) from recent collections from the Pacific North-west (California, Oregon) and the Gulf of California reveal a well supported assemblage of three corticated taxa: *C. codicolaj* Ag. 1894, *C. sinicola* and *C. sinicola* var. *interruptum*. Sequence divergence values among the three taxa are sufficient to warrant separate species ranking; hence, we reinstate *C. interruptum* as a widely distributed epiphyte for the region. *C. sinicolais* more closely related to *C. codicolathan* to *C. interruptum*, with the former two restricted to the host *Codium*. The molecule-based relationships are congruent with evolutionary trends in cortication pattern and attachment mode.

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PHYTOPLANKTON OF A MISSISSIPPI RIVER WATERSHED IN COASTAL LOUISIANA

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In July–August 2001, phytoplankton samples were collected from a coastal marsh south of New Orleans, LA. This wetland area is usually cut off from the Mississippi River by levees and other flood control devices, but periodically the Caernarvon Diversion is opened to allow fresh river water to flow into the marsh. As

part of a larger project, the present study seeks to determine the affect the periodic input of freshwater has on phytoplankton dynamics in the Caernarvon Diversion watershed. The first phase of the project - collecting and identifying algae during the summer when the Caernarvon Diversion is kept closed - has been completed. A total of 168 species from 76 genera representing 8 phyla were identified and digital images recorded. Little or no previously published data on the taxonomy and ecology of phytoplankton from this area are available. Sampling in spring 2002, when the diversion is open, will allow comparative analysis of phytoplankton composition and productivity. In a collaborative study, the phytoplankton data will be combined with information on nutrients, chlorophyll a, turbidity, salinity, etc. and used in a model to demonstrate how the ecosystem is affected by the diversion. Also, a database of digital images is available at <http://www.chapman.lsu.edu/digitalalgae>. In addition to the taxonomic identification data and images, the site will eventually include physico-chemical and GIS data on the collection sites. Supported in part by a grant from the Office of Sea Grant Development at LSU.

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ORIGIN AND EARLY EVOLUTION OF PLANT BODY SYMMETRY AND GRAVITY RESPONSES

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Land plant bodies exhibit both apical-basal and radial symmetry, and they are able to detect and respond to gravitational forces. These attributes were, likely important factors in the success of earliest plants on land. This study focuses on features of charophycean green algae likely to have been pre-adaptive to early establishment of plant symmetry and gravitational responses, though most modern charophyceans occupy aquatic habitats where the buoyancy of water counteracts the effects of gravity. Trait mapping suggests that even the earliest-divergent modern members of the streptophyte clade have bodies whose symmetry departs significantly from the spherical condition, and that cellular mechanisms defining aspects of radial symmetry and polarized tip growth originated early. Genes, cell biological approaches, and taxa are identified for which further exploration is likely to illuminate early evolution of plant body symmetry and gravity responses.

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EFFECTS OF SAMPLING SCALE AND ANALYSIS METHOD ON PERCEPTIONS OF PHYTOPLANKTON SPECIES ASSOCIATIONS

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Phytoplankton associations generally are identified by nonrandom co-occurrence of phytoplankton species, and are considered to be a result of similar species response to properties of the environment. Because an association is characterized by co-occurrence of species across samples, or synchrony of the abundance of species over time, we proposed that the perception of phytoplankton association is subject to spatial and temporal sampling scale and analysis method. We investigated this using an intensive phytoplankton dataset from three lakes of the Greater Yellowstone Ecosystem, with particular concentration on *Asterionella formosa* (AF) and *Aulacoseira subarctica* (AS). We compared numerical analyses, including Fager analysis of recurrent groups, rank correlation, and other measures of synchrony, for differences in assessment of co-occurrence. The analyses were conducted on the entire dataset, and then the dataset was reduced to assess the effect of different spatial or temporal sampling regimes on apparent association. While the two diatom species significantly co-occurred across samples, assessment of synchrony was affected by analysis method and sampling regime. AF and AS abundance were positively correlated across lakes and within each lake, but peaks in abundance of the two species did not significantly coincide over time. In addition, species abundance were negatively correlated across depths, and we argue that this is significant to the autecology of each species. These results indicate that the perception of phytoplankton association may simply be an artifact of the limits of our understanding of the distribution and physiology of the organisms.

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A GUIDE TO THE SEaweEDS OF FLORIDA

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A review of Florida seaweeds indicates a highly diverse flora within a large coastal zone (over 700 km)

spanning more than 7° of latitude, containing tropical, subtropical, and temperate communities. Surprisingly, there have been relatively few detailed floristic studies of Florida seaweeds, except for the Florida Keys and on the east coast. The patterns are a reflection of the early predominance of winter visitors and lack of resident phycologists. The seaweeds of the west coast of Florida were virtually ignored until the 1950s. We estimate that the state's seaweed flora consists of at least 670 taxa including 219 green, 97 brown, 349 red algae, and at least 3 species of *Vaucheria*. Such a compilation is significantly higher than the numbers of taxa recorded for Florida by Taylor (1960), as well as those for North Carolina (i.e. Schneider & Searles, 1991), and Caribbean reefs (Littler & Littler, 2000). The majority of Florida seaweeds have tropical/Caribbean affinities, although a significant temperate component also occurs, presumably reflecting the influence of the northern floras of the Gulf of Mexico and southeastern coast of the US. The tropical nature of the state's flora is supported by the R/P and R + C/P indices of Feldman (1937) and Cheney (1997) with these ratios being 3.6 and 5.8, respectively. Florida's seaweed diversity is enhanced by the extensive salt marsh and seagrass communities of the west coast, plus the coral reef habitats of the Florida Keys. The present review summarizes several important historical studies, conspicuous geographic patterns, the type of communities, and the structure of a guide to the seaweeds of Florida. The guide will also serve as a historical reference for possible anthropogenic impacts in the future.

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ONE SMALL STEP: WHY DID THE CHAROPHYTES HAVE THE RIGHT STUFF?

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The origin and evolution of land plants (i.e. embryophytes) represents one of the seminal events in the history of life on earth. When the land plant lineage took hold upon the continents, there were profound changes in the global environment including dramatic modification of the erosion regime, correlated changes in marine nutrient inputs, and striking fluctuations in atmospheric chemistry. These changes took place not only because of the ability of land plants to survive on land, but also because their structure and physiology permitted them to make efficient

use of resources, anchor themselves effectively and in so doing stabilize the substrate and permit the formation of modern soils, and maintain metabolic activity for long periods of time even in the absence of rainfall and surface moisture. Molecular phylogenetic analyses show the order Charales is the sister group to all land plants, with the Coleochaetales sister to the land plant/Charales lineage. Thus, in a very real sense, the embryophytes are 'drier algae.' Many groups of green algae live in the terrestrial environment, but only one of these – the land plants – has radiated into a wide range of habitats. It is not known why the land plant lineage has been so successful. Traits that are likely to have played a role in their success include cell wall biochemistry, desiccation resistance and tolerance, structural complexity, and various reproductive strategies. In all probability, the success of the land plant lineage was not the result of a single 'key innovation', but an emergent property resulting from complex interactions among these and other features of the lineage. Comparative study of the properties of diverse aquatic and terrestrial algae can be used to identify properties that were important in the colonization of the land and how they interact.

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DIVERSITY OF FUSIFORM MICROCHLOROPHYTE ALGAE FROM ITASCA STATE PARK, MINNESOTA

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Through the use of molecular techniques, several different types of fusiform and twisted-fusiform microchlorophyte algae (*Monoraphidium*, *Ankistrodesmus*, etc.) have been characterized from samples taken from lakes and ponds of Itasca State Park, MN. PCR-RFLP analysis of 18S ribosomal RNA genes was used to categorize 29 different isolates from the Itasca State Park lakes into nine different types. The 18S ribosomal DNA sequences were determined for one isolate from each type for phylogenetic analysis. Light microscopy was used so that morphological characteristics as well as molecular characteristics can be compared and contrasted. The morphological characteristics were consistent for each PCR-RFLP type. Further discussion will include comparisons of the distribution and diversity of these organisms from Itasca State Park to those from Arrowwood National Wildlife Refuge, ND.

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A TOTAL EVIDENCE APPROACH TO INFERRING THE PHYLOGENETIC RELATIONSHIPS WITHIN AULACOSEIRA (BACILLARIOPHYTA)

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Phylogenies provide the context in which hypotheses about character evolution can be tested. But the phylogenetic understanding of many diatom taxa, including some of the more spatially and temporally ubiquitous genera, is insufficient to fruitfully pursue questions about character evolution within them. Consequently, we have estimated the phylogeny of Aulacoseira using a combination of nucleotide sequence data and data derived from morphological characteristics of the silica cell wall. In addition to the use of traditional 'discrete' or 'qualitative' morphological characters, we have assessed the use in our cladistic analyses of 'continuous' or 'quantitative' characters, including descriptors of the ontogenetic trajectories of these characters over a species' life cycle (size range). Also, molecular characters gathered from a chloroplast genome marker (*rbcL*) as well as a nuclear genome marker (18S rRNA) were used simultaneously in the cladistic estimation of the phylogeny of approximately 12 species of Aulacoseira. In order to have more thorough taxon sampling for a better estimate of phylogeny, we included several fossil species, as well as taxa where cultures were unavailable, by utilizing the morphologically based characters despite the absence of molecular characters for these taxa.

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POST-EUROPEAN SEDIMENTATION AND NUTRIENT LOADING IN LAKE ST. CROIX: A NATURAL IMPOUNDMENT ON THE ST. CROIX RIVER, USA

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The final 37 km of the St. Croix River were naturally dammed by the Mississippi River to form Lake St. Croix approximately 9500 years ago. The St. Croix River is currently regarded as having 'exceptional resource value', as one of the least impacted large Midwest river systems. Twenty-four 2-m piston cores were recovered in 1999–2001 from Lake St. Croix sub-basins to identify post-European settlement signals of

land use, trophic change, and sedimentation using a whole-basin approach to reconstruct loading history of nutrients, sediments, heavy metals, and organics. Dating chronologies based on ²¹⁰Pb inventories indicated both cores recovered a sediment sequence dating from pre- and post-European settlement (*c.* 1850) in the St. Croix River basin. Select cores were subjected to magnetic susceptibility, loss-on-ignition, and diatom microfossil analysis. Sedimentary increases in magnetic susceptibility were indicative of increased erosion and transport of ferromagnetic mineral grains due to initiation of settlement, logging, and agricultural activities in the basin. A three-fold increase in sediment accumulation began in the mid-1800s in the northern basin and by 1900 in the southern basin. Diatom accumulation increased 20–50-fold since settlement with a shift from benthic- to planktonic-dominated assemblages. Simultaneous with the assemblage shift were the introduction and establishment of many planktonic diatoms considered ubiquitous indicators of eutrophy. The fossil diatom assemblages were further analyzed using weighted-averaging calibration and reconstruction of historical water column total phosphorus (TP). Reconstructed TP values showed that water column nutrient values have increased 2.5–3-fold since presettlement times. Presettlement values of about 0.02 mg/L TP were found in both cores with TP increases beginning *c.* 1910 and especially dramatic increases after World War II. Modern reconstructed TP values (about 0.055 mg/L) were similar to TP concentrations reported from monitoring during the last few decades; however, the river was clearly impacted well before monitoring efforts were begun.

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PHOSPHATASE EXPRESSION BY *CHLORELLA VULGARIS* (CHLOROPHYCEAE) IS MEDIATED BY INTERNAL PHOSPHORUS LEVELS AND EXTERNAL pH

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Phosphorus has been implicated as one of the causative agents of the eutrophication of lacustrine, estuarine and coastal waters. One of the major inputs of phosphorus into these waters comes from agricultural nonpoint sources in the form of dissolved organic phosphorus (DOP). In order to better understand how DOP may directly impact the microalgal community structure, it is necessary to understand the physiology of DOP uptake and utilization. To this end, cultures of *Chlorella vulgaris* were grown in custom pho-

tobioreactors in acid (pH 5.5) and alkaline (pH 7.5) media under phosphate replete and starved conditions in order to determine the types and triggers of phosphatase expression by this organism. Analysis of differential phosphatase expression during phosphate stress indicates that cultures of *C. vulgaris* grown under alkaline conditions derepressibly express two alkaline phosphatases, while cultures grown under acid conditions weakly express only one alkaline phosphatase. Analysis of internal and external phosphorus levels, whole-cell phosphatase activity and in-gel activity of protein extracts indicate that alkaline phosphatase expression is further mediated by internal, as opposed to external, phosphorus levels.

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BIODIVERSITY AND ASSOCIATION OF DINOFLAGELLATES IN CORAL REEF RUBBLE, CARRIE BOW CAY, BELIZE

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The species diversity and distribution of benthic dinoflagellates are illustrated from the back reef sand habitats at Carrie Bow Cay. Sand appears an ideal environment for nurturing a variety of species. Sand supported blooms of toxic, nontoxic, and parasitic assemblages of dinoflagellates. Morphology of dinoflagellates is illustrated in SEM and LM pictures; including the life cycle of a parasitic dinoflagellate's vegetative stage, sporogenetic stages, and the morphology of the feeding organelle. The feeding organelle of the primary dinoflagellate cysts is a hold fast and a peduncle-like infestation tube, a 'sucker organelle', that inserts itself into copepod eggs. The parasitic dinoflagellate continues feeding on crustacean eggs since it is the only food source during vegetative cell division within the cyst. This is the first observation of the presence of parasitic dinoflagellates in shallow coral reef water. The study attempts to provide new knowledge on dinoflagellate associations and morphology of sandwelling species in the microscopic food web of shallow warm tropical waters. Even, sand-inhabiting invertebrates are not exempt from parasitic dinoflagellates.

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DIFFERENCES AMONG COCCOID GREEN ALGAL COMMUNITIES IN MINNESOTA AND NORTH DAKOTA LAKES

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Very little is known about the diversity and distribution of freshwater unicellular coccoid green algae. The simple morphology and small size of these organisms make reliable identification extremely difficult. Current hypotheses suggest that species of coccoid algae are widely distributed, opportunistic organisms that are always present in aquatic systems. These hypotheses are based on the assumption that phytoplankton communities include only one or a few species of coccoid algae. We examined the diversity and distribution of green-colored autosporic coccoid algae in Itasca State Park in Minnesota and Arrowwood National Wildlife Refuge in North Dakota using molecular techniques and light microscopy. Our preliminary results indicate a higher level of diversity of coccoid green algae than has been reported in regional flora. Contrary to current hypotheses, communities of coccoid green algae vary among the different lakes.

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DIVERSITY OF COCCOID ALGAE IN MINNESOTA AND NORTH DAKOTA LAKES

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The coccoid algae (a.k.a 'little green balls') are important primary producers in freshwater communities, but very little is known about their diversity. This lack of knowledge results from the extremely small size and simple morphology of these organisms, which makes identification by light microscopy essentially impossible. However, molecular techniques allow differentiation of taxa at various levels and can ultimately lead to species identification. We have isolated several hundred cultures of coccoid algae from lakes and ponds in Itasca State Park in northwestern Minnesota and Arrowwood National Wildlife Refuge in central North Dakota. Many of these isolates have been examined by light microscopy, 18S rDNA PCR-RFLP analysis, and 18S rDNA sequence analysis. In addition, some taxa with multiple isolates have been examined by *rbcL* and ribosomal RNA ITS sequence analysis. Our results indicate a high level of diversity, including possible new species of *Chlorella*, *Muriella*, *Pseudomuriella* and *Mychonastes*, among others. We will discuss the results of our analyses, including implications for identification of taxa and higher level systematics. Supported by National Science Foundation Grants DBI-00703867, MCB-0084188 and DEB-0128952.

23

ASSESSING THE BIODIVERSITY OF MONORAPHIDIUM USING 18S rDNA SEQUENCESFawley, M. W.¹, Fawley, K. P.¹ & Dean, M. L.^{2,*}¹*Department of Biological Sciences, North Dakota State University, Fargo, ND 58105;* ²*8715 W. Mallard Ct., Franklin, WI 53132, USA*

The taxonomy of the genus *Monoraphidium* is unclear due in part to the absence of morphological features to clearly distinguish one species from another. Phytoplankton samples collected from lakes in the Arrowwood National Refuge in eastern North Dakota were found to contain several morphological species of *Monoraphidium*. Eighteen *Monoraphidium* isolates were examined with light microscopy and six morphological species were identified. PCR-RFLP of the 18S rDNA was used to type the isolates. Following digestion by *Hae* III and *Taq* I, the 18S rDNA PCR-RFLP patterns indicated 10 different types. Presently, the 18S rDNA product is being sequenced for each of the 10 types. By examining morphological characters and 18S rDNA sequences, congruence between morphology and sequence data may be compared. Also, because there is a lack of morphological characters defining *Monoraphidium* species, diversity within the 18S rDNA sequences may aid in the taxonomy of the genus and its place within the Chlorococcales. Supported by National Science Foundation Grants MCB-0084188 and DBI-0070387.

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SPATIAL AND TEMPORAL DISTRIBUTION OF LIFE HISTORY VARIANTS IN THE RED ALGA, MASTOCARPUS PAPILLATUS

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We investigated small-scale spatial structure in *Mastocarpus papillatus*, a red alga with sexual and apomictic life-history variants. Spatial structure and habitat partitioning can facilitate the coexistence of ecologically similar organisms, and in *M. papillatus* a large scale geographic spatial division is well documented. Variants of *M. papillatus* display geographic parthenogenesis where sexual fronds are common south of San Francisco Bay and apomicts dominate north of San Francisco Bay. Although geographic parthenogenesis in *M. papillatus* is well-documented small-scale spatial structure was not previously known due to the similar appearance of sexual and apomictic upright fronds.

