

Kingdom: Plantae

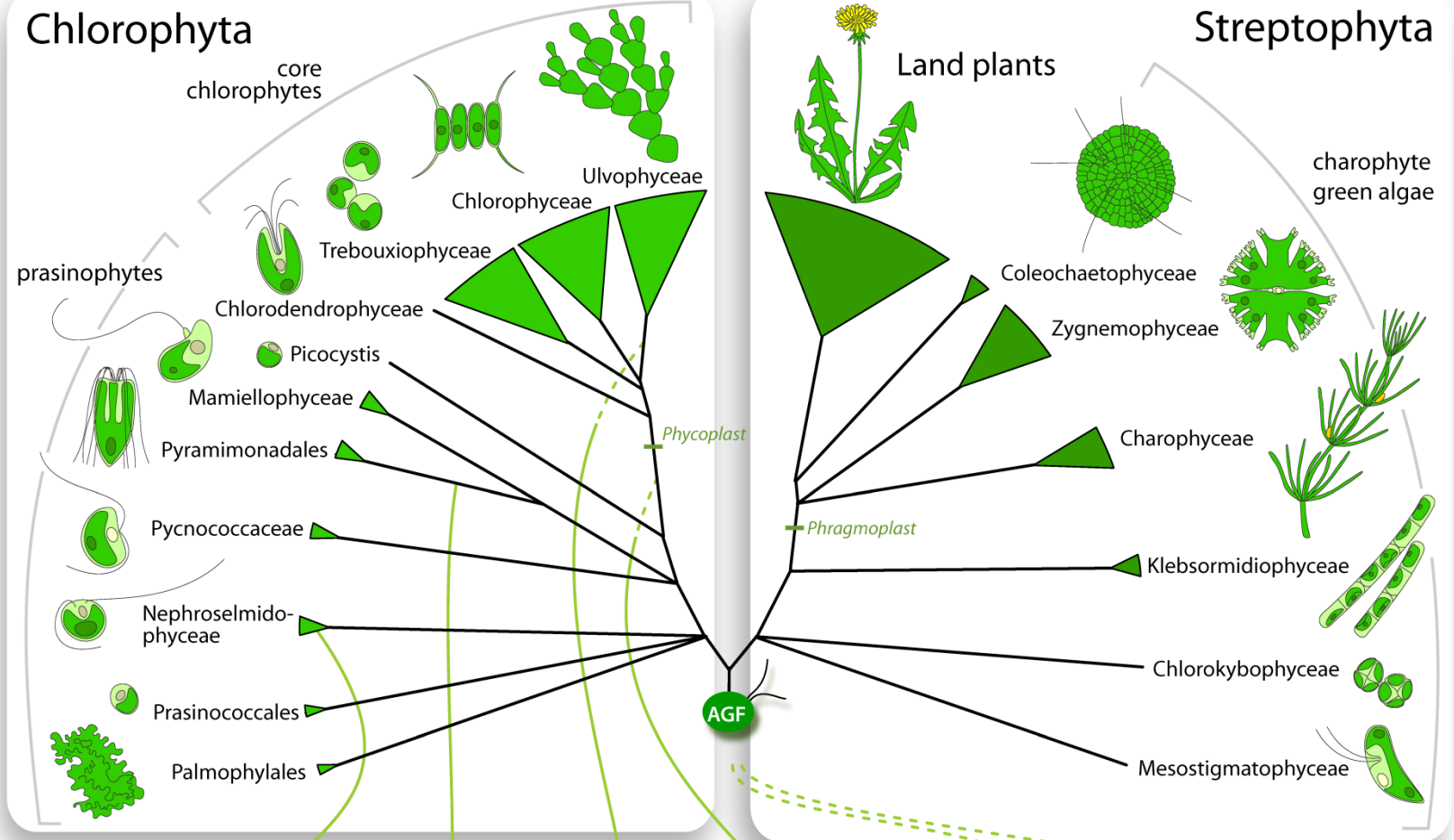
Order: Chlorophyta

Class: Trebouxiophyceae

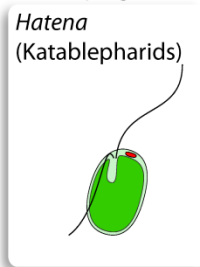


Chlorophyta

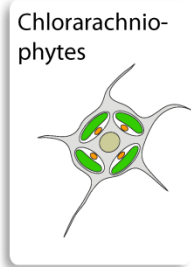
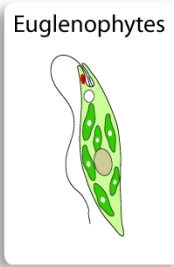
Streptophyta



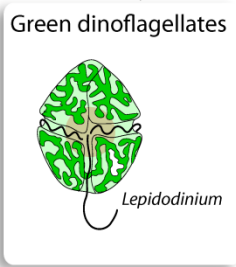
Putative secondary symbiosis in progress



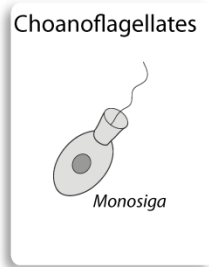
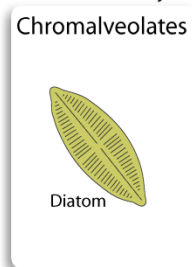
Secondary endosymbiosis



Serial secondary endosymbiosis

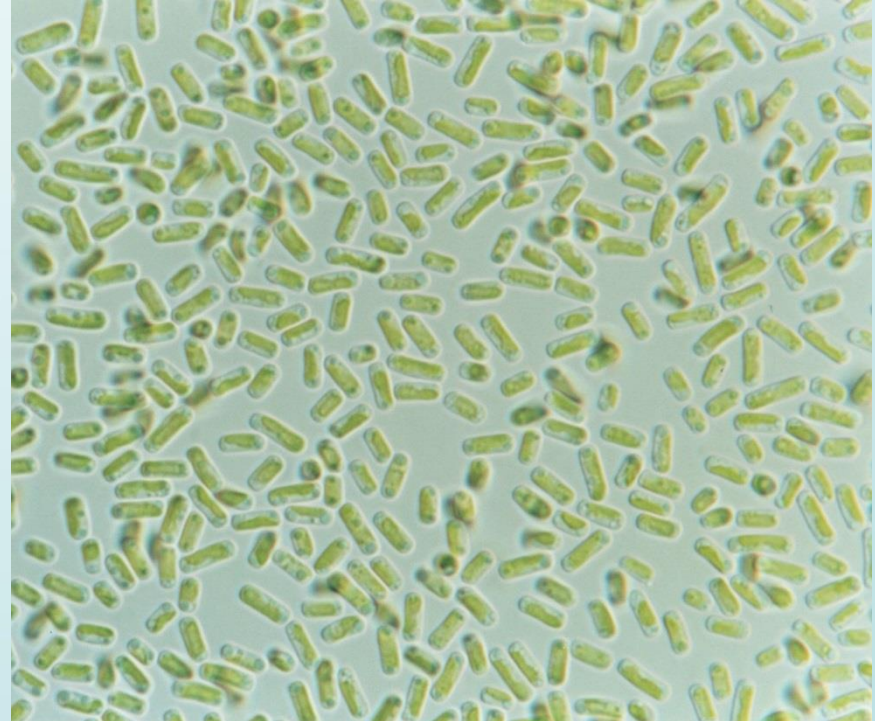
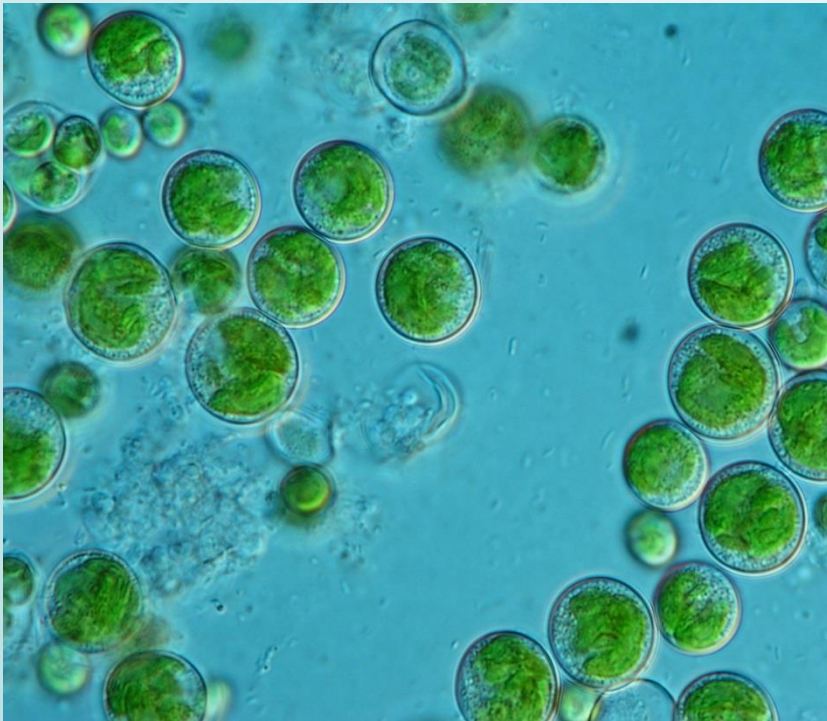


Putative ancient endosymbioses



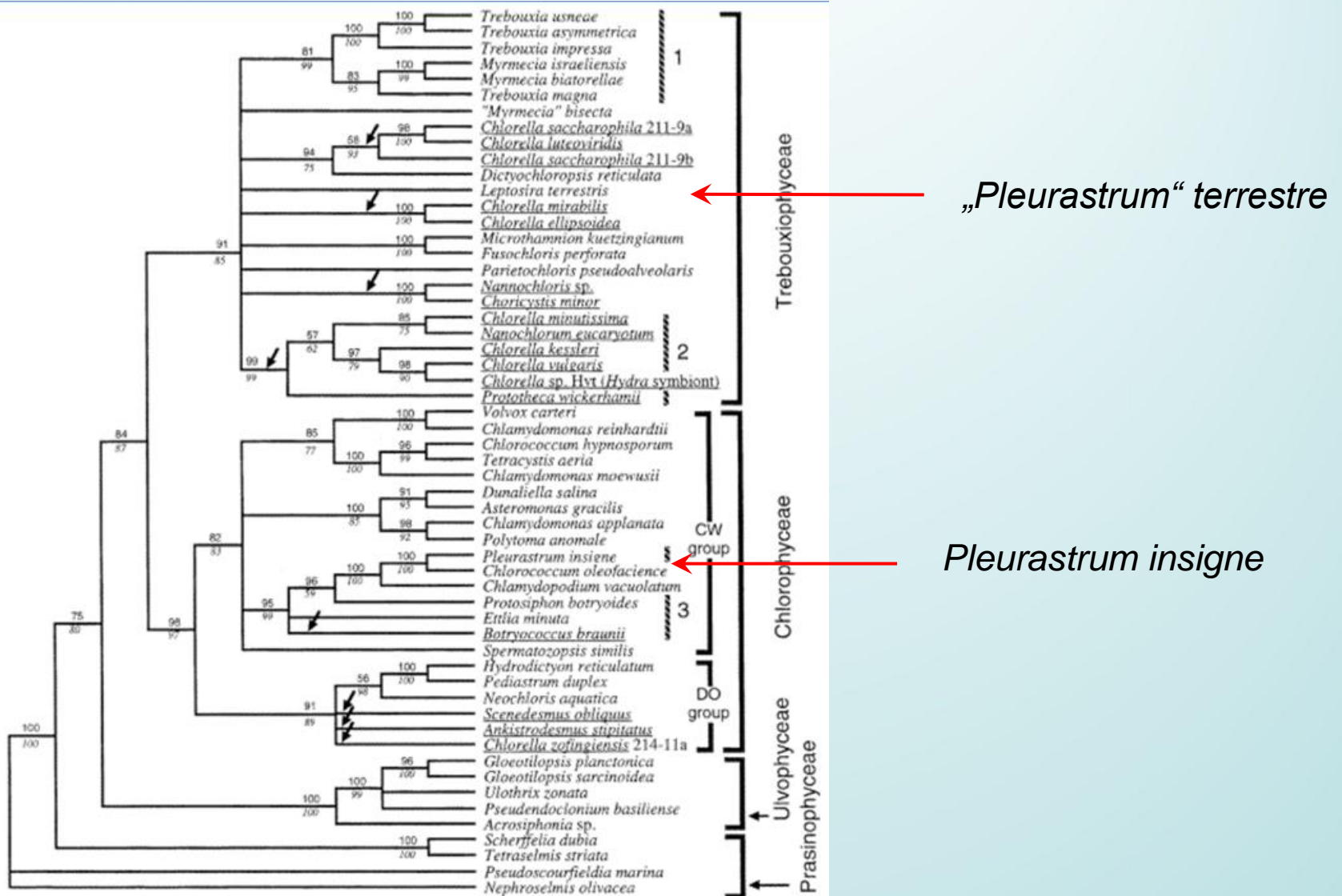
General features

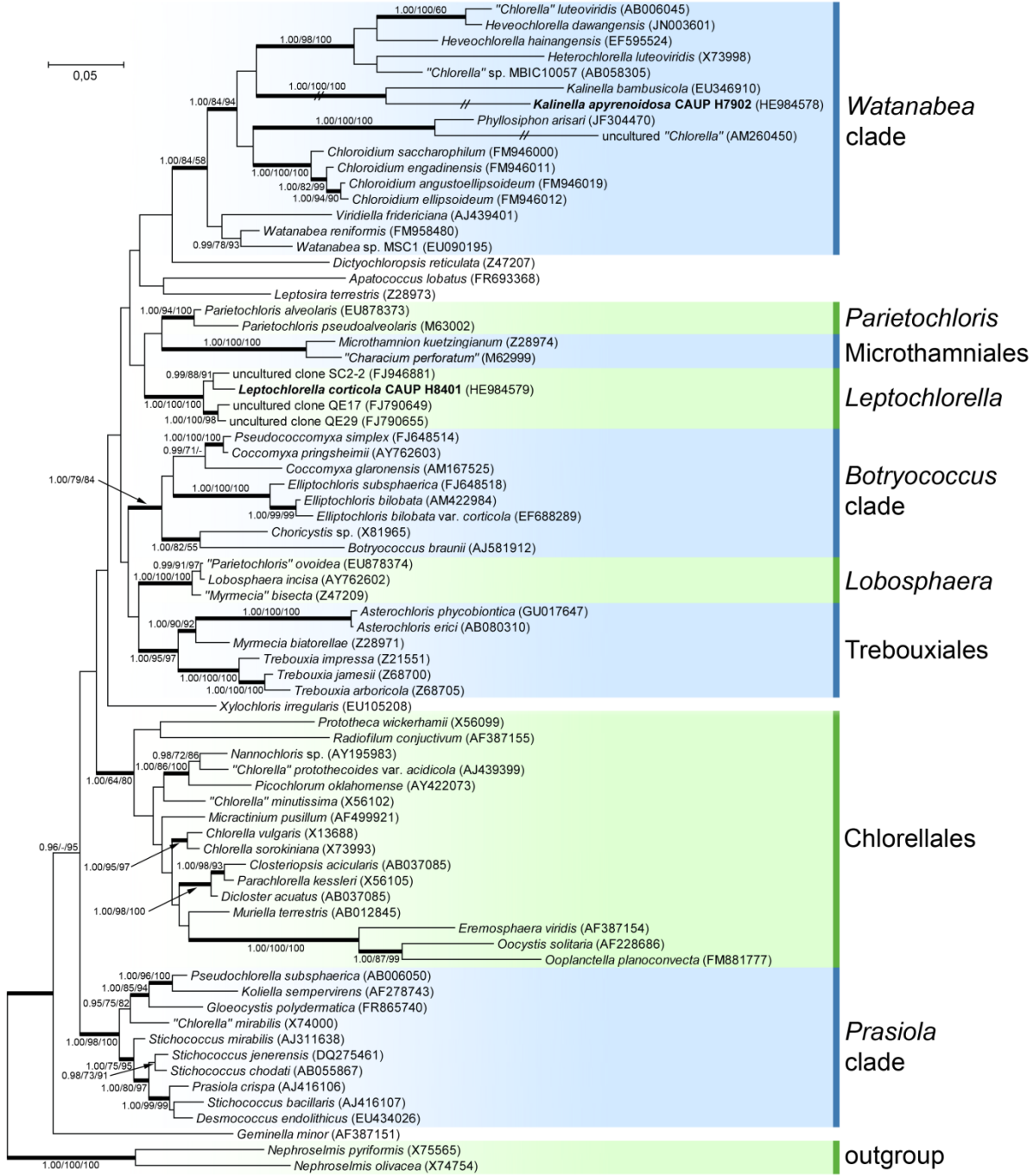
- the class was established in 1995
- unicellular and filamentous algae
- predominantly terrestrial, aerophytic
- a high proportion of symbiotic organisms



Mattox & Stewart (1984): Pleurostrophyceae (characterized by DO orientation of flagellar roots)

Friedl (1996): Trebouxiophyceae

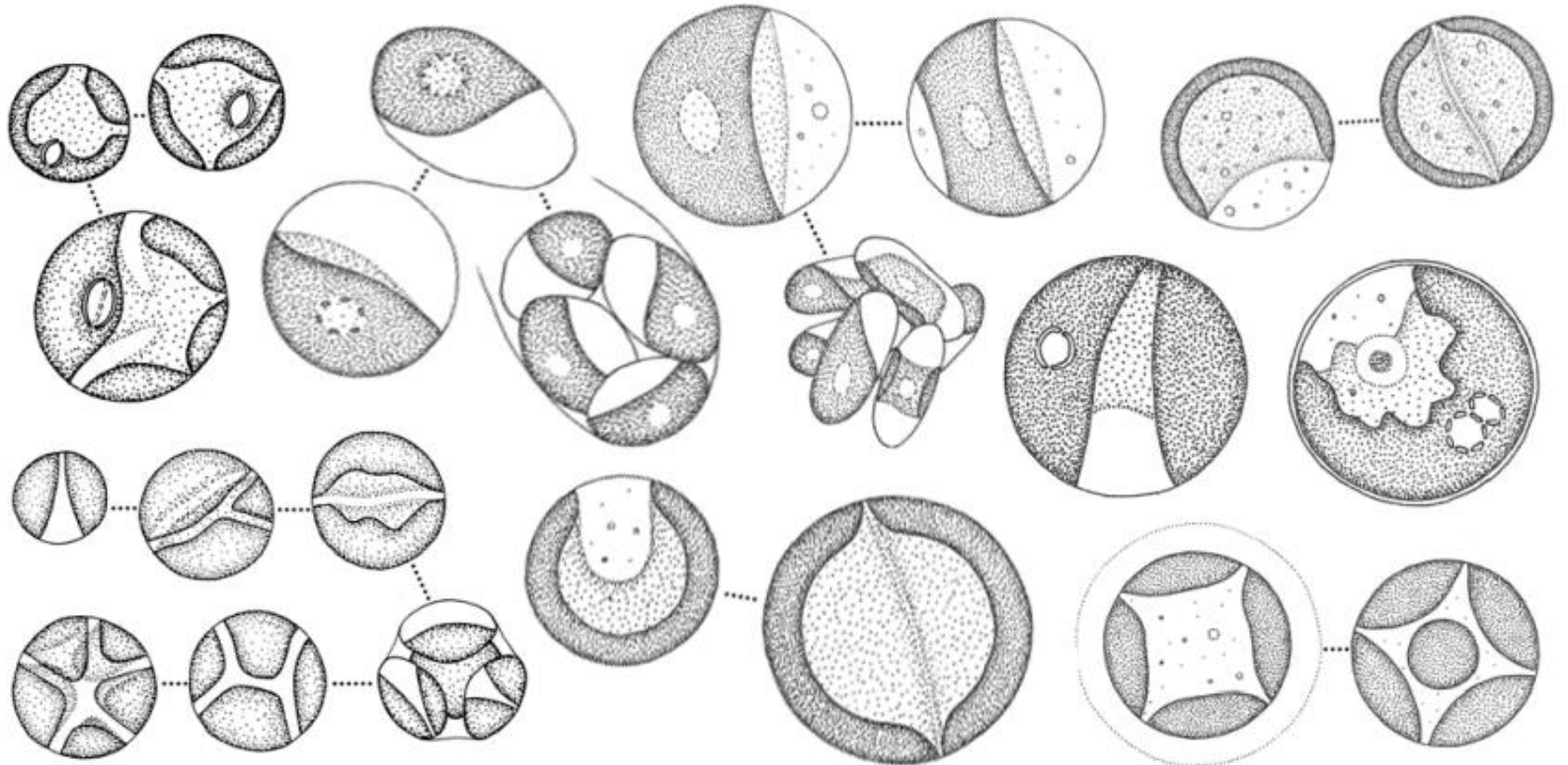




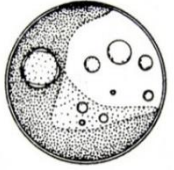
Trebouxiophyceae

Trebouxiophyceae, *Chlorella*

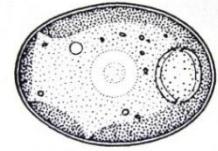
- Coccoid unicellular green algae with globular to oval cells that reproduce entirely by autospores
- Probably the most abundant and diversified group of aerophytic algae
- Beijerinck (1980):
„... ich werde unten die Algen derart beschreiben, dass jeder dieselben leicht erkennen kann.“
„... now I describe this alga in such a way that everybody will simply distinguish it... “



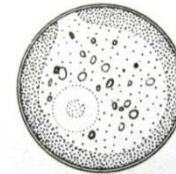
Trebouxiophyceae, *Chlorella*



Chlorella vulgaris



Chlorella fusca
v. fusca



Chlorella homosphaera



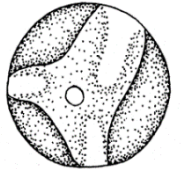
Chlorella sorokiniana



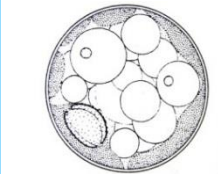
Chlorella fusca
v. rubescens



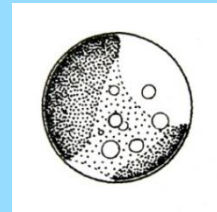
Chlorella zofingiensis



Chlorella lobophora



Chlorella fusca
v. vacuolatus

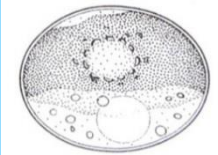


Chlorella ellipsoidea

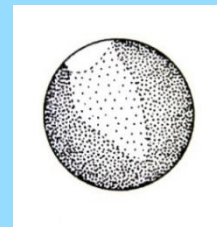
Chlorella protothecoides



Chlorella kessleri



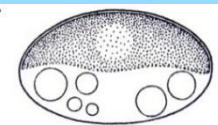
Chlorella trebouioides



Chlorella minutissima

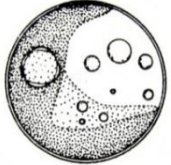
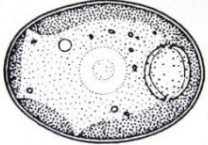
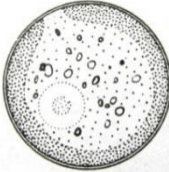

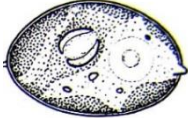
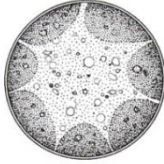
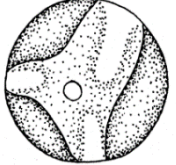
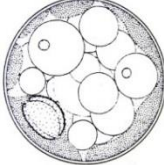


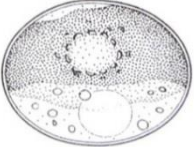


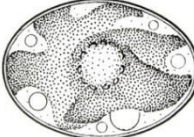
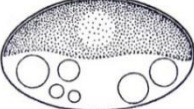


Chlorella luteoviridis



Chlorella saccharophila

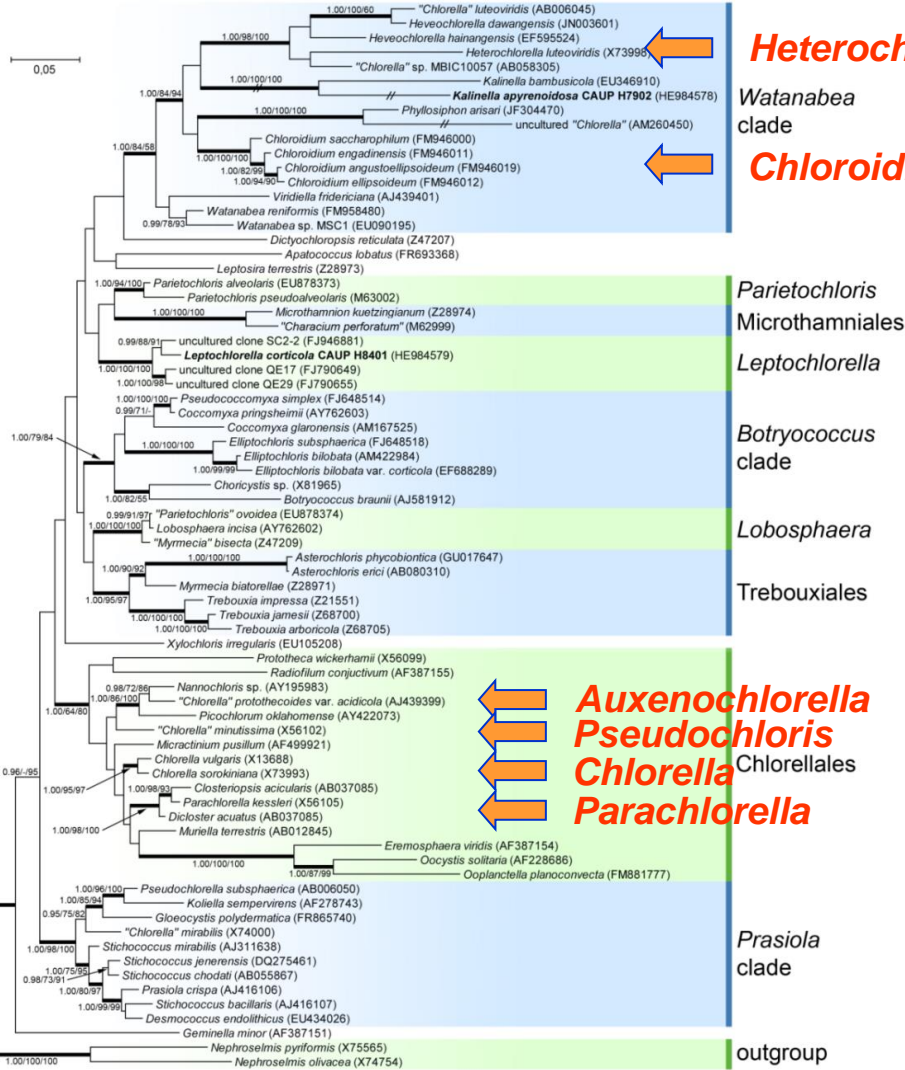
Trebouxiophyceae, *Chlorella*

	<p>glukosamin <i>Chlorella vulgaris</i></p>		<p><i>Desmodesmus abundans</i> (<i>Ch. fusca</i>, v. <i>fusca</i>) (<i>Scen. abundans</i>)</p>		<p><i>Mychonastes homosphaera</i> (<i>Ch. homosphaera</i>, <i>Ch. minutissima</i>)</p>
	<p><i>Chlorella sorokiniana</i> (<i>Ch. vulgaris</i>)</p>		<p><i>Scenedesmus rubescens</i> (<i>Ch. fusca</i>, v. <i>rubescens</i>) (<i>Halochlorella rubescens</i>)</p>		<p><i>Chromochloris zofingiensis</i> (<i>Ch. zofingiensis</i>)</p>
	<p><i>Chlorella lobophora</i></p>		<p><i>Scenedesmus vacuolatus</i> (<i>Ch. fusca</i>, v. <i>vacuolata</i>) (<i>Graesiella vacuolata</i>)</p>		<p><i>Auxenochlorella protothecoides</i> (<i>Ch. protothecoides</i>)</p>
	<p><i>Parachlorella kessleri</i> (<i>Ch. kessleri</i>)</p>		<p><i>Chloroidium ellipsoideum</i> (<i>Ch. ellipsoidea</i>)</p>		<p><i>Pseudochloris wilhelmii</i> (“<i>Ch. minutissima</i>”)</p>
	<p><i>Heterochlorella luteoviridis</i> (<i>Ch. luteoviridis</i>)</p>		<p><i>Chloroidium ellipsoideum</i> (<i>Ch. trebouxioides</i>)</p>		
			<p><i>Chloroidium saccharophilum</i> (<i>Ch. saccharophila</i>)</p>		

