Subaerial Algae in Tropical Rainforests

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Overview

- Biodiversity and systematics of subaerial algae
- Collection and study of subaerial algae
- Molecular systematics of subaerial microchlorophytes
- Evolution of subaerial microchlorophytes

Biodiversity and Systematics of Subaerial Algae

Over the past millions of years the land on our planet has been the testing ground for many experiments or, more dramatically, the battleground for many invasions. A myriad of ancestral plant forms came from the sea and lakes to exploit the terrestrial environment.

Those life forms were algae, that eventually prepared the land for the terrestrial flora and fauna that were to follow. They successfully conquered the land in terms of making it a useable new habitat for themselves and developed new forms and processes to adapt. Those plant “invaders” or “conquerors” are represented today by algae living among us populating soils and other terrestrial habitats.

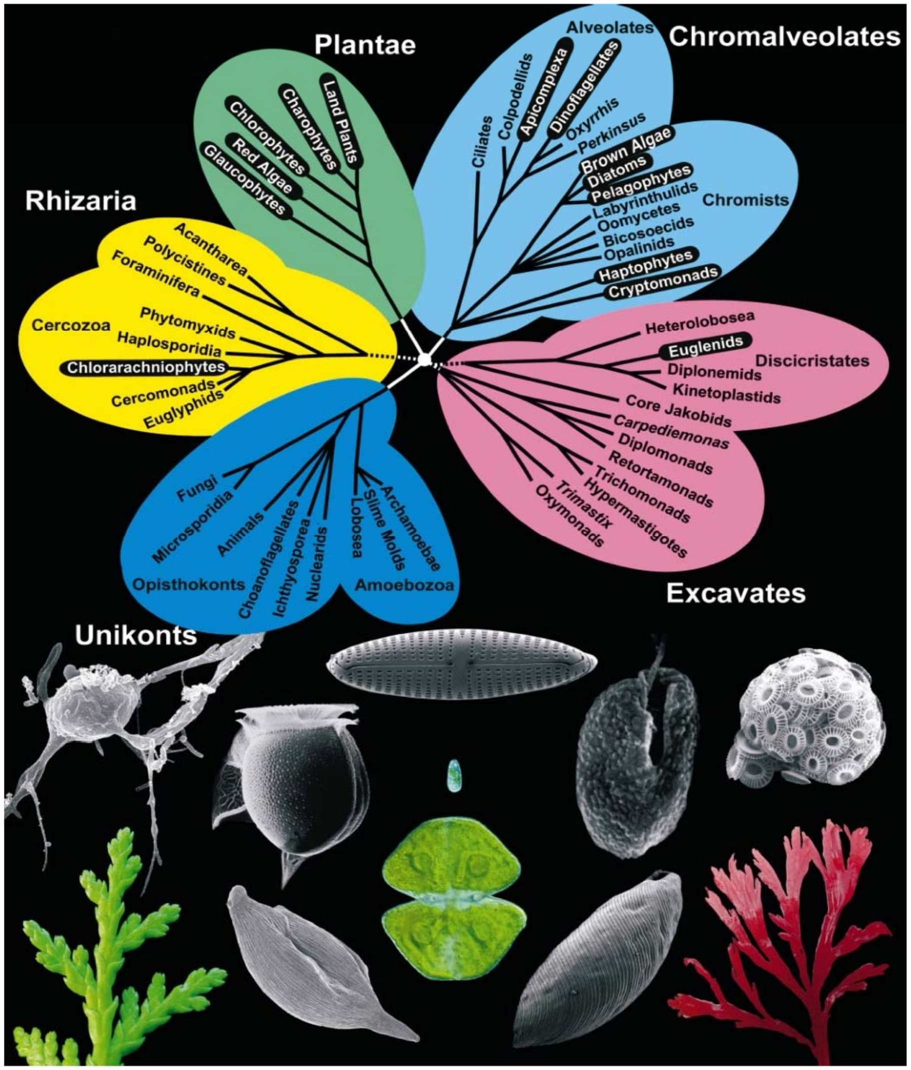
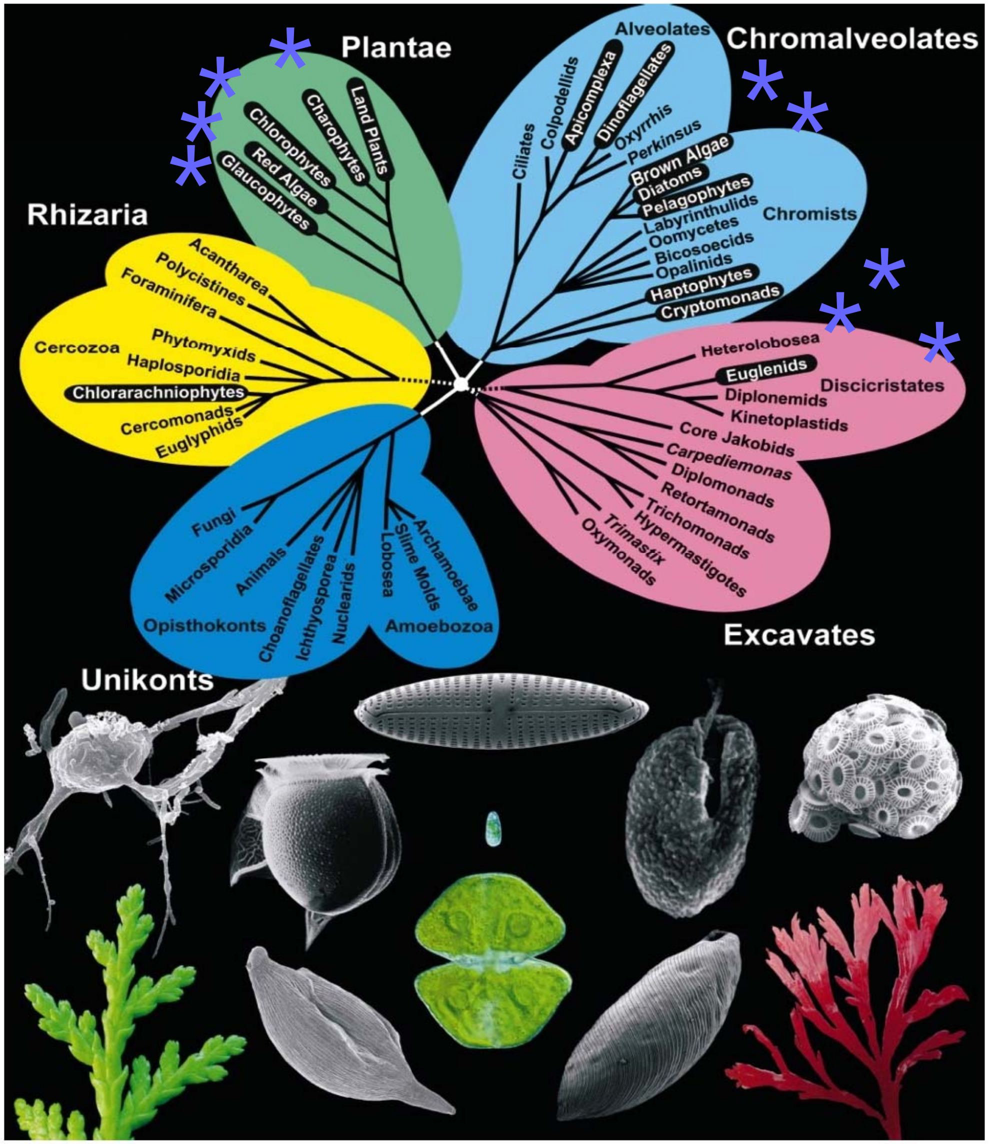


Fig. 1. Tree of eukaryotes and diversity of plastid-bearing eukaryotes. Top: an unrooted hypothetical phylogeny of eukaryotes based on a synthesis of many gene trees, protein insertions and deletions, and cellular and biochemical characters. In this tree, eukaryotes are divided into five large groups, or "supergroups," within which representatives of the major lineages are shown with their interrelationships as we know them. Dotted lines are plausible but more weakly supported parts of the tree. All groups in which plastids are known from at least a large number of species are indicated by white text on black. Bottom: a small taste of the diversity of plastid-bearing eukaryotes can be seen from one representative of each of the major "algal" lineages. Outside photographs, clockwise from

Keeling 2004

Most of the photosynthetic organisms that occur nowadays in aquatic habitats belong to this heterogeneous category generally called algae.