We investigated whether sexual and apomictic fronds differed in their spatial distribution by sampling for vertical differences in intertidal height and horizontal patchiness. Fronds were sampled when reproductive and cultured for life history. We found sexual variants were significantly aggregated at lower intertidal heights while apomicts were found throughout the intertidal range. Sexual fronds were strongly seasonal and reproduced October–December whereas apomictic fronds reproduced throughout the year. These results parallel the large-scale geographic distribution with apomicts inhabiting marginal areas, and suggest dispersal capability may also facilitate geographic parthenogenesis.

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MACROALGAE OF THE HAWAIIAN ISLANDS: 23 ADDITIONAL STREAM SEGMENTSFilkin, N. R.^{1,*}, Sherwood, A. R.² & Vis, M. L.¹¹*Department of Environmental and Plant Biology, Ohio University, Athens, OH 45701;* ²*Department of Botany, University of Hawaii, Manoa, HI, USA*

The Hawaiian Islands are located 3700 km from the nearest continental land mass and as such provide an interesting research opportunity to study freshwater organisms intolerant of seawater. The freshwater biota have been only partially studied with little research on the stream macroalgae. The only previous study to focus solely on stream macroalgae, reported 25 new species to the Hawaiian Islands from 34 stream segments sampled. The purpose of our study was to expand the sampling of streams to better determine macroalgal distributions and species richness. Twenty-three additional stream segments (seven on Oahu, eight on Kauai and eight on Hawaii) were sampled for macroalgae. The physical and chemical parameters of each stream were measured. Stream segments ranged greatly in size from 1.2 to 40 m in width. Water temperature was relatively uniform (about 21 °C) but other chemical parameters differed from site to site (pH 5.5–8.9, specific conductance 20–200 mS/cm). Mean species richness per stream segment was 3.9 with one to eight species collected per segment. Ninety populations of 44 infrageneric taxa were identified from the Cyanobacteria (20), Chlorophyta (18), Rhodophyta (3) and Chrysophyta (3). The most abundant taxa were *Spirogyra* sp. 2, *Audouinella pygmaea* and *Phormidium retzii*. All three of these species are cosmopolitan. Twenty-two of the taxa are new records for streams in the Hawaiian Islands. The large percentage (50%) of new taxa suggests that more research is needed to fully catalog the Hawaiian stream macroalgal diversity.

26**THE ECOLOGICAL SIGNIFICANCE OF MYCOSPORINE-LIKE AMINO ACIDS IN ALGAE**

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The links among mycosporine-like amino acids (MAAs), algal physiological ecology, and the environment may be viewed from two sides. The UV-absorption spectra of MAAs make them prime candidates for UV-screening compounds, and indeed, numerous studies have shown that a high concentration of MAAs is correlated with various degrees of protection of cellular processes from UV damage. Thus, it might be said that the structure of terrestrial, eulittoral and sublittoral algal communities depends in part on their ability to synthesize effective quantities of MAAs. Corollaries to this hypothesis might include the proposition that those species incapable of synthesizing MAAs will be excluded from UV-containing environments, and that UV is necessary to trigger MAA synthesis. However, a number of studies have demonstrated that triggers of MAA synthesis need not include exposure to UV, but may include the relative availability of nitrogen, osmotic stress, or high levels of photosynthetically active radiation. Furthermore, the response to these factors may vary as a function of other environmental conditions. These issues will be discussed with respect to elucidating the master switch for MAA synthesis.

27**BIOLOGICAL WEIGHTING FUNCTIONS FOR THE EFFECT OF UV RADIATION ON CARBON PARTITIONING IN MICROALGAE**

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UV radiation clearly inhibits phytoplankton photosynthesis, with direct effects on both photosystem II and the enzyme, Rubisco, that catalyses the first reaction of the photosynthetic carbon reduction cycle. The subsequent partitioning of fixed carbon into macromolecular pools (carbohydrates, lipids, and proteins) is also affected by UV exposure, although relative changes in allocation are not well-documented and vary considerably. The wavelength specificity of inhibition of photosynthesis has been described by spectral weighting functions, or biological weighting functions (BWFs) that combine the effect of UV radiation with interactive effects of longer wavelengths. BWFs permit one to

compare the effects of different radiation sources or various ozone depletion scenarios. However, no data are available from which BWFs for partitioning can be determined, as experimental techniques have varied widely. These BWFs are essential for predicting the effects of increased UV-B on other trophic levels, and for analyzing the cost and benefit of synthesizing protective compounds. Using a polychromatic approach, we are developing BWFs for the partitioning of ^{14}C in *Thalassiosira pseudonana* (^3H) at photosynthetically saturating irradiance. We are comparing these functions to BWFs calculated from simultaneous measurements of primary productivity. We have found consistent differences in sensitivity to UV exposure among the pools, and are testing these differences for significance using a variety of model and calculation approaches.

28**A SURVEY OF THE OFFSHORE MARINE MACROALGAE FROM THE NORTH-WESTERN GULF OF MEXICO HARD BANK COMMUNITIES**Fredericq, S.^{1,*}, Cho, T. O.¹, Gavio, B.¹, Gurgel, C. F.¹, Hickerson, E.², Lin, S. M.¹, Lopez-Bautista, J.¹, Phillips, N.¹, Viguerie, M. & Wysor, B.¹¹*Department of Biology, University of Louisiana at Lafayette, Lafayette, P.O. Box 42451, LA 70504-2451;*²*Flower Garden Banks National Marine Sanctuary, Bryan, TX 77803, USA*

The upper north-western Gulf basin is characterized by a relatively shallow but well-developed continental shelf with an extensive system of mid- and outer shelf hard banks formed on unique tectonically created salt diapirs of varying origin and composition. Observations done by our laboratory while participating in monitoring cruises to the National Marine Sanctuaries of the E and W Banks of the Flower Gardens (TX), Stetson Banks (TX), Sonnier Banks (LA) and dredging cruises off Louisiana reveal a surprisingly different floristic composition among the sites. Several range extensions, new macroalgal records for the Gulf of Mexico, and new species imply a much more complex algal community than previously documented. Our ongoing project includes the development of a modern taxonomic, phylogenetic and morphological database essential to informed management for preservation of biological diversity. It also provides a framework of seasonal algal composition against which long-term trends and anomalies in algal distribution and health of the Gulf of Mexico can be assessed at future dates.

29

NEW INSIGHTS IN THE SYSTEMATICS OF THE PHYLLOPHORACEAE (GIGARTINALES, RHODOPHYTA)

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The Phyllophoraceae, consisting of about 100 species worldwide, stands out in exhibiting a wide spectrum of unique life history types that makes it unusually interesting for assessing the phylogenetic importance of reproductive traits relative to classification criteria. Type of life history and position of the reproductive structures on the thallus have traditionally formed the basis for separating 11 genera in the Phyllophoraceae; however, phylogenetic analyses inferred from three sets of DNA sequences [chloroplast-encoded *rbcl*, nuclear large-subunit ribosomal RNA gene (LSU rDNA), and internal transcribed spacer regions (ITS) of nuclear ribosomal DNA], instead indicate a lack of correlation between type of life history and phylogenetic relationships among the established taxa. This lack of correlation dramatically challenges all of the traditional taxonomy, and we will present a revised classification for the family that downplays life-history features. The study will answer the question which morphological features can be used as meaningful indicators of phylogenetic relationships in the Phyllophoraceae. The results will also be addressed in light of global biogeographic hypotheses for the family.

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THE DIVERSITY AND AVAILABILITY OF CAULERPA SPECIES FOUND IN RETAIL AQUARIUM OUTLETS IN SOUTHERN CALIFORNIA, USA

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Biological invasions are recognized as a serious threat to marine biodiversity. Within the last 2 years, invasive populations of *Caulerpa taxifolia* were found in southern California's coastal waters as well as in New South Wales, Australia. In addition, two other seaweeds (*Undaria pinnatifida* and *Caulacanthus ustulatus*) also appear to have recently invaded southern California's warm temperate waters. The introduction of *C. taxifolia* has attracted much attention because this exotic seaweed is thought to have significantly altered

the structure of Mediterranean marine ecosystems following its 1984 invasion. The southern California inoculation of *C. taxifolia* is believed to have resulted from the release of aquarium specimens. Besides *C. taxifolia*, other species of *Caulerpa* being sold for aquarium use also may have the potential to invade temperate waters. As a first step towards making this determination, the availability (% frequency) of *Caulerpa* species being sold in southern California for aquarium use was ascertained. Fifty retail saltwater aquarium stores were visited in three southern California counties between November 2000 and August 2001. Sixteen *Caulerpa* taxa were identified from the retail aquarium outlets. *Caulerpa* species were sold in 52% of these stores. *C. taxifolia*, 'Mediterranean form', was offered for sale in 10% of the visited stores; *C. serrulata* var. *hummii* (18%), *C. racemosa* (14%), and *C. racemosa* var. *lamourouxii* (14%) were the most commonly sold species. These data indicate that the aquarium industry is bringing into the region other species of *Caulerpa* besides *C. taxifolia*. Some of these species may also have the potential to invade temperate waters.

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PHYLOGENETIC ANALYSES OF NUCLEAR 18S rRNA GENE SEQUENCES INDICATE THAT THE MASTOPHOROIDEAE (CORALLINALES, RHODOPHYTA) IS A POLYPHYLETIC TAXON

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Nuclear small subunit ribosomal RNA (18S rRNA) gene sequences were determined for six species representing three genera classified within the Mastophoroideae and also for two species of *Amphiroa* (Lithophylloideae). These data were combined with previously published 18S rRNA sequences for 37 other coralline species and analyzed to: (1) determine the phylogenetic position of the Mastophoroideae within the Corallinales; and (2) examine relationships among *Hydrolithon*, *Metamastophora*, *Neogoniolithon*, and *Spongites*. Trees derived from parsimony and maximum likelihood analyses of these data indicate that the Mastophoroideae is not monophyletic. Instead, our data suggest that group is polyphyletic and includes species belonging to three (or more) distinct evolutionary lineages. The nongeniculate genus *Neogoniolithon* is resolved as sister to the geniculate subfamily Corallinoideae. *Hydrolithon* is resolved as sister to the geniculate genus *Metagoniolithon*, an Australian endemic. In turn, *Spongites* is positioned at the

base of a clade including *Hydrolithon/Metagoniolithon* as well as species classified in the Lithophylloideae. Finally, *Metamastophora* is placed in a well supported, but isolated, position as sister to all other taxa bearing uniporate tetrasporangial conceptacles. Our results imply that some reproductive and vegetative characters among the suite of features used to delimit the Mastophoroideae were acquired independently and are not homologous. Significantly, our results suggest that genicula are nonhomologous structures that evolved independently in four separate coralline taxa and from four nongeniculate ancestors belonging to distinct evolutionary lineages. On the basis of our findings *Porolithon pachydermum* is transferred to *Hydrolithon* as *H. pachydermum* (Foslie).

32

NEW INSIGHTS IN THE CRYPTONEMIALES–RHODYMENIALES COMPLEX AND RESURRECTION OF THE ALLIED RED ALGAL ORDER NEMASTOMATALES KYLIN 1925 AS INFERRED FROM *rbcL* SEQUENCE ANALYSIS AND COMPARATIVE REPRODUCTIVE MORPHOLOGY

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In a global molecular phylogeny of florideophycean red algae inferred from chloroplast-encoded *rbcL* sequence analysis, a major monophyletic assemblage comprises the Cryptonemiales (=Halymeniales), the Rhodymeniales, the Schizymeniaceae (*Schizymenia*, *Titanophora*, *Platoma*) and the Nemastomataceae (*Nemastoma*, *Predaea*). The phylogenetic significance of the auxiliary cell and its interaction with the fertilized egg cell in this assemblage is discussed in relation to established and newly proposed classification schemes. The order Nemastomatales Kylin 1925 is reinstated and emended to contain the nonprocarpic Schizymeniaceae and Nemastomataceae. Unifying characters of the Nemastomatales include fertilized carpoconidia that may establish fusions with carpogonial nutritive cells prior to the formation of septate connecting filaments, and simple gonimoblasts developing outwardly from auxiliary cells or from connecting filaments in their vicinity. The auxiliary cell is a transformed vegetative intercalary cell (Sebdeniaceae), that becomes surrounded by either clusters of nutritive cells (Nemastomataceae), involucrel filaments (Schizymeniaceae) or by three-dimensional ampullary filaments (Halymeniaceae including the Corynomorphaceae), or is part of a procarp (Rhodymeniales). The homology of outward gonimoblast initiation and maturation into a simple ball of carposporangia in the Cryptonemi-

ales, Rhodymeniales and Nemastomatales will be illustrated.

33

GRATELOUPIA TURUTURU (HALYMENIACEAE, RHODOPHYTA): THE CORRECT IDENTITY OF THE INVASIVE SPECIES IN THE ATLANTIC KNOWN AS GRATELOUPIA DORYPHORA AS INFERRED FROM MOLECULAR AND MORPHOLOGICAL EVIDENCE

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Grateloupia doryphora (Montagne) Howe, originally described from Peru, has repeatedly been reported as an invasive species in Atlantic and Mediterranean waters. Various attempts to explain this species' route of invasion have, so far, been unsatisfying. In the present paper, we provide new evidence on the basis of comparative *rbcL* sequence analysis and morphology that this invasive species in the NE and NW Atlantic corresponds to *G. turuturu* Yamada, originally described from Japan. The true origin of this alga follows a well-recognized trend of invasive marine organisms that have on various occasions colonized the Atlantic and Mediterranean Sea from Pacific NE Asia.

34

EFFECTS OF OUTGROUP SELECTION ON PHYLOGENETIC HYPOTHESES OF THE HETEROKONT ALGAE

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Nuclear ribosomal SSU and chloroplast *rbcL* data for heterokont algae and potential outgroups were analyzed separately and together under parsimony. A series of taxon sampling experiments were performed. Traditional heterokont classes (e.g. diatoms, phaeophytes, etc.) were monophyletic in most analyses of either dataset and in both together. Relationships among classes and of heterokonts to outgroup taxa are sensitive to taxonomic sampling. Stability of heterokont algae relationships was analyzed by deletion of outgroup classes, starting with those presumably most distant from the heterokonts to those closest. In only three analyses using SSU data were the heterokonts monophyletic: with all available outgroup taxa, with the outgroup being hyphochytrids alone or with the outgroup being hyphochytrids plus *Developayella*. For *rbcL* data, heterokonts were monophyletic when all

available rhodophytes were used, when any combination of rhodophytes tested were used, and when rhodophytes plus bacteria were used. Any addition of cryptophyte and/or haptophyte sequence resulted in a nonmonophyletic heterokont algae. In both cases, distribution of heterotrophic stramenopiles among the heterokont algae changed as different outgroups were used. Combined data was similarly equivocal about monophyly of the heterokont algae. The literature suggests that this result is obtained regardless of whether parsimony or likelihood methods are used, and that is because taxon sampling affects all inference procedures. We suggest not only that more molecular data be obtained, but that morphological evidence for monophyly of the heterokonts be re-examined. Finally, a community effort needs to be made to synchronize character sampling across different datasets to increase the number of taxa sampled and to create parallel datasets for all data types.

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SPECIES DIVERSITY OF MICROBIAL COMMUNITIES IN A NORTHERN TEMPERATE HUMIC LAKE

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Species richness and species diversity of bacterioplankton, phytoplankton and protoplankton were examined in Crystal Bog, a humic lake in northern Wisconsin, as part of an NSF sponsored Microbial Observatory Program. Crystal Bog is part of the North Temperate Lakes Long-Term Ecological Research site. Automated ribosomal intergenic spacer analysis (ARISA) was used to assess bacterial diversity. Phytoplankton and protoplankton were enumerated in a settling chamber with an inverted microscope. Four distinct phases in the ice-free season of the bog can be recognized based on microbial populations. The first phase extends from ice-out until about the first week of April. During this phase three species of dinoflagellates come to dominate the phytoplankton, with *Glenodinium quadridens* representing 60% of total phytoplankton biovolume. By mid-April the spring dinoflagellate blooms collapse. The second phase is a period of instability as a series of species dominates the phytoplankton. In mid-July dinoflagellates again dominate the third phase, and *Peridinium limbatum* alone represents 90% of the bog phytoplankton. The fourth phase is a stable period as *P. limbatum* slowly declines in late summer and fall. A strong correlation

was found between bacterial and phytoplankton diversity (Shannon–Weaver Index) in this bog, suggesting the two microbial communities are linked. The protoplankton, however, were relatively uniform throughout the ice-free period. The ice-covered period may be treated as a fifth phase. When snow accumulates over the ice-covered bog, light penetration is blocked and photosynthesis shut off. Heterotrophic protozoa assume dominance in the plankton.

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PHYLOGENY OF THE GRACILARIACEAE (GRACILARIALES, RHODOPHYTA) INFERRED FROM *rbcL* SEQUENCE ANALYSIS: TAXONOMIC IMPLICATIONS

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This research promotes the correlation of molecular phylogenies and morphological data with biogeographic hypotheses for the economically important agarophyte family Gracilariaceae. The generic concepts are evaluated on the basis of chloroplast-encoded *rbcL* sequences from 150+ specimens worldwide. The results confirm the monophyly of the family and identifies three large clades, one of which corresponds to the ancestral, antiboreal genera *Curdiea* and *Melanthalia*, one to *Gracilariopsis*, and one to *Gracilaria sensu lato* which identifies at least nine distinct independent evolutionary lineages, including *Hydropuntia*. The species currently attributed to *Hydropuntia* comprise two well-supported clades with different biogeographic patterns, one composed of Indo-Pacific species and the other of atlantic species. At least the three most basal clades within *Gracilaria sensu lato* deserve to be considered distinct genera: (1) a new genus centered around *G. chilensis* and *G. tenuistipitata*; (2) *Hydropuntia sensu stricto* encompassing pacific species (*G. urvillei*, *G. eucheumatoides*, *G. edulis*), and (3) a new genus composed of atlantic species currently placed in *Hydropuntia* (*G. cornea*, *G. crassissima*, *G. usneoides*). Cystocarp features within the *Gracilaria sensu lato* clades are more phylogenetically informative than do male characters. The henriquesiana-type of spermatangial conceptacle appeared independently at least four times. The textorii-type of male apparatus is represented in two distinct clusters of *Gracilaria*. Several unknown and poorly described species are being described, resurrected from synonymy or re-circumscribed. *G. textorii* seems to be part of a major complex of flat species in the Pacific

Ocean. The current number of Gracilariaceae is underestimated in the Western Atlantic due to convergence in habit type and the apparent homoplasy in vegetative and reproductive anatomy.