An et al. (1999), Huss et al. (1999), Krienitz et al. (2004), Darienko et al. (2010), Neustupa et al. (2009), Fučíková & Lewis (2012), Somogyi et al. (2013)

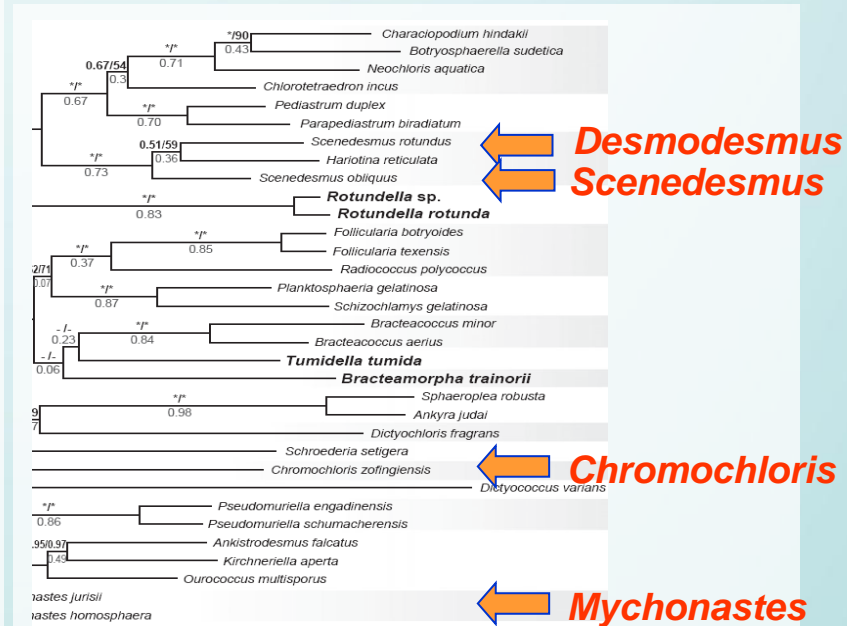
Trebouxiophyceae, *Chlorella*

Trebouxiophyceae:



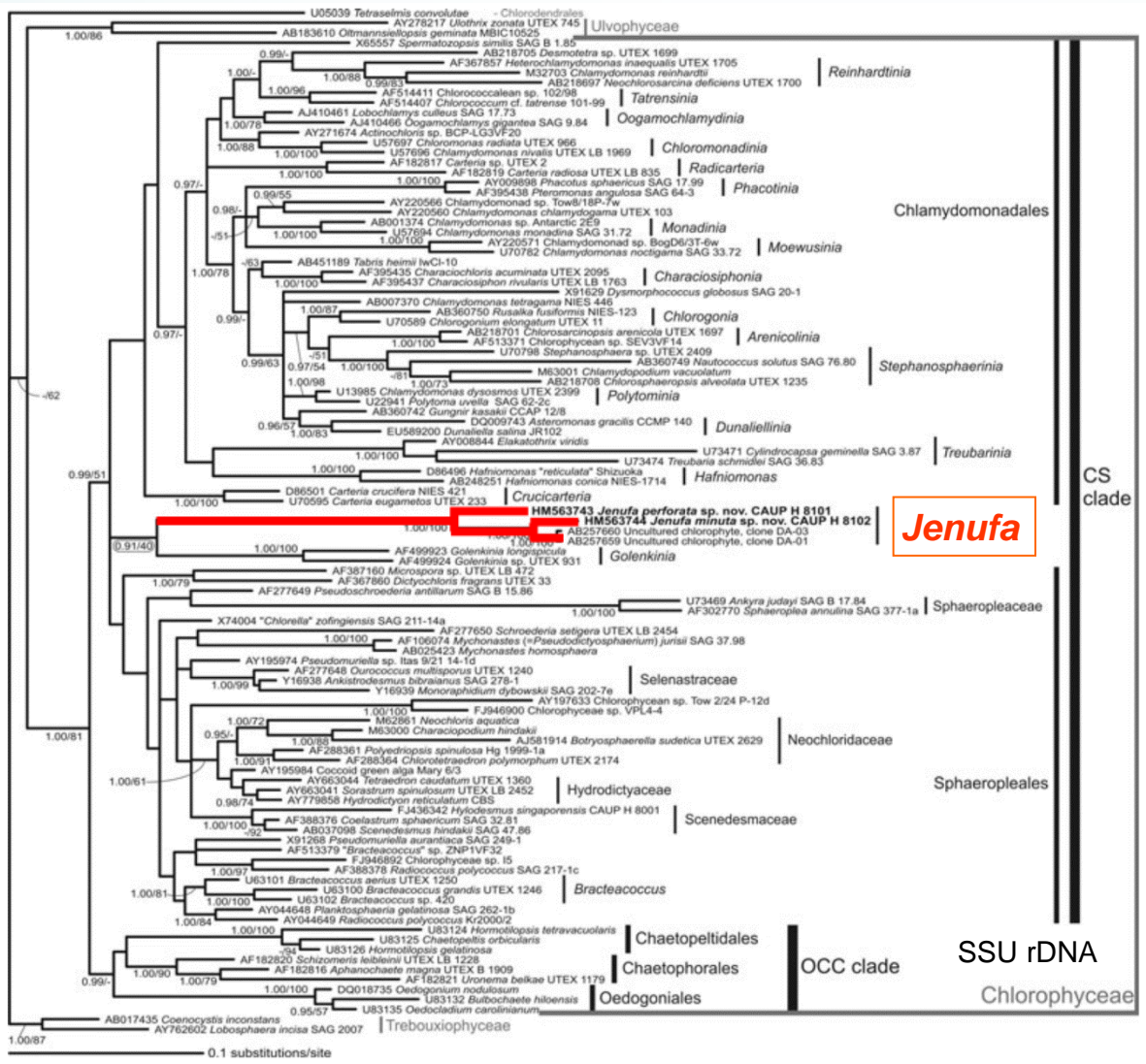
- In subaerial conditions, the drought stress drives selection towards the globular forms with low surface-to-volume ratio
- Traditionally circumscribed *Chlorella* species fall into at least 10 different lineages/genera

Chlorophyceae:



Trebouxiophyceae, *Chlorella* – cryptic genera

Chlorophyceae:



CS clade

Jenufa

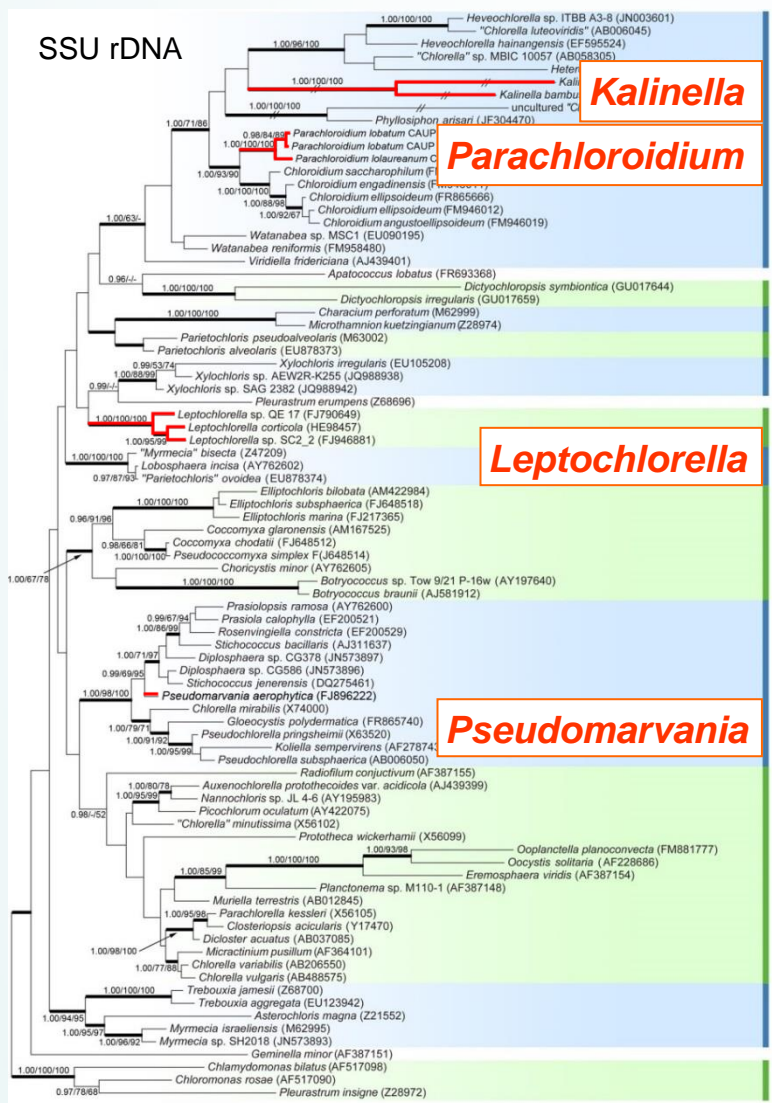
SSU rDNA

OCC clade

Chlorophyceae

Trebouxiophyceae, *Chlorella* – cryptic genera

SSU rDNA



Trebouxiophyceae:

Watanabea clade

Dictyochloropsis
Microthamniales
Parietochloris
Xylochloris

Leptochlorella
Lobosphaera

Botryococcus clade

Prasiola clade

Chlorellales

Trebouxiales

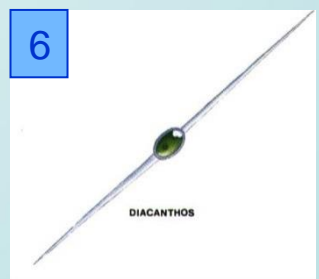
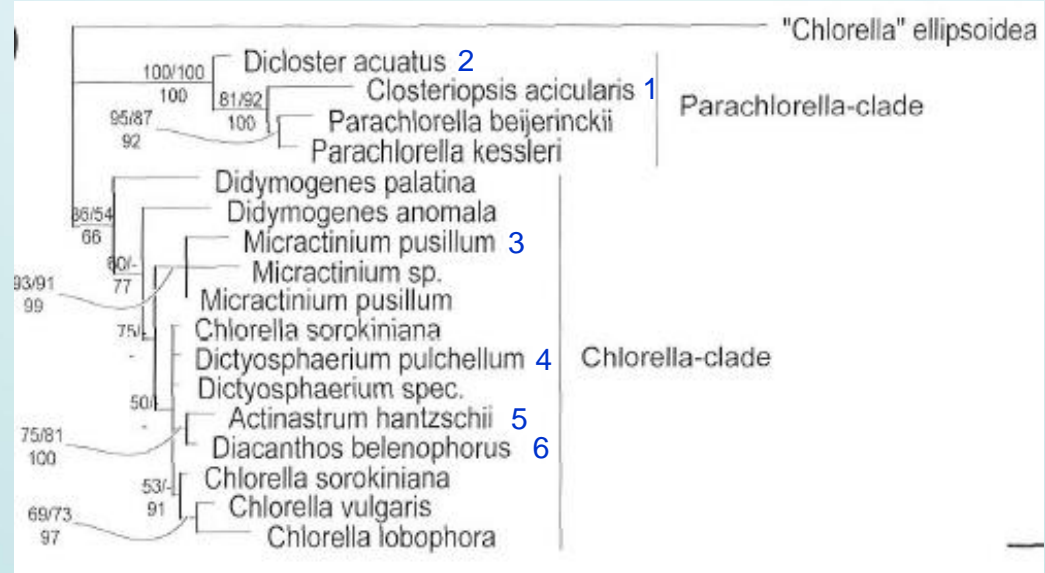
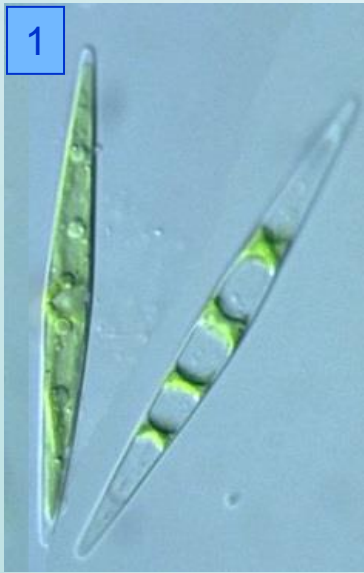
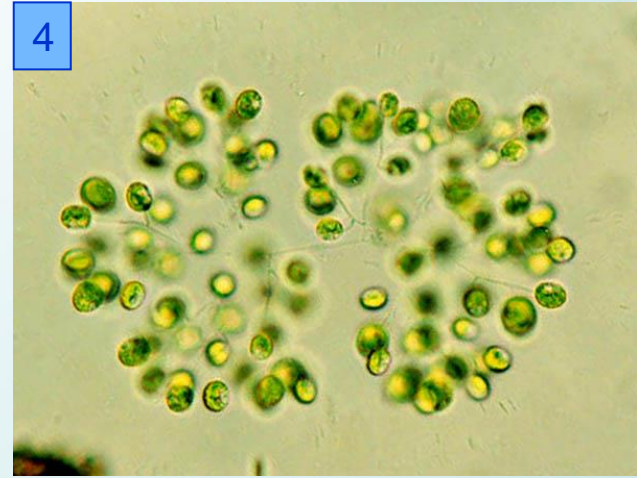
outgroup



Neustupa et al. (2013): *Phycologia* **52**, 411-421; Neustupa et al. (2013): *IJSEM* **63**, 377-387 ; Neustupa et al. (2009): *Phycological Research* **57**, 159-169; Eliáš & Neustupa (2009): *Fottea* **9**, 169-177

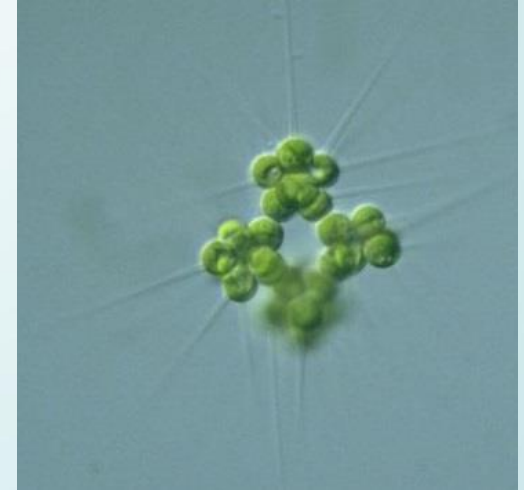
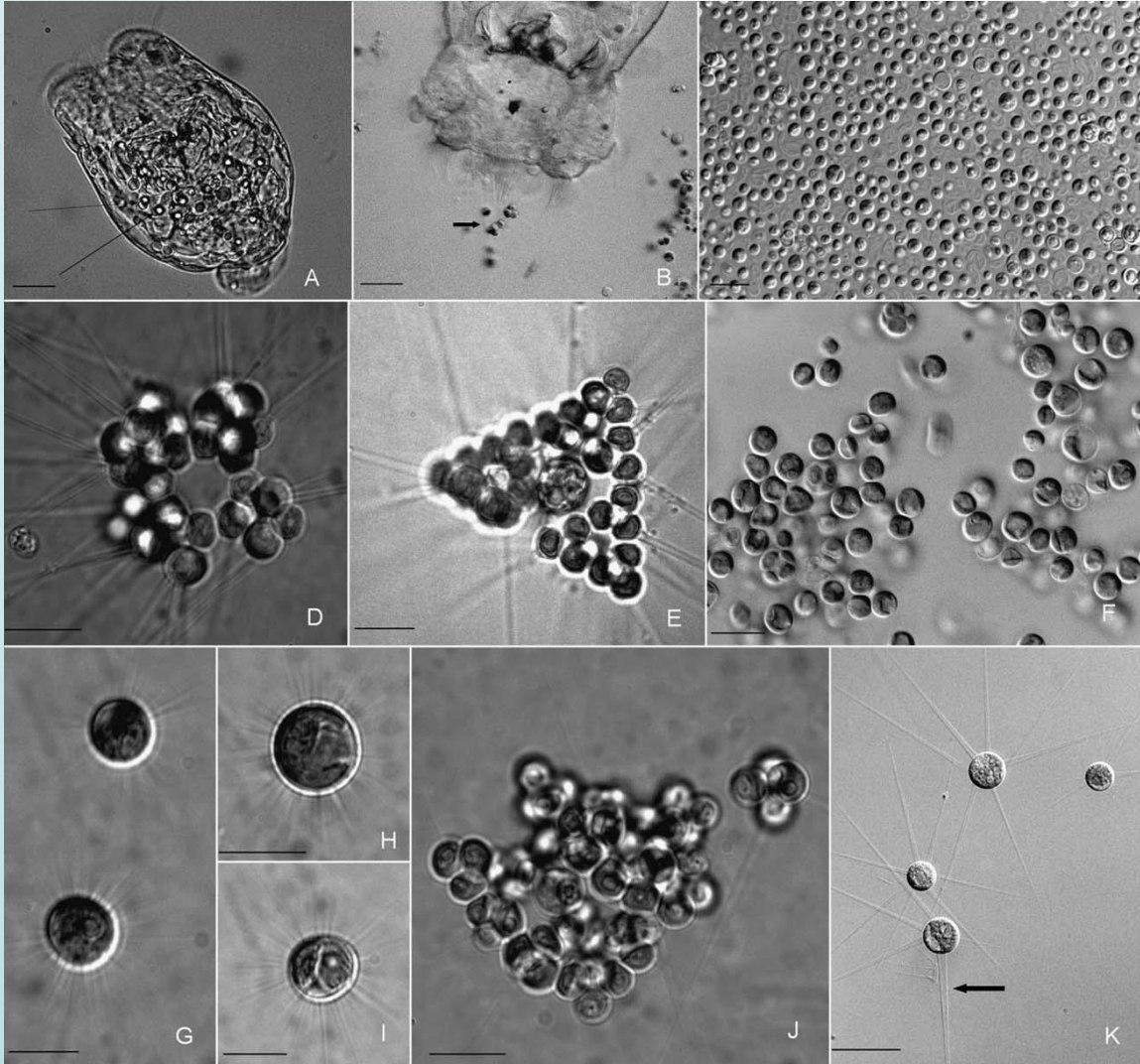
Trebouxiophyceae, Chlorellaceae

- morphologically distinct planktonic genera are close related to *Chlorella*



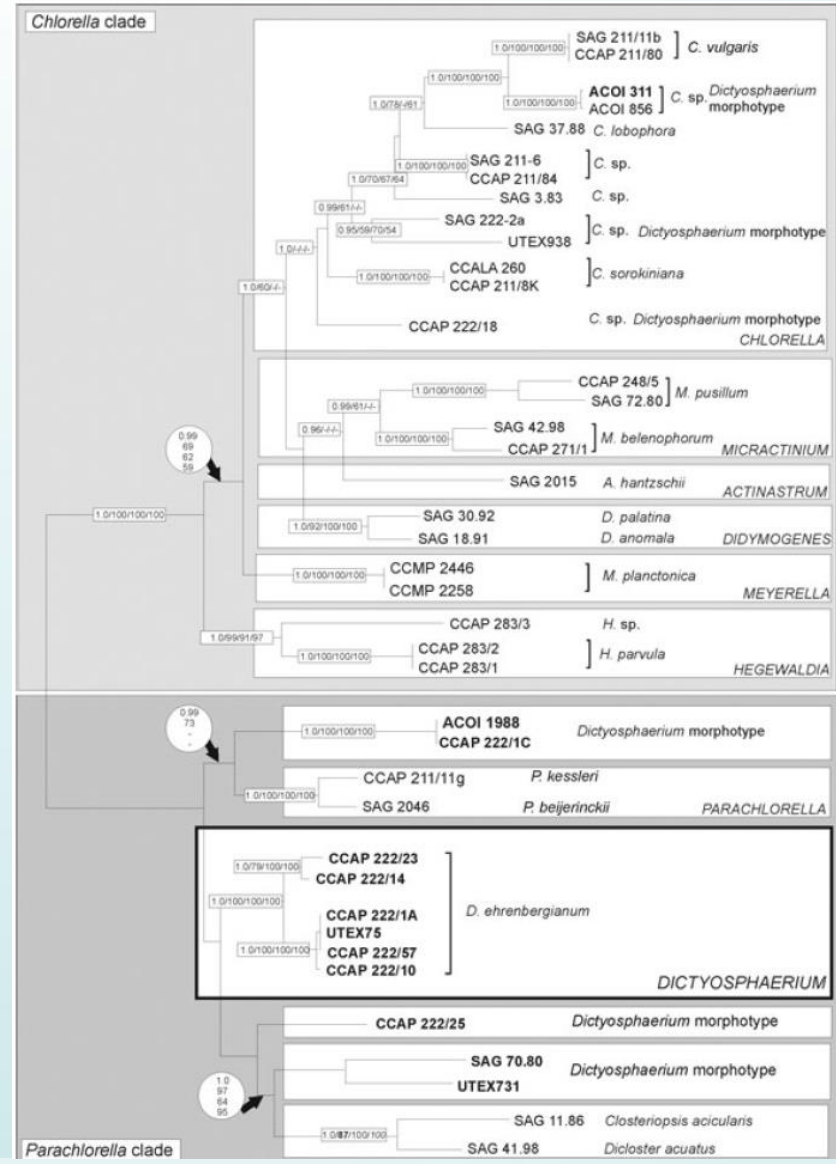
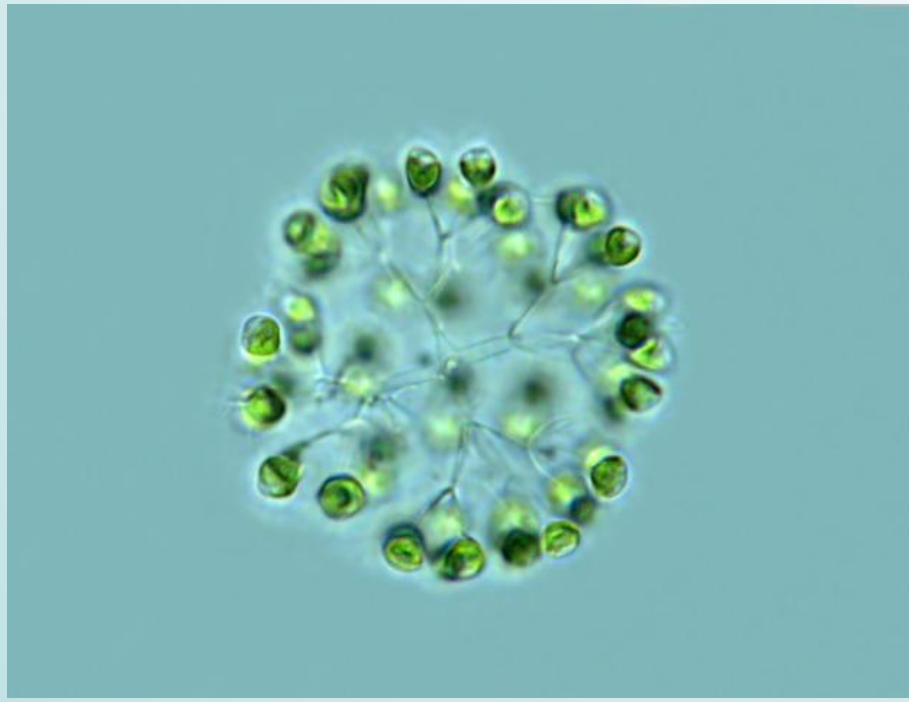
Trebouxiophyceae, Chlorellaceae

- Luo et al. (2006)
- production of spines in *Micractinium* as a result of predation pressure by *Brachionus*



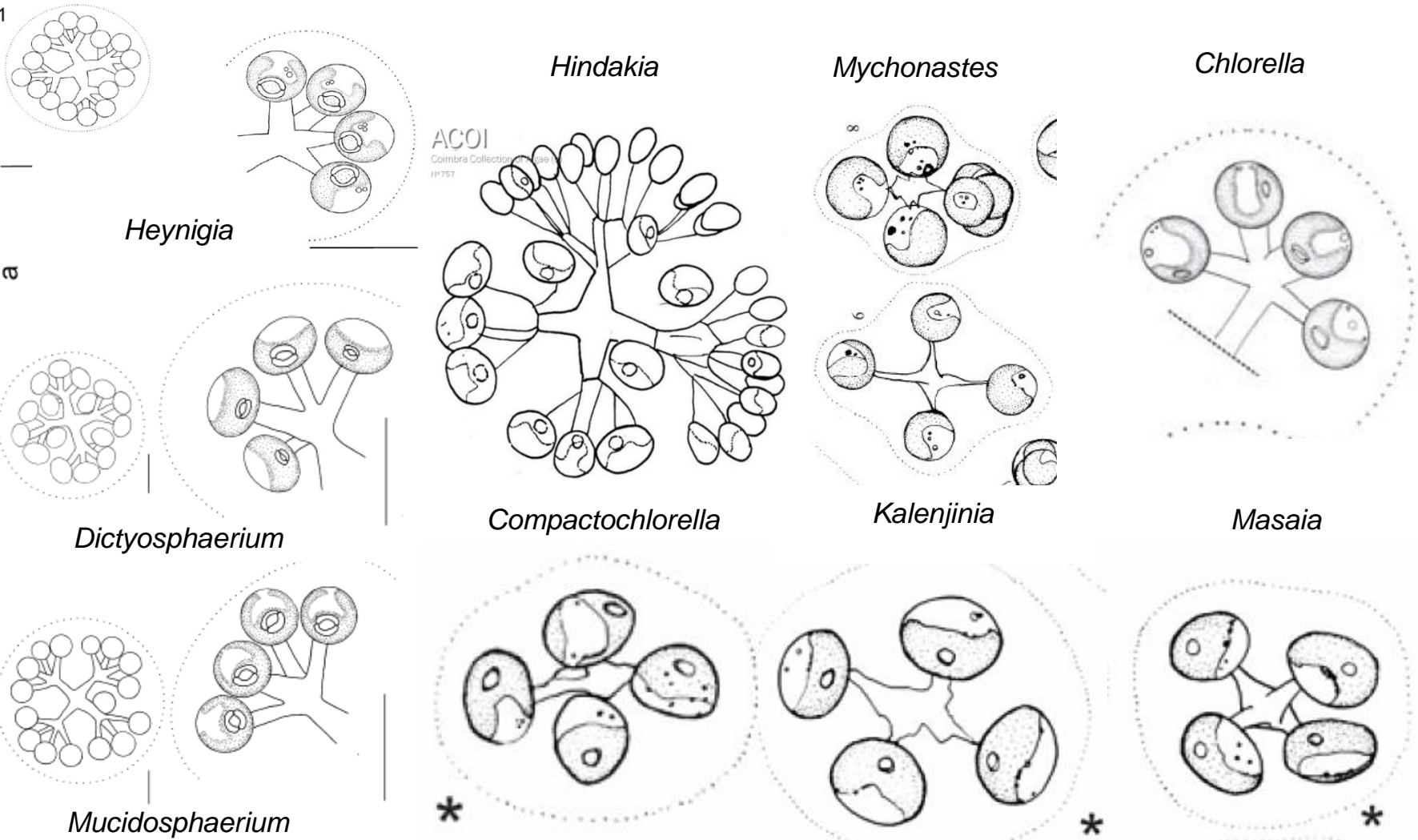
Trebouxiophyceae, Chlorellaceae

- Chlorella-clade + Parachlorella-clade
- *Dictyosphaerium* – highly polyphyletic genus