These organisms are phylogenetically unrelated, or only distantly related, and differ enormously among themselves (morphology, ultrastructure, biochemical, etc.)



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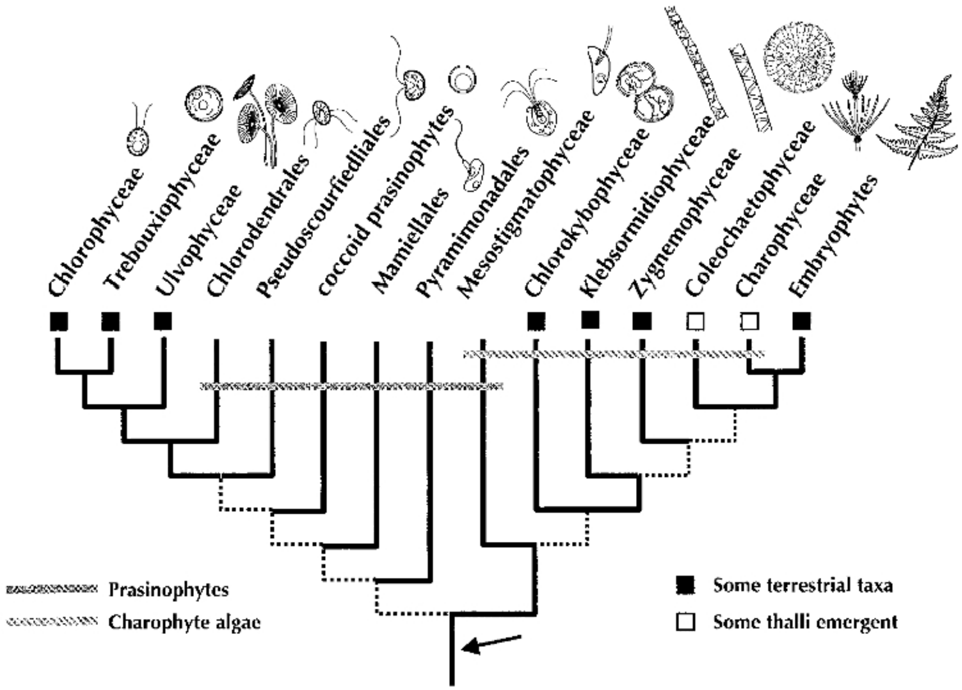
Several lineages of algae (*) successfully colonized terrestrial environments

Although the most important conquest of land was that of the green algae of the streptophytan lineage, several other groups did succeed in becoming terrestrial

Keeling 2004

There were multiple conquests of the land by different lineages of green algae that did not give rise to land plants, but nevertheless did succeed in making a permanent existence on land