37

THREE NEW SPECIES OF *GRACILARIOPSIS* (GRACILARIALES, RHODOPHYTA) FROM THE NORTH-WESTERN ATLANTIC

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A recent molecular phylogenetic study inferred from chloroplast-encoded *rbcL* sequence analyses based on 20 specimens of *Gracilariopsis*. Dawson 1949 worldwide confirmed the monophyly of the genus and revealed the existence of three new Caribbean species (Gurgel et al. J. Phycol., submitted). *Gracilariopsis* sp. 1 collected in the Mexican Gulf of Mexico is sister to *G. tenuifrons*. *Gracilariopsis* sp. 2 collected in Caribbean Panama (Colón City) and Venezuela (Paraguana Península) is morphologically closely related to *G. panamensis*. *Gracilariopsis* sp. 3 collected in Venezuela (La Vela de Coro) is distinct from all other terete and stringy species that characterize the genus by being a flat-foiose taxon; it also shows a basal topology with uncertain phylogenetic relationship to an undetermined species from Japan and China and to *G. heteroclada* from the Philippines. The lack of clear-cut vegetative and reproductive features among the species coupled with a high degree of thallus plasticity may account for the fact that genetically distinct species were not previously recognized as taxonomically distinct. Our molecular and morphological studies reveal that the diversity of *Gracilariopsis* species in the western Atlantic and probably in the rest of the world is currently underestimated. Morphologically detailed descriptions for all three new species are provided.

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THE PLASTID OF DINOPHYSIS (DINOPHYCEAE): PHYLOGENETIC EVIDENCE FOR A PERMANENT REPLACEMENT

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In the photosynthetic dinoflagellate *Dinophysis*, pigment composition and ultrastructural characters place the origin of the chloroplast with the cryptophytes. Presented here is the first molecular data from the plastid of *Dinophysis*. We cloned and sequenced the coding regions of *psbA* and small subunit (SSU) rDNA from four populations of *D. acuminata* and from *D. acuta*. These data were used to test two hypotheses about *Dinophysis* plastid evolution: (1) The plastid is of cryptophyte origin; and (2) The plastid is a permanent replacement. The *psbA*-coding region was identical among the populations of *D. acuminata* and between this species and *D. acuta*. On the other hand, the SSU rDNA showed polymorphisms, both between species and among populations. Phylogenetic analysis shows that both *psbA* and SSU rDNA sequences firmly place the *Dinophysis* spp. plastid within the cryptophytes, confirming its origin through plastid replacement. The polymorphic SSU rDNA sequences group together and are sister to a cryptophyte ultraplankton (OCS20). The SSU rDNA sequence heterogeneity between species (about 1%) is comparable to the heterogeneity among different isolates of *D. acuminata*, suggesting these are population-level differences and not indicative of different plastid captures by *Dinophysis*. Interestingly, a second class of cloned coding regions was also isolated from each population. These *psbA* and SSU rDNA sequences were evolutionarily more divergent and specifically related to florideophyte red algae. Given the mixotrophic habit of *Dinophysis*, the possibility arises that this second class may have come from red algal preys in the food vacuoles of our single-cell isolates. These results highlight the importance of sampling multiple genes and populations in determining the complex evolutionary history of the *Dinophysis* plastid.

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BIOASSESSMENT OF STREAM-WATER QUALITY USING BENTHIC AND PLANKTONIC ALGAE COLLECTED ALONG AN URBAN INTENSITY GRADIENT IN THE EASTERN CORNBELT PLAINS ECOREGION, OHIO, USA

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Algae possess many attributes that make them ideal organisms to employ in water-quality investigations. They are ubiquitous, ecologically important, and sensitive to a broad range of stressors; moreover, they respond rapidly to changes in water quality. As part of the USGS National Water Quality Assessment (NAWQA)

Program, two methods of analyzing stream-dwelling algae were used to assess relations among nutrient concentrations, algae, and overall biological integrity of streams along a gradient from agricultural to urban land cover. One method is based on algal production (chlorophyll *a*, biomass, and 48-h measures of dissolved oxygen), whereas the other is based on algal community assemblages (species abundance, biovolume, and autecology). Quantitative periphyton and phytoplankton samples were collected from 30 stream reaches in small watersheds (drainage area 7.7–188.7 km²) in south-west Ohio, during July 2000. Phytoplankton Chl *a* ranged from 0.5 mg/L in an urban area to 43 mg/L in an agricultural basin, whereas the epilithic periphyton Chl *a* ranged from 2 mg/m² in an urban area to 142 mg/m² in an agricultural basin. Spearman correlations were used to indicate relations between the physical, chemical, and biological factors and measures of algal production. Multivariate analysis of algal species abundance and metrics will be used to characterize gradients of algal community response relative to chemical and landscape factors. Results will be discussed in relation to the influence of urban land-use intensity and the US Environmental Protection Agency nutrient criteria proposed for this region. The extent to which the algal production and community structure results are useful for bioassessment of stream health will be evaluated with regard to understanding community responses to organic enrichment, dissolved oxygen, specific conductance, light availability, pesticide concentrations, and the monitoring of watershed management practices.

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A NEW METHOD FOR STUDYING NUTRIENT LIMITATION OF PERIPHYTON: A CASE STUDY FROM ACID MINE DRAINAGE STREAMS

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Acid mine drainage (AMD) affects about 16 900 km of streams in the Appalachian region, but little is known about the biology of these habitats. Typically, these habitats have low primary productivity most likely due to metal stress. However, these streams are also very low in nutrients. Traditionally, nutrient limitation studies of periphyton have been conducted employing terracotta pots. Recently, research has concluded that this methodology may have inherent flaws due to variable porosity. Therefore, development of new nutri-

ent diffusing substrates is needed. To test a new approach for nutrient diffusion, Petri dishes were filled with agar and the nutrient of choice. A Whatman glass microfiber filter (GF/F) was attached to the top of the dish, which served as the medium through which the nutrients diffused and as a uniform surface area for periphyton colonization. Before testing in a stream impacted by acid mine drainage, laboratory studies were conducted to simulate field conditions. The Petri dishes readily diffused the nutrients and were durable to the acid conditions. Six replicates with a control, nitrogen (0.5 M), phosphorus (0.5 M), and nitrogen + phosphorus (0.5 M of each) Petri dishes were deployed in four AMD impacted streams for the month of February. Samples were analyzed for chlorophyll *a*, ash-free dry mass (AFDM), and taxa present. The chlorophyll *a* analyses showed the phosphorus treatment to be significantly ($P < 0.05$) greater than all other treatments and the nitrogen treatment was significantly ($P < 0.05$) lower. However, these trends were not seen in the AFDM results with no difference among treatments detected. Seasonal assessment of this approach is currently under way.

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IMPACTS OF REDUCED SALINITY ON SEAGRASSES IN INDIAN RIVER LAGOON

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From November 1993 to November 1995, a study was conducted in Indian River Lagoon (IRL) to determine relationships of light attenuation, water quality, and seagrass at sites representing a range of water quality conditions. There were considerable interannual differences at the sites. The most striking event occurred at a station in northern Banana River, an area of IRL considered the least anthropogenically impacted. This station had a well-developed, multi-species seagrass bed (*Halodule wrightii* and *Syringodium filiforme*, with small quantities of *Halophila engelmannii*). Throughout the first year, salinity was stable (90% of weekly values ranged from 26 to 32 ppt). Beginning in November 1994, the cover, shoot density, and biomass of all three species declined precipitously due to sharply reduced salinities associated with high rainfall and run-off. Salinities during the following year varied little and remained low (90% of weekly values ranged from 13.5 to 19.8 ppt). The lack of recovery to earlier salinity levels was due to the long residence time (about 1 year) in that portion of IRL. Seagrass cover, shoot density, and biomass reached minima in May 1995. By August 1995, *Halodule* began to recover,

but nearly 8 years later, *Syringodium* has not recovered to 1994 levels.

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18S-BASED TAXONOMY AND INTRASPECIFIC ITS SEQUENCE VARIATION IN THE MARINE FUNGAL ENDOSYMBIONT *HALOUIGNARDIA IRRITANS* INFECTING *CYTOSEIRA OSMUNDACEA* ALONG THE CALIFORNIAN COAST

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Abstract withdrawn

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INTRASPECIFIC GENETIC VARIATION IN *H. IRRITANS*, A FUNGAL ENDOSYMBIONT OF MARINE BROWN ALGAE ON THE NORTH AMERICAN PACIFIC COAST

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The marine ascomycete genus *Haloguignardia* occurs endophytically in members of the marine brown algal family Sargassaceae globally. This example of endosymbiosis has been morphologically described: the fungal component internally infects the algal host resulting in prolific cell growth, forming galls composed chiefly of host algal cells but containing fungal reproductive structures and vegetative hyphae. *H. irritans* induces the formation of galls in the brown algae *Cystoseira osmundacea* and *Halidrys dioica* along the Pacific coast from Baja California, Mexico. Using culture-independent molecular techniques, I sequenced the 18S rDNA gene region for *H. irritans* and generated a 18S-based taxonomy consistent with the current taxonomy for this morphological species. In order to study intraspecific genetic variation in *H. irritans*, I have sequenced the ITS rDNA (ITS 1, 2 and the 5.8s) regions for five separate gall-tissue samples from Santa Rosa Island in southern California and for five samples from Monterey and Carmel in central California. Intraspecific DNA sequence variation in the ITS regions of *H. irritans* reveals consistent sequence divergence between sites sampled. The fungal ITS regions for *H. irritans* total 613 bp in length and contain 40 synapomorphic characters for a total of 6.5% variation in informative loci between southern and central Californian sites. This value is similar to

those found for the ITS and other gene regions previously used by researchers investigating species boundaries at the intraspecific level in symbiotic, terrestrial fungi. In addition to ITS 1, 2 and the 5.8s gene regions, I am currently using the 5' end of the EF1a coding region to construct intraspecific genealogies for *H. irritans*. By comparing these genealogies to each other and to the geographic distribution of samples, I aim to determine if more than one genetic species is present within the morphological species *H. irritans*.

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SYNCHROTRON-BASED INFRARED IMAGING OF *EUGLENA GRACILIS* SINGLE CELLS

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Infrared (IR) microspectroscopy is used to examine and identify the chemistry in individual living cells of *Euglena gracilis*. The spectra provide relative concentrations of lipids, proteins and carbohydrates in the specimen. Diffraction-limited, spatially resolved IR images of single cells of the microalga were measured using synchrotron radiation as a bright IR source. These contour maps reveal the distributions of the proteins, lipids and carbohydrates including the phospholipids, sugars and paramylon. We compare the images with the biochemistry for *E. gracilis*. We also present the IR images for two different cells, which were exposed to different culture conditions prior to the measurements. The overall trends observed for the two specimens are in qualitative agreement with the hypothesis that there was a significantly reduced concentration of nitrogen in the culture as a function of time. Importantly, this study demonstrates the potential to examine changes in the chemistry of living cells while modifying environmental stimuli.

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CELL SIGNALING IN THE CHEMOACCUMULATION RESPONSE OF THE COLONIAL GREEN ALGA *ASTREPHOMENE GUBERNACULIFERA*

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A. gubernaculifera can grow on acetate even in the dark. Interestingly, it displays a well-developed chemoaccumulation response to added acetate. We suggest that this chemoaccumulation results from a receptor-based signaling pathway because the alga responds to the structurally similar molecule propionate even though the latter compound cannot support growth. Based on suggestions about chemotactic signaling pathways in other eukaryotes and postulated phototactic response of some algae, we hypothesized that the signaling pathway involves heterotrimeric G-proteins. The effectiveness of the chemoresponse was determined by measuring the diameter of the chemoaccumulation cloud after addition of 10 mL aliquots of media containing acetate to colonies swimming in media without acetate. Neomycin, which inhibits the G-protein dependent activity of phospholipase C, completely inhibited chemoaccumulation at 100 mM, and markedly reduced the density of the accumulation response at 10 mM with no obvious changes in general colonial motility. EGTA, a calcium chelator, abolished the chemoresponse. Mastoporan, a G-protein activator, caused accumulation even in the absence of acetate. These results support the hypothesis that chemoaccumulation in *A. gubernaculifera* uses a G-protein mediated, calcium-dependent, cell-signaling pathway.

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MAA SYNTHESIS AND ACCUMULATION IN POLAR MACROALGAE ARE CONTROLLED BY ABIOTIC FACTORS

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Mycosporine-like amino acids (MAAs) are regarded as powerful sunscreens protecting the algae against harmful UV radiation. The MAA protection efficiency was tested in algal samples by measuring the optimum quantum yield of photosynthesis using photosystem II fluorescence. It could be demonstrated that the recovery of photosynthesis after exposure to enhanced UV radiation is faster in individuals with high MAA content. MAAs can be synthesized in several polar macroalgae in response to different radiation conditions. Although MAA induction patterns are very species-specific, some similarities can be found. Field studies indicate that plants from different growth habitats providing distinct radiation climate can be grouped into three physiological categories depend-

ing on their MAA content. The first group (I) includes mainly deep-water species, typically lacking MAAs. The second group (II), algal species found in a broad range of water depths (eu- and sublittoral), which are able to flexibly synthesize and accumulate MAAs. The third group (III) includes supra- and eulittoral taxa, which always contain high MAA concentrations. In laboratory studies, we showed that taxa of group II and III responded in three different ways based on MAA accumulation when exposed to different radiation conditions (PAR, PAR + UVA, PAR + UVA + UVB). Either they: (a) exhibit highest total MAA concentration under the full artificial spectrum; (b) increase their MAA concentration after exposure to PAR and PAR + UVA or (c) MAA concentration declines after exposure to the full spectrum. Our studies have indicated that when coupled with UVR, exposure to temperature fluctuations ranging from 0 to 10 °C also affect MAA biosynthesis.

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AN EXAMINATION OF THE EFFECTS OF A RESERVOIR, LAKE ASHTABULA, ON THE PERIPHYTIC ALGAE IN THE SHEYENNE RIVER, NORTH DAKOTA

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The Sheyenne River is a third order stream that drains a significant portion of central and eastern North Dakota. In 1950, the Baldhill Dam was built and as a result, the reservoir, Lake Ashtabula, was formed. The periphytic algae in this river were extensively studied from 1997 to 1999. During this research, the effects of differences in environmental variables on the algal communities were assessed with Canonical Correspondence Analysis (CCA). In this analysis, the periphytic algal communities in the downstream sites appeared to be quite different from those communities found upriver of Lake Ashtabula. Other research on different rivers has shown profound changes in the algal community after a dam was built along the river. Redundancy Analysis (RDA) was used to determine if the sites downstream are more statistically similar with Lake Ashtabula than with the upstream sites. Finally, CCA was used to examine which environmental variables explain the most variance in Lake Ashtabula and the downstream sites.

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RELATIONSHIP BETWEEN PERIODIC RESUSPENSION EVENTS AND PLANKTONIC DIATOM COMMUNITY STRUCTURE IN LAKE MICHIGAN: A FIELD AND LABORATORY INVESTIGATION

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Lake Michigan provides an ideal location for comparing episodic physical forcing events (storms) on phytoplankton processes and the more persistent seasonal variability of phytoplankton communities. This is due to the duration and extent of the highly turbid, recurrent coastal plume (RCP) in the lake during the winter/early spring. Although the RCP can coincide with the initiation of the basin wide spring diatom bloom, linkages between the duration and intensity of the plume and the prominent role of light availability in regulating Lake Michigan phytoplankton growth during the spring isothermal period have been postulated, but not verified. The concurrent physical and biological events provide a novel opportunity to examine phenomena associated with the RCP affecting the distribution and abundance of species in Lake Michigan's planktonic diatom flora. In this study, planktonic diatom assemblages from pre, post and active spring plume events were examined from stations along Lake Michigan's southern shoreline. Species abundance changed rapidly during storm events. Sediment resuspension via storm activity created a sub-optimal growth environment. Post-storm event diatom phytoplankton communities were floristically distinct from prestorm event communities, with resting cell-forming taxa playing a significant role in these community-restructuring periods. Laboratory simulations of resuspension events using Lake Michigan sediments were conducted under a variety of environmental conditions. Parameters varied included day length, temperature, and silica. The resulting assemblages were quantitatively counted. A statistically significant relationship was identified between day length and the vegetative growth of many resting cell-forming diatom species. When day length was calculated for poststorm event field data, it revealed a high correlation between poststorm event communities and those predicted by the laboratory simulations. Timing of storm events and latitudinal position of the aquatic system are then important elements for consideration when predicting diatom phytoplankton community structure due to the relationship these factors have with day length.