Dictyosphaerium

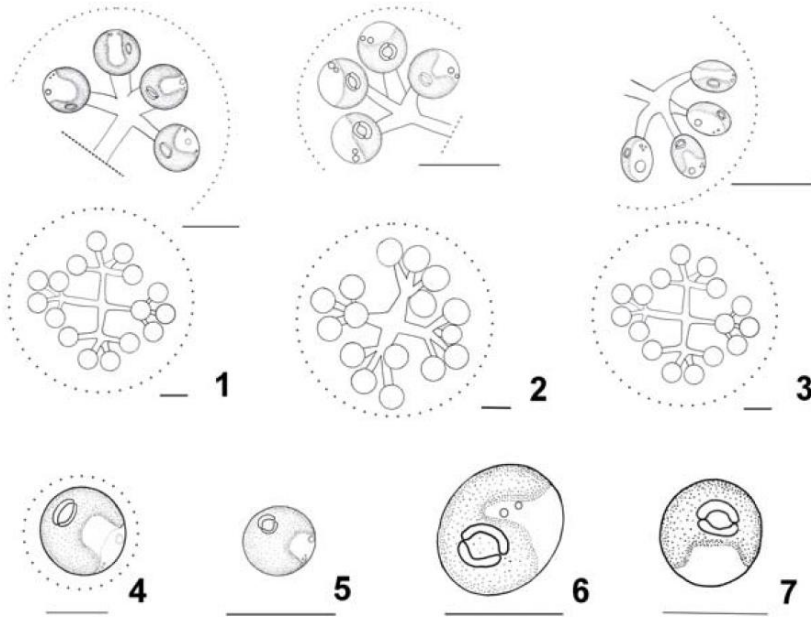
- 9 cryptic genera



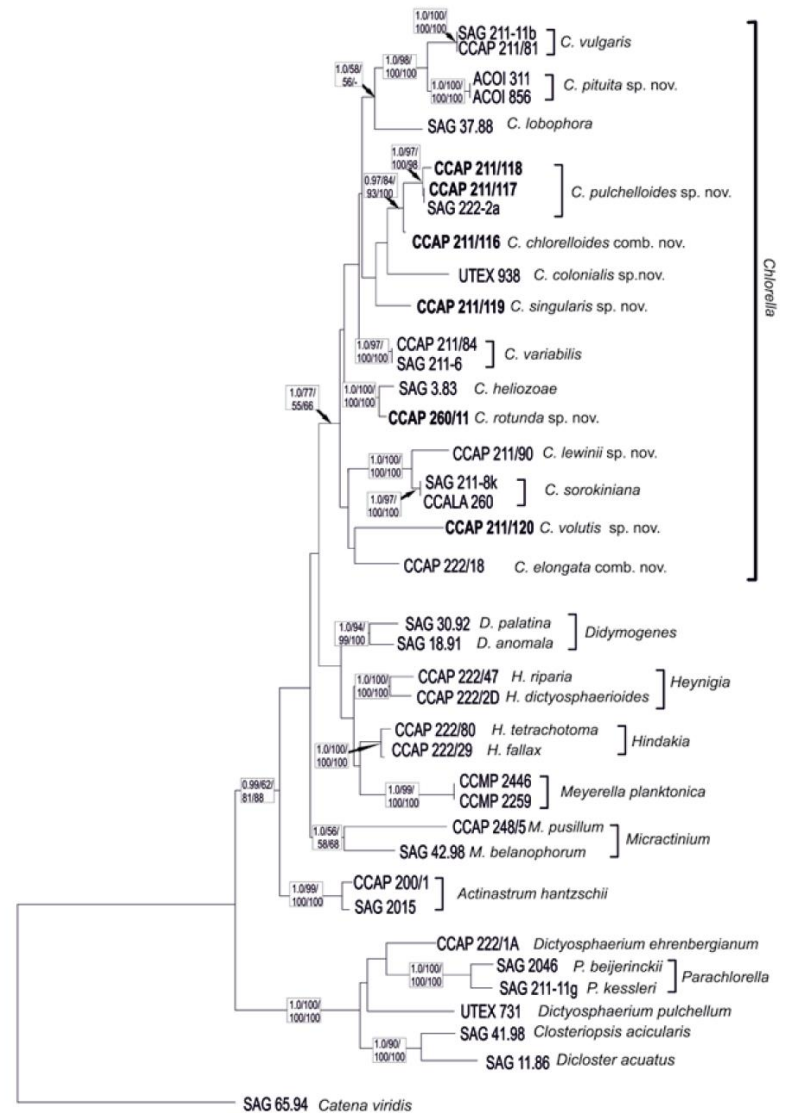
Krienitz et al. (2012): *Fottea* **12**, 231-253; Bock et al. (2010): *European Journal of Phycology* **45**, 267-277; Bock et al. (2011): *Journal of Phycology* **47**, 638-652; Krienitz et al. (2011): *Phycologia* **50**, 89-106

Trebouxiophyceae, Chlorellaceae

- Chlorella-clade
 - Chlorella* – unicellular and colonial (*Dictyosphaerium*-like) species



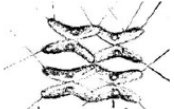
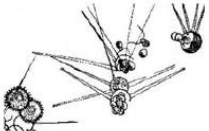
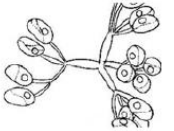
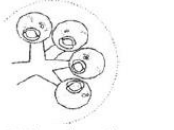




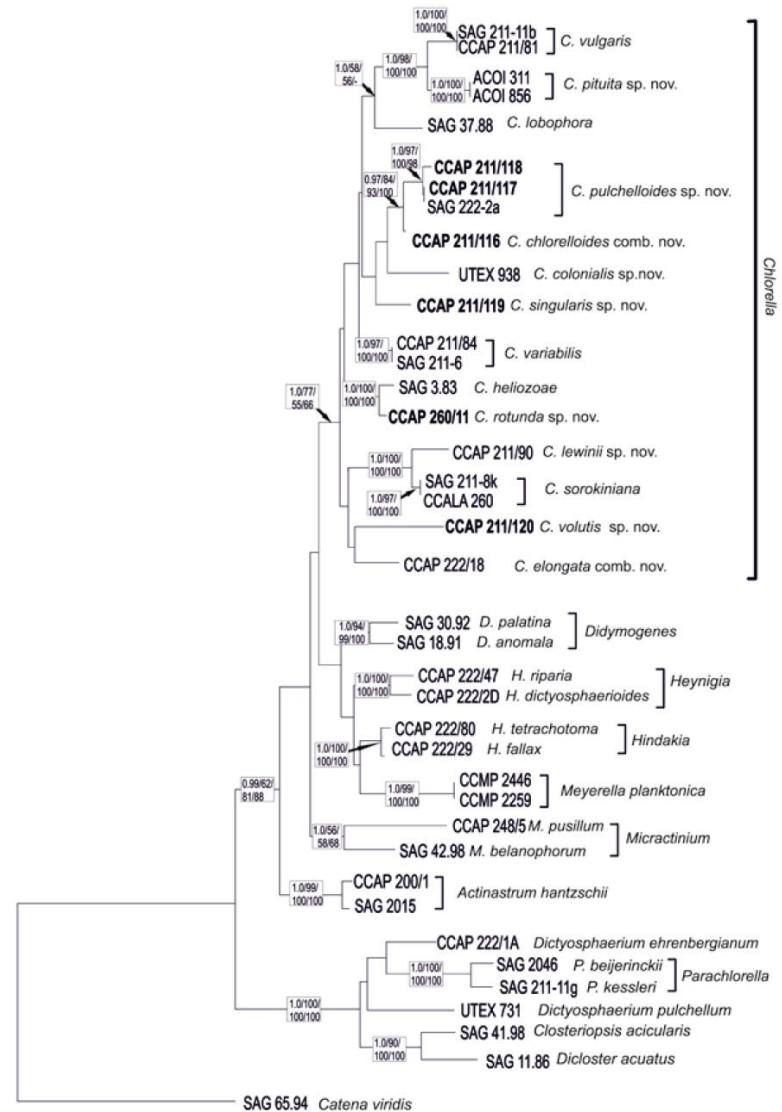
Figs 1–7. Drawings of light microscopical characters of *Chlorella* species. Iconotypes: (1) *Chlorella pituita*, authentic strain ACOI 311; (2) *Chlorella pulchelloides*, authentic strain CCAP 211/118; (3) *Chlorella coloniales*, authentic strain UTEX 938; (4) *Chlorella singularis*, authentic strain CCAP 211/119; (5) *Chlorella rotunda*, authentic strain CCAP 260/11; (6) *Chlorella lewinii*, authentic strain CCAP 211/90; (7) *Chlorella volutis*, authentic strain CCAP 211/120. Scale bars 10 μ m.



Trebouxiophyceae, Chlorellaceae

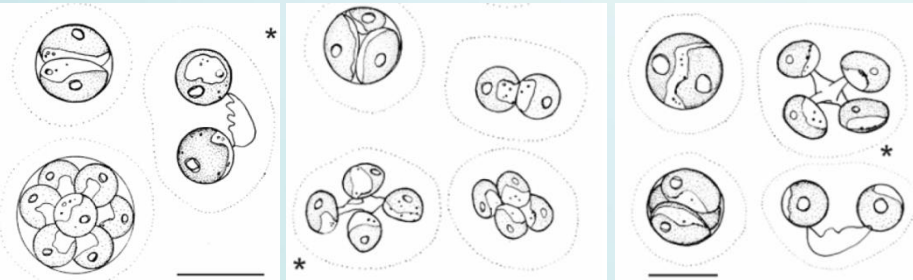
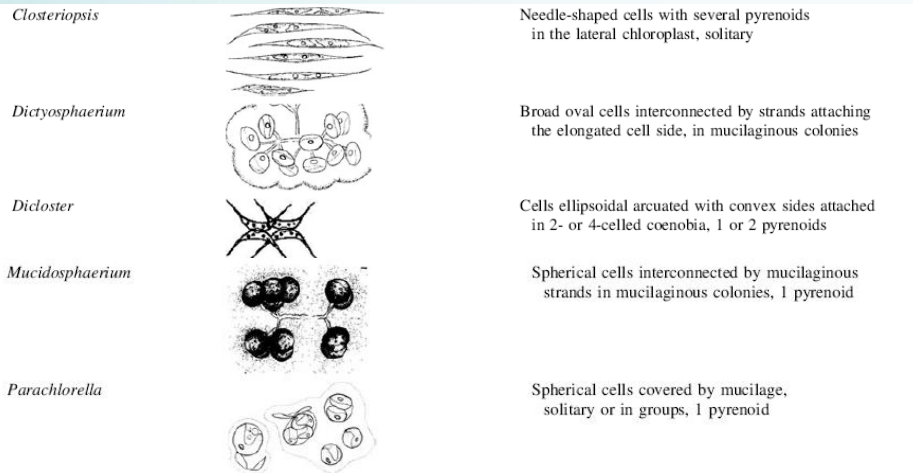
Chlorella-clade

Genus	Drawing	Main diacritic morphology
<i>Actinastrum</i>		Cells rod-shaped, elongated, radially arranged in coenobia
<i>Chlorella</i>		Spherical or broad oval cells, with one pyrenoid, solitary or in mucilage covered colonies
<i>Didymogenes</i>		Cells cylindrical curved with convex side attached in 2-, 4- or 16-celled coenobia, one pyrenoid, spines
<i>Hegewaldia</i>		Spherical cells with or without bristles, solitary or in colonies, 1 pyrenoid, facultative oogamy
<i>Hindakia</i>		Broad oval cells interconnected by strands attaching the apical pole, in mucilaginous colonies, 1 pyrenoid
<i>Heynigia</i>		Spherical cells interconnected by mucilaginous strands in mucilaginous colonies, 1 pyrenoid
<i>Micractinium</i>		Spherical cells with long bristles, in colonies, 1 pyrenoid
<i>Meyerella</i>		Cells short cylindrical, small, without mucilage, solitary, pyrenoid missing



Trebouxiophyceae, Chlorrellaceae

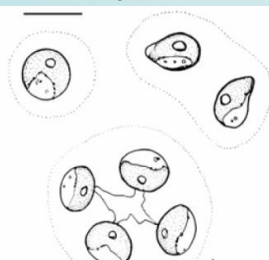
• Parachlorella-clade



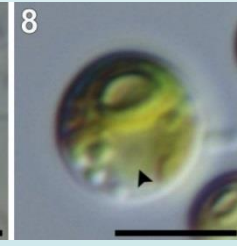
Marasphaerium

Compactochlorella

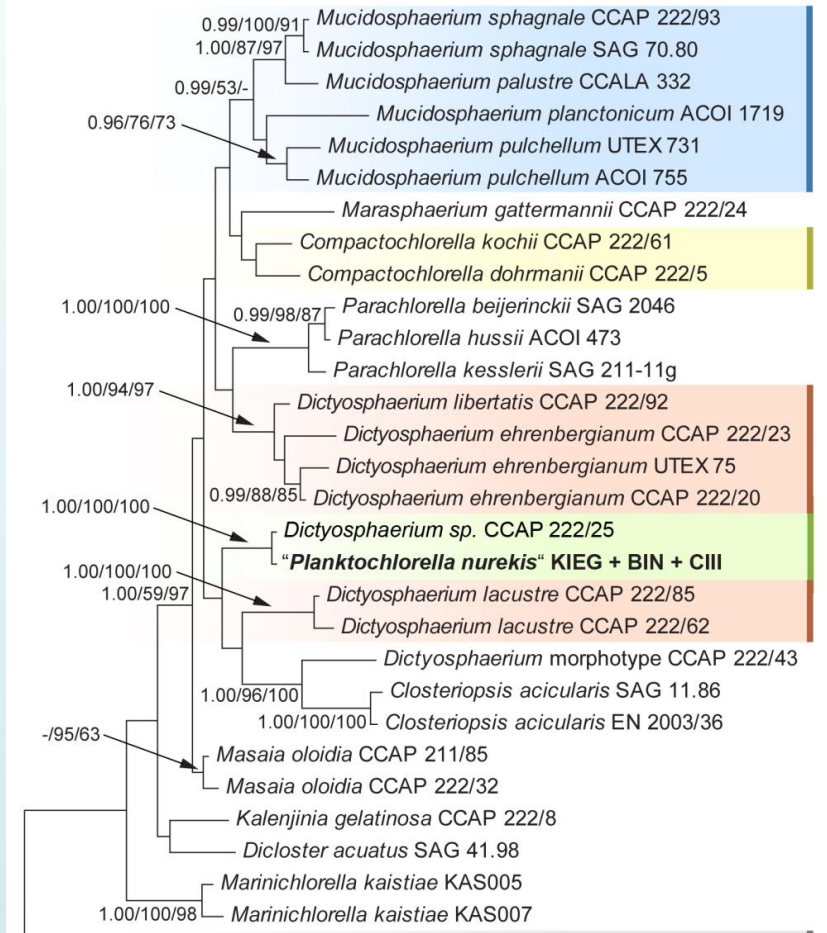
Masaia



Kalenjinia



Planktochlorella



Krienitz & Bock (2012): *Hydrobiologia* **698**, 295 – 326; Krienitz et al. (2012): *Fottea* **12**, 231-253; Škaloud et al. (2014): *Fottea* **14**, 53-62

Trebouxiophyceae, Chlorellaceae

- Parachlorella-clade
 - Marinichlorella kaistiae*

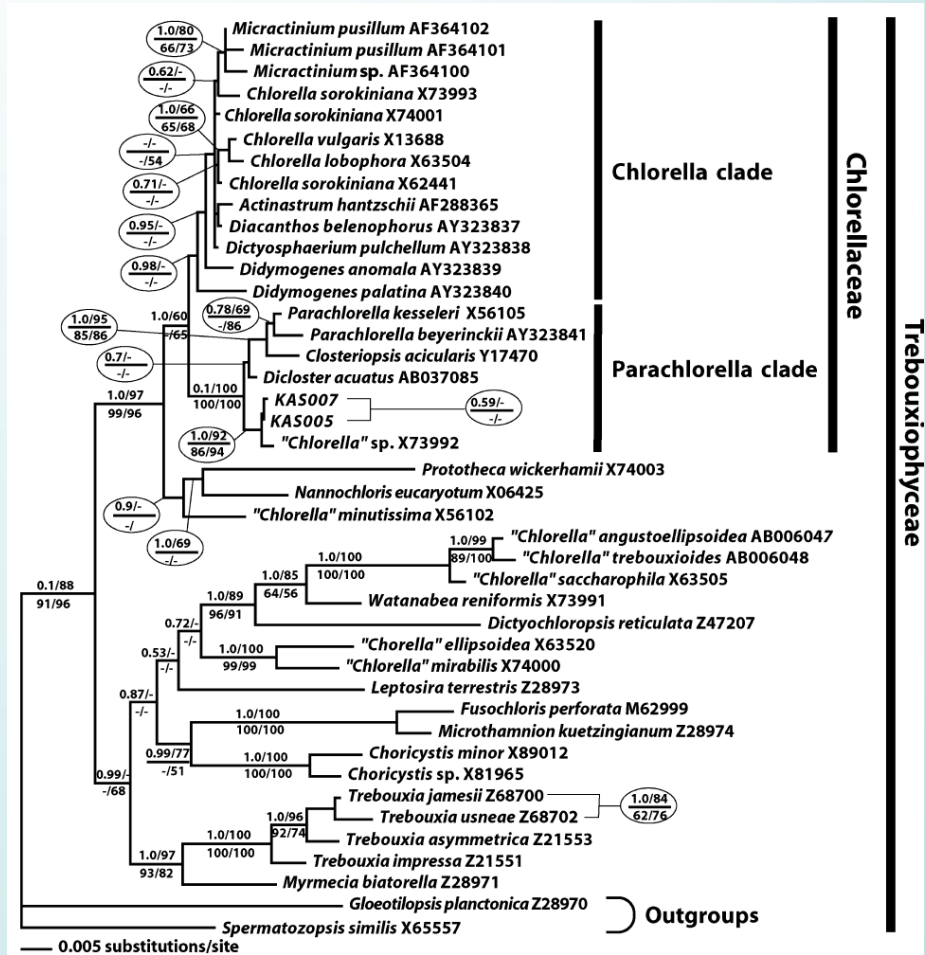
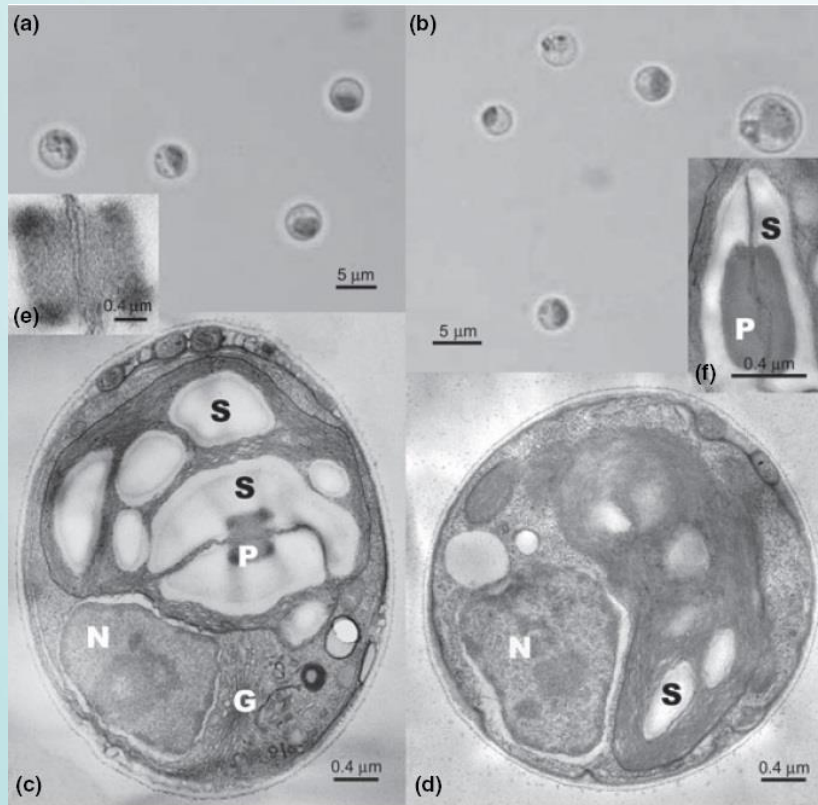


Fig. 2. Maximum-likelihood (ML) tree inferred from 18S rRNA gene sequences, with *Gloeotilopsis planctonica* and *Spermatozopsis similis* as outgroup taxa. The numbers above each branch show posterior probabilities (0.50 and higher) of Bayesian analysis (1,000,000 generations, left) and bootstrap values (50% or more) of ML (500 replicates, right). The numbers below each branch show bootstrap value (50% or more) of ML distance (1000 replicates, left) and maximum parsimony (1000 replicates, right). Some duplicate strains of some genera are not included in the tree because they have the same sequences. Branch lengths correspond to evolutionary distances. A distance of 0.005 is indicated by the scale.

Trebouxiophyceae, Chlorellles

- Chlorella-clade**

- symbionts

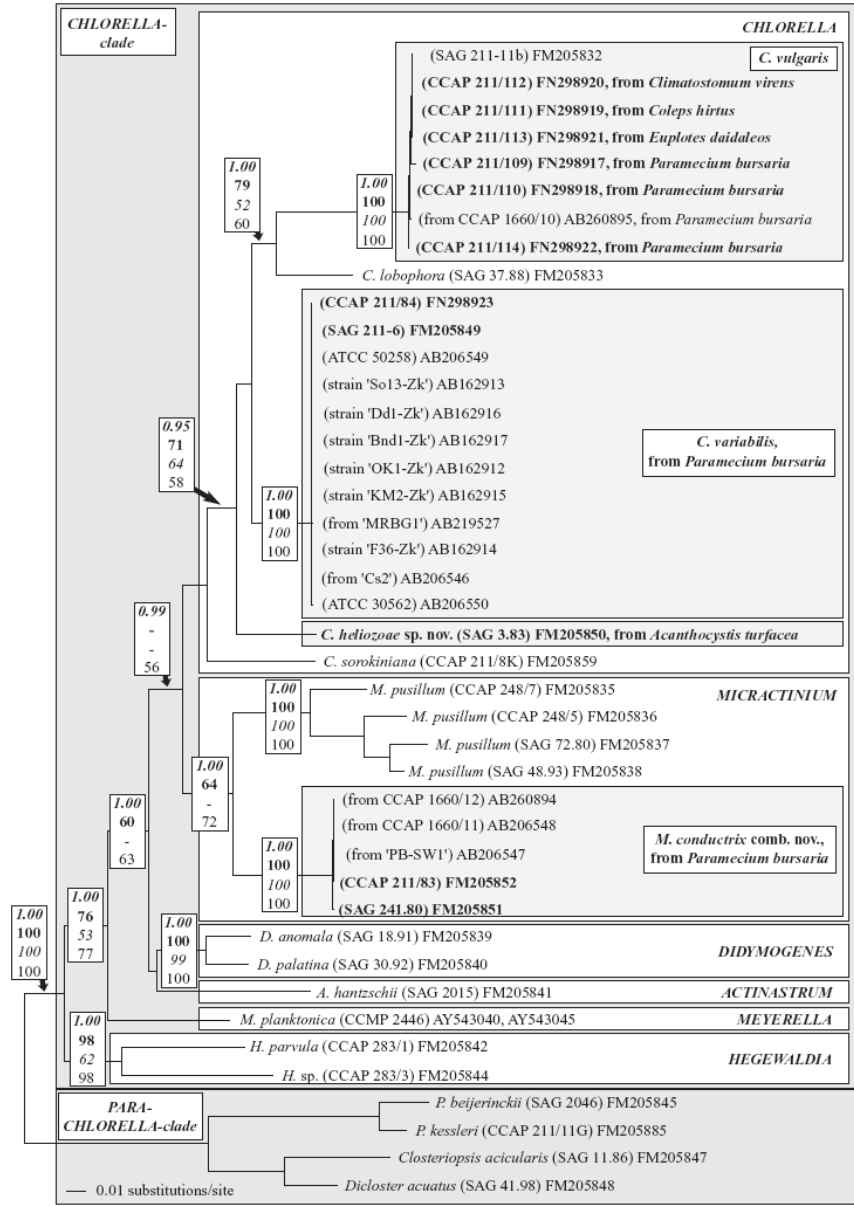
environmental microbiology



Environmental Microbiology (2010)

doi:10.1111/j.1462-2920.2010.02333.x

The systematics of *Zoochlorella* revisited employing an integrative approach



Chlorellales, practical use

- high growth rate – a popular model organism
- *Chlorella* has the highest chlorophyll content from all studied genera
- large scale cultivations



Chlorellales, practical use

- **chlorophyll:**

- anti-inflammatory effects, supports wound healing
- elimination of heavy metals and toxins
- anaemia therapy
- anti-tumor activity
- supports immune system
- reducing high blood pressure

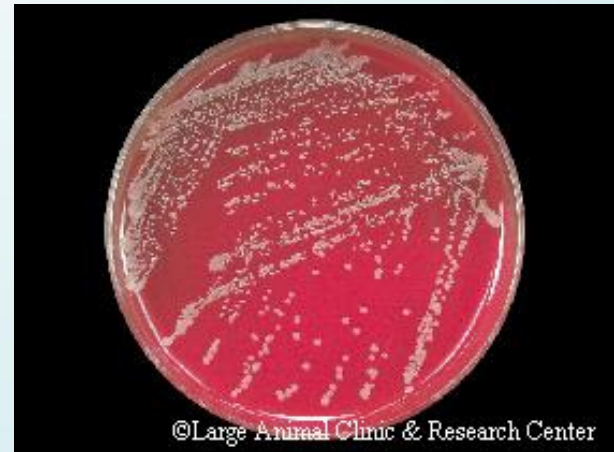
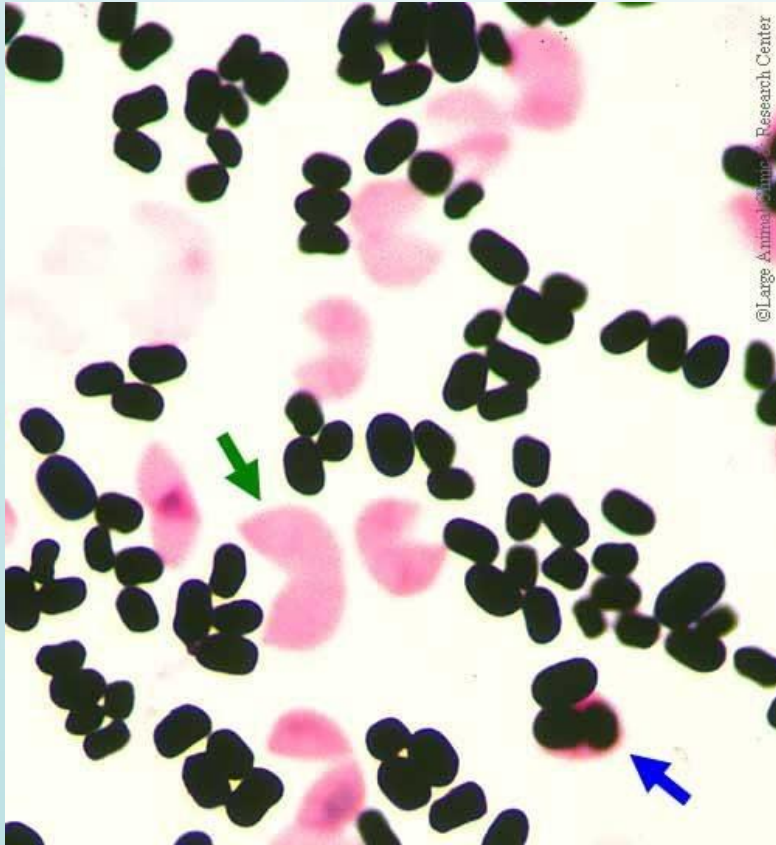