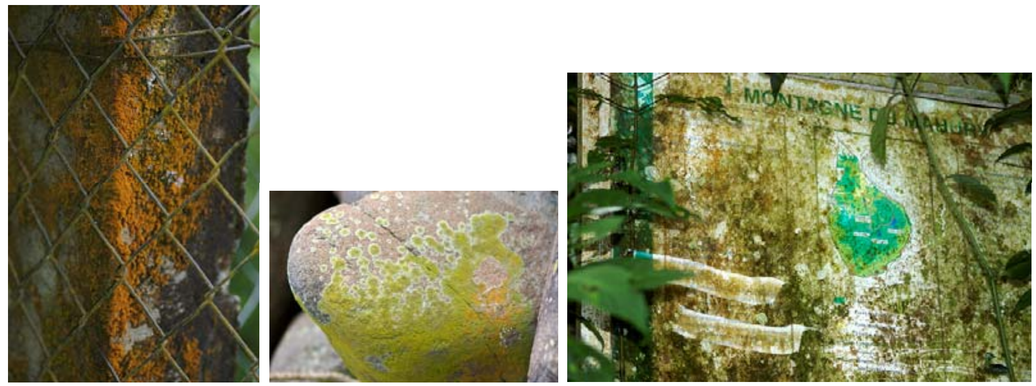


Lewis & McCourt 20040

Recently we started a research program at UA to unravel the evolutionary history of these algal quests for land; this session will highlight our most recent studies and the unexpected and interesting findings

Subaerial algae are terrestrial algae that live on stable exposed surfaces above the soil, they are perhaps the most obvious, and yet most overlooked, group of algae

Yucatan 2005



French Guyana, 2006

South Africa 2005

In countries with ancient and rich cultural heritage the effects of algal deterioration on monuments has been studied in detail and great efforts have been made to limit their development (Biodeterioration)

Mayan pyramid, 2005

Galway, Ireland 2004

Boboli Gardens, Florence 2006

Venice, Italy 2006

Species of *Cephaleuros* are common on the leaves of tropical trees and shrubs with economic importance causing death and perhaps injuring the host plants

In tropical countries subaerial algae are extremely abundant to damage concrete buildings and biocides for their control have been evaluated; in Singapore legislation forces owners of houses to keep their homes algae free!

But subaerial algae can also be useful

- ✓ Potential tools as bioindicators of air pollution
- ✓ Commercial production of carotenoids
- ✓ Potential use as biofuels

Often overlooked in the subaerial habitats, the total diversity of these taxa is typically underestimated

This is particularly problematic in the tropical rainforests, for which this flora is poorly known and in urgent need of further investigation

They may provide the keys to understand the struggles and solutions that primordial photosynthetic forms went through in order to conquer the land and become the most notable feature of our planet

• The subaerial habitat, although rich in other groups of organisms (*i.e.*, bryophytes and fungi) in tropical and subtropical regions, has been long neglected

• The general public is unaware of these organisms

• Even scientists are usually unaware of this algal group; for example, *Veralucia brasiliensis* was erroneously described as a new genus and species based on a fungus from the Amazon in Brazil

• EVEN Phycologists confuse vascular plants with these algae: *Trentepohlia allorgei* is the protonema phase of an aquatic fern!

Floristics

- Subaerial algal studies are available mostly from Europe and recently Antarctica
- In terms of habitats, natural rock surfaces are the most extensively studied
- Due to their biodeterioration effect some algae have gained attention
- Epiphytic algae are best known in temperate areas
- Little information exists about epizoic algae; although sloths, spiders, lizards, polar bears, prosimians, and birds have all been found to support subaerial algae!

Biodiversity and Systematics

The vast majority of the photosynthetic microorganisms occurring in terrestrial habitats belong to five groups

- Cyanobacteria
- Chlorophyta
- Charophyta (*sensu* Lewis and McCourt 2004)
- Heterokontophyta (*sensu* Andersen 2004)
- Rhodophyta

From a numerical point of view, the prokaryotic Cyanobacteria and the eukaryotic Chlorophyta account for the largest numbers of species currently described for the subaerial habitat

Account of terrestrial algae (soil, subaerial and lichenized) according to Ettl & Gartner 1995

Algal group	Genera	Species
Chlorophyta	182	608
Rhodophyta	4	8
Cryptophyta	1	1
Dinophyta	3	4
Chrysophyta	64	418
Eustigmatophyta	5	13
Euglenophyta	5	13
Total	264	1,065

Cyanobacteria not considered

Cyanobacteria are classified into five groups according to Bergey's *Manual of Systematic Bacteriology*

- The Chroococcales are unicellular and colonial cyanobacteria (Prochloron, *Chroococcus* and *Microcystis*)