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ESTIMATING DIVERGENCE TIMES WITHIN THE CHAROPHYTA: A BAYESIAN APPROACH USING FOSSIL AND DNA SEQUENCE DATA
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Morphological and molecular studies have advanced our understanding of charophycean green algal relationships. A recent multigene phylogenetic analysis has identified the Charales as the closest living relatives of land plants with the Coleochaetales sister to the Charales/land plant lineage. With a robust phylogeny now in hand, it is possible to explore phylogeny-dependent questions that were previously difficult to assess. Estimating the divergence time of the land plant lineage is one such question. The most recent time estimate for the colonization of land by plants is 1061 ± 109 million years ago and 703 ± 45 million years ago for the divergence of vascular plants and bryophytes, a result much older than that found in the fossil record (roughly 470 million years ago). Several problems are inherent in estimating divergence times using DNA sequence data; these include the assumption of a molecular clock and choosing from a sparse fossil record for an external calibration date. Unlike most algae, a rich fossil record exists for the Charales in the form of calcified oospores termed gyrogonites. Representative gyrogonites that can be attributed to all six of the extant genera in the Characeae have been identified with reasonable accuracy. These six calibration points, identified from a closely related lineage of land plants, can be used in conjunction with a multigene DNA dataset to estimate the time that the land plant and Charales lineages diverged from their common ancestor.

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PUBLIC OUTREACH MATERIALS REGARDING HARMFUL ALGAL BLOOMS AND THEIR POSSIBLE EFFECTS ON HUMAN HEALTH
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The potential interactions between marine and freshwater harmful algal blooms (HABs) and humans are increasing. Humans are exposed through food, drinking water, and recreational and occupational water use to an increasing number of organisms and their toxins. Nevertheless, the amount of clinical and epidemiologic research concerning acute and chronic human health effects from the HAB organisms and their toxins is relatively sparse. At the same time, the public is increasingly aware of and interested in the potential dangers associated with exposure to HABs. Public health authorities and researchers must respond to these public health concerns. The development of appropriate educational and outreach materials based on limited scientific databases is the challenge of informing the public concerning the possible human health effects of HAB organisms and their toxins, and their prevention. Educational materials developed by a group of researchers and public health personnel for general HABs, Florida Red Tide Toxins (brevetoxins), Cyanobacteria, and Ciguatera are discussed, as well as methods for their dissemination.

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ENDEMISM AND INVASION IN THE GREAT LAKES DIATOM COMMUNITY

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Population dynamics and health for individual Great Lakes diatom species is explored. Information dealing with how these taxa compete with introduced species and rapid environmental changes in modern times is examined. Diatomists have identified a number of these taxa, but discussion is limited with little or no information covering the ecological range of the taxa over time. Some of these include *Cyclotella americana* Fricke; *C. bodanica* var. *stellata* Skvortzow; and *Stephanodiscus superiorensis* Theriot. A number of taxa endemic to the Great Lakes undescribed in the literature also exist. These are species wedged into taxonomic categories from the European taxonomic system. Recent advances in the understanding of diatom species boundaries, suggest these names are inappropriately used for a number of taxa identified in paleolimnological investigations of the Great Lakes. This problem developed because taxonomic information outside of the European flora is not readily available to researchers, and because the focus of paleolimnological studies is primarily environmental reconstruction minimizing the time that can be spent unraveling taxonomically troublesome species. Examples of these

are *Cyclotella bodanica* var. *glabriscula* and *C. bodanica* var. *oligactis*. Great Lakes populations of these taxa are significantly different from the original populations described from alpine lakes in Austria and Switzerland and merit taxonomic separation. Many of the endemic taxa are no longer present in the modern Great Lakes assemblages. The precise time of their disappearance from the system is not known, but modifications in Great Lakes diatom populations clearly coincided with the development of substantial European settlement of the Great Lakes region. *C. americana*, for example, was always limited to Lakes Erie and Ontario but disappeared from both of these lakes prior to 1900. With the disappearance of these taxa, came a number of introductions to the lakes. This work represents the first attempt to document these floristic changes.

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SYSTEMATICS OF COCCOID GREEN ALGAE: MORPHOLOGY VS. 18S rRNA GENE PHYLOGENY

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Classical approaches using morphological characters for the circumscription of taxa in coccooid green algae do not adequately reflect the phylogenetic relationships. Phylogenetic trees based on 18S rRNA gene sequence analysis provide new insights into the systematics of these algae. We give a review and discuss the content of orders of coccooid green algae and evaluate the usefulness of some traditionally used diacritic features. Whereas the content of the orders Chlorococcales and Chlorellales needs to be reduced, the order Sphaeropleales comprises more families than in the traditional circumscription. According to molecular biological investigations the Neochloridaceae, Selenastraceae and Scenedesmaceae formerly classified in the Chlorococcales and later into the Chlorellales belong to the Sphaeropleales. The Hydrodictyceae, formerly a member of the Protosiphonales, are also to be grouped in the Sphaeropleales. Some members of the Ulotrichales, which propagate by binary fission (e.g. *Nannochloris* and *Catena*) cluster together with autospore coccooid taxa within the Trebouxiophyceae. With the example of members of the Selenastraceae,

Oocystaceae and Chlorellaceae it is shown that the morphology of cells as well as the formation of coenobia, spines, mucilage and incrustations cannot be used to separate genera.

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UV-ACCLIMATION OF CULTURED PHYTOPLANKTON EXPOSED TO NATURAL AND ENHANCED UVB RADIATION AND METHODOLOGICAL ASPECTS OF MAA QUANTIFICATION

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Bloom-forming dinoflagellates often synthesize high concentrations of mycosporine-like amino acids (MAAs) as a protection from high PAR and UV radiation, which may afford them a competitive advantage over other species. Photoprotection and long-term acclimation to natural and enhanced UVB radiation were examined during 4 weeks in three dinoflagellates and one diatom. Semi-continuous, nutrient-replete cultures were maintained outdoors under natural light, with or without additional UVB (from lamps). The photosynthetic performance (XE-PAM fluorometry) and the concentration and composition of MAAs and photoprotective carotenoids (PPCs; comprising xanthophylls) were followed weekly. A great variety of MAAs was found in the dinoflagellates (up to 10, identified by cochromatography and LC-MS). Large differences between sp. (and between two strains of the same sp.) were observed. In most cases, the composition and concentration of MAAs and PPCs increased under enhanced UVB. Growth rate and photosynthetic performance were sometime lower under enhanced UVB, while in other cases photoacclimation seemed to occur. The least affected sp. was *Alexandrium tamarense* who responded most rapidly to enhanced UVB. The algae also responded to changes in natural incident radiation. Some methodological aspects of MAA quantification were also investigated (with four species of dinoflagellates). Our results indicate how freezing (liquid-N) likely breaks the cells, mobilizing the water-soluble MAAs in the filter moist (otherwise highly packaged in fresh cells), while the organelles containing the pigments remain relatively intact. The implications of standard sample handling on the evaluation of MAA concentration in algae and on the estimation of the absorption of suspended ma-

terial in water from *in vivo* spectroscopy (filter pad technique) will also be discussed.

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PRESERVATION OF NOSTOCACEAN HORMOGONE MOTILITY IN DESICCATED CALCIUM CARBONATE AGAR PATCHES

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Filamentous nitrogen fixing cyanobacteria are resistant to desiccation, nutrient depletion, adverse temperature and challenging photic environments. This is generally thought to account for their role as pioneering forms of life on denuded strata. It has also lent credence to the possibility that cyanobacteria with prolonged viability may have been able to survive conditions of interplanetary space to implement a panspermian hypothesis for the origin of life on earth. Prolonged retention of viability had been noted in our collection of myxotrophically grown strains of nostocaceans cultured in liquid or solid media containing calcium carbonate. Such media permitted satisfactory recovery and regrowth after storage at low light intensities for 2 years or more. Moreover, even the oldest calcium carbonate cultures contained motile hormogonia when observed microscopically. This phenomenon can be used to advantage in sending cultures to other laboratories. Not only are organisms maintained during passage through the postal system but since the cultures formed hormogonia readily, it was possible to produce preservable homogeneous agar lawns that generated hormogonia as needed for demonstration or experiment. A practical technique consists of seeding axenic strains of *Nostoc* species grown in liquid shake culture under cool-white fluorescent illumination into solid media containing 0.7% sucrose, 0.05% finely divided calcium carbonate and 1.4% purified agar. The preparations are incubated under red fluorescent illumination to produce several crops of hormogonia in successive cycles of development, within the agar. The heavily grown lawns are cut into disks or squares, then transferred to dishes and dried in desiccators with anhydrous calcium sulfate. The dry patches produce swarms of hormogonia in 2 or 3 h, depending upon the rate of rehydration. The patches are facile tools for purifying cultures through the isolation of hormogonia and studying cyanobacterial physiology by the rapid response of motile hormogonia.

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FATTY ACID AND STEROL COMPOSITION OF A *KARENIA BREVIS* BLOOM IN THE GULF OF MEXICOLeblond, J. D.^{1,*} & Chapman, P. J.²

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In the Gulf of Mexico, recurring algal blooms caused by *Karenia brevis* (formerly known as *Gymnodinium breve*) have significant adverse health and economic impacts. *K. brevis* is one member of a small group of dinoflagellates, related morphologically and by DNA-based phylogenetic analysis, that synthesize the carotenoid, gyroxanthin diester, in place of the more widely distributed peridinin. While this novel photopigment has been proposed as a biomarker, especially for remote-sensing imaging technologies, to detect the emergence of *K. brevis* blooms, other chemicals such as sterols and triglycerides, respectively, with potential to report the distribution and physiological condition of *K. brevis* are required. Recent work from our laboratories characterizing the lipids of dinoflagellates has confirmed that *K. brevis*, together with those few close relatives lacking peridinin, possesses a relatively simple sterol profile comprised of two unusual primary 4-methyl sterols, designated ED and NED, each with an ergosterol-type side chain. A recent dinoflagellate bloom in the waters of the north-west Gulf of Mexico near the Gulf Breeze EPA laboratory provided an opportunity to examine the usefulness of these sterols and other lipids as indicators of *K. brevis* in phytoplankton communities. Lipid extracts of filtered bloom samples, fractionated to separate free and esterified sterols, were examined by GC-MS of trimethylsilyl ether derivatives. ED and NED were the major sterols found in all bloom samples. Fatty acids found in lipid fractions containing membrane phospholipids, chloroplast-associated glycolipids, and storage triglycerides, respectively, differed significantly. The glycolipid fraction was found to contain octadecapentaenoic acid [18:5(n-3)], a fatty acid commonly associated with dinoflagellates. The phospholipid fraction was found to contain small amounts of the recently described highly unsaturated fatty acids, octacosaoctanoic acid [28:8(n-3)] and octacosahptaenoic acid [28:7(n-6)]. Fatty acids from the triglyceride fraction were more abundant than those associated with glycolipids or phospholipids.

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LIPID BIOMARKER ANALYSIS OF THE TOXIC DINOFLAGELLATE *PFIESTERIA PISCICIDA*: DISTRIBUTION OF STEROLS AND FATTY ACIDS WITHIN THE CLASS DINOPHYCEAELeblond, J. D.^{1,*} & Chapman, P. J.²

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Within US waters, regular blooms of harmful dinoflagellates occur in the Gulf of Mexico and Chesapeake Bay regions. Although the causes of blooms are not fully understood, events in Gulf of Mexico waters have been recorded for over 30 years, and are almost exclusively caused by the photosynthetic dinoflagellate, *Karenia brevis*. Conversely, blooms in the Chesapeake Bay region, caused by the heterotrophic dinoflagellate *Pfiesteria piscicida*, appear to be a relatively new phenomenon, and are hypothesized to be linked to an increase in nutrient-rich runoff from chemical plants and livestock farms along tributaries leading into the Chesapeake. Despite the environmental, economic, and human health relevance of *P. piscicida*, there appears to be no published work on the characterization of its lipid composition. The objective of this study was, therefore to examine the sterol and fatty acid composition of cultured *P. piscicida* isolates as a precursor to eventual field studies of blooms of this organism. GC-MS analysis of trimethylsilyl ether derivatives of sterols in free sterol and sterol ester fractions revealed that the overwhelming majority of sterols was found as free sterols. Furthermore, free sterols were found to resemble those of other dinoflagellates, with the dominant compound being the common dinoflagellate sterol, dinosterol; a number of other 4-methyl sterols common to other dinoflagellates were also identified. The fatty acids of *P. piscicida* were found to be primarily associated with a fraction containing cellular membrane phospholipids; small amounts of the recently described highly unsaturated fatty acids, octacosaoctanoic acid [28:8(n-3)] and octacosahptaenoic acid [28:7(n-6)], were observed in this fraction. The dominant fatty acid (approximately 40–60% of the phospholipid fatty acids) was docosahexanoic acid [22:6(n-3)]. Unlike *K. brevis* (see accompanying poster), *P. piscicida* does not appear to possess significant amounts of unusual sterols or fatty acids which may serve as potential biomarkers for this organism.

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THE TRANSITION FROM ALGAE TO EMBRYOPHYTES: CHLOROPLAST PHYLOGENOMIC EVIDENCE (II)

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The transition of plant life from aquatic algae to land plants was one of the major events in the history of life. However, in hypothesizing the evolutionary path of the transition, limited shared phenotypic characters in aquatic algae and land plants (embryophytes) have been a major hinderance. Chloroplast genomes contain characters useful in tracing evolutionary histories. Embryophyte chloroplast genomes are distinguished from algal cpDNAs by the presence of over 20 group II introns and three ribosomal protein operons (*rpl23*, *clpP* and *3CERps12* operons). These phylogenomic features indicate a phylogenetic relationship of charophytes and embryophytes. In addition to these operons and introns, the evolution of rRNA and *psbB* operon evolution of streptophyte lineages will be incorporated with major biological phenotypic features to produce a phylogenetic tree. Basal embryophytes, the antithetic hypothesis, monophyly of embryophytes, and paraphyly of charophytes will be discussed. Streptophytes are classified into three major groups (basal streptophytes, mid-divergent streptophytes and late divergent charophytes-embryophytes).

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ACTIN PHYLOGENY IN THE CHAROPHYTA

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Photosynthetic green plants, the Viridiplantae, are composed of two distinct lineages, the Chlorophyta and Charophyta. The Chlorophyta include the Chlorophyceae, Trebouxiophyceae, Ulvophyceae and most prasinophytes. The Charophyta include the Mesostigmatales, the traditional charophycean green algal orders Chlorokybales, Klebsormidiales, Zygnematales, Coleochaetales and Charales, and land plants, referred to here as Embryophyceae. The Embryophyceae are embedded within the paraphyletic charophycean green algae, so land plants are, therefore best considered a specialized group of green algae adapted to terrestrial life. Due to the close evolutionary relationship between green algae and Em-

bryophyceae, characterizing the early evolution and diversification of land plants depends on understanding phylogenetic relationships among the less extensively studied green algal members of the Charophyta. Relationships within the Charophyta have previously been based on morphology and inferred through phylogenetic analyses of molecular data that include ribosomal DNA and several genes from the chloroplast and mitochondrial genomes. To this point, however, few phylogenetic analyses of nuclear, protein-coding genes have been conducted. We are attempting to isolate and sequence actin-coding regions from representative members of the Charophyta. Actin is a highly conserved protein that is ubiquitous among eukaryotes; these features make actin an ideal subject for comparative sequence analysis. A major component of the cytoskeleton, actin plays a number of important roles in plant cell processes such as cell division and cytoplasmic streaming. Hence, in addition to being potentially useful for resolving organismal relationships, the evolution of the actin protein itself is intrinsic to the study of morphological evolution and development within green plants.

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NUMEROUS TRANSITIONS TO LAND IN GREEN PLANTS: THE 'OTHER' LAND PLANTS

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In addition to the more conspicuous embryophyte group, numerous other green plant lineages have made successful transitions to land. Phylogenetic analyses using molecular data indicate that these lineages are primarily derived from freshwater ancestors, and include free-living and lichen photobiont algae in the class Trebouxiophyceae, and several distinct lineages of free-living algae in the class Chlorophyceae. In all cases, these independently derived land plants must possess adaptations for environments with reduced water availability, oxidative stress, and different light regimes than experienced by their aquatic ancestors. In the most extreme cases, these algae are living in very arid habitats. Given the monophyly of green plants and the evolution of independent terrestrial lineages from aquatic ancestors, these lineages represent natural experiments in the transition of green photosynthetic organisms to terrestrial living, and offer a rich source of taxa that can be compared experimentally with the embryophytes.

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CHLORORESPIRATION IN GREEN ALGAE ISOLATED FROM DESERT CRUSTS

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Photosynthetic organisms enduring extreme temperatures, low water availability, or high light require photoprotective mechanisms to prevent sustained damage to photosynthetic machinery. Green microalgae living in desert crust communities of the south-western US experience all these environmental stresses, yet photophysiological studies of green algae in the literature have focused on only a handful of common aquatic and marine species. We are examining the variation in green algal photoprotective mechanisms that is the result of natural selection acting independently in multiple lineages of highly diverse desert green algae (Chlorophyta) within the classes Chlorophyceae and Trebouxiophyceae. We have found that unusually extensive dark reduction of the plastoquinone pool is a prominent photophysiological feature among these desert algae; this reduction may be linked with enhanced chlororespiration. Recently, chlororespiration in higher plants has been linked through mutant analysis to control of the carotenoid synthetic pathway, heat stress, and starch metabolism among other pathways, though the function of chlororespiration remains controversial. Given that green algae and higher plants are monophyletic, analysis of potential chlororespiration in desert green algae may help decipher the evolution of the chlororespiratory process as well as its potential role in photoprotection in desert habitats.