Chlorella tabs



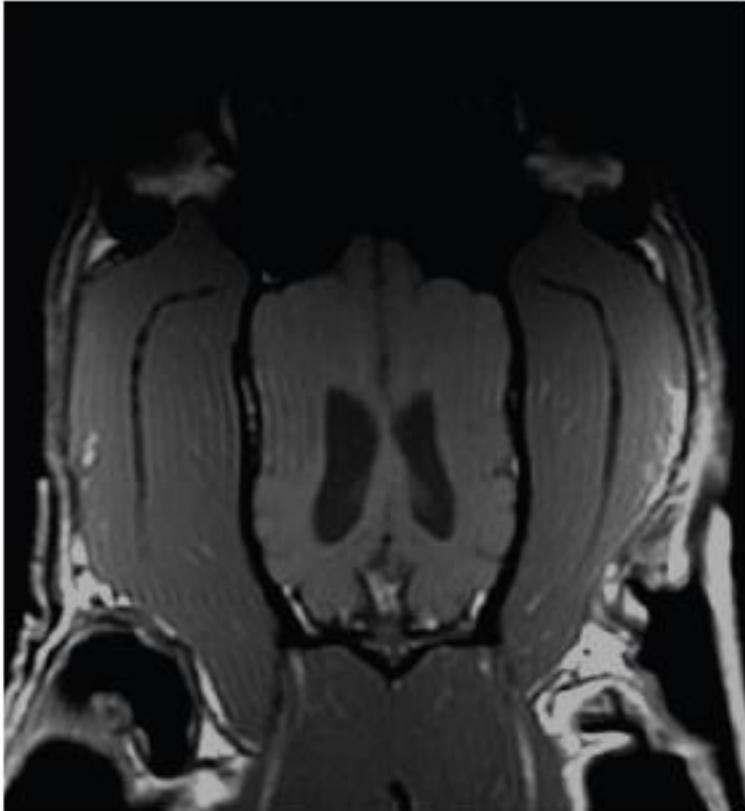
Chlorellales, *Prototheca*

- heterotrophic, pathogenic organism
- *protothecosis*



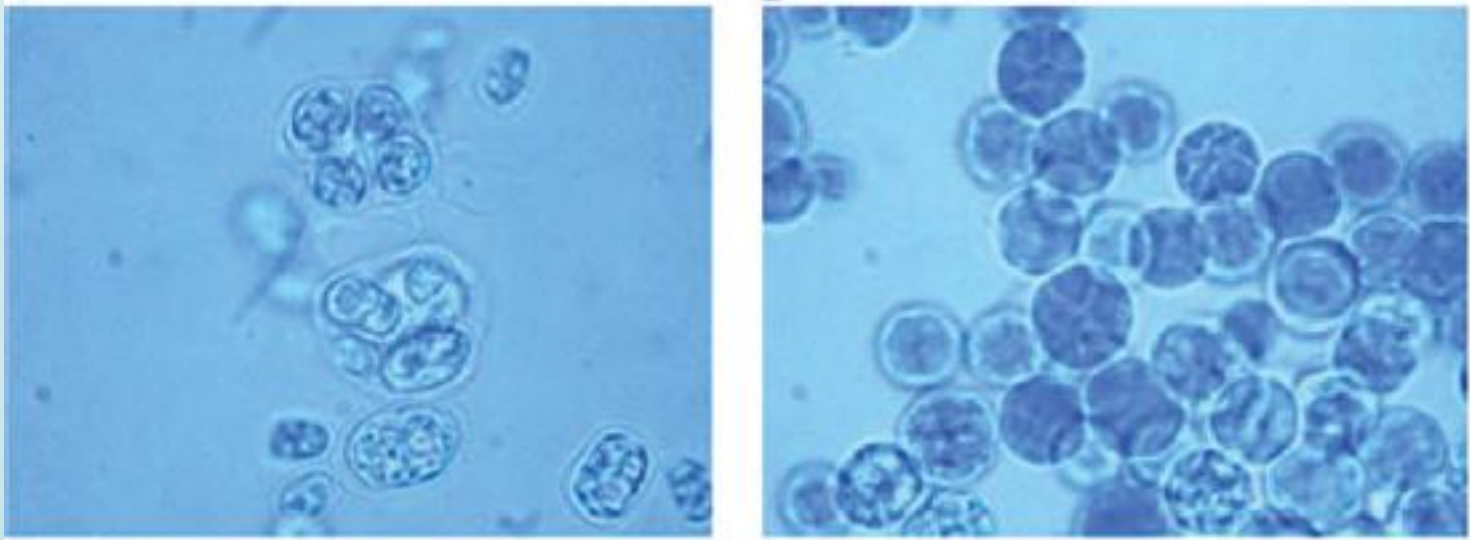
Protothecosis

- 6-year-old dog: malfunction of coordination
- surgery of cranial tendon prior the disease
- magnetic resonance – brain lysis (meningoencephalitis)
- the dog was put to death

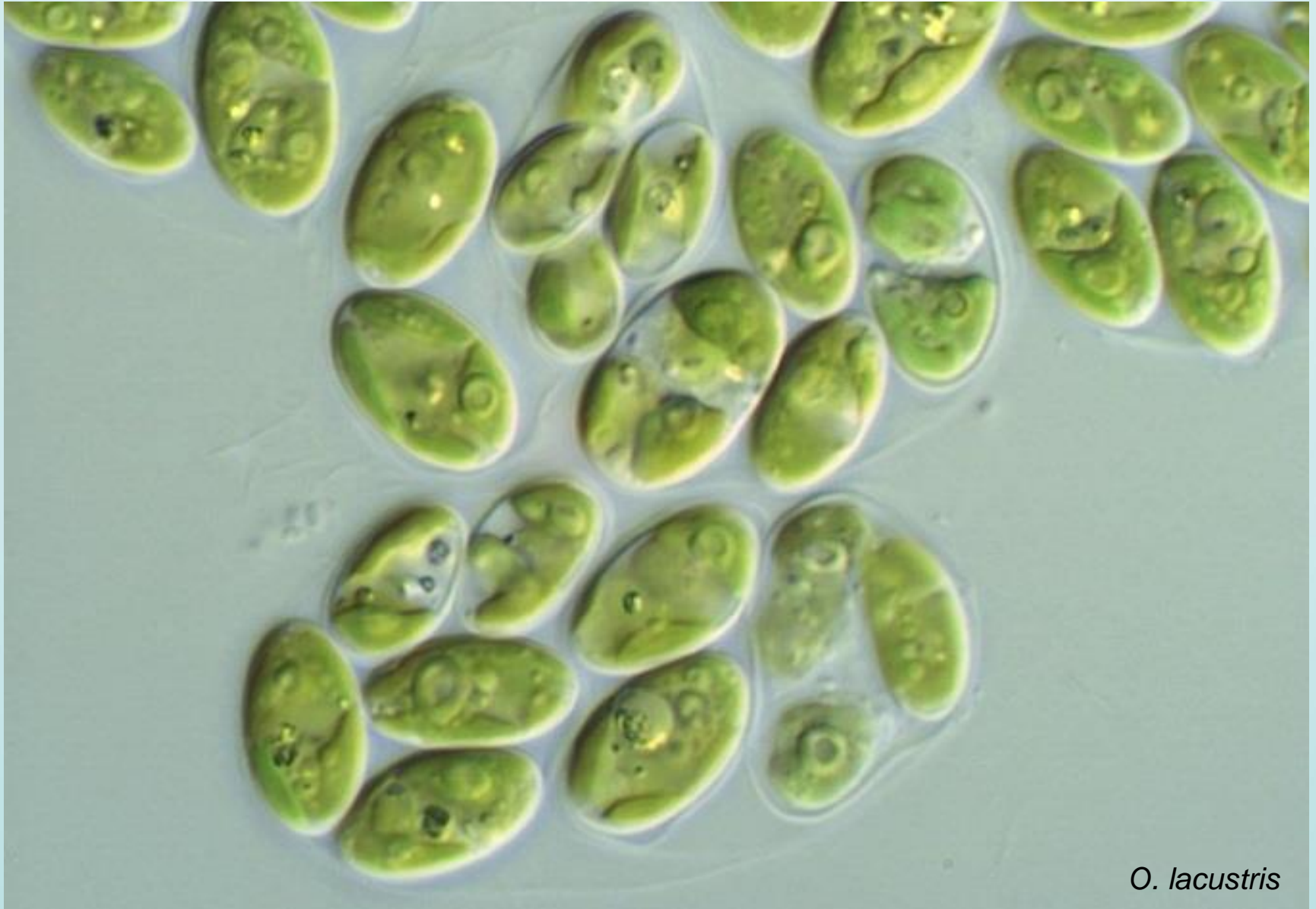


Protothecosis

- humans – infections from soil, water, ...
- skin at immunosuppressive patients
- first incidence - 1964 (Sierra Leone – foot lysis)
- mortality 2,2%

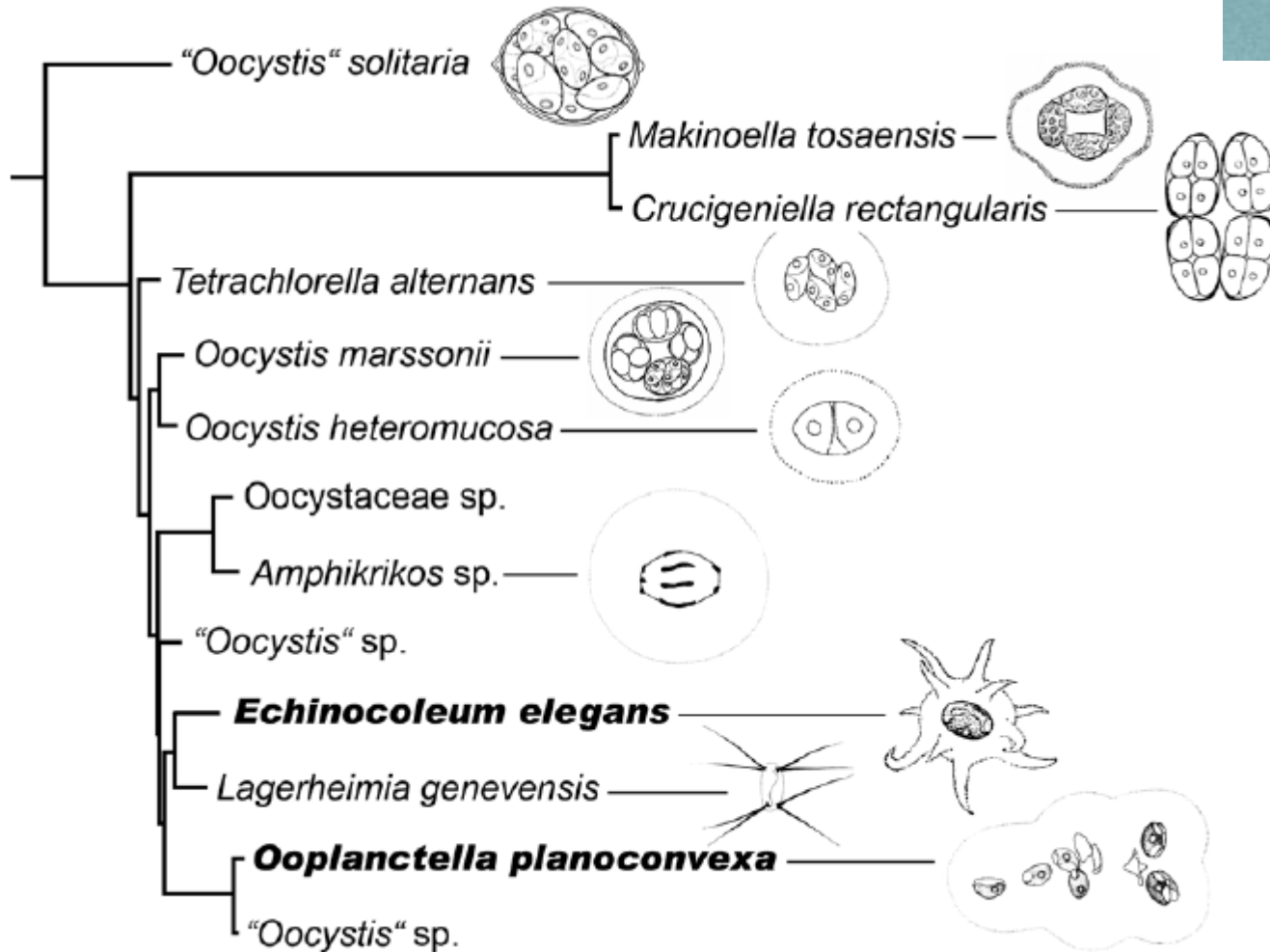


Chlorellales, Oocystaceae, *Oocystis*

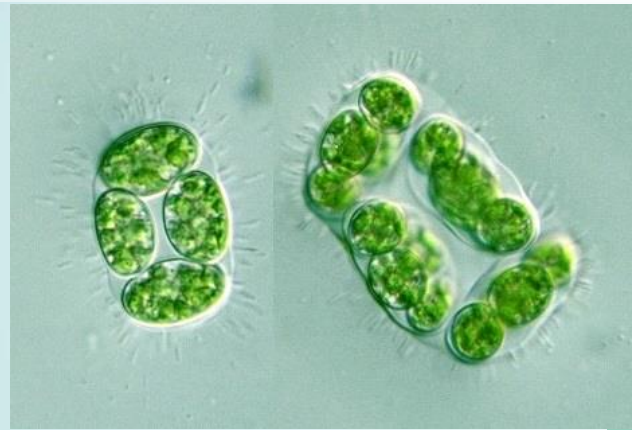


O. lacustris

Chlorellales, Oocystaceae, *Oocystis*



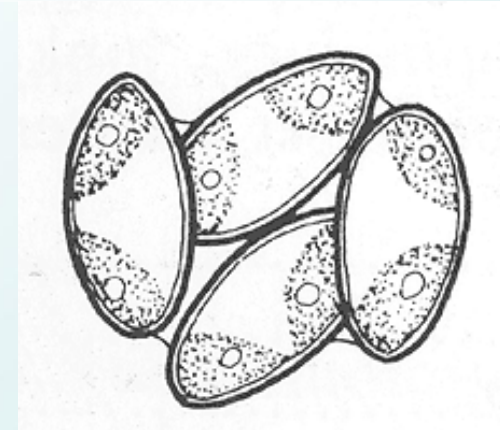
Chlorellales, Oocystaceae



Makinoella



Crucigeniella



Tetrachlorella



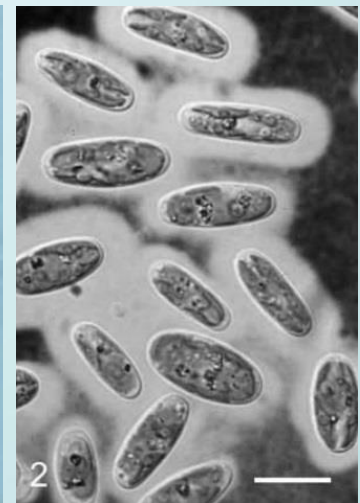
Amphikrikos



Lagerheimia

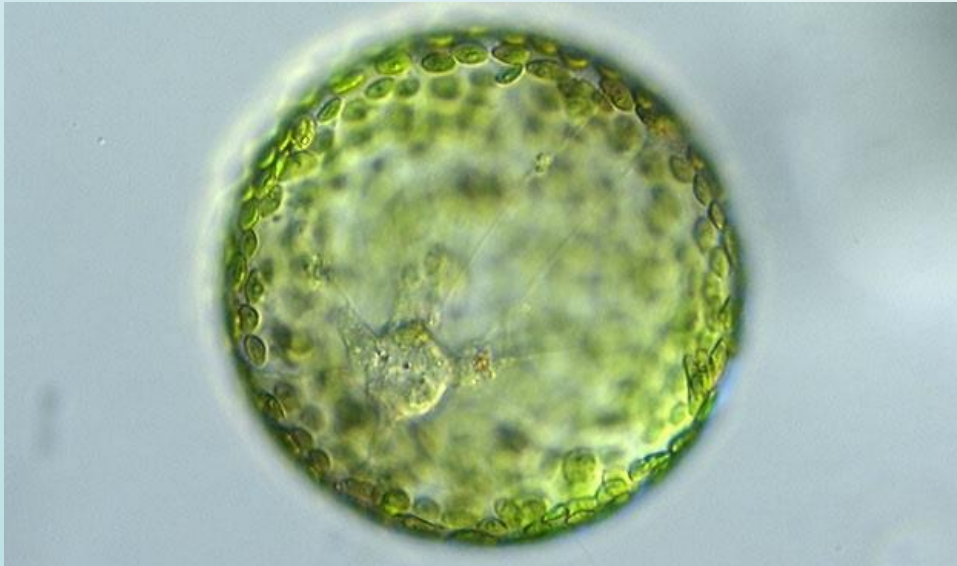
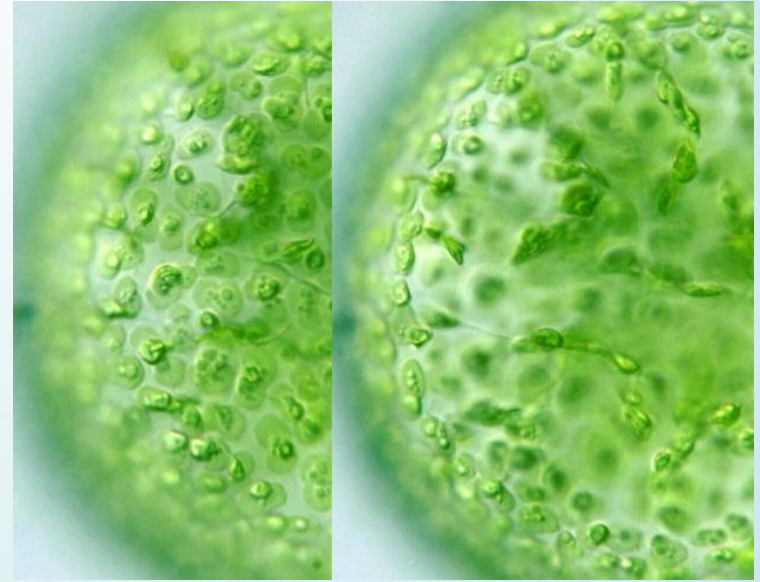


Echinocoleum



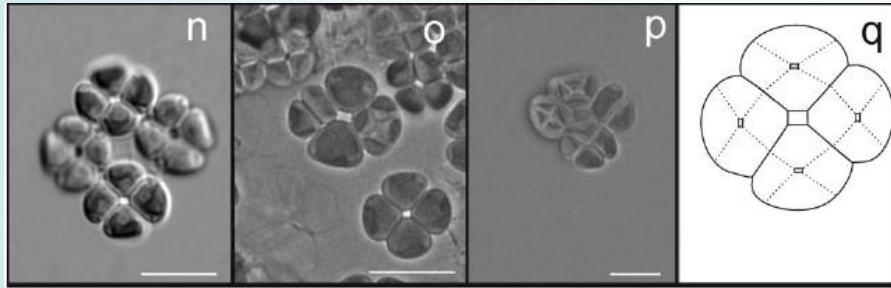
Elongatocystis

Chlorellales, Oocystaceae, *Eremosphaera*

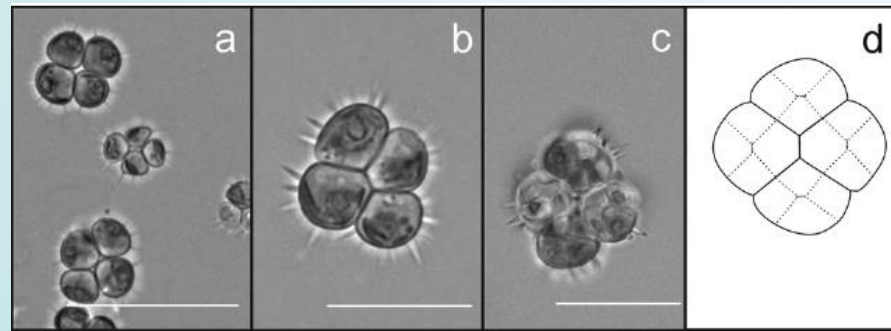


Trebouxiophyceae, tetraedrické kolonie

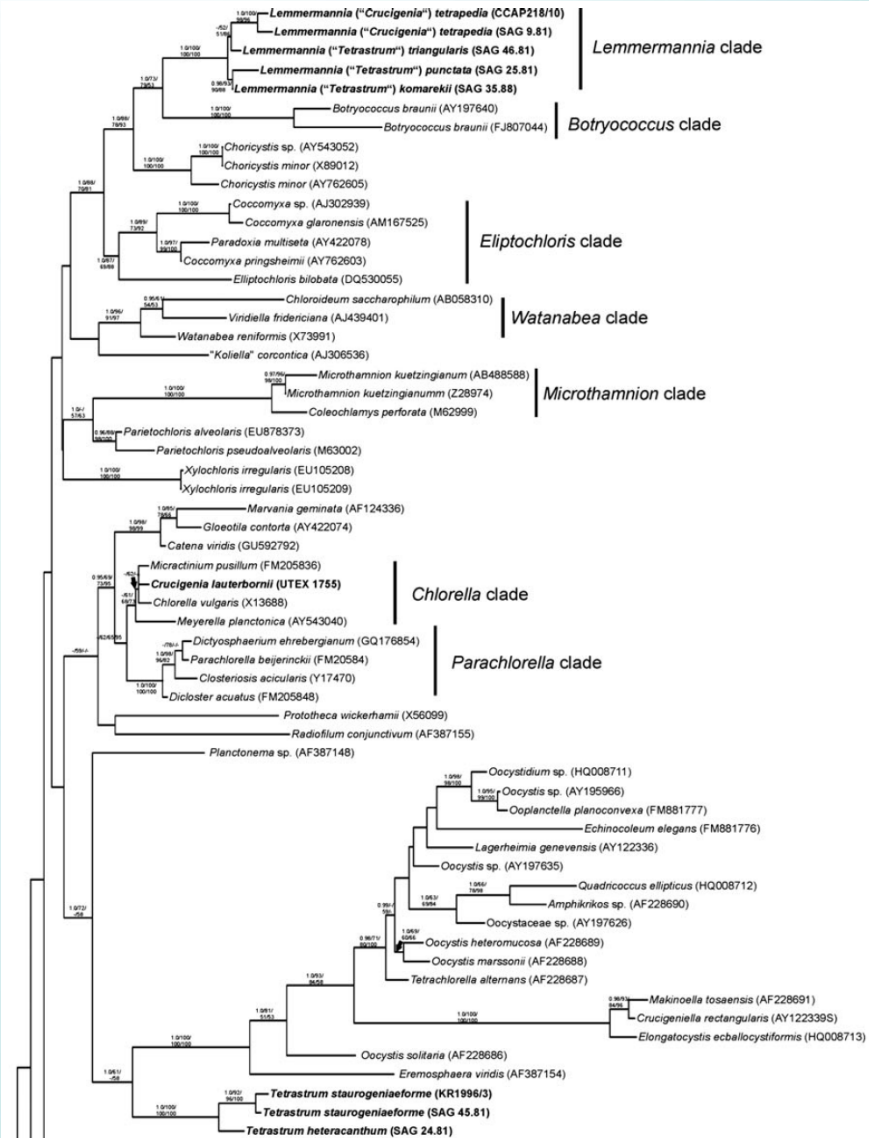
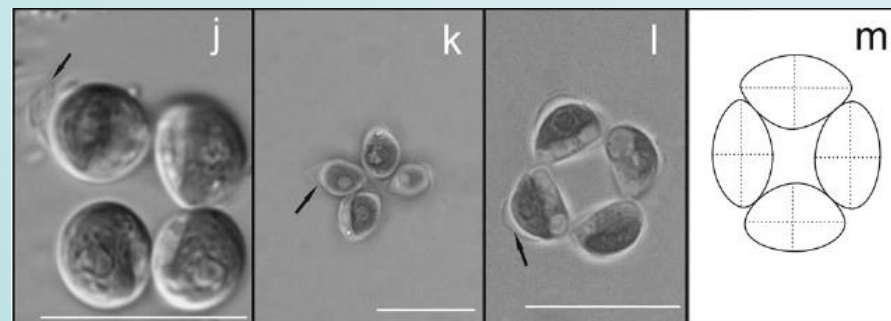
Lemmermannia – smooth colonies, small aperture



Tetrastrum – spiny colonies

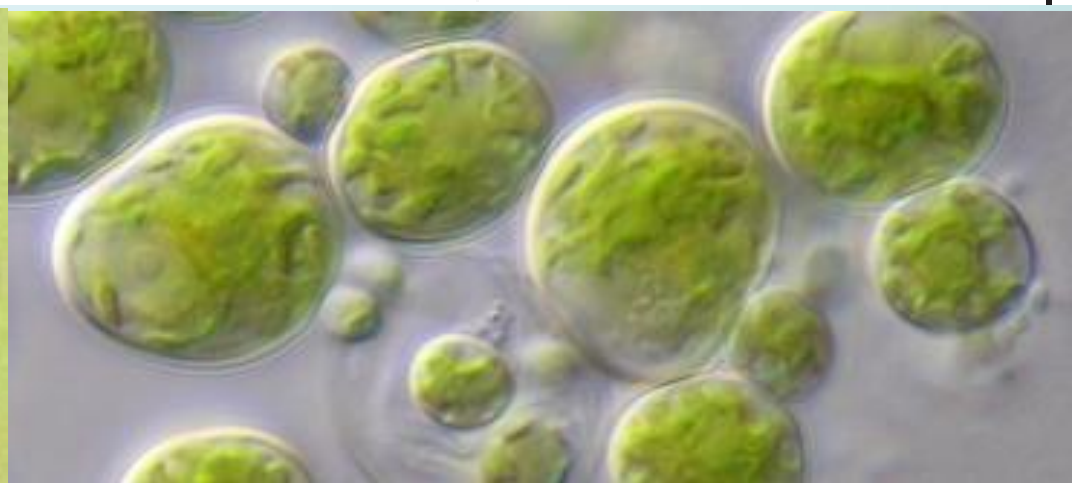
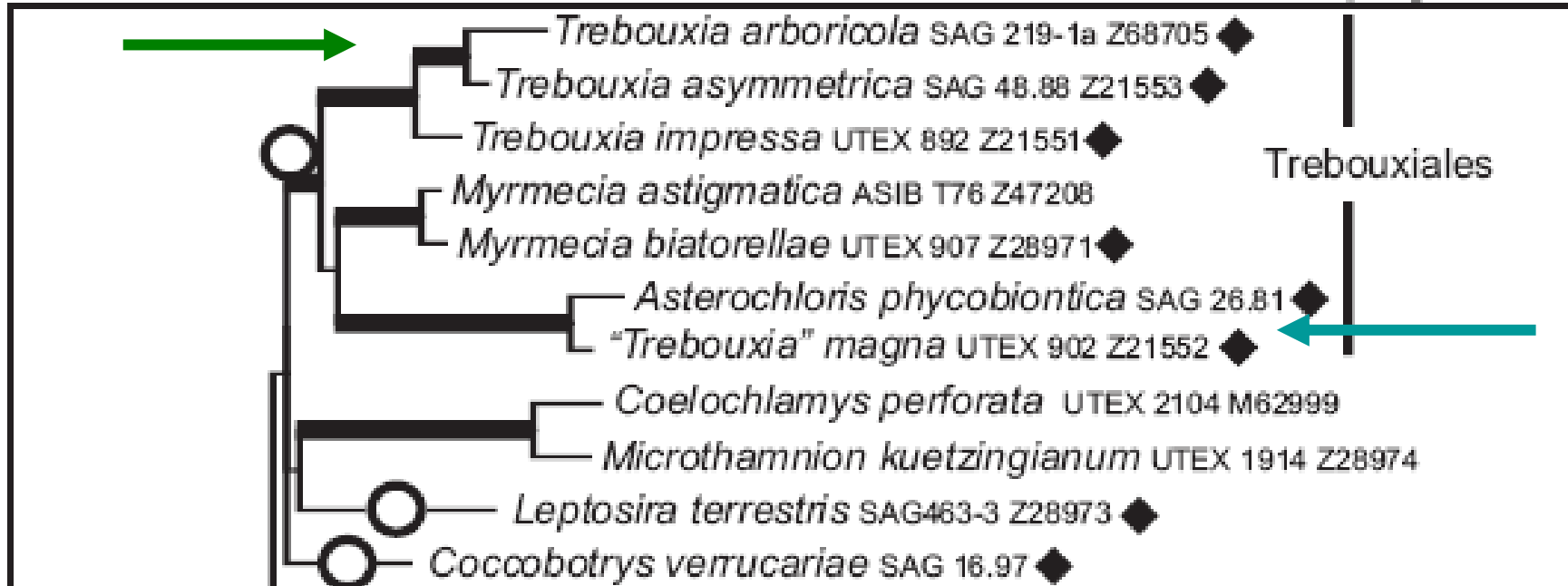