- Pleurocapsales, are coccoid forms with division in more than one plane (*Pleurocapsa*)

- The Oscillatoriales includes simple filamentous forms such as *Lyngbya, Microcoleus* and *Oscillatoria*

- The Nostocales is largely formed by filamentous forms with heterocysts (*Nostoc, Anabaena,* and *Rivularia*)

- The Stigonematatales are consisting of multiseriate branched filaments with heterocysts (*Stigonema* and *Fischerella*)

SYSTEMATICS OF SUBAERIAL CYANOBACTERIA

- Although the Cyanobacteria are some of the most widely distributed and ubiquitous organisms on the planet they are also historically understudied
- Growing on such diverse habitats as rocks, soils, tree bark and dripping walls, the taxonomy and biodiversity of these taxa is still mostly unknown
- Among the oldest recorded fossils, the Cyanobacteria were significantly involved in the formation of life on the planet as some of the earliest colonizers
- Unfortunately, they are also among the most poorly characterized organisms, in part due to the prevailing belief among classic monographers that many species are cosmopolitan
- However, recent studies have noted that these organisms may not be as cosmopolitan as previously assumed and endemic taxa are currently being described
- Cyanobacterial diversity is still underreported due to difficulties in employing morphological characters to identify species and a lack of specialists exploring novel habitats
- Thus, the subaerial habitats are a nearly unexplored treasure of cyanobacterial biodiversity

SYSTEMATICS OF SUBAERIAL CYANOBACTERIA

•The taxonomy of Cyanobacteria has gone through major rearrangements in the last two decades, culminating in the recent revisions of Komárek and Anagnostidis

•Their approach is based on a combination of morphological, ultrastructural, biochemical, and (in part) genetic data and has proposed a substantial rearrangement in comparison with the traditional Geitlerian- and the simplified Drouetian-system

•Many new genera have been erected and the circumscription of many traditional general (including some widespread in subaerial habitats, such as *Gloeocapsa, Lyngbya* and *Phormidium*) have been substantially rearranged

•In the last 15 years, an increasing number of investigations have studied the phylogeny of the Cyanobacteria, mainly using 16S rDNA gene sequences

•This molecular approach, coupled with careful ecological and morphological assessments, has enabled researchers to begin cataloguing the great biodiversity undoubtedly present in this lineage

Preliminary observations on subaerial cyanobacterial taxa have revealed:

- First, there are many more species of Cyanobacteria (particularly Oscillatoriales and Chroococcales lineages) than are evidenced by employing traditional taxonomic keys, and consequently there are numerous new species to be described

- Second, in a number of broadly defined genera, such as *Leptolyngbya, Microcoleus,* and *Nostoc*, the 16S rRNA and ITS data show that more genera must be recognized

- Third, ITS regions vary widely between strains, and have been informative for making systematic decisions at both the genus and species levels.

- Fourth, in *Leptolyngbya* the ITS is not always congruent with phylogenies based on 16S sequences, possible due to multiple operons within genomes

- Fifth, when habitats of geographically isolated regions are studied closely, they have endemic species of cyanobacteria. For example, the Nostocacean lineage, is among the most common components of subaerial algal communities, and two new endemic genera in this group have recently been described: *Rexia* and *Mojavia*

- Thus, as these habitats are explored, more new taxa as well as emendations of some existing genera of Cyanobacteria are expected from the subaerial habitat

The Chlorophyta consist of four main lineages

- The Prasinophytes, is basal and non-monophyletic group with marine members

- Most subaerial Chlorophyta are in the Trebouxiophyceae, with several widespread genera (e.g., *Chlorella*, *Prasiola*, *Stichococcus*, and *Trebouxia*)

- The Chlorophyceae are mostly freshwater algae, but the class includes some common subaerial genera (i.e., *Bracteacoccus* and *Chlorococcum*)

- The Ulvophyceae is largely formed by the marine green algae

The Charophyta are members of the other major lineage of green plants (the Streptophytes)