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BRANCHING MORPHOLOGY OF GAMETOPHYTES AND TETRASPOROPHYTES OF *CERAMIUM CODICOLA* IS RELATED TO PHASE AND REPRODUCTIVE STRUCTURES

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Most red algae with a *Polysiphonia*-type life history are regarded as having isomorphic phases. However, some show minor deviations in morphology, including differences in branching reported in female gametophytes and tetrasporophytes of *Ceramium*. By sampling populations of *Ceramium codicola*, an epiphyte of *Codium fragile*, it was possible to characterize the morphology of female and male gametophytes

and tetrasporophytes. Branching pattern was characterized by the Strahler method, in which terminal branches are counted as primary branches, and higher order branches are counted where lower order branches meet. Since *Ceramium* shows dichotomous branching, this method can be used to characterize the degree in which branching is dichotomous. The angle of branching was also measured. Female gametophytes had a higher ratio of primary to secondary branches (3.6–5.1) than male gametophytes (2.5) and tetrasporophytes (2.5–2.6), which have a similar ratio to each other. This indicates that males and tetrasporophytes have a more dichotomous pattern of branching while female gametophytes have more proliferative primary branches. The differences in branching pattern appear to be related to the location of reproductive cells, which occur along the surface of branches in male gametophytes and tetrasporophytes, but occur near the apices in female gametophytes. The branching angle of female and male gametophytes is similar to each other, and greater than that of tetrasporophytes. Therefore, gametophytes and sporophytes differ in branching angle.

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APOGLOSSEAE TRIB. NOV. (DELESSERIAEAE, RHODOPHYTA) BASED ON TWO MOLECULAR DATASETS AND CYSTOCARP DEVELOPMENT

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The genus *Apoglossum* J. Agardh has traditionally been placed in the tribe Delesserieae. Our morphological observations on *Apoglossum* and the 'Delesseria' species belonging to the resurrected genus *Paraglossum* from the Southern Hemisphere show that they possess a distinct procarp. *Apoglossum* has two one-celled sterile groups that divide after fertilization and *Paraglossum* consists of two one-celled sterile groups that do not divide after fertilization, but in which the nuclei divide to produce four to eight nuclei in the undivided sterile groups. In contrast, the procarp in *D. sanguinea*, the type species of the tribe Delesserieae, consists of two 2–6-celled sterile groups before fertilization and the number of sterile cells double after fertilization. The development of the carposporophyte in both *Apoglossum* and *Paraglossum* is shown to be unique in the subfamily Delesserioideae. The pit connections between the segments of the gonimoblast filaments

broaden without fusion and secondary carposporangia are produced. This pattern is absent in other members of the Delesserioideae, but has been reported for the tribes Myriogrammeae and Schizoserideae in the new subfamily Phycodryioideae Lin, Fredericq et Hommersand. Based on both LSU rDNA and *rbcL* sequence analyses, the Delesserieae is restricted to species of *Delesseria* and *Membranoptera* from the North Atlantic and North Pacific Oceans. In contrast, the species of *Apoglossum* and *Paraglossum* form a distinct cluster having a center of distribution in the Southern Hemisphere with only a few species, such as *A. ruscifolium*, reaching the Northern Hemisphere. In this study, details of postfertilization development leading to the cystocarp are illustrated for the type species of *Apoglossum* and *Paraglossum* and a new tribe, the Apoglosseae, is recognized in the Delesserieaceae.

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PHRAGMOPLASTINS, THE TRENTEPOHLIALES, AND THE EVOLUTION OF THE CYTOKINESIS IN GREEN PLANTS

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Green plants have been shown to have a common ancestor and to contain two lineages: the Chlorophytes and the Streptophytes, both encompassing representatives of the original 'green algae'. The phragmoplast-mediated cell division characterizes the land plants in the Streptophyte lineage and some basal green algal orders (Coleochaetales, Charales and Zygnematales). However, a well-developed phragmoplast-type cell division has been documented in two subaerial green algae (*Cephaleuros parasiticus* and *Trentepohlia odorata*) belonging to the order Trentepohliales, an order that molecular sequence data place unequivocally within the Chlorophytes (rather than Streptophytes). Is the phragmoplast-mediated cell division of the Trentepohliales a case for homology or nonhomology? In order to reveal the answer to this question, we are exploring the potential phylogenetic information inferred from gene sequences of phragmoplastin, a dynamin-like protein which has been demonstrated to be associated with cell plate formation during the phragmoplast-mediated cytokinesis in land plants. Primers were designed based on an available phragmoplastin sequence from soybean, and yielded PCR amplifications from the green algae (*Trentepohlia* and *Cephaleuros*), a bryophyte (*Bazzania*) and land plants (*Glycine* and *Arabidopsis*). The evolutionary his-

tory of the development of the phragmoplast may remain in the analysis of the gene sequences for this group of proteins.

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MOLECULAR SYSTEMATICS OF THE SUBAERIAL GREEN ALGAL FAMILY TRENTEPOHLIACEAE (CHLOROPHYTA; TRENTEPOHLIALES) INFERRED FROM 18 SSU rDNA SEQUENCES

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Representatives of the monophyletic Trentepohliales are widely distributed in the tropics, subtropics, and temperate regions worldwide. They grow in soil, or are epilithic, epiphytic or endophytic. The family comprises approximately 70 species placed in at least four genera (*Trentepohlia*, *Cephaleuros*, *Phycopeltis* and *Stomatochroon*), with *Trentepohlia sensu lato* (including *Printzina* and *Physolinum*) accounting for half of the species in this family. PCR amplification and sequencing of the 18 SSU rDNA of 18 isolates of the Trentepohliaceae were used to assess the monophyly of the genus *Cephaleuros* and to determine the phylogenetic relationships among species of *Trentepohlia sensu lato*. Distance, Parsimony, and Maximum Likelihood analyses indicate that *Trentepohlia sensu lato* is basal and includes two species recently transferred to *Phycopeltis* (*P. umbrina*) and *Printzina* (*P. lagenifera*). In contrast, *Cephaleuros* is a derived monophyletic clade. Analysis of isolates of *C. virescens*, from the USA, Taiwan, and South Africa indicate that this taxon may consist of different species sharing a convergent morphology. The results of this study have implications for the taxonomy of the genera.

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BIODIVERSITY AND POTENTIAL USE OF MARINE MACROALGAE FROM THE OFFSHORE OIL PLATFORMS IN THE GULF OF MEXICO

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In the north-western Gulf of Mexico, more than 3000 oil and gas platforms are supporting a significant community of marine organisms in an area wherein natural hard substrata are scarce. This preliminary study is designed to provide information on the algal component in terms of species diversity of marine red, brown and green macroalgae and cyanobacteria colonizing these artificial reefs. The vertical, horizontal and seasonal distribution and relative abundance are being documented. Taxonomic results are being evaluated for potential use of some of the algae present as sources of natural products for the biotechnological industry. The island character of this steel archipelago in the north-western Gulf of Mexico provides an ideal environment to test biogeographical relationships with adjacent floras. This research is supported in part by the Minerals Management Service, US Department of the Interior.

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CHARACTERIZATION AND POSITION OF THE GENUS *STENOGRAMME* IN THE PHYLLOPHORACEAE (GIGARTINALES, RHODOPHYTA)

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The red algal genus *Stenogramme* was described by Harvey (1840) for entities having flattened, dichotomously branched thalli with cystocarps borne in narrow, linear sori resembling an interrupted midrib on blades, and with isomorphic tetrasporophytes with scattered circular to irregular tetrasporangial nemathecia. In the current literature, the most common species listed is *Stenogramme interrupta* (C. Agardh) Montagne ex Harvey 1848, said to have a worldwide distribution in temperate seas. DNA sequences from three molecular datasets (chloroplast-encoded *rbcL*, and nuclear-encoded ITS and LSU rDNA) instead reveal that the genus is more species-rich than is currently appreciated, and that several species go under the name *S. interrupta*. The latter species, described from Cadiz, Spain, is found to be restricted to the Eastern Atlantic. The name *Stenogramme californica* Harvey 1840, the type species described from the Pacific coast of North America, is reinstated for members from

California and Oregon. *S. rhodymenioides* Joly et Alveal 1969 is reinstated for the entity from Chile. The range of *S. phyllophoroides* (J. Agardh) Millar 1990 described from northern NSW, Australia, is expanded to accommodate the taxon from South Australia referred to as *S. interrupta*. *S. leptophylla* J. Agardh 1885 is a distinct South Australian species. A new name needs to be provided for the New Zealand taxon. The genus also comprises a new entity (sterile) from Alaska, and a new (sterile) species from offshore Louisiana. *Stenogramme* is a well-defined monophyletic genus, but its position within the Phylloporaceae remains equivocal.

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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF TWO SEAWEEDS TO NUTRIENT OR LIGHT DEPRIVATION

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The algae *Fucus vesiculosus* (brown) and *Enteromorpha compressa* (green) were cultured in long-term experiments in which natural seawater without nutrient enrichment was used, or in which the plants were kept in complete darkness. Measurements of variable fluorescence (F_v/F_m), an indicator of photosynthetic competence, showed a gradual decrease of F_v/F_m in nutrient-deprivation experiments, varying from 0.64 to 0.25 (*E. compressa*) and from 0.65 to 0.40 (*F. vesiculosus*) after 6 months of cultivation. *F. vesiculosus* was virtually unaffected after more than 2 months in darkness, showing values of F_v/F_m around 0.65 throughout the experiments, but cultures of *E. compressa* collapsed after 45 days in darkness. In both species lower concentrations of chlorophyll *a* resulted of nutrient-deprivation experiments, decreasing three times in *F. vesiculosus* and 25 times in *E. compressa* by the end of the experiments. *F. vesiculosus* cultured in darkness showed 50% more chlorophyll *a* than the control, while plants of *E. compressa* kept in dark and in light showed similar values of chlorophyll *a* during the first 30 days of cultivation. The tissue-N content of both species decreased dramatically in nutrient-deprivation experiments, establishing variations in C:N ratio from 20:1 to 79:1 (*F. vesiculosus*) and from 8.6:1 to 75:1 (*E. compressa*). The algae showed small variations in C:N ratio throughout the experiments in darkness. Such as previously identified for phytoplanktonic species, the variations in the physiological responses exhibited by the seaweeds tested suggest that these conventional diagnostics of limitations may

be misleading in natural algal assemblages, which are composed by mixed species. Gel electrophoretic is currently being performed to identify possible proteins that may respond specifically to these experimental conditions.

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DISTRIBUTION AND MORPHOLOGY OF DIATOMS IN HIGH ALTITUDE STREAMS ON KAUAI (HAWAII)

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Diatom samples collected from Kawai'oi Stream differ in species composition and relative abundance from those in Kokee Stream. Kuwai'oi collects drainage from the Alakai Swamp, which developed on a 1200–1500 m elevation ridge of Mt. Waialeale extending at a right angle to the NE winds. Kokee lies about 2000 m further west at about 1100 m elevation. *Frustulia* and *Eunotia* species characteristic of low conductivity waters predominate. Greater diversity of *Achnanthes*, *Navicula*, *Pinnularia* and *Surirella* taxa occurs in the somewhat more conductive waters of Kokee. The morphological species observed bear a close, but not exact, resemblance to mainland taxa described in the literature. Morphological variations are documented using light and scanning electron microscopy.

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PHYLOGENY OF THE CONJUGATING GREEN ALGAE (ZYGNEMATALES AND DESMIDIALES) BASED ON PLASTID AND MITOCHONDRIAL GENE SEQUENCES

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The conjugating green algae (Order Zygnematales and Desmidiaceae) are charophyceans, that is, they are among the green algae most closely related to land plants. Compared to the five other groups of charophyceans (Mesostigma, Klebsormidiales, Chlorokybales, Coleochaetales, Charales), the conjugating greens are by far the most diverse taxonomically (6 families, about 55 genera, 3–4000 species) and

very widespread in distribution (including common species such as *Spirogyra*, *Zygnema*, *Cosmarium*, and *Staurastrum*). Thallus shape ranges from simple, oblong unicells (saccoderm desmids, or Mesotaeniaceae), to unbranched filaments of cells with smooth walls (Zygnemataceae), to elaborately ornate and deeply incised unicells or filaments made of such cells (placoderm desmids, in four families). Prior analyses of sequences of the chloroplast-encoded gene *rbcl* (1354 bp, or 95% of the gene) sampled from all six families showed that two of the traditional families (saccoderms and filamentous forms) are paraphyletic, i.e. they do not form two independent clades, although the component genera may together constitute a monophyletic group. In contrast, three families of placoderm desmids formed three monophyletic groups within a larger clade. New analyses using partial sequences from the mitochondrial gene *coxIII* (608 bp, or 76% of the gene) for 21 species of conjugating green algae from all six families supported the conclusions of the *rbcl* analysis, although bootstrap support for each gene along was weak for many branches. The *coxIII* sequences are more variable than *rbcl* and have proportionately more parsimony-informative sites (43% vs. 34%, respectively). Combining the two genes improved the resolution of the resulting tree: (1) bootstrap support increased for all branches, particularly in regards relationships of placoderm desmids; and (2) monophyly of a clade containing filamentous and saccoderm desmids was strongly supported. We conclude that phylogeny of conjugating green algae will be much improved by sampling from additional genes.

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THE ROOTS OF LAND PLANTS: RECENT RESEARCH ON EARLY DIVERGING LINEAGES IN THE EVOLUTION OF HIGHER DRIER ALGAE

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Recent multigene phylogenetic analyses of charophycean algae *sensu* Mattox and Stewart have advanced our understanding of the relationships of the green algae that are most closely related to land plants. Several clades of green algae are monophyletic with land plants (i.e. liverworts, mosses, nonvascular and vascular plants that arose from an aquatic ancestor) to the exclusion of other green algae: Mesostigma (a flagellate unicell); the Chlorokybales (small pack-

ets of cells); Klebsormidiales (unbranched filaments); Zygnematales (conjugating unicells, filaments, and colonies); Coleochaetales (filamentous and parenchymatous thalli); and Charales (branched filamentous thalli with complex nodal structure). The morphological and molecular diversity of the extant members of this nearly exclusively freshwater clade is remarkable. Moreover, the taxon diversity within clades is highly asymmetric, having yielded several distinctive albeit small groups of green algae, as well as one of the major evolutionary success stories on earth, viz. land plants. The most recent analysis (Karol et al. (2001) *Science* 294: 2351–2353) of four genes from plastid, mitochondrial, and nuclear compartments strongly support the sister relationships of Charales (stoneworts) to the most primitive land plants. The analysis also shows that within this clade there has been a trend toward increasing complexity of thallus form (from unicells, to filaments, to branching and parenchymatous thalli) and reproduction (absence of sexual reproduction to iso- or anisogamy, to oogamy). Although the major clades mentioned above are well supported by molecular analyses, relationships among these groups are less well-resolved, especially for the Klebsormidiales, Chlorokybales, and Mesostigma. This paper reviews the morphological and molecular evidence for the relationships among and within these groups. More sequence data from a broader sampling of taxa at the base of the charophyceans (including perhaps some algae not now known to be charophytes) should provide a clearer picture of the evolution of this group and the reasons for its success.

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PHYLOGENETIC ANALYSIS OF THE MISCHOCOCCALES, TRIBONEMATALES, AND VAUCHERIALES (XANTHOPHYCEAE) INFERRED FROM 18S rRNA GENE SEQUENCES

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The Xanthophyceae have traditionally been classified using a phenetic classification system based upon vegetative cell structure. Species belonging to the class are placed in one of six orders corresponding to amoeboid, coccoid, filamentous, flagellate, palmeloid, or siphonous organization. Nuclear 18S rRNA gene sequences were determined for 29 species and aligned with 10 previously determined sequences. Parsimony and maximum likelihood trees inferred from these data imply that the orders Mischococcales (coc-

coid), Tribonematales (filamentous), and Vaucheriales (siphonous) are not monophyletic. The majority of species are divided between two clades defined by the presence or absence of a bipartite cell wall. Also, several families, particularly those placed in the Mischococcales, are resolved as polyphyletic. Results indicate that coccoid and filamentous life forms may have arisen independently at least three times, and some coccoid and filamentous life forms are resolved as closely related. For example, *Heterococcus* (filamentous) is more closely related to *Mischococcus* and *Pseudopleurochloris* (coccoid) than it is to other members of the Tribonematales. Our data strongly support the contention that *Bumilleriopsis* and *Pseudobumilleriopsis* are congeneric with *Bumilleria*. The siphonous xanthophytes including *Asterosiphon*, *Botrydium*, and *Vaucheria* probably do not form a monophyletic group although robust bootstrap support for this conclusion is low. Our results indicate that the generic, familial, and ordinal classification of the Xanthophyceae requires major revision.

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PHYLOGENETIC RELATIONSHIPS AMONG *PEDIASTRUM* sPP. AND *HYDRODICTYON* sPP.

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Within the class Chlorophyceae, the family Hydrodictyaceae (order Sphaeropleales) consists of taxa that form flat or net-like coenobia and reproduce asexually by way of biflagellated zoospores. Two taxa within this family, *Pediastrum* and *Hydrodictyon*, share many features during development, especially the manner of daughter colony formation. The colonies of *Pediastrum* differ from *Hydrodictyon* in that growth is planar resulting in two-dimensional colonies, while three-dimensional nets are formed in *Hydrodictyon*. Studies of the Chlorophyceae using morphological and ultrastructural data, as well as molecular sequence data, have supported the close relationship of *Pediastrum* and *Hydrodictyon* and they have remained in the family Hydrodictyaceae throughout major classification revisions. However, in these studies only single species of *Pediastrum* (*P. duplex*) and *Hydrodictyon* (*H. reticulatum*) were included, therefore the exact relationship of these two taxa could not be explored. Preliminary molecular data from a second species of *Pediastrum*, *P. boryanum*, indicate that *Hydrodictyon* may be derived from *Pediastrum*. In this analysis, *H. reticulatum* resolves as sister taxon to *P. duplex* with *P. boryanum* the ancestral taxon. Further molec-

ular studies of the family Hydrodictyaceae allow exploration of the relationships between *Pediastrum* spp. and *Hydrodictyon* spp., and assist in determining whether they are monophyletic. Preliminary analyses include *H. reticulatum* and *H. africanum* and several species of *Pediastrum*, *P. duplex*, *P. boryanum*, *P. tetras*, *P. biradiatum* and *P. simplex*. An understanding of the phylogenetic relationships among the species of *Pediastrum* and *Hydrodictyon* will allow a more complete comparison of colony formation.