Crucigenia – smooth colonies, big aperture



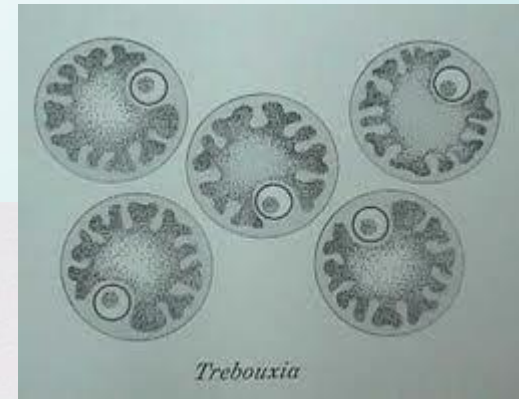
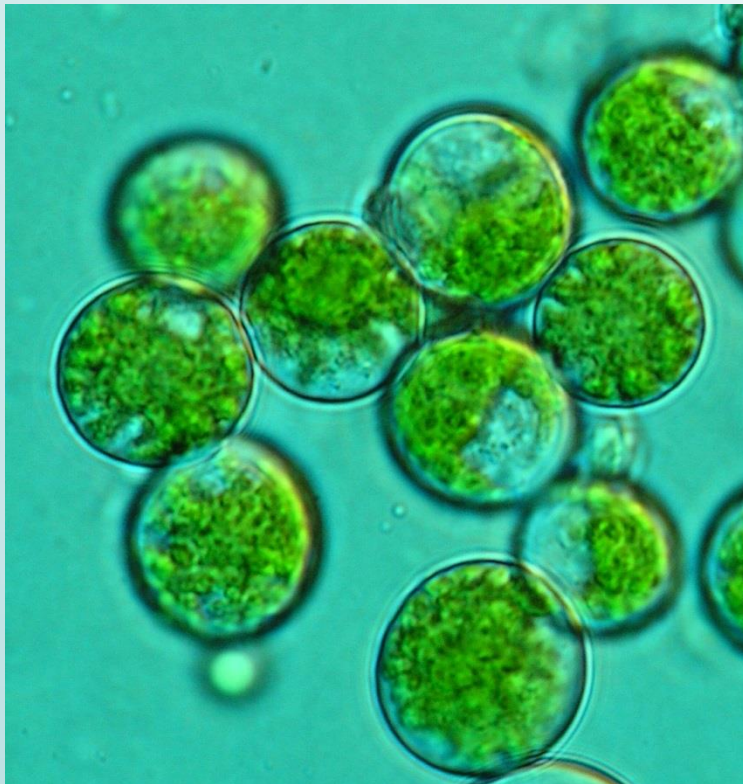
Trebouxiales

Trebouxiophyceae



Trebouxiales, *Trebouxia*

- lichen endosymbiont, probably free-living, as well (bark)
- coccoid green alga with a central, asteroid plastid
- autospores, zoospores



Trebouxiales, *Trebouxia*



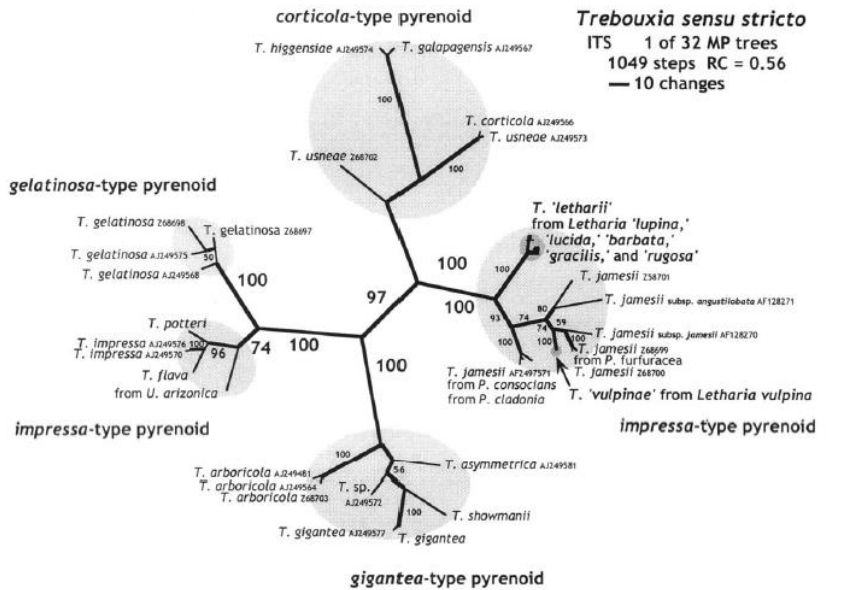
Trebouxiales, *Trebouxia*



Phylogenetic Species, Reproductive Mode, and Specificity of the Green Alga *Trebouxia* Forming Lichens with the Fungal Genus *Letharia*

Scott Kroken; John W. Taylor

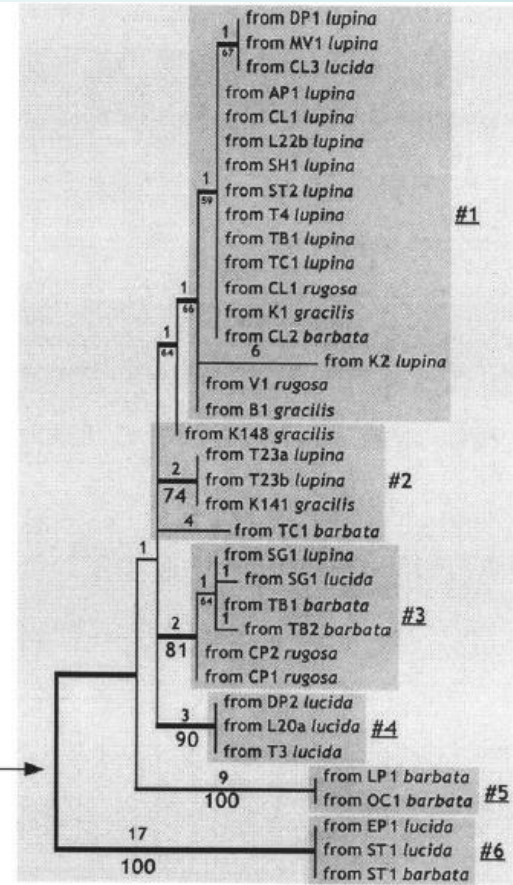
The Bryologist, Vol. 103, No. 4. (Winter, 2000), pp. 645-660.



Cryptic diversity – e.g. *T. jamesii*

***Trebouxia jamesii* species complex**
actin I intron 569
1 of 8 MP trees
51 steps
RC = 0.95

from
Letharia 'lupina'
Letharia 'gracilis'
Letharia 'rugosa'
Letharia 'barbata'
Letharia 'lucida'



Trebouxiales, *Trebouxia*

Lichenologist 31(5): 501–510 (1999)

Article No. lich.1999.0232

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PHOTOBIONT INVENTORY OF A LICHEN COMMUNITY GROWING ON HEAVY-METAL-RICH ROCK

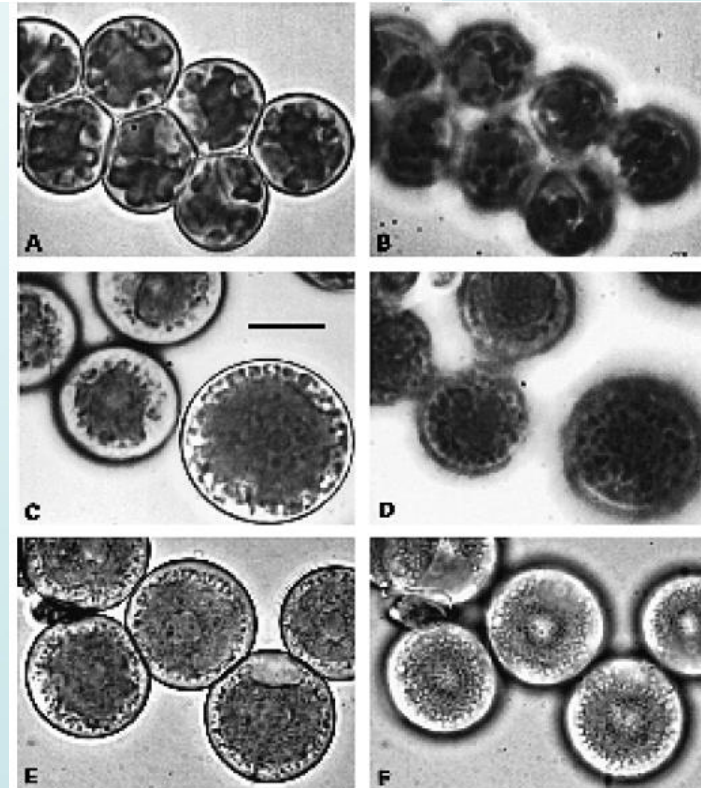
Andreas BECK*

Lichen species

Acarospora rugulosa
Bellemeria diamartha
Lecanora polytropa
L. subaurea
Lecidea silacea
Rhizocarpon geographicum
Umbilicaria cylindrica
Acarospora sinopica
Lecidea lapicida

symbiont population
on a single rock:

- 9 different lichens
- 1 alga (*T. jamesii*)



Trebouxiales, *Trebouxia*

Casano et al. (2011):
co-existence of two physiologically distinct algae

- TR1: cold-requiring
- TR9: warm-requiring (thermophilic)

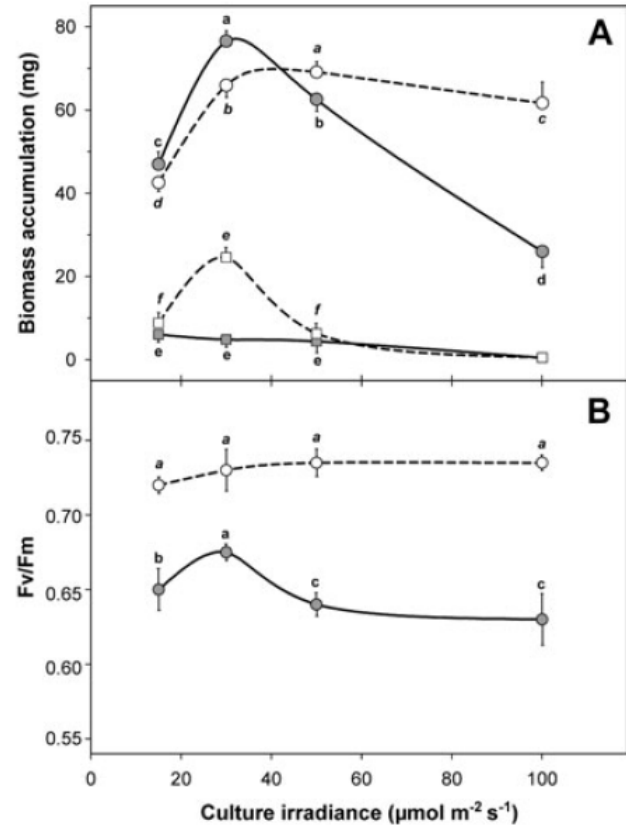
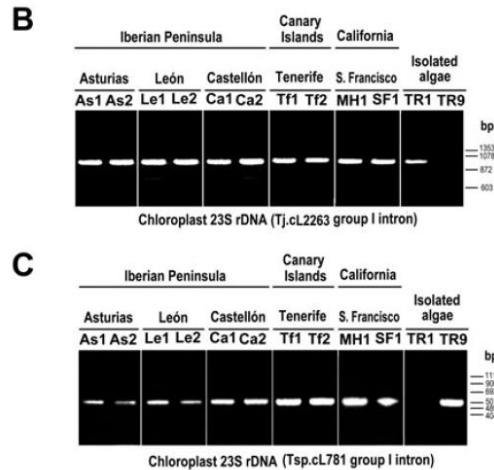
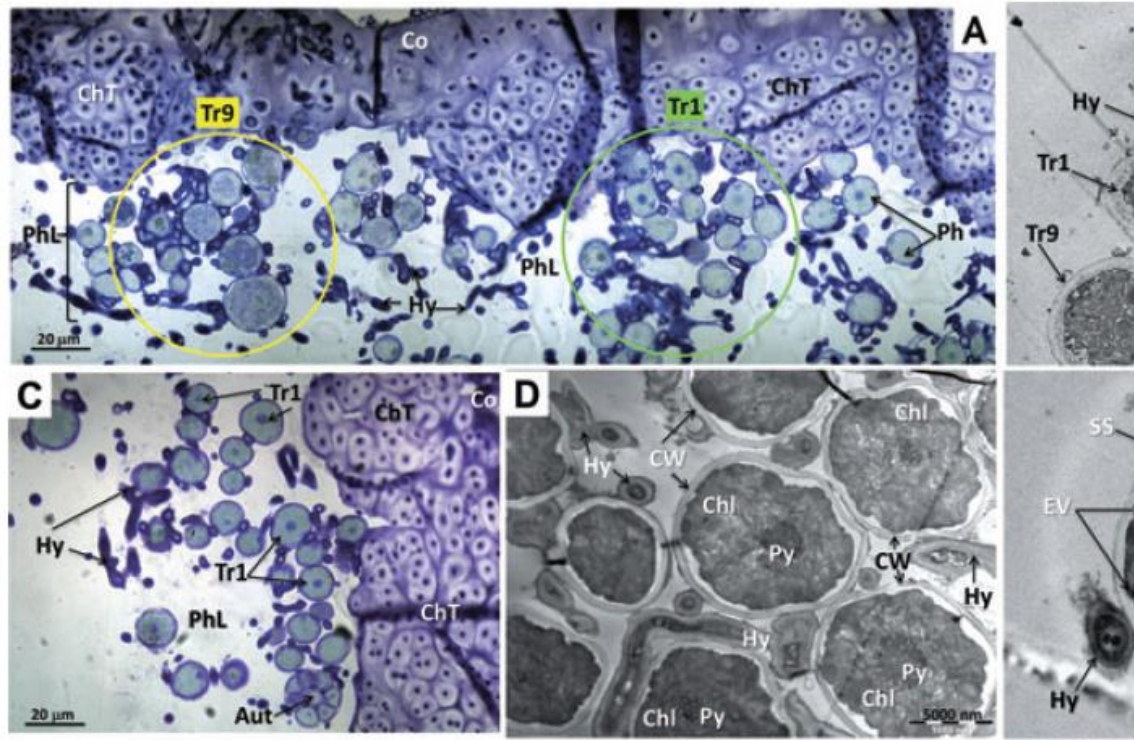


Fig. 4. Effects of temperature and irradiance during culture on growth and photosynthesis in isolated TR1 and TR9 phycobionts. **A.** TR9 and TR1 biomass accumulation after 30 days of culture at 17°C or 20°C and the indicated irradiances. Grey and white circles represent TR1 and TR9 phycobionts cultured at 17°C respectively. Grey and white squares represent TR1 and TR9 phycobionts cultured at 20°C respectively. Data are the mean values of five independent replicates (\pm SD).

B. Maximum quantum yield of PSII (F_v/F_m) of TR1 and TR9 phycobionts cultured at 17°C and four different light intensities during 30 days. Data are the mean values of five independent replicates (\pm SD). Grey and white circles represent TR1 and TR9 phycobionts respectively. Different normal and italic letters indicate significant differences among culture conditions for TR1 and TR9 phycobionts respectively (LSD test).



Trebouxiales, *Trebouxia*

Del Campo et al (2013) – ecological speciation of mycobionts and photobionts

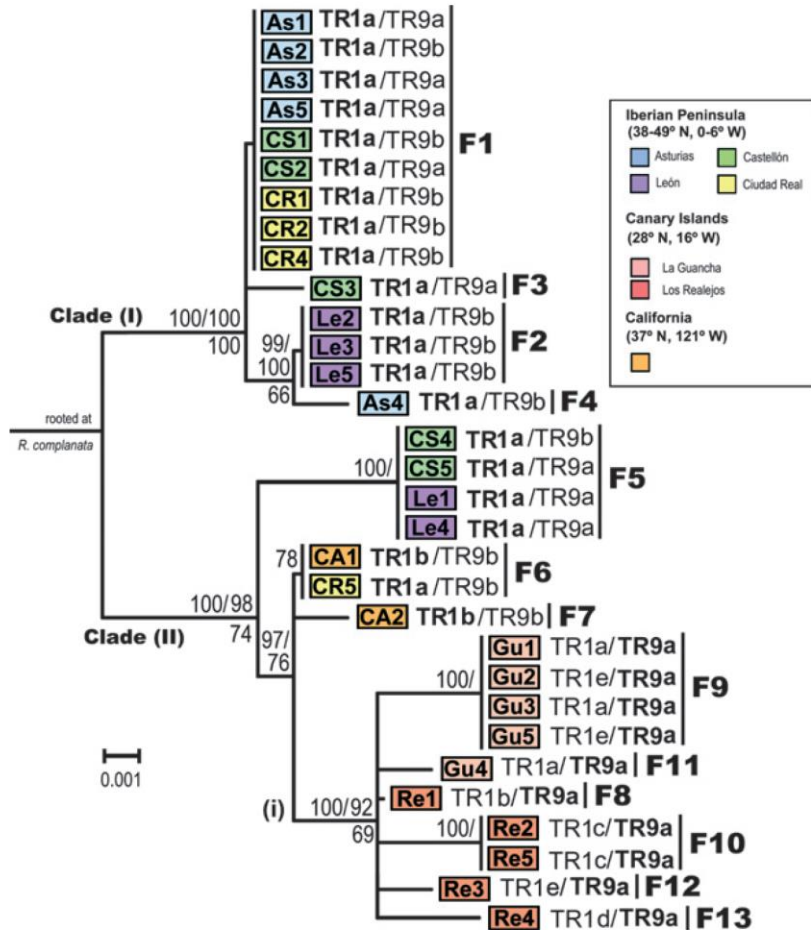


Fig. 1. Phylogram based on the analysis of fungal nrITS and *rpb2* gene sequences in specimens of *Ramalina farinacea*, with *Ramalina complanata* as out-group (accession numbers FJ356152 and DQ883762 for nrITS and *rpb2*, respectively). Vertical bars on the right indicate different fungal haplotypes of F1–F13. Algal haplotypes are indicated at the right of each sample: TR1 haplotypes based on nrITS sequences (TR1a–TR1e) and TR9 haplotypes based on nrITS sequences (TR9a–TR9b) are indicated at the left and the right of the slash-marks, respectively. The predominant phycobiont associated with each fungal haplotype is noted in bold letters. Subclade (i) includes mycobionts from the Canary Islands. The tree was obtained using a Bayesian approach with BEAST. Values at the left and right of the slash-mark on the tree branches correspond to posterior probabilities inferred with Bayesian and MP methods, respectively. Below the branches are the bootstrap values recorded only for those lineages supported by 50% of all (1000) replicates. Subpopulations within the studied populations (the Iberian Peninsula, the Canary Islands and California) and their coordinates are shown in the inset.

Trebouxiales, *Asterochloris*



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Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris* (Trebouxiophyceae, Chlorophyta)

Pavel Skaloud^{a,*}, Ondrej Peksa^{a,b}

^aDepartment of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-12801 Praha 2, Czech Republic
^bThe West Bohemian Museum in Pilsen, Kopeckého sady 2, CZ-30100 Plzeň, Czech Republic

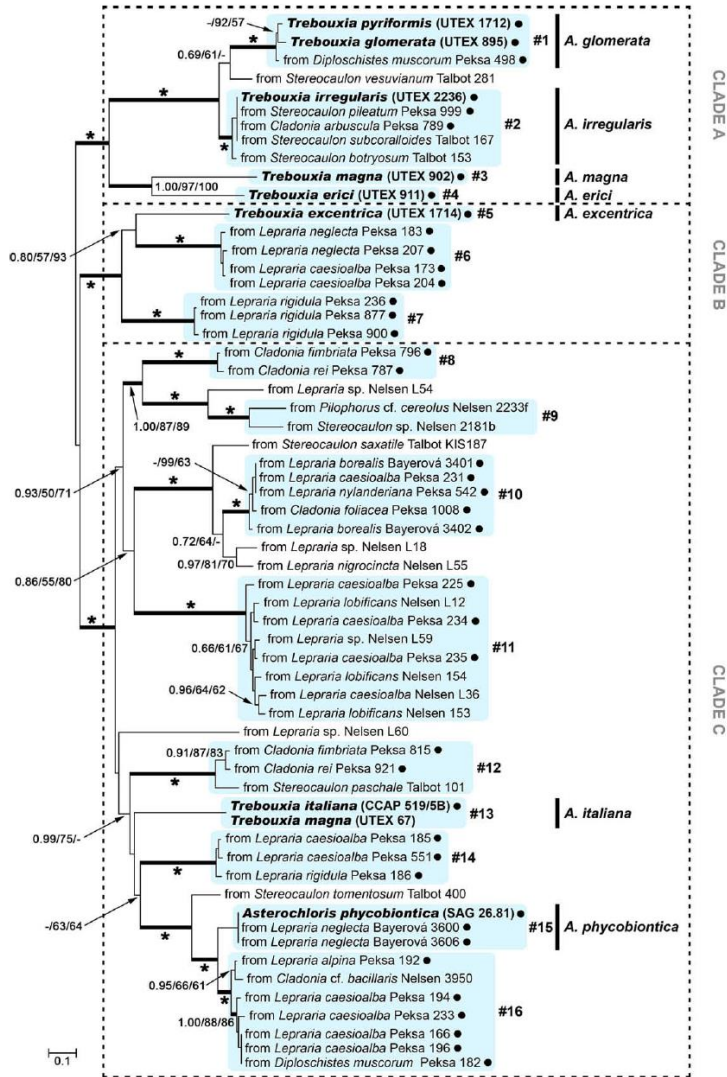
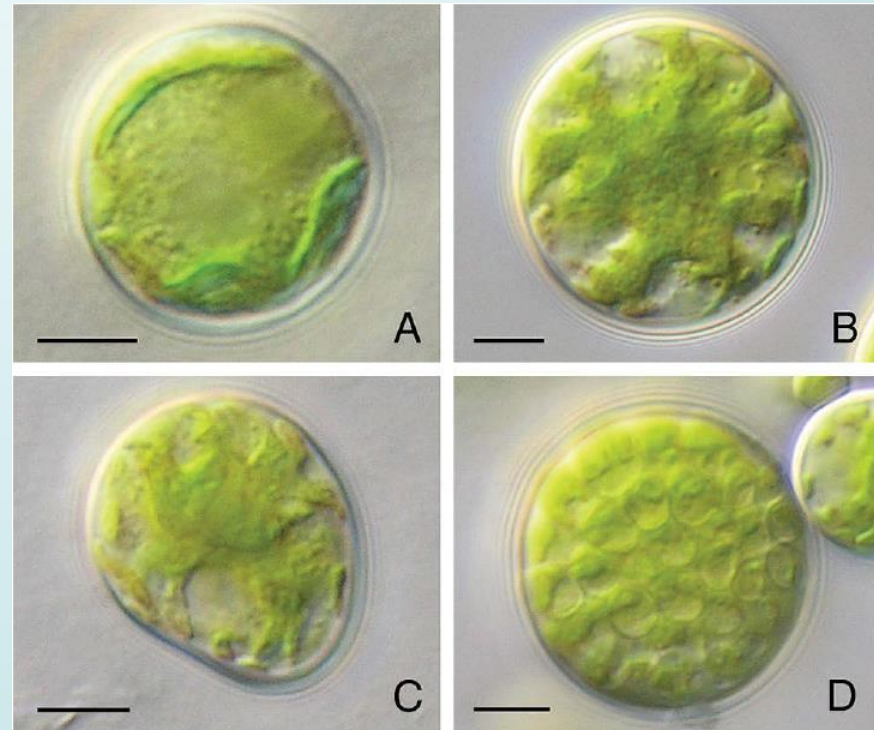


Fig. 3. Unrooted BI analysis based on the combined ITS + actin dataset using a GTR+ Γ model for ITS1, K80+ Γ model for ITS2, and JC model for 5.8 rRNA partition; and HKY+ Γ model for the actin-intron 206, HKY+ Γ model for the actin-intron 248, and K80+CP_{1,2,3}+ Γ model for the actin-exon partition. The doublet model is applied for the stem regions in all ITS rDNA partitions. Values at the nodes indicate statistical support estimated by three methods – MrBays posterior node probability (left), maximum likelihood bootstrap (in the middle) and maximum parsimony bootstrap (right). Full statistical support (1.00/100/100) is marked with an asterisk. Thick branches represent nodes receiving the highest PP support (1.00). Sequences determined in this study are marked by full circles. Authentic strains of *Asterochloris phycobiontica* and several former *Trebouxia* species are given in bold. Strain affiliation to 16 lineages (#1–16) and three major clades (A–C) is indicated. Scale bar – substitutions per site.

Trebouxiales, *Asterochloris*



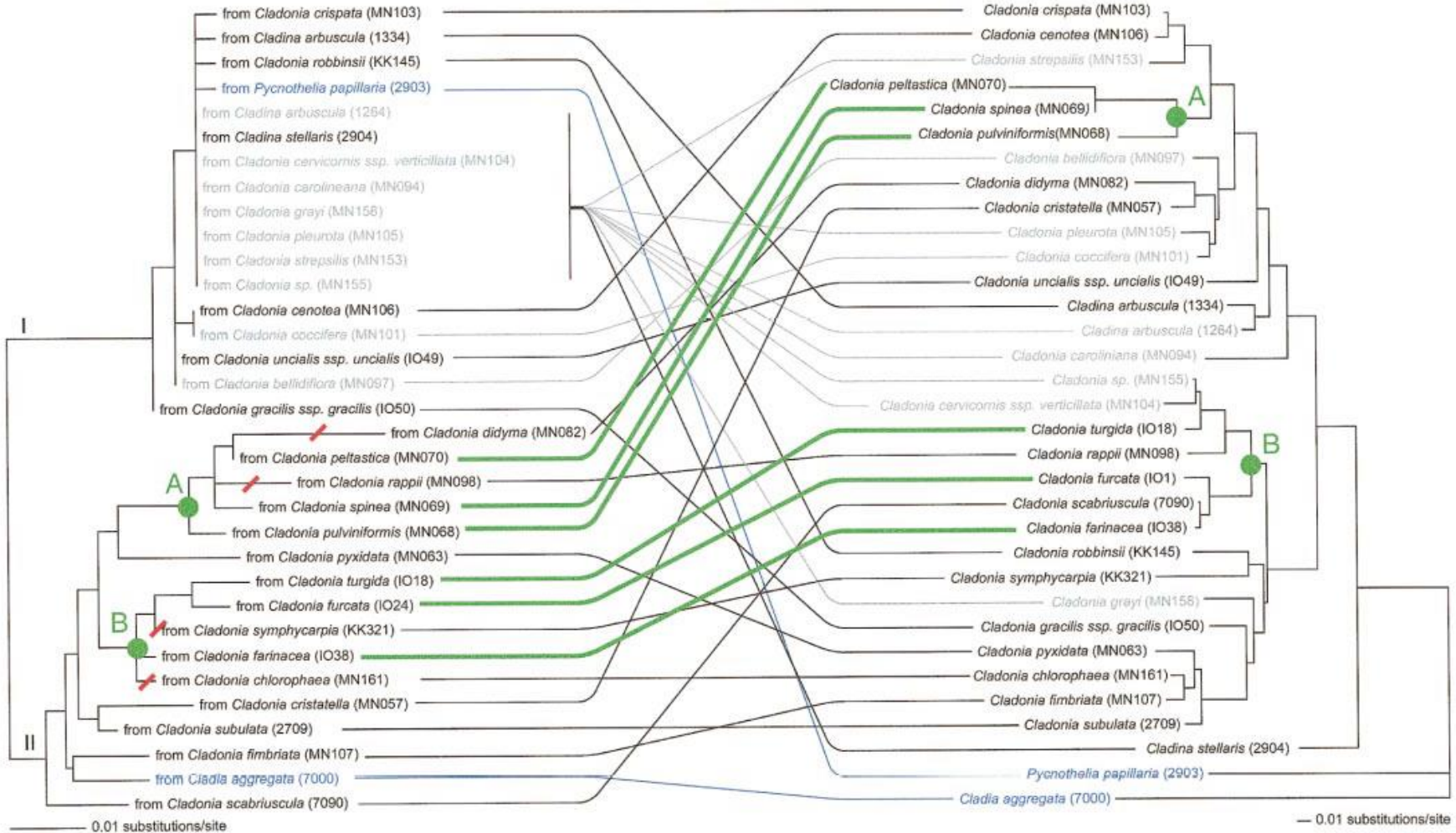
Trebouxiales, *Asterochloris*

American Journal of Botany 88(8): 1490–1498. 2001.