•The phylum includes the closest relatives to the algae that gave rise to the land plants. The main lineages are:

•Mesostigmatophyceae, represented only by the marine *Mesostigma viride*

•Chlorokybophyceae, a single representative, *Chlorokybus atmophyticus,* found on soil

•Klebsormidiophyceae, with subaerial representatives (*Klebsormidium*)

•Zygnematophyceae, mainly freshwater forms (Desmids and conjugating algae)

•Coleochaetophyceae, freshwater forms (*Coleochaete* and *Chaetosphaeridium*)

•Charophycea or stoneworts, mainly found in freshwater but also in brackish and semi-terrestrial habitats.

SYSTEMATICS OF SUBAERIAL CHLOROPHYTA

•The taxonomy of green algae is in a phase of major rearrangements

•The existence in the Chlorophyta of four main groups, three of which represent monophyletic lineages (Chlorophyceae, Trebouxiophyceae and Ulvophyceae), is now widely accepted

•Arrangement at all other taxonomic levels, however, is still unclear

•molecular data collected in the last two decades has given rise to dramatic modifications at all level of classification, and chlorophytan taxa in terrestrial habitats have been the most affected

•Some traditional orders (Chlorellales, Chlorococcales, Chlorosarcinales) originally circumscribed using vegetative morphology, contain phylogenetically unrelated taxa

•Reclassification into different groups has occurred even at lower taxonomic levels, especially the genus level

•Several common genera of subaerial chlorophytes, such as *Chlorella*, *Chlorococcum*, *Neochloris* and *Trebouxia*, include species that in fact belong to different classes!

•Splitting of groups described on traditional morphological basis and erection of new genera is a general trend in the current microchlorophyte taxonomy

•We will review specific cases of subaerial microchlorophytes during the afternoon session

Morphological Convergence

- It is now generally accepted that gross morphology and reproductive features do not reflect phylogenetic patterns

- Furthermore, in several different lineages of subaerial algae, the thallus has converged in a limited number of morphological types

- Consequently, a great deal of genetic diversity is often hidden behind identical or very similar morphologies.

- For terrestrial green algae this makes their taxonomy particularly problematic, due to the limited number of characters useful for a reliable morphological identification

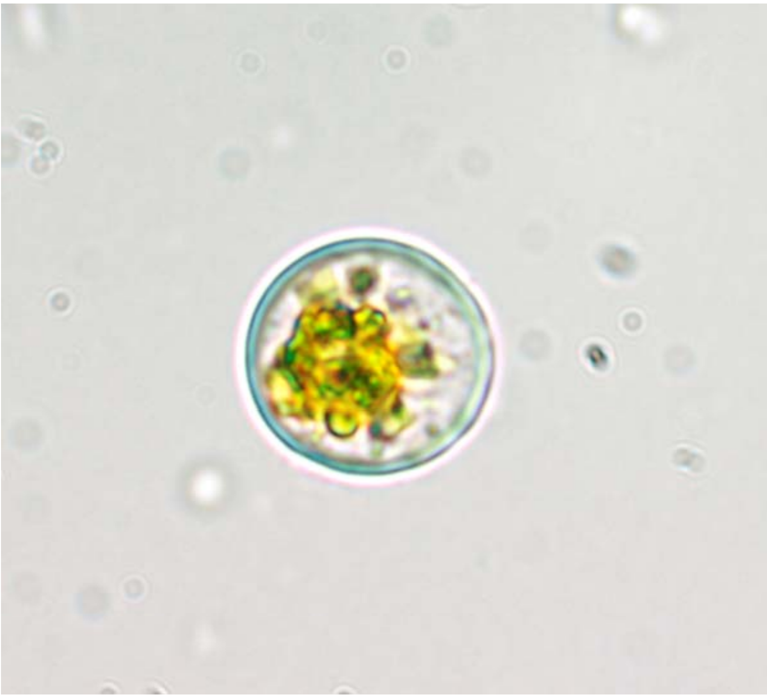
Three main types of thallus morphology are found in subaerial algae:

1. Unicellular

2. Sarcinoid (regular packets formed by a small number of cells)

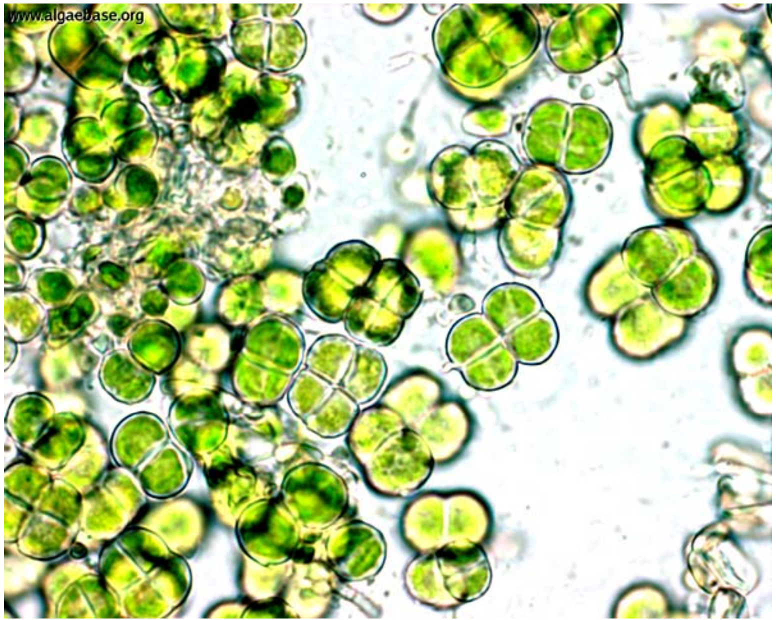
3. Uniseriate filaments

The unicellular morphology is clearly the most widespread ; this is typical of many common genera shown to be polyphyletic: *Bracteacoccus*, *Chlorella*, *Chlorococcum*, *Muriella*, *Myrmecia*, *Stichococcus*, *Tetracystis*, and *Trebouxia*



Spongiochrysis Hawai'i 2005

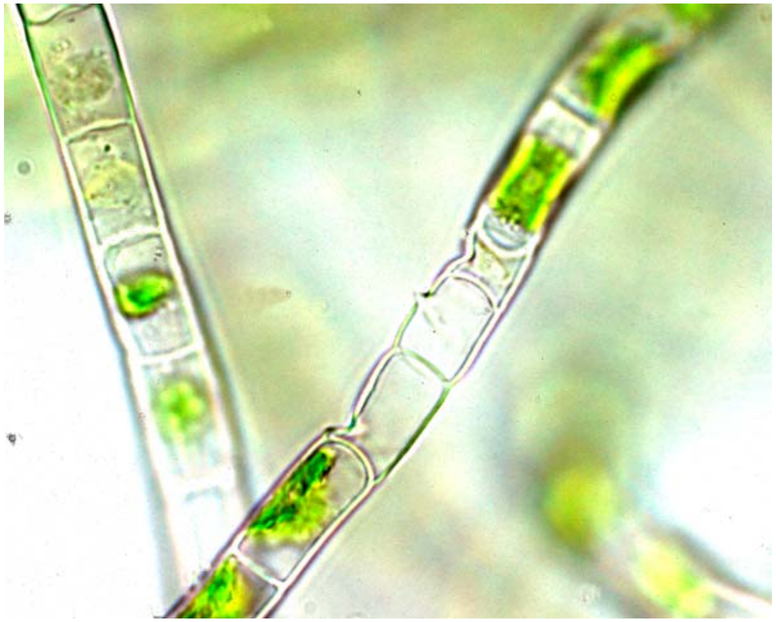
The sarcinoid morphology occurs in a more limited number of genera: e.g., *Apatococcus*, *Chlorokybus*, *Chlorosarcina*, *Desmococcus*, and *Prasiococcus*