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RELATING PHYTOPLANKTON DYNAMICS TO ENVIRONMENTAL FORCING IN THE LOWER ST. JOHNS RIVER ESTUARY: A MULTIVARIATE ASSESSMENT

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The St. Johns River, a 300-mile riverine and estuarine system located along the north-eastern coastline of Florida, has undergone extensive eutrophication through point and nonpoint source nutrient inputs. Moreover, recent reports of sudden fish kills and a high incidence of fish with lesions suggest the potential for harmful algal blooms. As part of a NOAA-funded project involving the characterization of water quality parameters in relation to water inflows and nutrient inputs and the development of species-specific markers/probes for instrumental-based monitoring efforts, we used primer-based multivariate analyses to examine the relationship between abiotic variables and both total and phylogenetic-group chlorophyll *a* concentrations (derived from photopigments and ChemTax matrix factorization of diagnostic carotenoids) during 2001. Seven sampling sites (identified through principle components ordination of physical/chemical parameters as ranging from oligo- to mesohaline) were sampled intensively over 2-week periods on a seasonal basis. Chlorophyll *a* concentrations typically ranged from 5 to 35 µg/L with the greatest concentrations occurring at the oligohaline sites. Phytoplankton assemblages were dominated by diatoms, cryptophytes, and cyanobacteria, and together typically comprised up to 90% of the total chlorophyll *a*. Temporal variability in phytoplankton assemblages followed seasonal trends impacted by meteorological and hydrological forcing. Spatial variability in phylogenetic-group abundance (illustrated through multidimensional scalar ordination of sample

dissimilarity) was dramatic and associated with differences in abiotic variables along the estuarine gradient.

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CHARACTERIZATION OF *sufB* SEQUENCES FROM NONGREEN PHOTOAUTOTROPHS

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Partial sequences for the hypothetical chloroplast frame 24 (*ycf24*) were determined for 13 species of algae representing the classes Bacillariophyceae, Chrysophyceae, Pelagophyceae, Pinguicophyceae, Prymnesiophyceae, Raphidophyceae, and Xanthophyceae. In many databases *ycf24* is considered an ATP-binding cassette (ABC) transporter. Our analyses of translated amino acid sequences demonstrate that these sequences lack Walker A and B boxes, a conserved ABC signature sequence motif, and transmembrane domains that characterize ABC transporters. Our data, as well as those of others, convincingly indicate that *ycf24* is not an ABC transporter and is instead homologous to the *sufB* gene first described from prokaryotes. The function of the *sufB* gene product in eukaryotic plastids is not known with certainty but it likely plays a key role in iron-sulfur (2Fe-2S) cluster formation. Our data, unpublished results and database searches imply that *sufB* is plastid encoded in the 'red' plastid lineage, but has been transferred to the nucleus or lost within the streptophyte lineage. Phylogenetic analyses of all available eukaryotic *sufB* sequences indicate that this gene is a useful marker for tracing the ancestry of plastids and their hosts. For example, our *sufB* analyses indicate that the 35-Kb circular plastid genome of *Plasmodium falciparum* is a member of the 'red', not 'green', plastid lineage and was acquired via secondary endosymbiosis. Our results also support the hypothesis that, despite their similarities, the plastids of prymnesiophytes and heterokont algae were independently obtained by separate secondary endosymbiotic events.

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ARE 'GREEN TIDES' HARMFUL ALGAL BLOOMS? ALLELOPATHIC PROPERTIES OF EXTRACTS FROM *ULVA FENESTRATA* AND *ULVARIA OBSCURA*

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Toxic properties are rarely associated with macroalgal blooms. A few studies have demonstrated inhibition of invertebrate larval development by extracts from *Ulva* spp. and we have observed substantial discoloration of the seawater in small bays following mass desiccation-induced mortality of *Ulvaria obscura*. We examined the effects of extracts from *Ulva fenestrata* and *U. obscura* on enzyme systems, larval oyster development, *Fucus gardneri* zygote development, epiphytic diatom growth, and growth of *Ulva* and *Ulvaria*. Extracts from *Ulvaria* inhibited trypsin activity, but not pepsin or amyloglucosidase activity when tested at pH optimized for each enzyme system (pH 8, 2, and 5.5, respectively). When tested at neutral to slightly alkaline pH (7 or 8), *Ulvaria* extracts also inhibited amyloglucosidase activity. 'Aged' extract caused greater enzyme inhibition than fresh extract. Maximum inhibition occurred in 6-h-old-extract. Oyster larval development was inhibited by extracts from both algae, although the visible effects were much greater when the larvae were exposed to extracts of *Ulvaria*. *Fucus* zygote development was inhibited by extracts of both species, although the effect was measurably greater in extracts from *Ulvaria*. Epiphytic diatom growth and growth of *Ulva* and *Ulvaria* were inhibited by extracts from both species. Thus, extracts from both *Ulva fenestrata* and *U. obscura* have allelopathic properties, although the impacts are more widespread for *Ulvaria* extracts. These properties could alter competitive interactions by inhibiting germination or development of algal and animal competitors. Multiple toxins are probably involved, since the two species tested cause visibly different responses.

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THE EFFECTS OF NITROGEN REGIME ON GROWTH, [CHN], [DMSP], [CHLOROPHYLL], [PROTEIN] AND NITROGEN UPTAKE RATE IN *ULVARIA OBSCURA* AND *ULVA FENESTRATA*

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Ulva fenestrata and *Ulvaria obscura* are major constituents of ulvoid macroalgal blooms in the north-eastern Pacific. These blooms have been associated with a variety of environmental problems and are thought to be caused by increased nitrogen concentration. We grew these algae at several nutrient concentrations in incubator and seawater table experiments. Growth rate and the concentrations of DMSP, C, H, and N in the tissues were determined for both kinds of

experiments. Incubator experiments alone were used to measure nutrient uptake rate, chlorophyll *a* and *b* content, and protein content (as determined by far-UV and bicinchoninic acid (BCA) techniques). Increasing nutrient concentration caused significantly more growth in the seawater table experiment but only a trend toward greater growth in the incubator. There was no significant interspecific difference in growth rate in the seawater table experiment, but *Ulva* grew significantly faster than *Ulvaria* in the incubator experiment. Tissue nitrogen increased significantly with increasing nutrient content in the growth medium in the incubator experiment and showed a similar (albeit not significant) trend in the seawater table experiment. *Ulvaria* had higher tissue nitrogen, protein, and chlorophyll *a* concentrations than *Ulva* in all experiments. The ratio of chlorophyll *b*:*a* was also higher in *Ulvaria* than in *Ulva*. Far-UV and BCA techniques yielded equivalent protein concentration in *Ulva* but protein concentration determined by BCA was 2.5 times higher in *Ulvaria* than when determined by far-UV. Nutrient uptake rates were similar between both species. DMSP concentration was not affected significantly by nutrient regime, but [DMSP] was higher in *Ulvaria* than in *Ulva*. These results help explain previous observations of these species. *Ulvaria* generally grows more slowly than *Ulva* because it requires more nitrogen to make chlorophyll and protein but has similar nitrogen uptake rates. *Ulvaria* is also shade adapted, while *Ulva* can acclimate to various conditions.

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THE EFFECTS OF THALLUS TOUGHNESS AND NUTRITIVE QUALITY ON PALATABILITY OF ANTARCTIC MACROALGAE

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Palatability of macroalgae can be affected by many different factors including thallus toughness, nutritional quality (carbohydrates, lipids, proteins, ash), and the presence of deterrent secondary metabolites. Palatability bioassays were performed using two different antarctic algal grazers (the antarctic rockfish *Notothenia coriiceps* and the sea star *Odontaster validus*). These data were then compared to toughness and chemical composition data. Toughness was determined using a penetrometer and a wide range of thallus toughness

was observed in individuals of differing species as well as of the same species. Some of the tougher individuals required more than 30 N of force to cut through the thallus while others required less than 1 N to break through. Each algal species is also being tested for percent composition of carbohydrates, lipids, proteins, as well as ash. It is hypothesized that the algal species with greater nutritional quality and/or less thallus toughness are more likely to be acceptable foods to consumers (NSF OPP9814538, OPP9901076).

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SURVIVAL OF *CHLAMYDOMONAS* SUBSEQUENT TO CRYOPRESERVATION IS PREVENTED BY A SUBSTANCE RELEASED FROM DAMAGED CELLS

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Storage of algae in liquid nitrogen offers a convenient way to permanently preserve many cultures with minimum genetic drift. We have successfully cryopreserved over 1400 strains in the Culture Collection of Algae at the University of Texas (UTEX), but over one-fourth of UTEX cultures remain recalcitrant. Our previous studies demonstrate that unicellular algae frozen at a high cell density (>2000000 cells/mL) do not survive cryopreservation well. We now show that viability during cryopreservation of *Chlamydomonas reinhardtii* UTEX #89 at high cell densities is not affected by the physical proximity of cells. Instead, when cells are damaged, a small organic molecule(s) is produced and released into the medium preventing cryopreservation of other cells in the culture. Healthy *C. reinhardtii* cultures were treated with extracts prepared from damaged cells or culture medium separated from damaged cells, then cryopreserved by a standard method (Crutchfield et al. (1999) *Eur J Phycol* 34: 43–52). Viability was measured after thawing by the ability of a cell wall to preclude Evan's blue dye, and was confirmed by quantitative agar plating. The inhibitory substance from these fractions appears to affect living cells only during cryopreservation, but can be produced from cells by a variety of treatments. The inhibitor is heat-stable, but heating cells to 80 °C precludes its production. The substance is stable when separated from cellular particulate matter, but gradually becomes inactive in the presence of cell membrane fractions. The inhibitor is a water-soluble and heat-stable organic compound(s), with a molecular mass less than or equal to 3500. Characterizing the inhibitory substance and the pathway that leads to its

formation may explain why most multicellular algae remain recalcitrant to cryopreservation, and lead to protocols that allow the cryopreservation of a broader range of organisms.

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PROTIST ORIGIN OF THE ULVOPHYCEAE (CHLOROPHYTA) REVEALED BY SSU rDNA ANALYSES OF MARINE COCCOID GREEN ALGAE

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Traditionally, brackish and marine coccoid green algae are placed as isolated species of common trebouxiophycean or chlorophycean genera (e.g. *Chlorella*, *Chlorococcum*); exclusively the marine genera *Chlorocystis* and *Halochlorococcum* are characterized by quadriflagellated zoospores and a 'Codiolum-stage' (characteristic for the Ulvophyceae) in their life histories. In *Chlorocystis cohnii*, sexual reproduction could only be induced by presence of the tube-dwelling diatom *Berkeleya rutilans*. Phylogenetic analyses of the SSU rDNA sequences of 32, mostly marine, coccoid or sarcinoid strains confirmed the monophyly of the Ulvophyceae. The class contain 11 independent well-supported lineages; of which eight contain coccoid species. (1) The basal branch of the ulvophytes is the *Oltmannsiellopsis* clade (in agreement with the order Oltmannsiellopsidales emend.) which contains the quadriflagellated monads (*O. viridis* and *O. unicellularis*) and the type species of the coccoid genus *Halochlorococcum* (*H. marinum*). (2) The other species of *Halochlorococcum* (except *H. saccatum*) formed together with *Chlorocystis* and *Chlorella salina* a significant clade corresponding to the order *Chlorocystidales* in emended form. (3) *Gomontia polyrhiza* is closely related to *Urospora penicelliformis* within the *Acrosiphonia* group (Acrosiphoniales). (4) The sarcinoid *Trichosarcina polymorpha* and *T. mucosa*, and the freshwater planctonic *Helicodictyon planctonicum* are member of the *Ulothrix* clade together with filamentous taxa. (5) The type species of *Pseudoneochloris* (*P. marina*) form together with three unidentified marine coccoid strains an independent lineage within the Ulvophyceae (called *Pseudoneochloris* clade). (6–8) *Ignatius tetrasporus* (isolated from soil), the strain CCMP 250 originating from desert soil and the marine strain CCMP 1293 isolated from Palau Islands represent three single lineages within the ulvophytes. From our results, the class Ulvophyceae can be subdivided taxonomically into at least 11 orders (including the

Ulvales, Cladophorales and Dasycladales) with the largest biodiversity at the coccooid level.

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CALCIFICATION AND DECALCIFICATION DURING EPITHALLIAL CELL TURNOVER IN CORALLINE RED ALGAE

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Epithallial cells of the coralline red algae are characterized by unusual structural specialization, which include deep invaginations of the distal cell surface, and by unique development, which culminates in senescence, shedding, and replacement of the cells. Electron microscopic study of epithelial cell differentiation in morphologically and taxonomically disparate species suggests that the unusual features of epithelial cell structure and development stem from the fact that these dynamics occur within a calcified matrix. Distal wall ingrowths begin to form on the initial cells, cells whose cleavage eventually gives rise distally to new epithelial cells. After the distal wall ingrowths form, the overlying crosswall becomes rich in organic material. For this organic wall material to be deposited into the existing crosswall, the wall must first be decalcified; therefore, the presence of abundant organic material in the crosswall provides a marker of localized decalcification. We propose that the location and time of origin of distal wall ingrowths indicate a connection between the ingrowths and two coordinated processes: localized secretion of wall material, and decalcification of the overlying cell wall in preparation for the movement of the young epithelial cell into a new location relative to the surrounding calcified matrix. The large plasmalemmal surface area associated with the distal wall ingrowths allows for a greater abundance of membrane-associated components, such as proton pumps, that could drive localized cell wall decalcification.

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INTERANNUAL VARIATION IN CORAL REEF PLANT COMMUNITIES OF CENTRAL BELIZE

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Algal and seagrass community composition were monitored biennially along three transect lines in Belize from 1997 through 2001. Two sites were on South Water Caye. The first was on the seaward side, between the reef crest and the caye. The second was on the landward side of the caye. The third site was on Long Caye, Glover's Reef Atoll. This transect line ran from a coral rubble filled embayment, through a small channel (formed by hurricane Hattie in 1960, bisecting Long Caye), and into a seagrass meadow in the interior of the atoll. After a 38-year hiatus, hurricanes struck Belize in 1998, 2000 and 2001, giving us the opportunity to examine changes in plant community structure before and after the storm sequence. Minimal interannual changes were seen at South Water Caye. The seagrasses at Long Caye were nearly eliminated along the transect line due to burial by sand. Peak density of *Thalassia testudinum* at Long Caye was over 60 shoots/m² in 1997 and less than 2 shoots/m² in 1999 and 2001. *Halodule wrightii* and *Syringodium filiforme* were both completely eliminated between 1997 and 1999. *Padina* and *Dictyota* spp. flourished in the embayment following the hurricane, but had been virtually absent prior to the sequence of storms. Future monitoring will be conducted to follow recovery of the meadow and look for other long-term changes.

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SEQUENCE DIVERSITY OF THE GENE *tufA* IN COLEOCHAETE: AN ALGAL MODEL FOR HORIZONTAL GENE TRANSFER

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Studying the organellar gene transfer of *tufA* in Coleochaete serves as a model system for understanding the effect of functional horizontal gene transfer on sequence conservation and divergence. Previous work indicates that *tufA*, which codes for protein synthesis elongation factor TU, is encoded only in the chloroplast genome in most green algae, red algae, and glaucocystophytes, but only in the nuclear genome of embryophytes. In the charophytes, the lineage of green algae that gave rise to embryophytes, *tufA* is present variously within the chloroplast and/or nuclear genome. This implies that *tufA* was probably transferred to the nucleus from the chloroplast after the charophytes diverged from other green algae but before the diversification of the group. The chloroplast copy has undergone three separate fates within the charophytes: maintained in Charales, divergent in Coleochaetales,

and lost in Zygnematales. Previous research indicates the presence of multiple nuclear copies and a highly divergent chloroplast encoded *tufA* in *Coleochaete orbicularis*. This research aims to isolate the chloroplast-encoded *tufA* from a representative panel of *Coleochaete* in order to characterize patterns of conservation in the divergent chloroplast *tufA*. Putative chloroplast copies of *tufA* have been isolated through PCR and subsequent molecular cloning. By comparing the DNA sequences from copies of the chloroplast-encoded *tufA* from a representative panel of *Coleochaete*, we hope to gain insight into patterns of sequence conservation and from this infer the current function of the divergent chloroplast *tufA*.