ALGAL SWITCHING AMONG LICHEN SYMBIOSES¹

MICHELE D. PIERCEY-NORMORE² AND PAULA T. DEPRIEST

- incongruent evolution of algae and fungi
- algal domestication



Trebouxiales, *Asterochloris*

MOLECULAR ECOLOGY

Molecular Ecology (2011) 20, 3936–3948

doi: 10.1111/j.1365-294X.2011.05168.x

Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae)

ONDŘEJ PEKSA*† and PAVEL ŠKALOUD†

*The West Bohemian Museum in Pilsen, Kopeckého sady 2, CZ-30100 Plzeň, Czech Republic, †Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-12801 Prague, Czech Republic

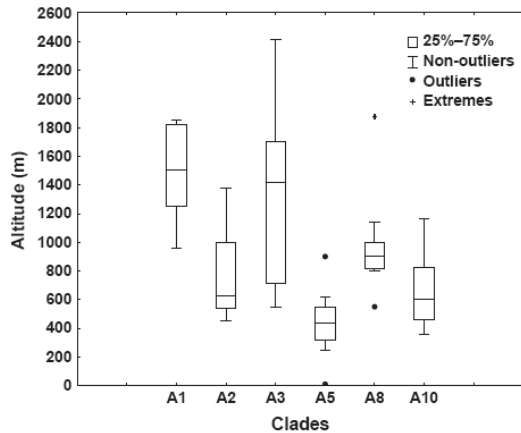


Fig. 3 Differences in the distribution of selected *Asterochloris* clades along the altitudinal gradient. Box and whisker plots are based on altitudinal data from six clades (only those represented by at least eight samples). All samples were collected in similar latitudes in Europe (43–58°N, i.e. somewhere in the temperate belt). The approximate upper borders of vertical vegetation belts for central Europe are as follows: 200 m a.s.l.—lowland, 600 m—colline, 1000 m—submontane, 1400 m—montane, 1800 m—subalpine, 2400 m—alpine.

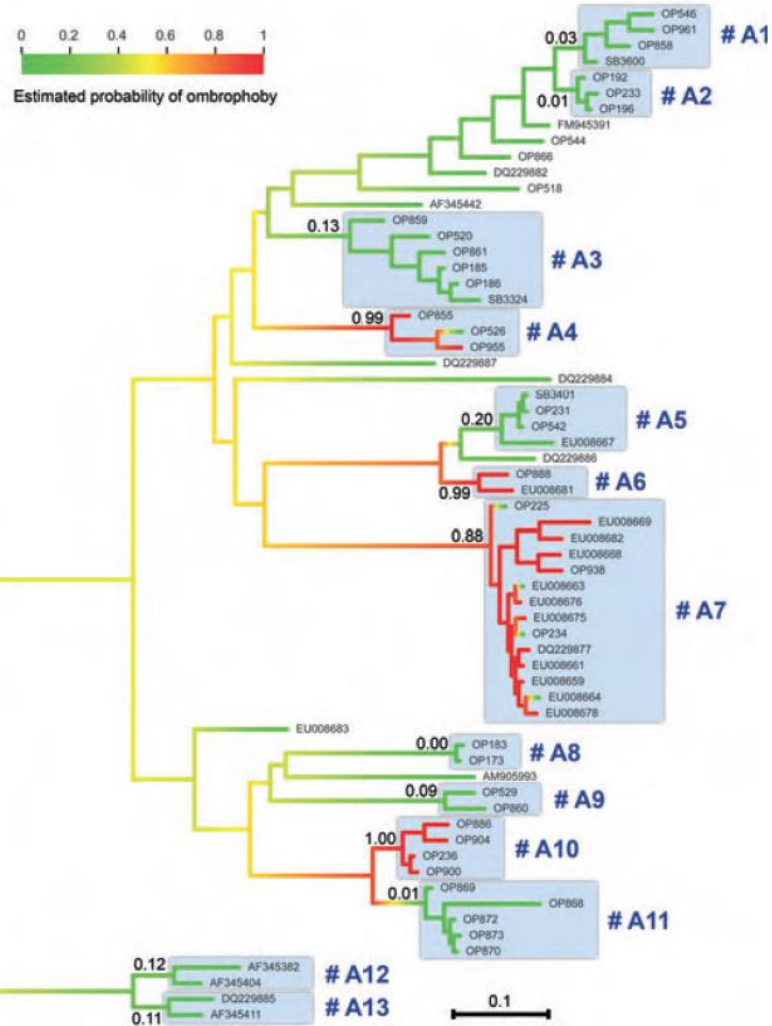
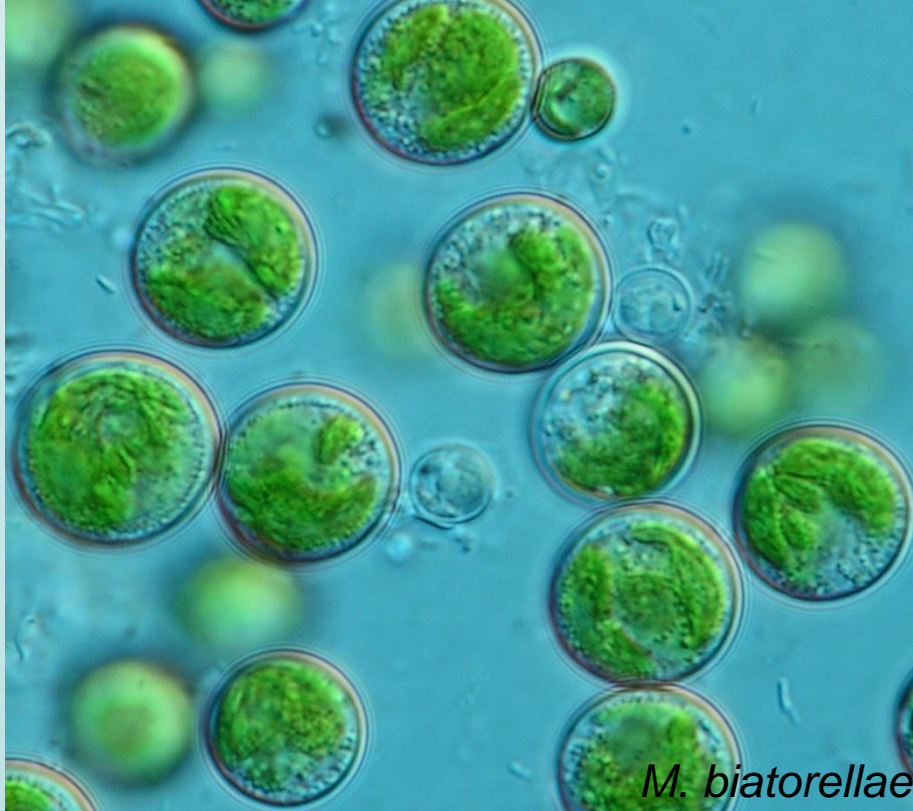


Fig. 2 The evolution of the selected ecological character—the ombrophoby (ombrophily) of lichens—mapped onto the photobiont phylogenetic tree. Colours are used to visualize estimated probabilities of the presence of ombrophoby along the phylogenetic tree. Red indicates a high probability of ombrophoby, whereas green denotes a low probability of ombrophobic preference. The estimated probabilities for ombrophoby are indicated for ancestors of each significantly supported clade (see Fig. 1). The topology of the tree corresponds completely to the topology of the phylogenetic tree in Fig. 1.

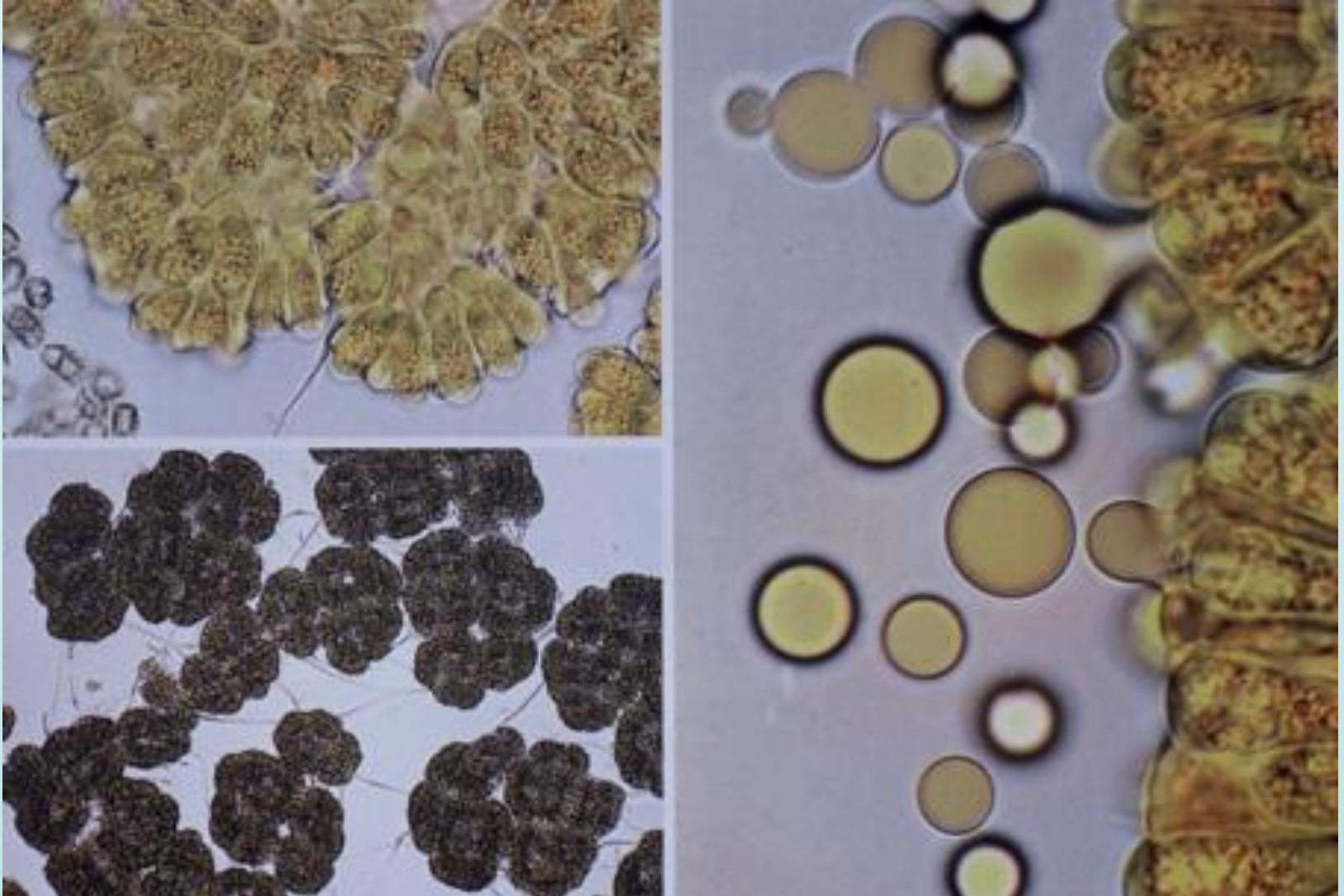
Trebouxiales, *Myrmecia*

- terrestrial, aerophytic
- lichen symbiont



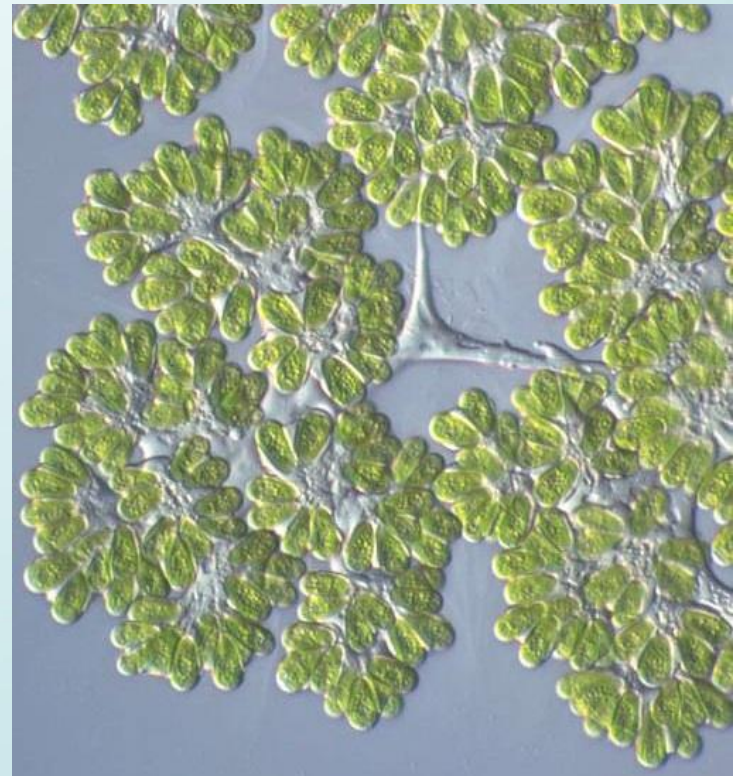
Botryococcus-clade, *Botryococcus*

- *Botryococcus braunii* – colonial green alga



Botryococcus-clade, Botryococcus

- water blooms
- botryococcon = hydrocarbon, 35% of the dry weight
- fuel?



Botryococcus-clade, Botryococcus

- Senousy et al. (2004) – polyphyletic *Botryococcus*

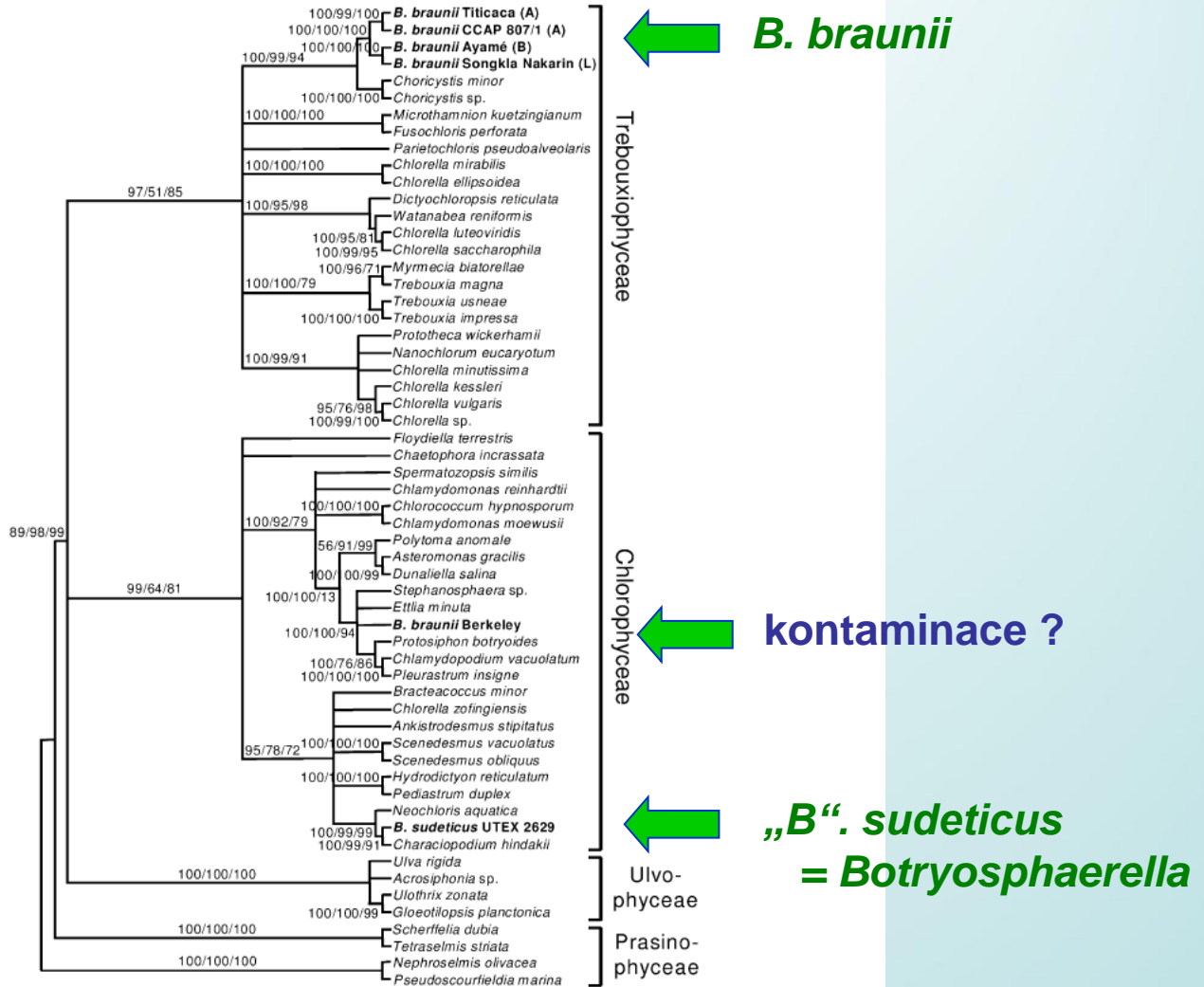
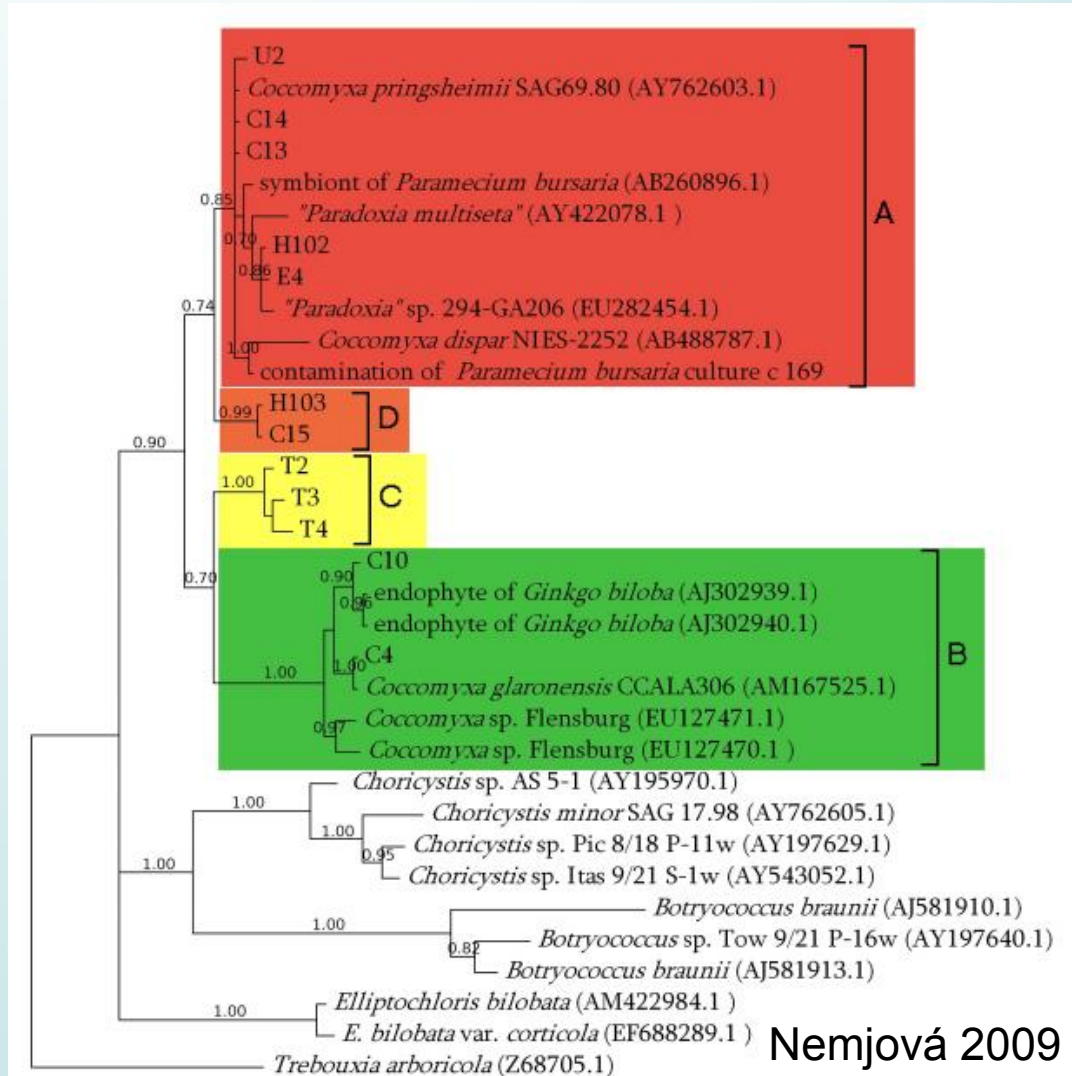
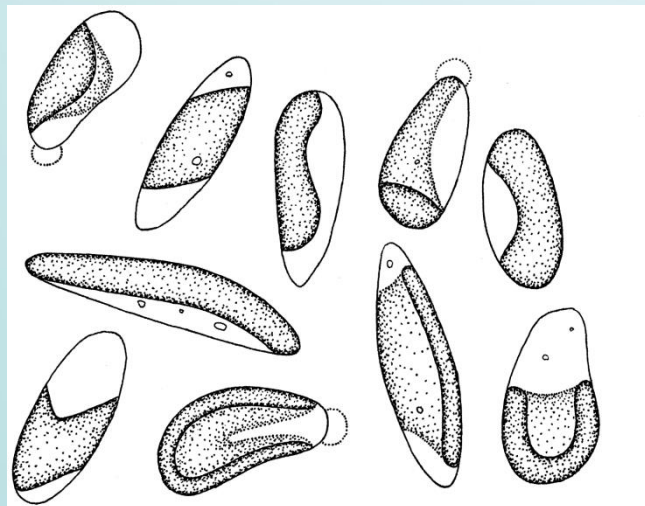
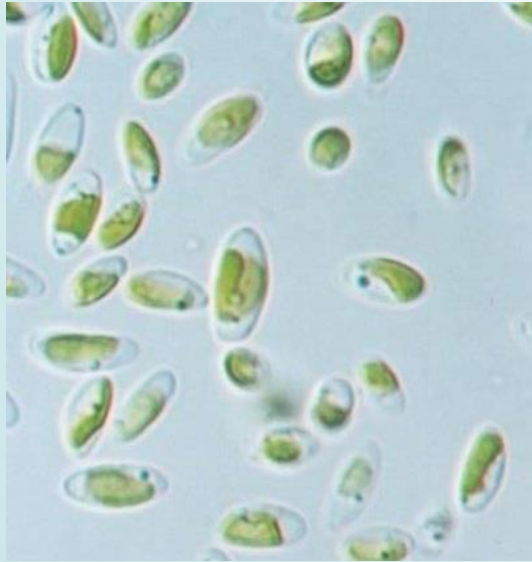


FIG. 3. Consensus tree from trees inferred with different methods of analysis. The numbers shown at the branches are credibility percentages determined using Bayesian inference (left) and bootstrap values determined using weighted parsimony (middle) and minimum evolution (right). Taxa shown in bold letters are the isolates whose 18S rRNA sequences were determined in this study.

Botryococcus-clade, (*Pseudo-*)*Coccomyxa*

- ellipsoid cells, frequent in aerophytic substrate



Botryococcus-clade, (*Pseudo-*)*Coccomyxa*

- lichen photobionts

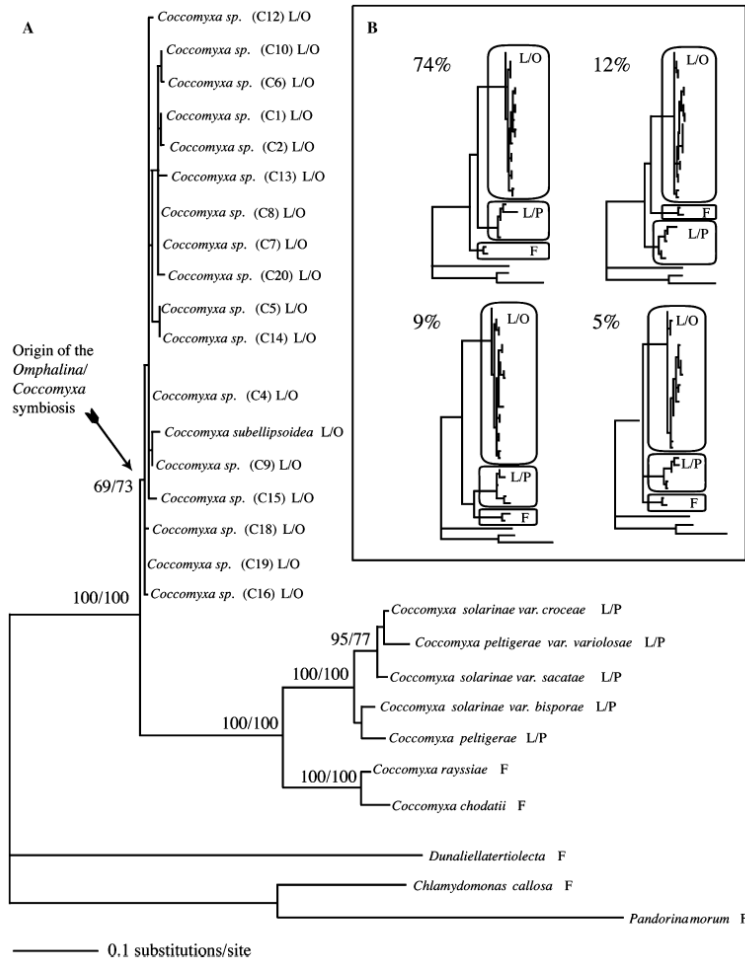


Fig. 3. Phylogenetic relationships among lichenized and non-lichenized *Coccomyxa* algae (dataset AC) as revealed by an ML search using ITS (ITS1-5.8S-ITS2) sequences. *Coccomyxa* isolated from lichenized *Omphalina* species (Basidiomycota) are denoted by a bold L/O after the name. *Coccomyxa* isolated from members of the Peltigerales (Ascomycota) are annotated with a bold L/P. Free-living species are denoted by a bold F. (A) Single most likely tree with maximum parsimony and maximum likelihood bootstrap support values greater than 50% given above internal branches, before and after the backslash, respectively. (B) Frequency of the four topologies recovered by the ML analysis from 100 simulated datasets. The topology under which the data were simulated (monophyly of the lichenized *Coccomyxa* species) was recovered only 74% of the times (top left).