Desmococcus Galway Ireland 2004

But it is characteristic of some of the most successful taxa; i.e., *Desmococcus olivaceus,* reported as the most common green alga in the world

Uniseriate filaments are found in a relatively limited number of species, belonging to two different groups: *Rosenvingiella* and *Prasiola* in the Trebouxiophyceae and *Klebsormidium* in the Charophyta



Klebsormidium Galway Ireland 2004

Klebsormidium, however, is one of the most successful and widespread genera on a global scale and occurs in a wide range of habitats

Although the causes for this morphological convergence are not well understood, it is clear that a simplification of the thallus from aquatic to subaerial habitats has generally been favoured

Other adaptations include, but are not restricted to:

- Capacity to utilize water in the form of vapour
- Mucilaginous envelopes retaining moisture
- Resistance stages such as akinetes
- Pigments acting as a protection from solarization
- Anti-freezing compounds
- Mycosporine-like amino acids as protection from UV radiation

It is considered that a spheroid unicell up to 12 µm in diameter, as found, for example, in species of *Chlorella* and *Stichococcus*, is the ideal airborne alga

Subaerial Algae and Tropical Rainforests

- Investigations by earlier naturalists in tropical rainforests were not specifically focused on subaerial habitats and did not attempt to provide an inventory of their algal diversity

- Until recently, no studies have focused specifically on these habitats and the information available is based entirely on a classical morphological approach

- No investigations have thus far attempted to characterize the subaerial algal flora of these forests and to examine in detail their biodiversity using state-of-the-art methods

- This is unfortunate since tropical rainforests are considered specialized chemical factories, with many molecules with valuable compounds

- Gaps in the biology and diversity of subaerial algae are even more substantial for microalgae from tropical rainforests

- To present a synthesis of the biodiversity status for subaerial algae of tropical forests is difficult or even impossible

The Urgency

•Rainforests are highly humid and wet, thus particularly suitable for subaerial algae

•It has been demonstrated that tropical rainforests are among the most diverse ecosystems on the planet

•They are repositories of a large number of endemic taxa and the necessity of their conservation is largely accepted and justified

•Several subaerial taxa that have been described in the last decade were originally discovered in tropical forests

•Rainforests are centres of diversity, and it is reasonable to expect that they host a much higher diversity of subaerial algae than currently appreciated

•Unfortunately, tropical rainforests are endangered ecosystems and rapidly disappearing due to deforestation, climatic changes and other human activities

•The risk that many tropical algal lineages will become extinct before they are discovered is largely acknowledged

Systematics, Biodiversity and Phylogeny

BIODIVERSITY: Subaerial algae from tropical rainforests. Discovery science.

SYSTEMATICS: Traditional morphology-based classifications are working hypotheses to be tested with molecular techniques.

PHYLOGENY: Uncover evolutionary histories of species and algal groups in order to clarify their classification and understand their current distribution

At the PhycoLab we use of chloroplast-encoded markers (*rbc*L), nuclear-encoded (SSU, ITS and LSU ribosomal DNA) and mitochondrial-encoded markers (COX1), as well as detailed comparative morphological data to generate phylogenies in order to answer questions on biodiversity, systematics, evolutionary relationships, and biogeography.

In order to accomplish our goals we must to go out and collect some algae

1. GOALS: What do you want to accomplish? Is it a biodiversity or taxon-based expeditionary work?
2. PLANNING PROCESS: What is necessary to do in order to accomplish your goals? Where, when, how and by who the expedition should be carried out to be cost effective?
3. FIELD WORK: When in Rome….
4. ASSESSMENTS: Lab work, tests, analyses
5. AFTERWORK: Publications, grants, dissertations, outreach, service
6. THE NEXT ADVENTURE

RESEARCH EXPEDITIONS

EUROPE

NORTHAMERICA: ALASKA, SE USA, MEXICO

AFRICA: SOUTH AFRICA, GABON, MOROCCO

CENTRAL AND SOUTH AMERICA: FRENCH GUIANA, PANAMA, SURINAM, CUBA