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EFFECTS OF ENVIRONMENTAL PARAMETERS ON THE GROWTH CHARACTERISTICS OF SYMBIODINIUM SPP. ISOLATED FROM CORALS

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Symbiodinium spp. were isolated from *Porites porites* (JR02F2 and RD03), *Montipora capitata* (JR12A7), *Madracis mirabilis* (RD02), *Montastrea faveolata* (RD04), *Pocillopora damicornis* (JR13E1), and an unknown coral (RD01). Growth rates and the distribution between motile gymnodinoid cells and sessile coccoid cells were determined in 200 µL cultures in 96-well dilution plates. Clones of each isolate were maintained in L1 medium (prepared with 34 ppt sterile Gulf of Mexico water), at 26 °C with illumination provided at 100–150 mE/m²/s PAR (400–700 nm) during a 10:14 (light:dark) photoperiod. Under these conditions, generation times of nearly once per 24 h were observed for clones, JR02F2, RD01 and RD02, in log growth. Clone RD04 had a generation time of approximately 5 days. The fastest growing clones produced the highest percentage of motile cells (>90) during the daylight portion of the diurnal cycle. Growth characteristics of selected clones were examined over a range of salinities, temperatures and PAR intensities. Salinities ranging from 25 to 45 ppt had little if any affect on growth rate. At salinities less than 25 ppt significant decreases in growth were generally observed; no growth was common at 5 and 10 ppt. In most cases inhibitory effects of temperature were observed at temperatures greater than 29 °C. For example, generation times for JR02F2 were slightly

greater at 29 °C than 24 °C, but maximal growth rates were reduced approximately 50–90% at temperatures of 32 and 34 °C, respectively. The most sensitive clone, RD04, stopped dividing after two divisions at 29 °C. The growth rate of JR02F2 and RD01 reached a maximum at PAR levels greater than 100 mE/m²/s and remained within a range of 0.75–0.85 divisions/day up to 390 mE/m²/s.

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PHOTOPROTECTIVE COMPOUNDS IN RICE-FIELD CYANOBACTERIA

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Cyanobacteria are cosmopolitan in distribution, forming a prominent component of microbial populations in both aquatic as well as terrestrial ecosystems. They are the dominant microflora in rice-fields, where they significantly contribute to fertility as a natural biofertilizer. The fluence rates of UV-B radiation impinging on the natural habitats is of major concern since being photoautotrophic organisms, cyanobacteria depend on solar radiation as the primary source of energy. UV-B radiation causes reduction in growth, survival, pigment and protein content, heterocyst frequency and fixation of carbon and nitrogen in cyanobacteria. However, a number of cyanobacteria synthesize water soluble colorless mycosporine-like amino acids (MAAs) and the lipid soluble yellow-brown colored sheath pigment, scytonemin to counteract the damaging effects of UV-B. *Anabaena* sp., *Nostoc commune*, *Scytonema* sp. and *Lyngbya* sp. were isolated from rice-fields in India and screened for the presence of photoprotective compounds. Spectroscopic and biochemical analyses revealed the presence of shinorine, a bisubstituted MAA containing both glycine and serine group with an absorption maximum at 334 nm in all cyanobacteria except *Lyngbya* sp. There was a circadian induction in the synthesis of this compound by UV-B. Polychromatic action spectra for the induction of MAAs in *Anabaena* sp. and *N. commune* show the induction to be UV-B dependent peaking at 290 nm. Another photoprotective compound, scytonemin, with an absorption maximum at 386 nm (also absorbs significantly at 300, 278, 252 and 212 nm) was detected in all cyanobacteria except *Anabaena* sp. In addition, two unidentified, water-soluble, yellowish and brownish compounds with an absorption maximum at 315 nm were recorded only in *Scytonema* sp. The spectral characteristics of these compounds neither matches with MAAs nor with scytonemin and seems to be a novel

compound acting as an effective UV screens. In conclusion, a particular cyanobacterium having photoprotective compounds may be a potent candidate as biofertilizers for crop plants.

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PHYLOGENY OF *KLEBSORMIDIUM* (KLEBSORMIDIALES; CHAROPHYTA) BASED ON *rbcL*, *atpB*, *coxIII* AND *nad5* SEQUENCE DATA

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The charophycean order Klebsormidiales consists of two unbranched filamentous lineages, the fresh water *Entransia* and the semiterrestrial *Klebsormidium*. Molecular data have supported the close relationship Klebsormidiales have with land plants. A monograph exists for the European species of *Klebsormidium*; this monograph provides a cladistic analysis of morphological characteristics for eight *Klebsormidium* species, but a species level molecular phylogenetic analysis has not yet been performed for the group. We have obtained 50 strains of *Klebsormidium*, representing 11 morphological species, both from nature and from culture collections. Phylogenetic analyses using *rbcL*, *atpB*, *coxIII* and *nad5* gene sequence data from these strains and 40 outgroup sequences suggest that several traditional *Klebsormidium* species may not be monophyletic.

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A NOTE OF CAUTION ON USING ABSOLUTE METHANOL CONCENTRATIONS AND LOW TEMPERATURES TO QUANTIFY MYCOSPORINE-LIKE AMINO ACIDS IN ALGAE

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Mycosporine-like amino acids (MAAs), a family of UV-absorbing compounds, are widespread among many marine and freshwater organisms, however, no standard method to analyze these compounds is available. Consequently, protocols to extract MAAs largely differ, for example, in the type of solvent, concentrations, temperatures, and times used. In this study, we tested whether the concentrations of MAAs are affected by extraction at different temperatures and methanol (MeOH) concentrations. Natural assemblages of freshwater phytoplankton and the marine alga *Porphyra* sp. were used as test organisms. The

MAAs shinorine, palythine, MAA 331, asterina-330, and porphyra-334 present in the organisms examined were generally best extracted at 45 °C in 25% aqueous MeOH. In *Porphyra* sp. and natural freshwater phytoplankton, the mean total MAA concentrations obtained with this protocol were, respectively, about 13 and 3 times higher than when extracted in 100% MeOH at 4 °C. Depending on the organism examined, both MeOH concentration and temperature affected extraction efficiency and final MAA concentration. Our results stress the need for *a priori* testing the influence of these variables to assure that the highest concentration is obtained without altering the qualitative MAA composition. Based on these results, direct comparison of MAA concentrations reported in the scientific literature should be done with caution.

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TRACING INVASIONS OF *CAULERPA TAXIFOLIA* WITH MOLECULAR MARKERS

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Comparative sequence data from the rDNA ITS have proven very useful in species identification and identifying major biogeographic groups of *Caulerpa taxifolia*. However, the low variability of the sequence, and the need to screen hundreds of samples has limited its utility for population level studies. Here we report on the performance of two new markers suitable for population level screening: the chloroplast rDNA 16S intron-2 and intersimple-sequence-repeat fingerprints (ISSRs). A new analysis of ITS1 insertion-deletion patterns was also conducted using all available sequences. A number of new insights have emerged. First, the invasive aquarium strain (Mediterranean, California) is clearly from Brisbane, but the Brisbane populations themselves may be the result of an introduction from Northern Australia. Second, an analysis of the new data in conjunction with an ITS1-insertion-deletion analysis further suggests that the Mediterranean populations may be the result of not one, but two separate introductions. Third, intrapopulation genetic diversity between invasive Mediterranean and 'native' Australian populations revealed the occurrence of two divergent and widespread clades. The first clade grouped nontropical invasive populations with coastal

inshore populations of Australia while the second clustered all offshore/oceanic populations so far studied. *C. taxifolia*, therefore exists as a complex of independent ecotypes that probably represent nascent species. Fourth, despite our finding of nine distinct nuclear and five distinct chloroplast profiles, strong linkage disequilibrium was found in most specimens, which indicates a predominance of asexual reproduction. However, nucleo-cytoplasmic recombination was detected in one case, supporting hybridization both within and between populations.

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THE CASE FOR A SINGLE-PLASTID ORIGIN REVISITED: CONVERGENT EVOLUTION IN ORGANELLAR GENE CONTENT

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Three eukaryotic lineages generally are believed to have plastids that are primary in origin; that is, descended directly from a cyanobacterial endosymbiont. The recovery of these plastids as a monophyletic group in most molecular phylogenetic analyses, along with similarities in genome content and protein targeting mechanisms, have been cited as strong evidence in support of the hypothesis of a single endosymbiotic origin of all plastids. Although these data indeed are consistent with a single plastid origin, they also are consistent with the proposition of multiple endosymbiotic origins. Each hypothesis requires certain evolutionary assumptions in order to be reconciled with all existing data; at present, it is unclear which of these assumptions most likely reflect the historical process that gave rise to plastid diversity. Here we examine similarities in gene content among representatives of the three primary plastid lineages, using as a control the genome of a mitochondrion that almost certainly originated as an independent endosymbiotic association. To minimize metabolic constraints on gene retention we focus on two datasets, ribosomal protein and transfer RNA genes, neither of which is tied directly to specific organellar functions. Analyses of all possible pair-wise comparisons among the three plastids and mitochondrion indicate that genomic similarities are most consistent with convergent evolution due to constraints on gene loss, rather than with hypothesized shared evolutionary histories. We find no evidence of phylogenetic signal in the pattern of gene loss overlying this convergence. In light of these results, we address other lines of evidence and arguments

that have been raised in support of a single plastid origin.

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ULTRASTRUCTURE AND PHYLOGENY OF A NEW SPECIES BELONGING TO THE PRYMNESIOPHYCEAE

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Ultrastructural and molecular data were used to examine a new alga (CCMP2000) collected from the Arabian Sea. Nonmotile vegetative cells are typically oblong to ovate, solitary and lack a gelatinous envelope. Ultrastructural analyses of these cells revealed the presence of a nucleus located in the posterior portion of the cell. A single large Golgi body with numerous cisternae is located anterior to the nucleus as are numerous profiles of mitochondria possessing tubular cristae. Two peripheral plastids are present having immersed pyrenoids that are traversed by one or more thylakoids. In some cells, the plastids are conspicuously lobed forming finger-like extensions. Nonmotile vegetative cells possess reduced mature and immature flagella as well as a haptonema that are 1 micron long or less. A distinct internal covering and external wall surround the cell protoplasm. The outer wall is apparently composed of simple scales, but the composition (organic or mineralized?) of these scales is unknown. The outer wall may form a protuberance, or 'foot', at the posterior end of nonmotile cells. Interestingly, many vegetative cells are infected by a labyrinthulid. Flagellate stages have been observed and possess roughly equal length flagella and a haptonema. Phylogenetic analyses of nuclear 18S rRNA and plastid-encoded *rbcL* genes indicate CCMP2000 is most closely related to *Phaeocystis*. However, CCMP2000 does not fit the description of *Phaeocystis*, and therefore, probably warrants recognition as a new taxon.

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THE EFFECTS OF BIOGENICALLY ACTIVE COMPOUNDS ON *CYCLOTELLA MENEGHINIANA* (BACILLARIOPHYTA)

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Biogenically active compounds have been discovered in many surface water samples in Europe and North

America. Wastewater effluent is identified as a major source of contamination and found to revert much of the metabolic products of these biologically active compounds back to their original form. This presentation explains methodology for determining the effects of these contaminants through a newly developed bioassay, examining the physiological response of a diatom to these compounds. Diatoms represent an important aspect of the primary production community. They are a desirable food source over other members of the primary production community through storage of photosynthetically produced sugars in the form of lipids rather than starch. Therefore, members of higher trophic levels selectively feed on diatoms when present with other members of the primary production community. This study examines the effects of select biogenically active compounds on the gross morphology and physiological development of the diatom species *Cyclotella meneghiniana*. Clearly, unicellular protists such as diatoms are not susceptible to these contaminants in a manner directly analogous to that expressed in vertebrates. However, many of these compounds are lipophilic making them particularly effective in entering the diatom cell membrane. Comparing cell density, lipid composition, fatty acid concentrations, and the electron microscope ultra-structure in control and exposed cultures achieve this study's experimental objectives. This diatom was selected because it commonly occurs in most freshwater environments and has been the subject of other toxicological studies. An adequate literature base also exists for evaluating results of this experiment. The species grows rapidly and is easy to maintain in culture. The use of a phytoplankton species as a test organism complements other investigations involving vertebrate models at the apex of the trophic hierarchy by considering effects at the base of the food web.

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DEVELOPMENT OF VALVES IN THE MARINE DIATOM GENUS *TRIGONIUM*

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Trigonium arcticum and *T. formosum*, marine diatoms epiphytic on *Ectocarpus* sp., were collected from the sublittoral off Point Loma, California. Forming valves of these two sympatric species were studied using scanning electron microscopy. The earliest developing valve of *T. arcticum* encountered is a porous basal layer of silica, triangular, with pseudocelli just starting to form at each corner. The central annulus, the site

of the earliest valve formation, appears to be about the size of a single areola. Early rimoportulae (labiate processes) are interspersed between central areolae and are simple oval openings. Internally these form their labiate lip-like structures just before cribrum formation. Ridges form on the external side of the basal layer in a honeycomb pattern and produce the walls of the loculate structures. The ridges expand outward until cribrum formation is initiated. Small protuberances grow out from the edge of the ridge-like structures and then become anvil-shaped as they grow toward the center of the pores and form the 'roofs' of the loculi. In external view of the mature valve, the pores are smoothly covered over except for a ring of C-shaped slits. These cribra are flush with the surface of the valve. At about the time the cribra are completing, rotae are formed within the porelli of the pseudocelli by extensions from two or three sides. The quadrangular *T. formosum* develops along much the same pattern. Differences are the less numerous, more centrally located rimoportulae, which possess external tubes and the formation of highly domed cribra.

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THE CHLOROPLAST GENOME SEQUENCE OF *CHLOROKYBUS ATMOPHYTICUS*: EVIDENCE THAT CHAROPHYCEAN GREEN ALGAE FROM AN EARLY DIVERGING LINEAGE ADAPTED TO TERRESTRIAL LIFE

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Land plants and green algae belonging to the Charophyceae *sensu* Mattox and Stewart form the Streptophyta, a lineage sister to the Chlorophyta which comprises most or all of the other green algae. A third green plant lineage, at the base of the split of the Chlorophyta and Streptophyta, is possibly represented by the green alga *Mesostigma viride*; however, this remains controversial because some phylogenetic analyses place *Mesostigma* within the Streptophyta. Five orders have been recognized in the Charophyceae: the Chlorokybales, Klebsormidiales, Zygnematales, Coleochaetales, and Charales. Based on its sarcinoid thallus, the only species in the *Chlorokybales*, *Chlorokybus atmophyticus*, has long been regarded as the earliest-diverging member of the Charophyceae. This rare species has been collected only in terrestrial habitats. In the present study, we have determined the complete chloroplast DNA (cpDNA) se-

quence (149 681 bp) of *Chlorokybus* to gain insight into its phylogenetic position and also to better understand how the chloroplast genome evolved in the Streptophyta. We have found that *Chlorokybus* cpDNA closely resembles *Mesostigma* cpDNA in showing a high degree of ancestral features. It displays a quadripartite structure, encodes 138 genes (only two extra genes compared to *Mesostigma* cpDNA), and contains a single intron (in trnL(UAA)). The gene order in *Chlorokybus* cpDNA is similar to that of *Mesostigma* cpDNA; we estimate that 14 inversions would be required to convert the gene order of one alga into that of the other. Phylogenetic analyses of combined chloroplast protein sequences from *Mesostigma*, *Chlorokybus*, other streptophytes, and a number of chlorophytes will be presented. Overall, our results support the idea that the *Chlorokybales* represent an early diverging lineage.

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RECIRCULATING CULTURE FOR *CHONDRACANTHUS EXASPERATUS*

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The red alga *Chondracanthus exasperatus* is a source of the phycocolloid carrageenan as well as an ingredient referred to as 'intralamellar gel' in a recently developed cosmetic formula (US Patent 6136 329). The high value of the cosmetic product has sparked renewed interest in cultivation of this species. Previous cultivation methods for this species include open water culture on nets and immersed cultivation in tanks supplied with flow-through pumped seawater. The installation of a high capacity seawater supply, pumping and drain system is a major cost for flow through systems. Recirculating or re-use seawater systems that minimize seawater turnover may offer significant cost savings over single-pass, flow through seawater systems. In this research several options for minimizing seawater use have been tested: recirculating batch culture in which nutrient replenished (carbon dioxide and mineral nutrients) natural or artificial seawater is used with minimal turnover and spray culture in which plants are suspended in air saturated with nutrient replenished natural or artificial seawater medium. Small volume (<2 L), single-plant bioreactors and larger multiplant, 20, 80 and 320 L (sea water volume) immersion and spray systems have been developed and tested. Results from these systems will be presented. Research supported by Washington Sea Grant, Washington Biotechnology Center and Soliv International Corporation.

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PHYLOGENETIC ANALYSES OF THE SPECIES OF *CHLOROSARCINOPSIS* AND *NEOCHLOROSARCINA* (CHLOROPHYCEAE)

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The genus *Chlorosarcinopsis* forms sarcinoid packages of ?@ cells and reproduces by forming naked biflagellate zoospores. The genus *Neochlorosarcina* is distinguished from *Chlorosarcinopsis* in having a thin cell wall around zoospores. We analyzed phylogenetic relationships of seven species of *Chlorosarcinopsis* and four species of *Neochlorosarcina* using 18S rDNA sequence data. The species of the *Chlorosarcinopsis* were separated into two clades, and those of *Neochlorosarcina* formed a monophyletic branch, which was far from the *Chlorosarcinopsis* clades. Groover and Bold (1969) investigated physiological abilities of species of *Chlorosarcinopsis* (which included species of *Neochlorosarcina*), and showed that the species were roughly divided into three groups by usage of nitrogen and carbon components. These groups correspond two clades of *Chlorosarcinopsis* and a clade of *Neochlorosarcina*. This result gave a phylogenetic meaning to the differences in the physiological abilities, and supported the separation of *Neochlorosarcina* from *Chlorosarcinopsis* based on a morphological attribute.