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Molecular Phylogenetics and Evolution 29 (2003) 629–640

MOLECULAR
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Slow algae, fast fungi: exceptionally high nucleotide substitution rate differences between lichenized fungi *Omphalina* and their symbiotic green algae *Coccomyxa*

Stefan Zoller* and François Lutzoni



Botryococcus-clade, (*Pseudo-*)*Coccomyxa*

Vol. 81: 231–240, 2008
doi: 10.3354/dao01956

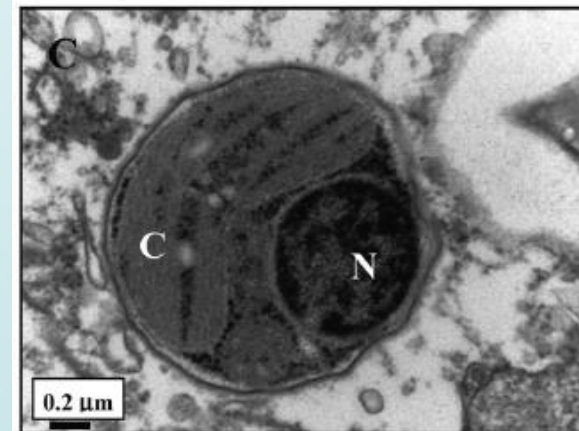
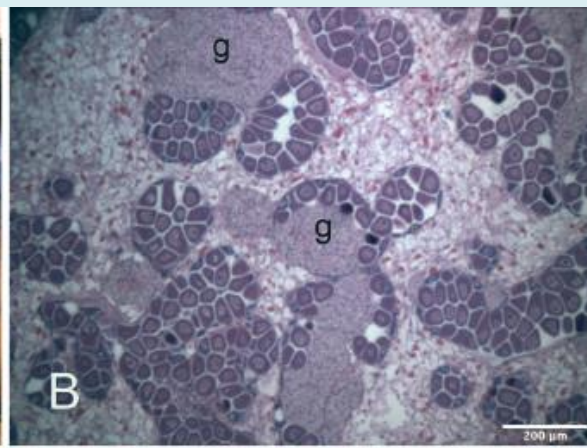
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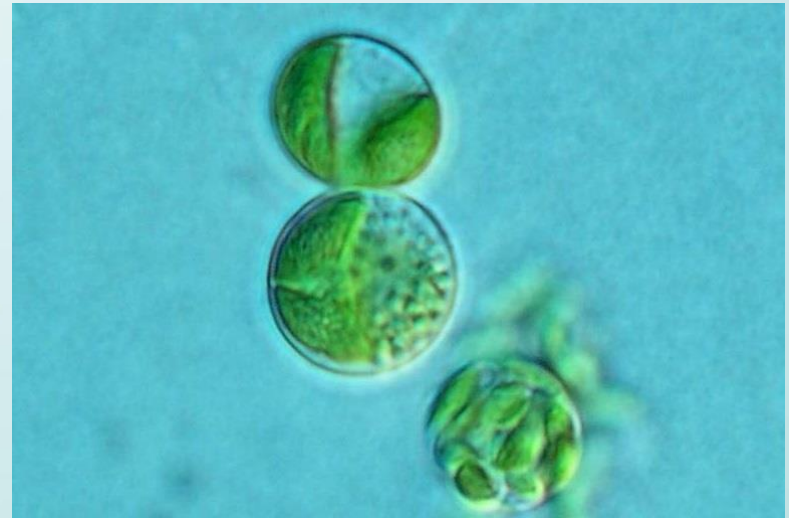
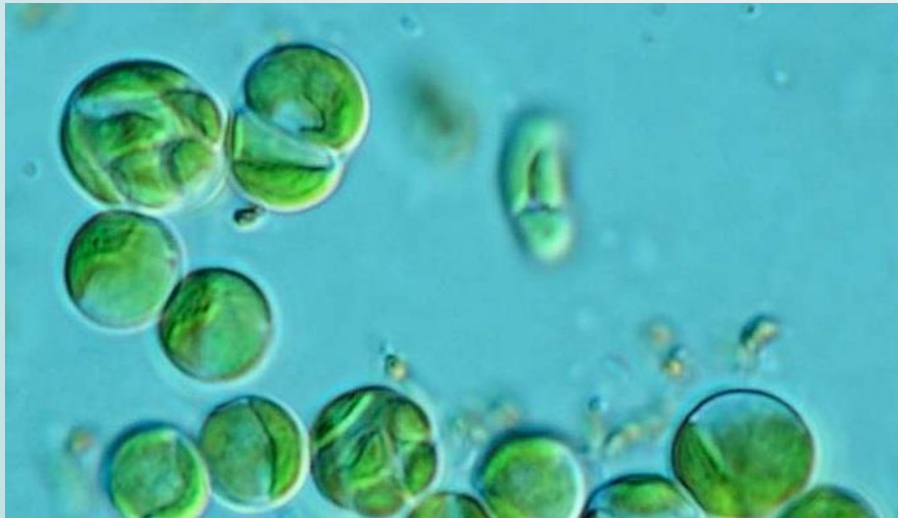
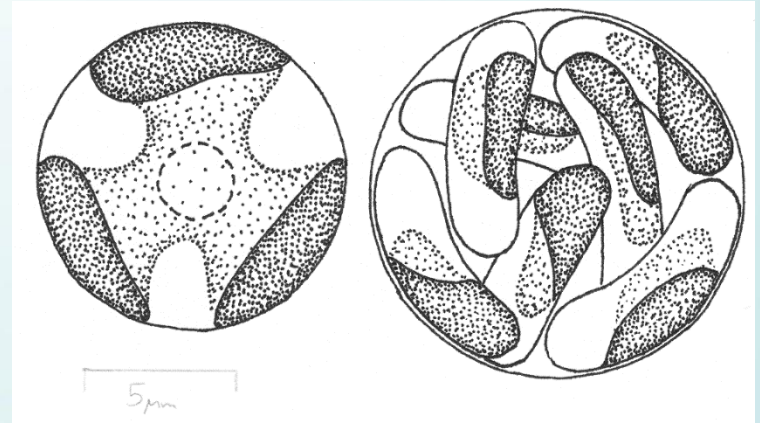
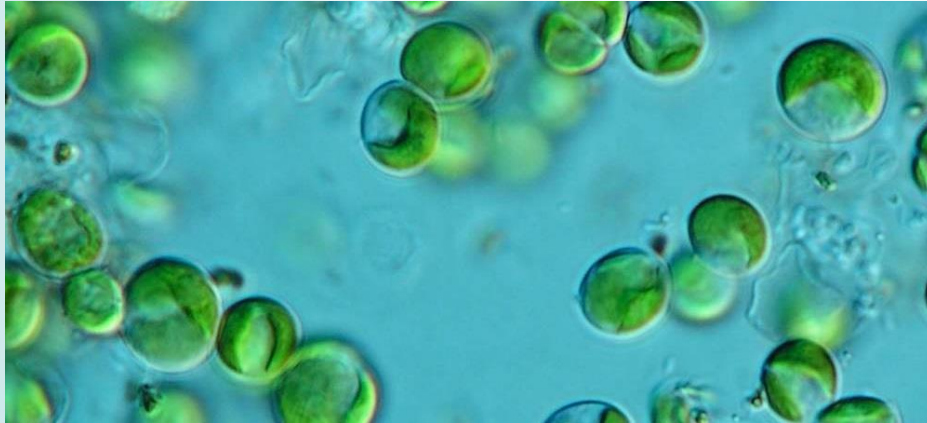
Phylogenetic and morphological characterisation of the green algae infesting blue mussel *Mytilus edulis* in the North and South Atlantic oceans

Francisco Rodríguez^{1,5}, Stephen W. Feist², Laure Guillou¹, Lisbeth S. Harkestad³,
Kelly Bateman², Tristan Renault⁴, Stein Mortensen^{3,*}



Botryococcus-clade, Elliptochloris

- 2 autospore types



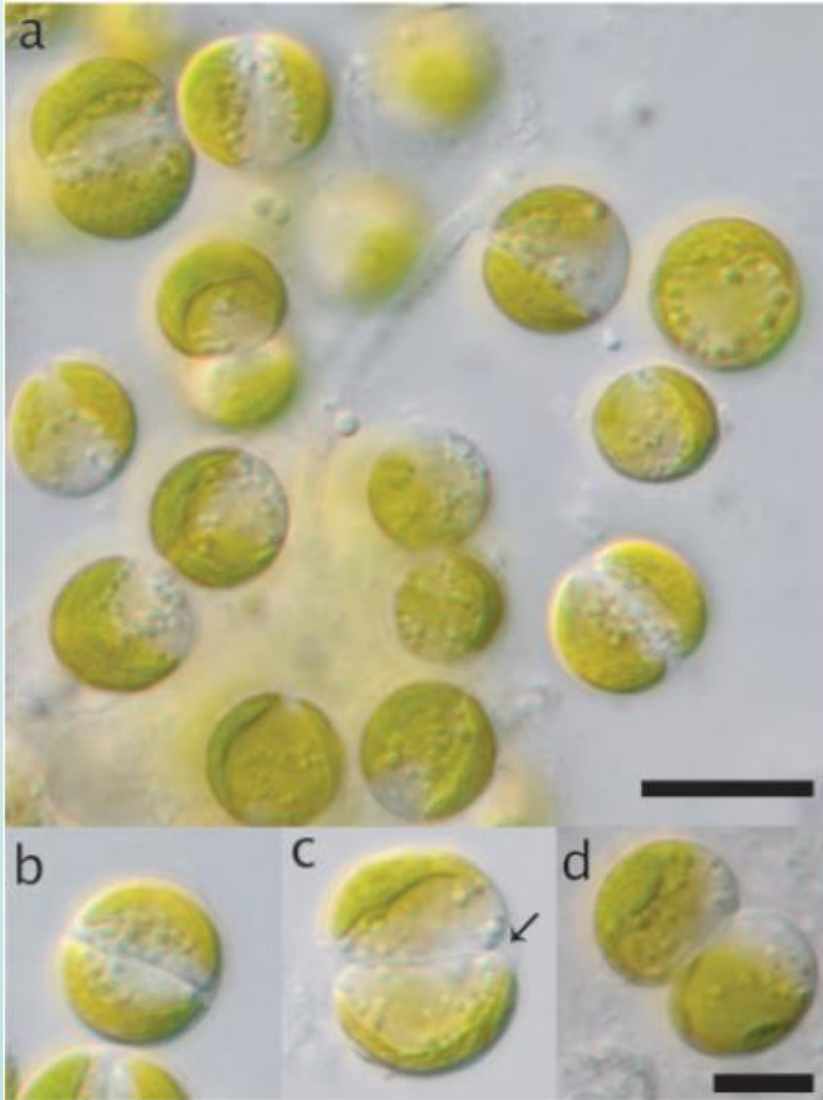
Botryococcus-clade, *Elliptochloris*

- symbiont of lichens (*Baeomyces*, *Catolechia*), sea anemones (*Anthopleura*)



Botryococcus-clade, *Elliptochloris*

- symbiont of lichens (*Baeomyces*, *Catolechia*), sea anemones (*Anthopleura*)



J. Phycol. 45, 1127–1135 (2009)
© 2009 Phycological Society of America
DOI: 10.1111/j.1529-8817.2009.00727.x

ELLIPTOCHLORIS MARINA SP. NOV. (TREBOUXIOPHYCEAE, CHLOROPHYTA),
SYMBIOTIC GREEN ALGA OF THE TEMPERATE PACIFIC SEA ANEMONES
ANTHOPLEURA XANTHOGRAMMICA AND *A. ELEGANTISSIMA*
(ANTHOZOA, CNIDARIA)¹

Molly R. Letsch²

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269, USA

Gisèle Muller-Parker

Department of Biology and Shannon Point Marine Center, Western Washington University, Bellingham,
Washington 98225-9160, USA

Thomas Friedl

Experimental Phycology and Culture Collection of Algae, Georg-August University Göttingen, Untere Karspüle 2,
37073 Göttingen, Germany

and Louise A. Lewis

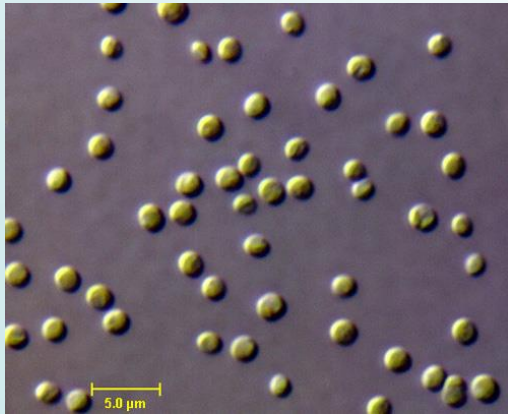
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269, USA



Trebouxiophyceae, picoplankton

- green balls up to 3 μm in diameter

Nannochloris

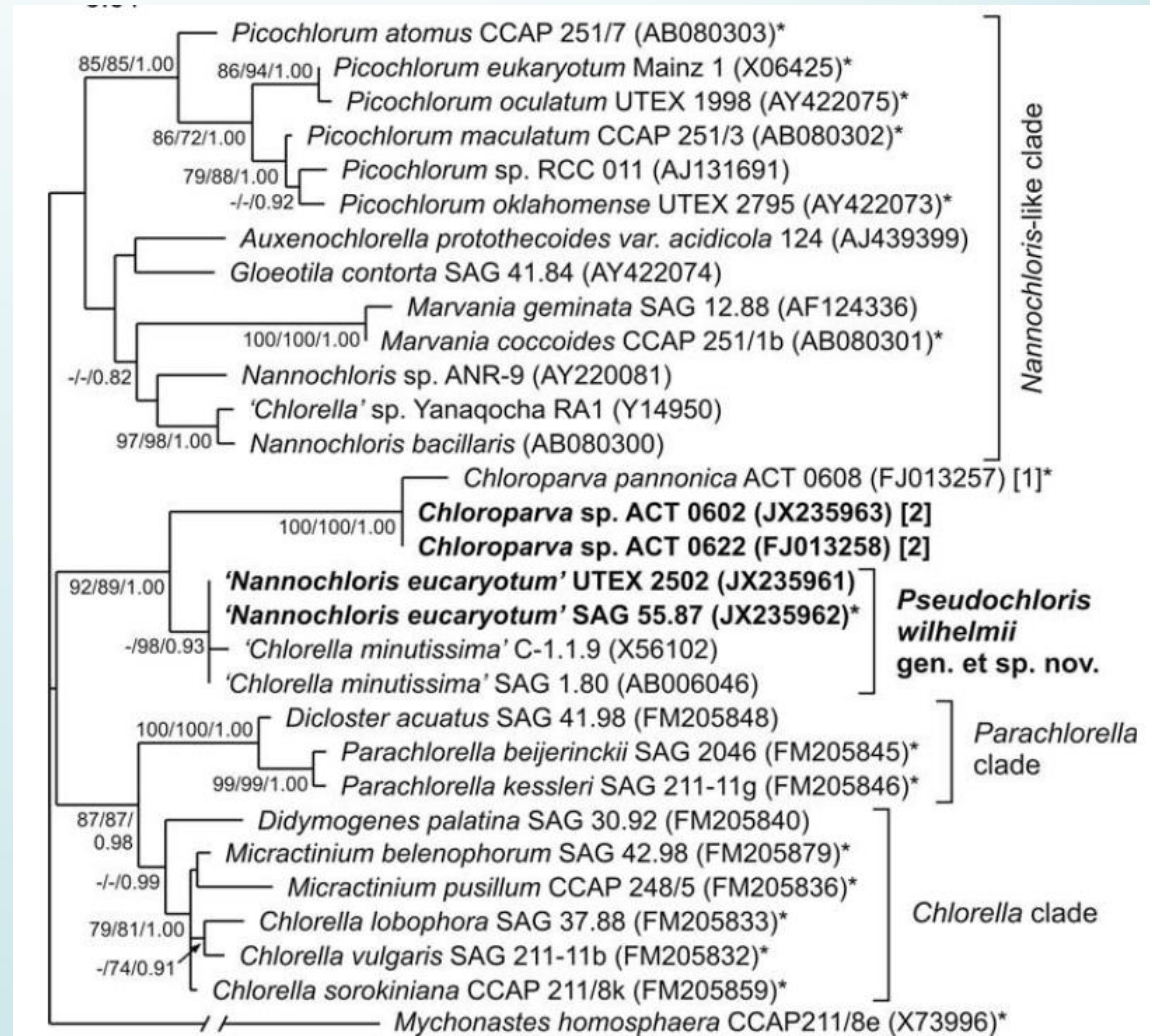
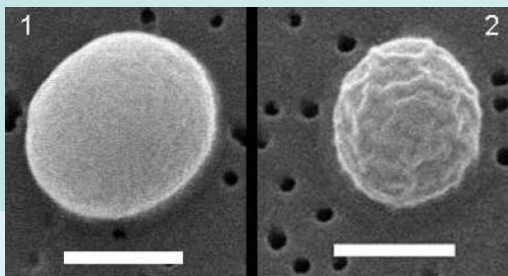


Picochlorum



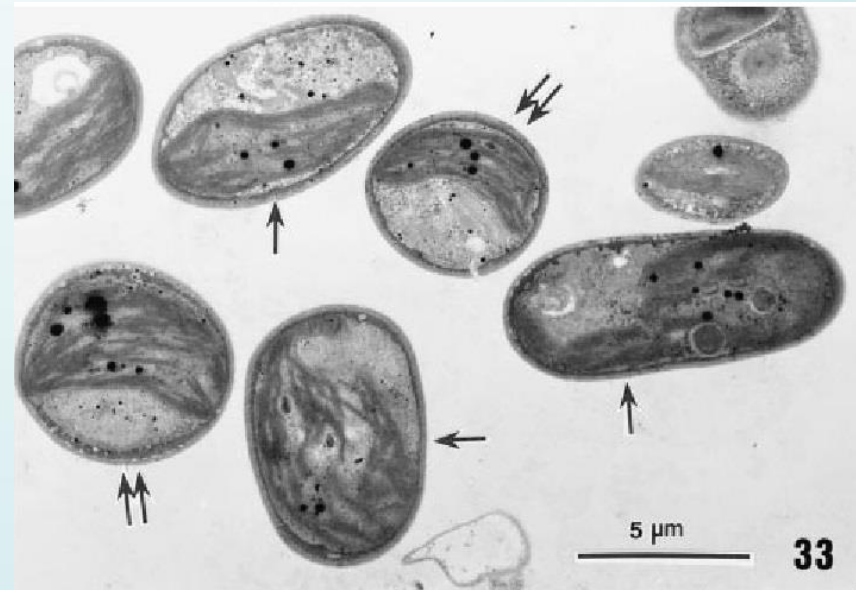
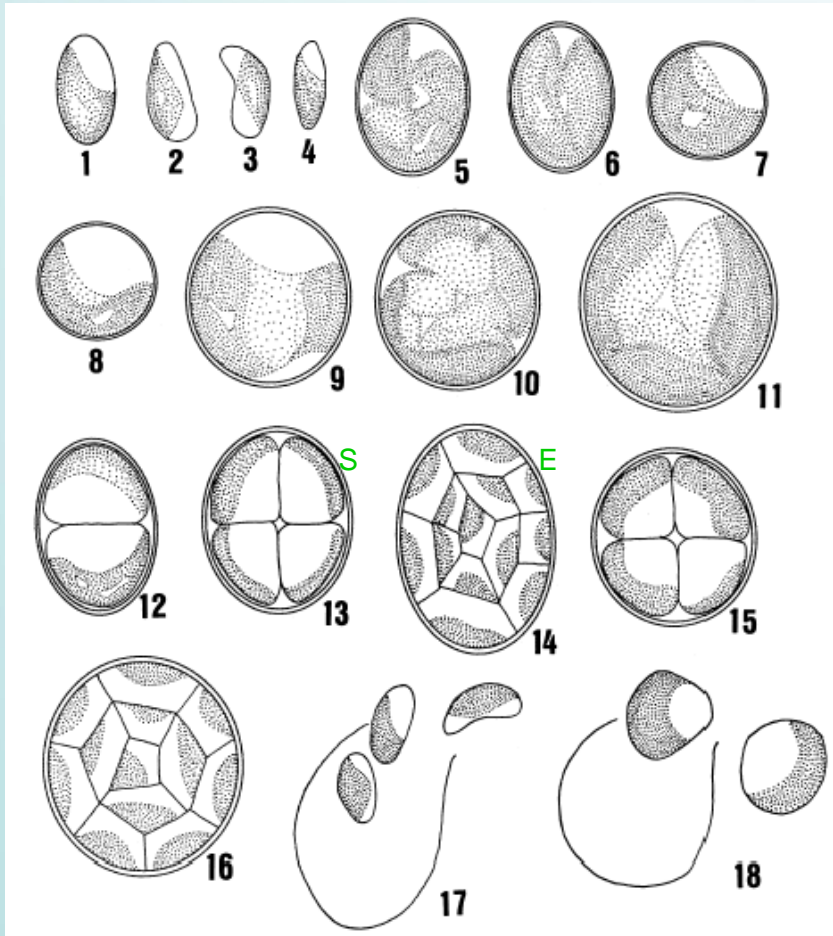
Pseudochloris

Chloroparva



Watanabea-clade, *Watanabea*

- *W. reniformis* (Hanagata et al., 1998) – 2 autospore types
- characteristic cell wall (different from *Elliptochloris*)



- unilayered CW (*Elliptochloris* – trilaminar CW)

Watanabea-clade, *Chloroidium*

Eur. J. Phycol., 2010, 1–17, iFirst



Chloroidium, a common terrestrial coccooid green alga previously assigned to *Chlorella* (Trebouxiophyceae, Chlorophyta)

TATYANA DARIENKO¹, LYDIA GUSTAVS², OPAYI MUDIMU^{3,5}, CECILIA RAD MENENDEZ⁴, RHENA SCHUMANN², ULF KARSTEN², THOMAS FRIEDL³ AND THOMAS PRÖSCHOLD⁴

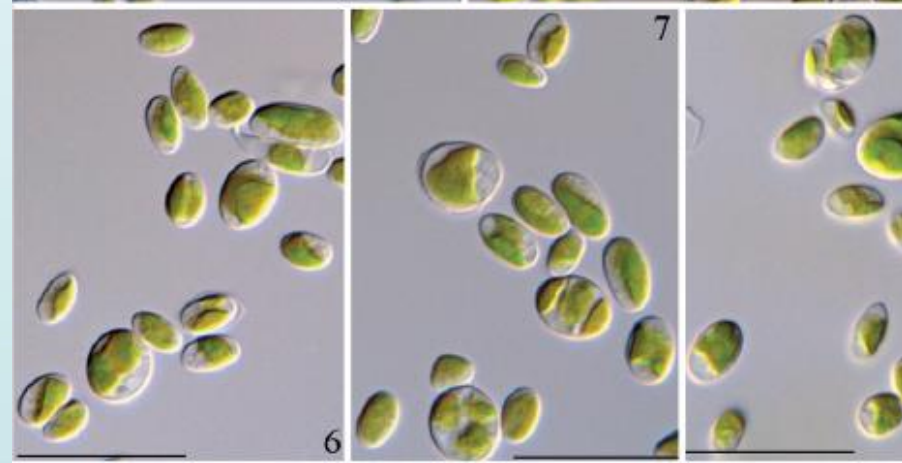
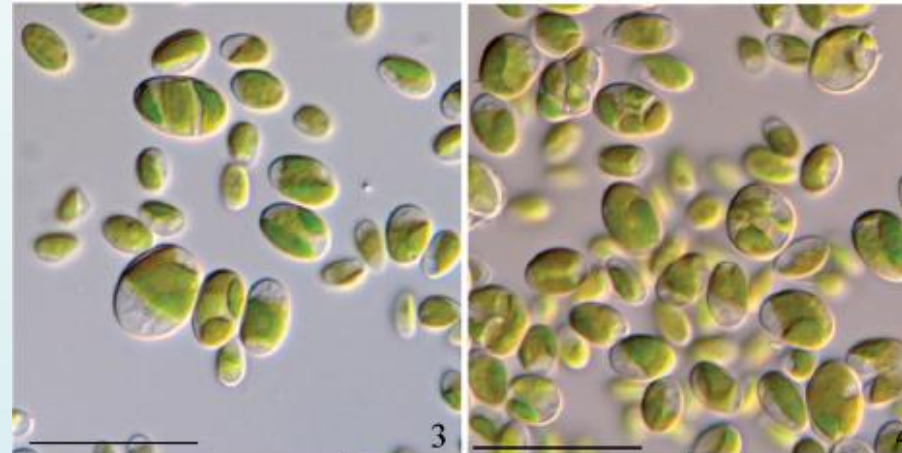
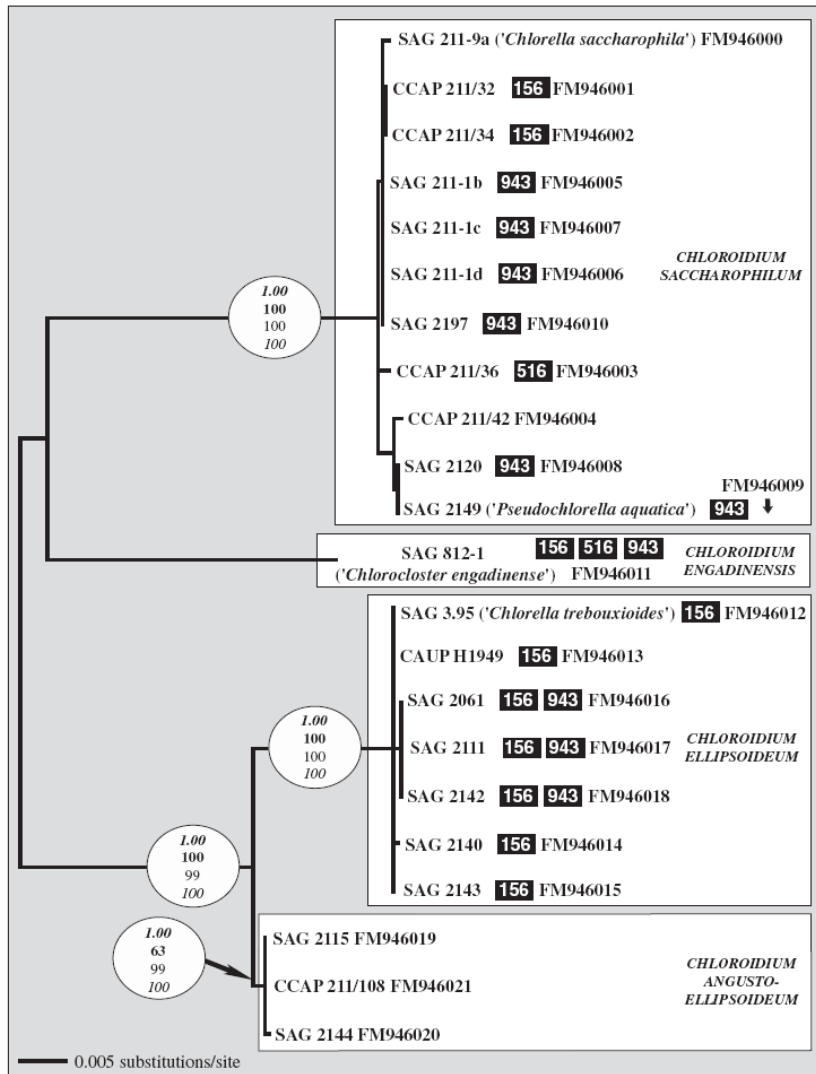
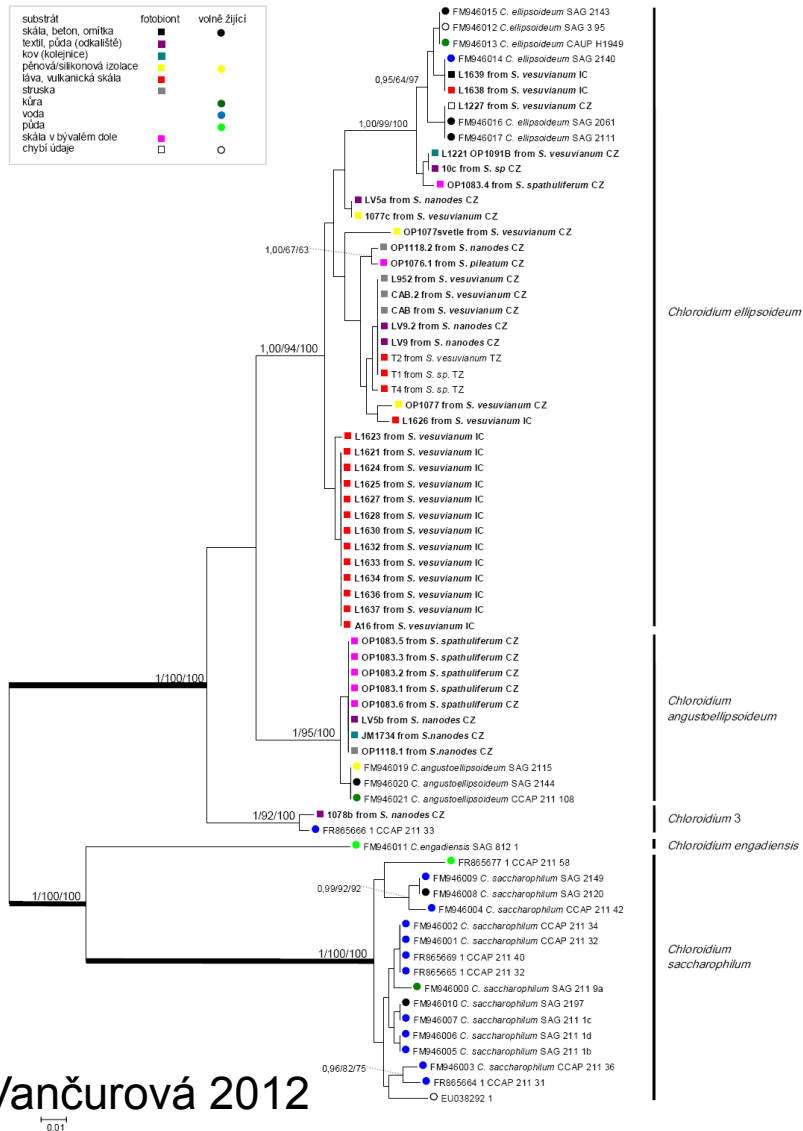


Fig. 2. Molecular phylogeny of *Chloroidium* based on SSU and ITS rDNA sequences, inferred by maximum likelihood (ML) based on a data set of 2500 aligned positions of 22 taxa using PAUP 4.0b10, using GTR model, with proportion of invariable sites $I=0.86$. Bayesian values (>0.95 ; bold italic) using the covarion model (5 million generations) and bootstrap values ($>50\%$) of the ML (using the GTR+I model, 100 replicates; bold), neighbor-joining (using the GTR+I model, 1000 replicates; not bold), and maximum parsimony (1000 replicates; italic) are shown in boxes on the tree. Strains marked in bold are new sequences, and strain and accession numbers, as well as intron position (white number in black box), are given.