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DIGITAL ALGAE: A BOON TO RESEARCH AND TEACHING

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As part of a larger coastal ecology project, we have initiated a web-accessible, digitized phytoplankton image and taxonomy database of algae collected in a coastal wetland south of New Orleans, Louisiana. Examining phytoplankton dynamics is a large component of the project, titled 'Pulses—The Importance of Pulsed Physical Events for Watershed Sustainability in Coastal Louisiana' (a joint NSF/EPA/USDA Water and Watershed program). A total of 168 species of algae from 76 genera in 8 phyla have been identified from the phytoplankton samples, and 130 high-resolution (300 dpi) digital images taken. These taxonomic identification data are included in a database with collection information (i.e. location, date). Ecological data (i.e. tur-

bidity, salinity, and nutrients such as nitrate and nitrite, ammonium, phosphorous, and silica) will be added to the database. Having this information available on the web is an important resource for continued research in coastal Louisiana and perhaps other regions with similar ecosystems and hydrology. The database can also be a useful tool for teaching high school and college students. Microscopic phytoplankton are notoriously difficult to identify, and the images can be used in conjunction with keys to help students confirm their algal determinations. Students would also be able to see the main features of different groups of algae and learn some ecological aspects of the wetland habitat where they were collected. Please visit the web site at chapmanlab.lsu.edu/digitalalgae. This project was supported in part by a grant from the Office of Sea Grant Development at LSU.

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ALGAL POLYUNSATURATED FATTY ACIDS: FOOD, PHEROMONES AND FOUL ODOUR

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Algal polyunsaturated fatty acids (PUFAs) are known to be high-energy, often-essential resources to freshwater aquatic food webs. On the other hand, high PUFA cell content in some algal taxa has been linked with the production of derivatives that may act as pheromones, allelopathogens or toxins. It has been known for some time that these compounds function in marine chemical ecology, but recent evidence indicates that they may play similar roles in freshwater ecosystems. This paper presents field and laboratory studies of planktonic and periphytic communities from freshwater systems, and shows that these communities are rich sources of certain PUFAs and derivatives which may function in both positive and negative foodweb interactions. This also has important implications for surface water quality, as these compounds are potent sources of rancid fishy odours.

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THE USE OF LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC–MS) IN THE IDENTIFICATION AND CHARACTERIZATION OF MYCOSPORINE-LIKE AMINO ACIDS (MAAS)

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Mycosporine-like amino acids (MAAs) are a class of UV-absorbing compounds synthesized by a variety of marine and freshwater algae and cyanobacteria with absorption maxima between 310 and 360 nm. To date, ~25 different MAAs have been characterized with several new compounds identified each year. A major difficulty in the analysis of MAAs is the lack of commercial standards. Thus far, MAAs have been identified by their retention time and UV absorption after separation via high performance liquid chromatography (HPLC). Complementing the HPLC method with the use of mass spectrometry (LC–MS) offers greater sensitivity and reduces the hindrance standard unavailability presents by providing detailed molecular information (molecular weight and fragmentation patterns). The molecular weight information adds one more level to our ability to characterize MAAs and any new UV-absorbing compounds discovered. The fragmentation patterns obtained via LC–MS also contributes diagnostic information for structure elucidation and identification. LC–MS analysis has been used to examine UV-absorbing compounds in methanolic extracts from *Phaeocystis antarctica*. When samples were analyzed using isocratic HPLC elution, several UV-absorbing peaks were eluted. However, molecular weight and fragmentation patterns of some UV-absorbing compounds were not commiserate with those expected for MAAs. Additionally, palythenic acid was detected rather than mycosporine-glycine:valine as had been previously reported for this organism. Gradient elution provided further separation into MAA and ‘unknown’ groupings. The unknown UV-absorbing component has longer retention times and different mass spectral characteristics with wavelength maxima between 315 and 325 nm. This study benefited immensely by the addition of MS techniques, as many of the compounds would have remained either misidentified or entirely unidentified. The increased analytical capability afforded by LC–MS allows us to further investigate the structural diversity and biochemistry of MAAs aiding our study of the role MAAs play as sunscreen agents.

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MYCOSPORINE-LIKE AMINO ACIDS IN HARMFUL MARINE MICROALGAE

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Two current environmental issues are the increasing occurrence of harmful algal blooms and the increasing level of ultraviolet radiation. The objective of this ongoing project is to investigate whether

there is a coupling between these two phenomena, i.e. to test the hypothesis that UV-B radiation functions as a selective pressure favoring surface-blooming toxic/harmful marine microalgal species capable of producing UV-absorbing compounds (mycosporine-like amino acids, MAAs). A series of experiments were designed to test if the light environment induces the production of MAAs. Treatments were PAR with and without the addition of UV-B radiation. Preliminary results show a different response for different species. Most species increased their production of MAAs when exposed to an increased PAR intensity (300 $\mu\text{mol photons/m}^2/\text{s}$) and in, e.g. *Alexandrium ostenfeldii* and *Gyrodinium aureolum* a further increase was observed upon exposure to UV-B radiation. The content and composition of MAAs for a number of potentially harmful marine microalgae will be shown. In addition, the outcome of small changes in the HPLC method used will be discussed.

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HIGHLIGHTS OF RECENT COLLECTIONS OF MARINE ALGAE FROM THE SULTANATE OF OMAN

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Collections of benthic marine algae made over the past 3 years on the Dhofar coast of the Sultanate of Oman have continued to provide a more complete picture of the floristic affinities of this up-to-now poorly explored region. This Algal Biodiversity Project of Oman was funded by the British Government's Darwin Initiative grant for the 'Survival of Species' and managed by HTS Development Ltd., of the UK. A rich and floristically complicated flora is emerging, with connections to subtropical and warm temperate regions. Many new algal taxa have been discovered and described, and numerous new records for Oman or for the Indian Ocean have been reported. Some species newly reported for Oman (and the Indian Ocean) had previously been known from Japan (*Distromium decumbens*, *Kallymenia crassiuscula*, *Dudresnaya japonica*, and *Chondria crassicaulis*). *Sporochnus pedunculatus* and *Pedobesia simplex* had been known from the North Atlantic and the Mediterranean and are new records for the Indian Ocean. Some new species of red algae have been described, such as a *Dipterocladia* and a *Plocamium*. Some brown algal novelties have also been discovered, including new species of *Turbinaria* and *Jolyana*, and a new genus of *Chordariaceae*. The macroalgal flora of

southern Oman is rich with representatives of *Codium*, *Scinaia*, *Galaxaura*, *Melanothamnus*, and the families Gelidiaceae and Dictyotaceae. Several representative marine algae from southern Oman will be depicted and discussed.

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THE CENTRAL AMERICAN ISTHMUS: IMPLICATIONS FOR INTRASPECIFIC PHYLOGENY AND BIOGEOGRAPHY OF A PANTROPICAL GREEN ALGA

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An intraspecific phylogenetic study was undertaken to resolve the evolutionary relationship of isolates of the green alga *Phyllocladon anastomosans* (Harv.) Kraft et Wynne that occur on Atlantic and Pacific coasts of the Central American Isthmus. Patterns of vicariance related to the emergence of the Central American Isthmus were evident, but numerous examples of recent trans-oceanic and trans-isthmian dispersal obscured the underlying pattern. This study, one of the first studies to assess the impact of the emergence of the Central American Isthmus in seaweed phylogeography, provided an ideal opportunity to estimate the rate of sequence evolution using a single time point. Using this newly calibrated molecular clock the timing of an historical introduction across the isthmus is shown to be concordant with a shallow water breach of the isthmus dated at approximately 2.3–2.0 million years ago. This work contributes to a growing body of literature that suggests marine algae are fairly successful at dispersing over long distances in recent times.

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COMPARATIVE PHYLOGEOGRAPHY OF RETICULATE CLADOPHORALEAN ALGAE

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The study of species distributions requires well-defined species concepts and well-resolved species relationships. Ideally, the biogeography of taxa will be concordant with their genetic signatures identified by molecular markers. For groups with poorly defined species (or generic) concepts, taxon sampling can be highly problematic because morpholog-

ical plasticity may deter sample collection of a species when it expresses alternative morphologies. This may give the impression of a disjunct species distribution when it is actually continuous across a particular geographic range. Likewise, morphological convergence can obscure biogeographic patterns, but with the opposite effect. To avoid taxon sampling problems in a phylogeographic study of the green alga *Phyllocladon anastomosans* (Harv.) Kraft et Wynne, a species known for its morphological variability, we included taxa across four genera (*Boodlea* G. Murray & De Toni, *Cladophoropsis* Børgesen, *Phyllocladon* J. E. Gray, *Struveopsis* Rhyne et H. Robinson) often characterized by overlapping character states. The inclusion of isolates outside the taxonomic boundaries of *P. anastomosans* allowed us to extend earlier phylogeographic studies of the closely related *C. membranacea* (*C. agardh*) Børgesen and compare patterns between two closely related taxa. Results suggest that the Central American Isthmus represents a formidable but permeable barrier to dispersal.

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THE AQUACULTURE OF *PORPHYRA LEUCOSTICTA* (RHODOPHYTA) FOR AN INTEGRATED FINFISH/SEAWEED RECIRCULATING AQUACULTURE SYSTEM IN AN URBAN APPLICATION

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Aquaculture represents an excellent opportunity to help rejuvenate blighted coastal urban areas on the north-east coast. Aquaculture requires relatively little space, often acquired at reduced cost in ungentrified city areas, and can represent an attractive, environ-

mentally benign form of commerce. However, finfish and shellfish aquaculture operations are a source of an effluent with high concentrations of dissolved inorganic nutrients (N, P). To prevent eutrophication, the EPA is developing stringent guidelines for the release of N and P into coastal waters. An integrated recirculating aquaculture system, coupling the growth of seaweed and fish, can solve these problems for urban aquaculture facilities; not only is the effluent remediated but an additional multiproduct, high-value crop can be generated. One tank-based (on land) marine aquaculture operation is GreatBay Aquaculture, LLC (Portsmouth, NH), a land-based hatchery and grow-out facility for high value summer flounder and cod. Our work is to develop an integrated finfish/seaweed recirculating aquaculture system (RAS) suitable for urban aquaculture. Our RAS system will integrate the culture of summer flounder and native species of seaweed (i.e. *Porphyra*). BRVAS students are working along side undergraduate and graduate students in the construction and operation of these systems. There are at least seven recognized species of *Porphyra* in the north-east. We have begun mesoscale evaluation of *P. leucosticta*, since it may be a good candidate for the food (sushi) and for r-phycoerythrin industries. The mass culture techniques (in both free culture and attached to nets) for this *Porphyra* species are developing. We will report on the mass seeding technologies that we have developed and the specific growth rates of *P. leucosticta* at the BRVAS culture facilities.

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MOLECULAR CHARACTERISTICS OF *CAULERPA* (CHLOROPHYTA) POPULATIONS IN TAIWAN

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Tropical and subtropical *Caulerpa* species are epidemic to northern and southern Taiwan seashore. We here describe the molecular characteristics of rRNA gene, including the internal transcribed spacers (ITS) in this genus and a comparison was made with associated species sequences registered in the GenBank. A total of nine specimens (*C. racemosa* var. *microphysa*, *macrophysa*, *peltata*, *laetevirens*, *C. serrulata* and *C. elongata*) were investigated. Samples were collected and distinguished first from morphological characteristics. The genomic DNA was individually extracted using CTAB protocol. To amplify the 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA sequences, a primer pair NS7 and ITS4, sequences located in nuclear rRNA region, was used. The range of 980–1100 bp DNA frag-

ment from genomic DNA of individual plant was generated from polymerase chain reaction (PCR). Amplified fragments were sequenced automatically with ABI sequencer. The sequences were aligned with those of other *Caulerpa* species retrieved from the GenBank. The phylogenetic tree was constructed using Clustal method. The comparison of whole sequence and 18S rRNA and ITS1 region among these specimens shows that the interspecies divergence within *C. racemosa* var. *microphysa*, *macrophysa*, *peltata* and *laetevirens* is larger than those of intraspecies of *C. taxifolia*, *C. serrulata* and *C. elongata*. It is supposed that they (*C. racemosa* var. *microphysa*, *macrophysa*, *peltata* and *laetevirens*) should be in the position of different species in *Caulerpa* genus from the present rRNA sequences analysis. The sequences of 5.8S region are shown more conserve than that of 18S region. The identity of ITS1 and ITS2 regions in present species are low, comparing with *C. taxifolia*, *C. maxicana*.

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THE MONOPHYLETIC ORIGIN OF THE PERIDININ-, AND FUcoxANTHIN-CONTAINING DINOFLAGELLATE PLASTID THROUGH TERTIARY REPLACEMENT

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The dinoflagellates contain diverse plastids of uncertain origin. To determine the origin of the peridinin- and fucoxanthin-containing dinoflagellate plastid, we sequenced the plastid-encoded *psaA*, *psbA*, and *rbcL* genes from various red and dinoflagellate algae. The *psbA* gene phylogeny, which was made from a dataset of 15 dinoflagellates, 22 rhodophytes, five cryptophytes, seven haptophytes, seven stramenopiles, two chlorophytes, and a glaucophyte as the outgroup, supports monophyly of the peridinin-, and fucoxanthin-containing dinoflagellates, as a sister group to the haptophytes. The monophyletic relationship with the haptophytes is recovered in the *psbA* + *psaA* phylogeny, with stronger support. The rubisco tree utilized the 'Form I' red algal type of *rbcL* and included fucoxanthin-containing dinoflagellates. The dinoflagellate + haptophyte sister relationship is also recovered in this analysis. *Peridinium foliaceum* is shown to group with the diatoms in all the phylogenies. Based on our analyses of plastid sequences, we postulate that: (1) the plastid of peridinin-, and fucoxanthin-containing dinoflagellates originated from a common ancestor; (2) the ancestral dinoflagellate acquired its

plastid from a haptophyte though a tertiary plastid replacement; (3) 'Form II' rubisco replaced the ancestral *rbcL* after the divergence of the peridinin-, and fucoxanthin-containing dinoflagellates; and (4) we confirm that the plastid of *P. foliaceum* originated from a *Stramenopiles* endosymbiont.

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THE SINGLE, ANCIENT ORIGIN OF CHROMIST PLASTIDS

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Phylogenetic comparisons suggest that plastid primary endosymbiosis, in which a single-celled protist engulfs and 'enslaves' a cyanobacterium, likely occurred once and resulted in the primordial alga. This photosynthetic cell diversified, through vertical evolution, into the ubiquitous green (Chlorophyta) and red (Rhodophyta) algae, and the more scarce Glaucophyta. However, some modern algal lineages have a more complicated evolutionary history involving a secondary endosymbiotic event, in which a protist engulfed an existing eukaryotic alga (rather than a cyanobacterium), which was then reduced to a secondary plastid. Secondary endosymbiosis explains the majority of algal biodiversity, yet the number and timing of these events is unresolved. Here we analyzed a five-gene plastid dataset to show that a diverse group of chlorophyll *c*-containing protists comprising cryptophyte, haptophyte, and stramenopiles algae (*Chromista*) share a common plastid that most likely arose from a single, ancient (about 1260 million years ago) secondary endosymbiosis involving a red alga. This finding is consistent with *C. monophyly* and implicates secondary endosymbiosis as a driving force in early eukaryotic evolution.

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ACCUMULATION OF TRIACYLGLYCEROLS IN HAEMATOCOCCUS PLUVIALIS IS CORRELATED WITH THAT OF ASTAXANTHIN ESTERS

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The chlorophyte *Haematococcus pluvialis* accumulates large quantities of astaxanthin under stress conditions. Under either nitrogen starvation or high light, the proportion of each picogram of astaxanthin was accompanied by that of 5 or 3–4 pg of fatty acids, respectively. In both cases, the newly formed fatty acids, consisted mostly of oleic (up to 34% of fatty acids in comparison to 13% in the control), palmitic and linoleic acids, were deposited mostly in triacylglycerols. Furthermore, the enhanced production of oleic acid was linearly correlated with that of astaxanthin. Astaxanthin which is mostly monoesterified, is deposited in globules made of triacylglycerols. We suggest that the production of tailor-made oleic acid-rich triacylglycerols on the one hand and the esterification of astaxanthin on the other hand, enable the oil globules to maintain the high content of astaxanthin esters.

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MYCOSPORINES: DETECTION METHODOLOGIES AND ASSESSMENT

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Mycosporine-like amino acids (MAAs) are found in a variety of prokaryotic and eucaryotic algae, as well as higher plants, fungi, and animals. These compounds function as a photoprotective sunscreen to prevent ultra-violet light damage. MAAs may thus be one of the competitive advantages that facilitated development of ozone (by oxytrophs), and thereby may be a competitive advantage for the proliferation of cyanobacteria and other harmful algal species. Numerous difficulties exist with assessment of MAAs, including identification of the compounds, conversion of isomers

during HPLC preparation as a result of pH shifts, as well as the ecological implications of the presence, concentration, and forms of these compounds (see *J Phycolgy* 1999; 35, for relevant papers). This symposium will provide opportunities for intercalibration of laboratories involved in MAA analyses, suggestions regarding standardization of extraction protocols, as well as results from field- and laboratory-based studies.

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MYCOSPORINE-LIKE AMINO ACID INTERCALIBRATION EFFORT USING REPLICATE SAMPLES

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The measurement of mycosporine-like amino acids (MAAs) has several inherent problems, including the lack of commercial standards for identification and quantification. This symposium provided the opportunity to coordinate analyses with six laboratories actively involved in MAA research. Two samples were provided to each laboratory and included freeze-dried nori (*Porphyra* sp.) and a freeze-dried (filtered) sample of the cyanobacteria *Microcystis aeruginosa* Kützing. Each laboratory provided extraction methodologies, chromatograms of the identified peaks, as well as estimates of the concentration of each analyte. All laboratories were able to identify major chromatographic components of the samples (*Porphyra*: shinorine, porphyra, mycosporine 2-glycine, asterina, palythine; *Microcystis*: shinorine, porphyra). Sequential cold (4 °C for 18 h) and hot (45 °C for 2 h) extractions of the same sample resulted in differing analyte recovery. Several currently unidentified compounds were observed in freshwater samples.