Watanabea-clade, *Chloroidium*



Chloroidium ellipsoideum



Chloroidium angustellipsoideum

Chloroidium 3

Chloroidium engadiensis

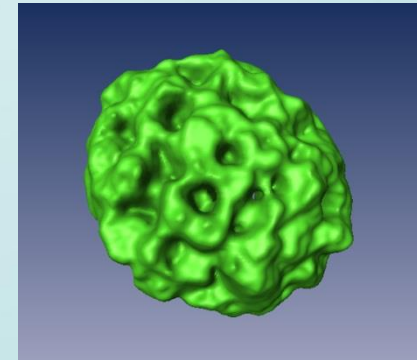
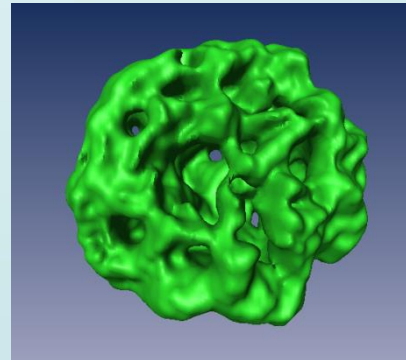
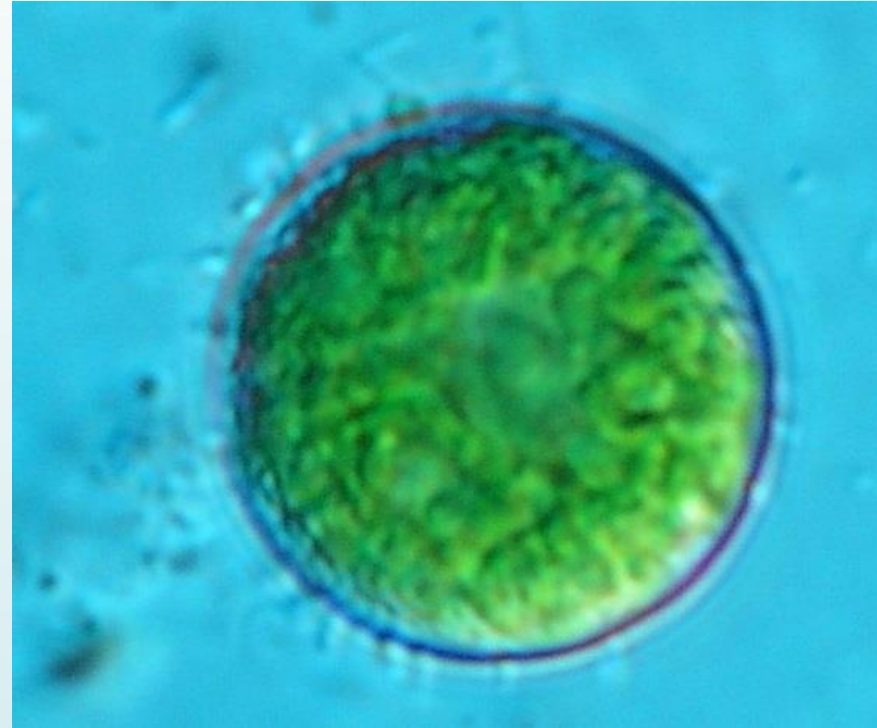
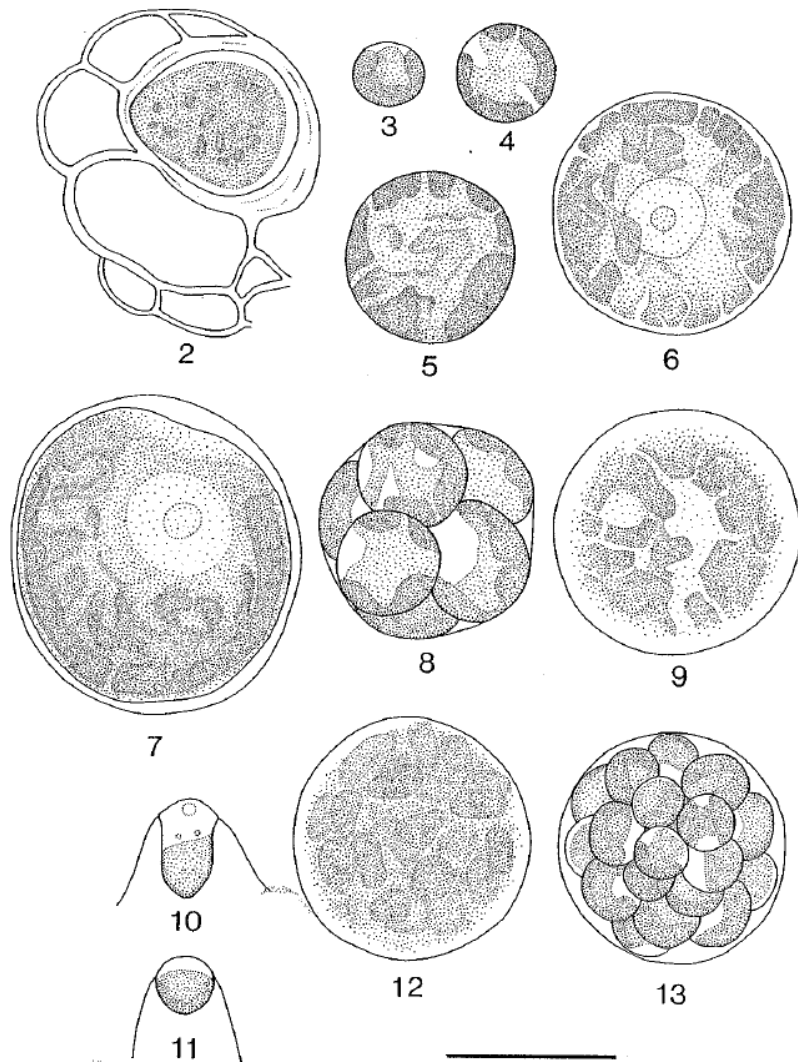
Chloroidium saccharophilum

Vančurová 2012

Obrázek 15: Nezakořeněný BI fylogenetický strom založený úseku ITS rDNA. Hodnoty v uzlech vyjadřují statistickou podporu určenou třemi metodami: MrBayes posteriori pravděpodobnost/maximium likelihood bootstrap/maximum parsimony bootstrap. Zobrazeny jsou pouze hodnoty >0,89 pro BI a >49 pro ML/MP. Popisky sekvencí fotobiontů obsahují označení DNA, druh mykobionta a geografický původ (CZ – Česká republika, IC – Kanárské ostrovy, TZ – Tanzanie). Popisky sekvencí volně žijících zástupců obsahují číslo sekvence v GenBanku, druh díle (Darienko et al., 2010) a číslo sbírkového kmene.

Watanabea-clade, *Dictyochloropsis*

- aerophytic alga, lichen symbiont



Watanabea-clade, *Dictyochloropsis*

- aerophytic alga, lichen symbiont

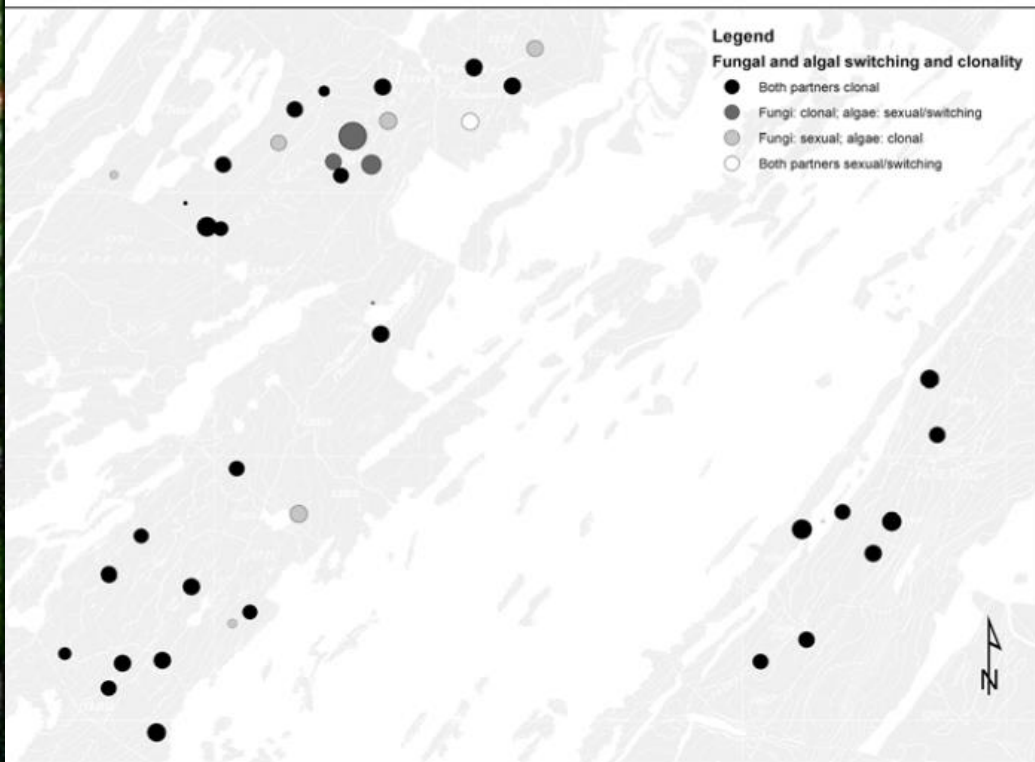


Congruent Genetic Structure in the Lichen-Forming Fungus *Lobaria pulmonaria* and Its Green-Algal Photobiont

Silke Werth and Christoph Scheidegger

Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

Submitted 31 March 2011. Accepted 26 October 2011.



Watanabea-clade, *Phyllosiphon*

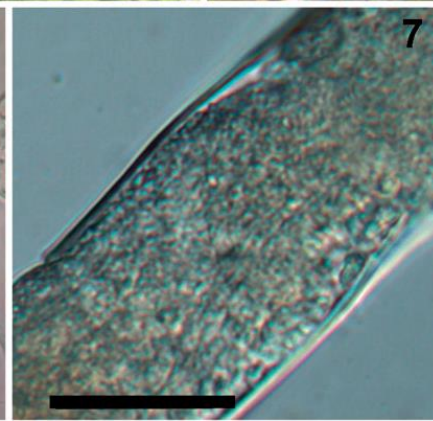
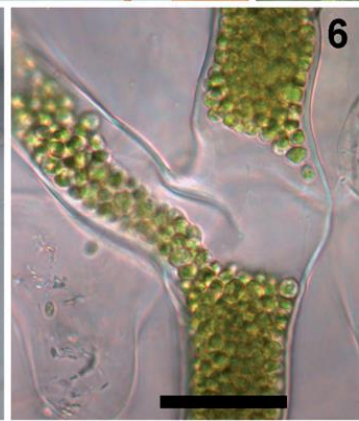
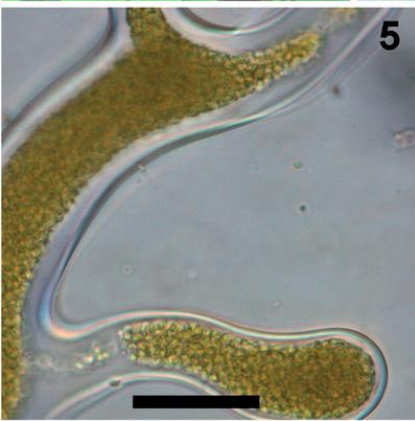
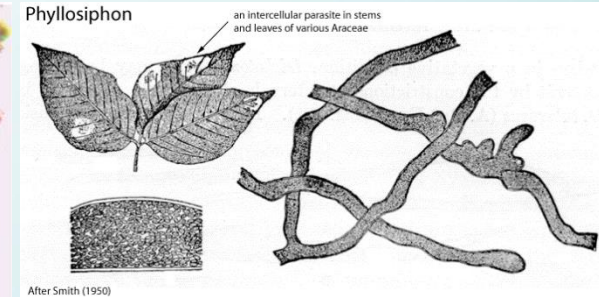
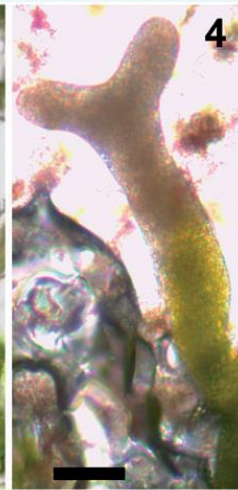
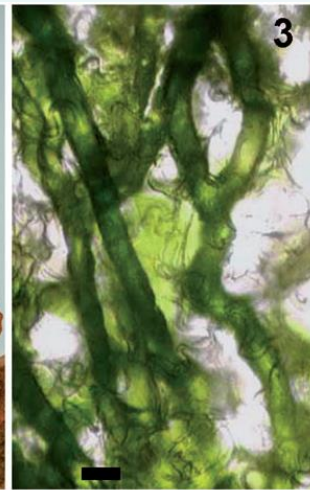
- sifonal parasitic alga
- leaves of *Arisarum*

Eur. J. Phycol. (2011) 46(3): 181–192



Morphology, fine structure, life cycle and phylogenetic analysis of *Phyllosiphon arisari*, a siphonous parasitic green alga

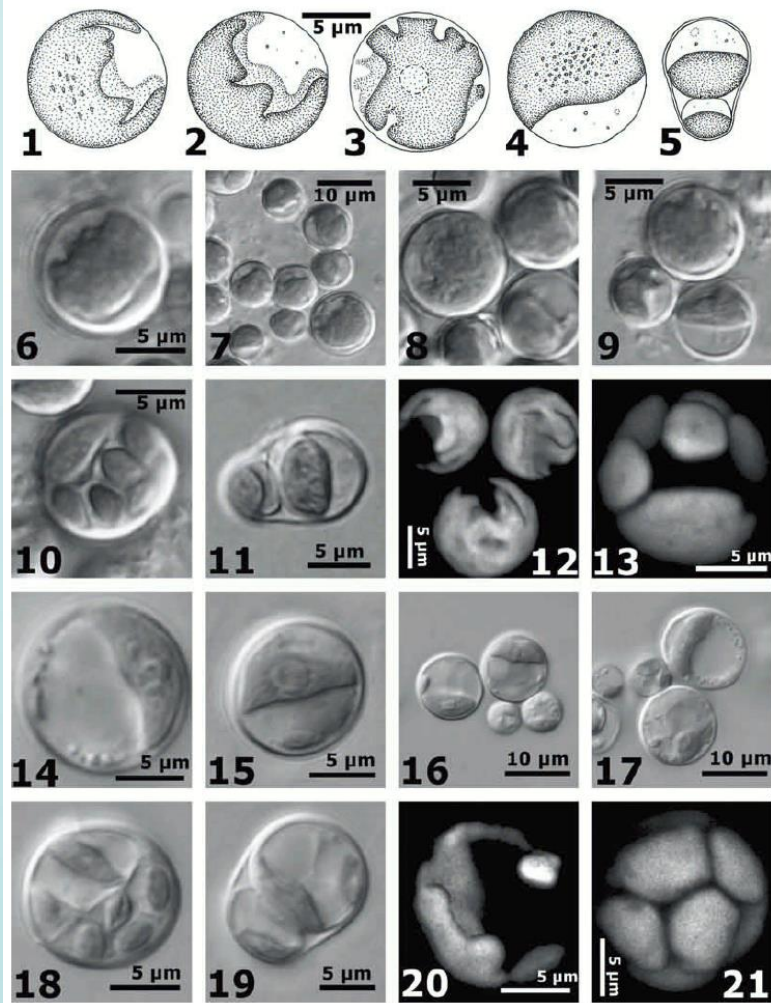
MARINA ABOAL¹ AND OLAF WERNER²



Watanabea-clade, *Kalinella*

Phycological Research

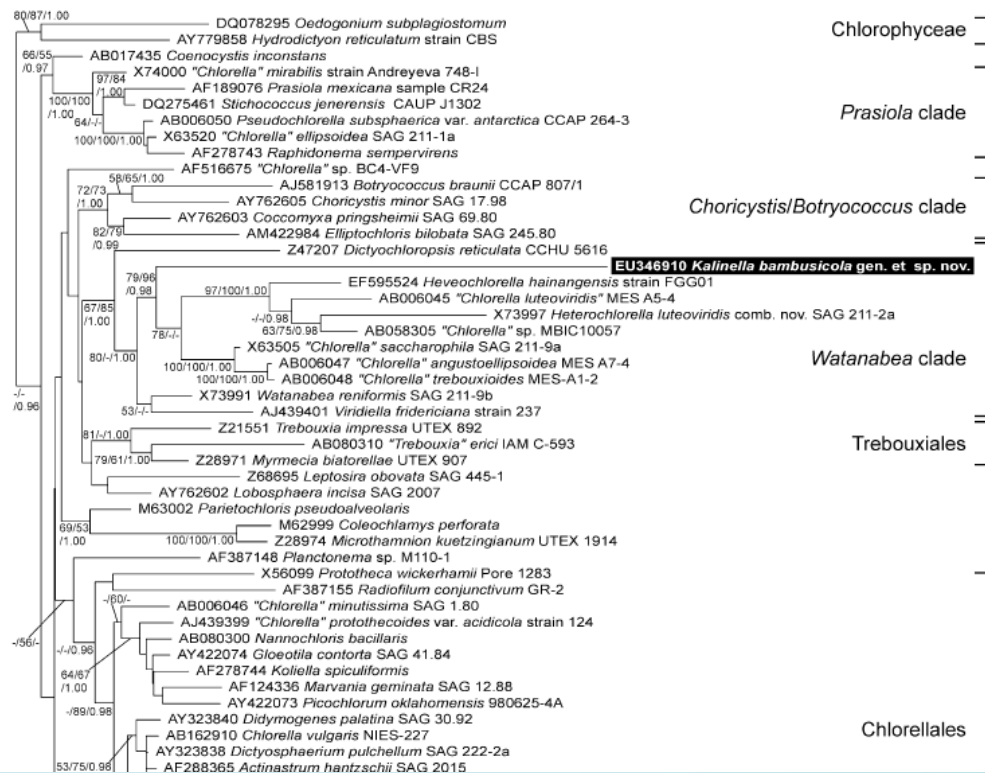
Phycological Research 2009; 57: 159-169



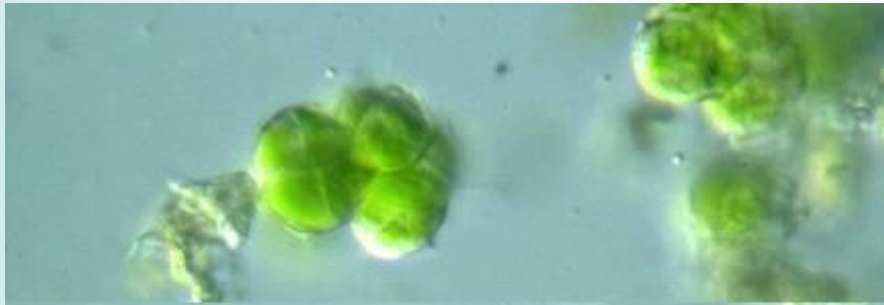
Kalinella bambusicola gen. et sp. nov. (Trebouxiophyceae, Chlorophyta), a novel coccoid *Chlorella*-like subaerial alga from Southeast Asia

Jiří Neustupa,* Yvonne Němcová, Marek Eliáš and Pavel Škaloud

Department of Botany, Faculty of Science, Charles University in Prague, Benatska 2, Prague, 12801, Czech Republic



Apatococcus

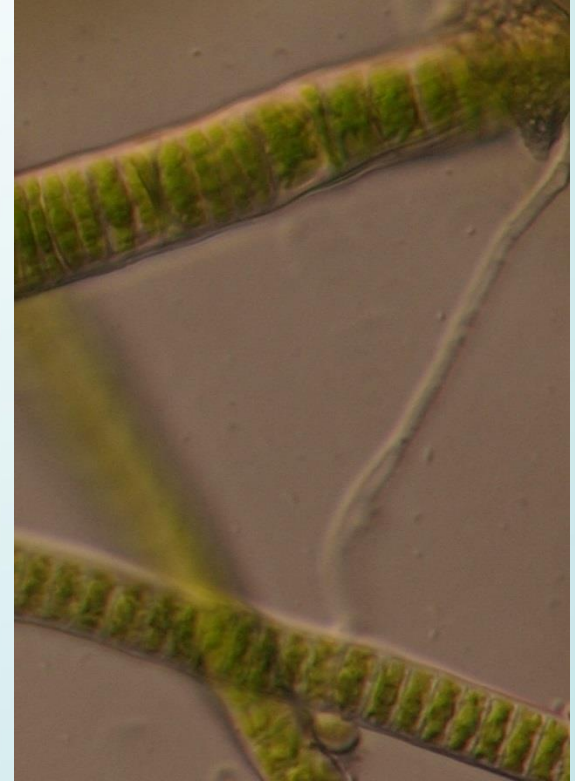
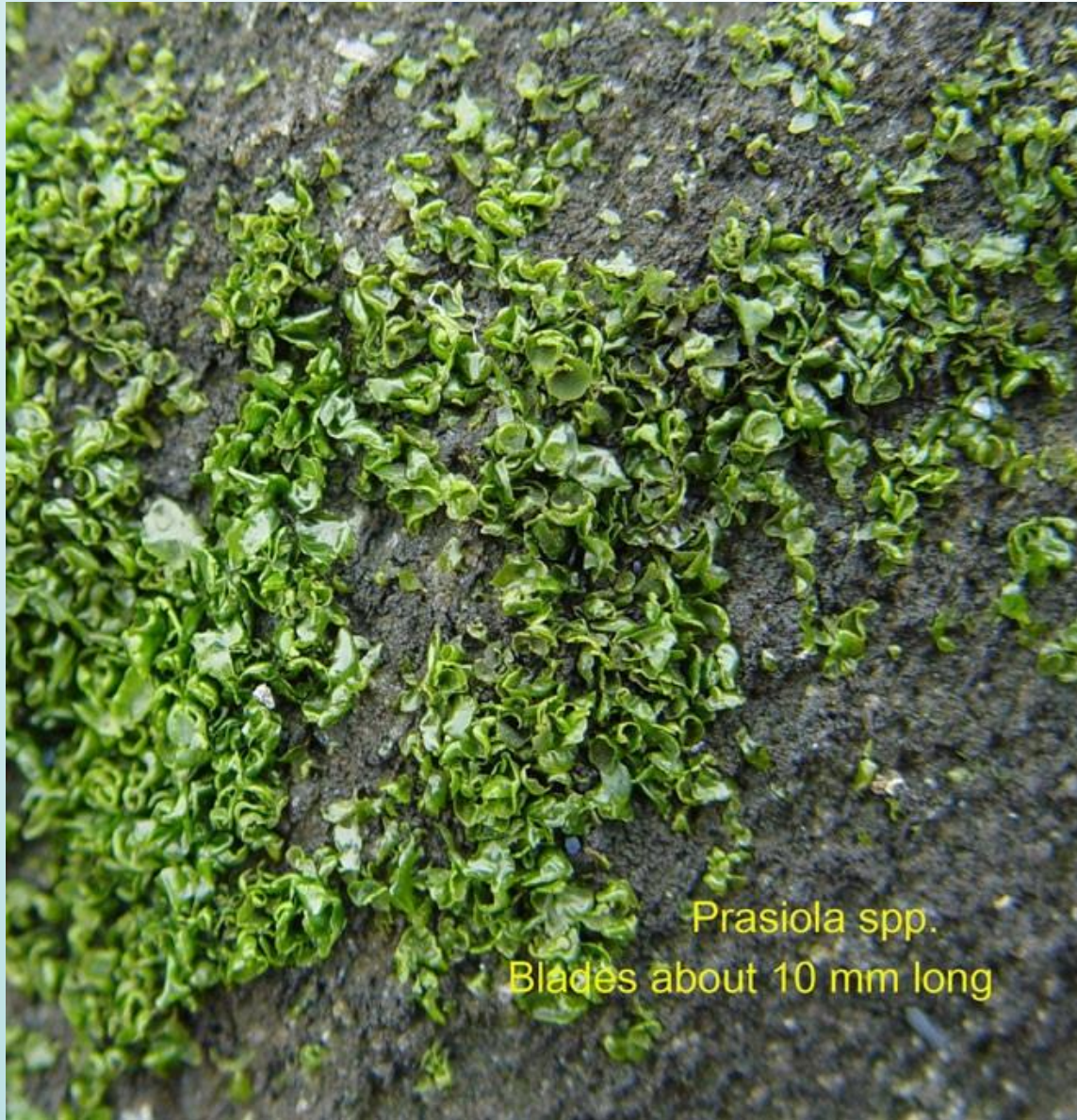


Microthamniales, *Microthamnion*



M. kuetzingianum

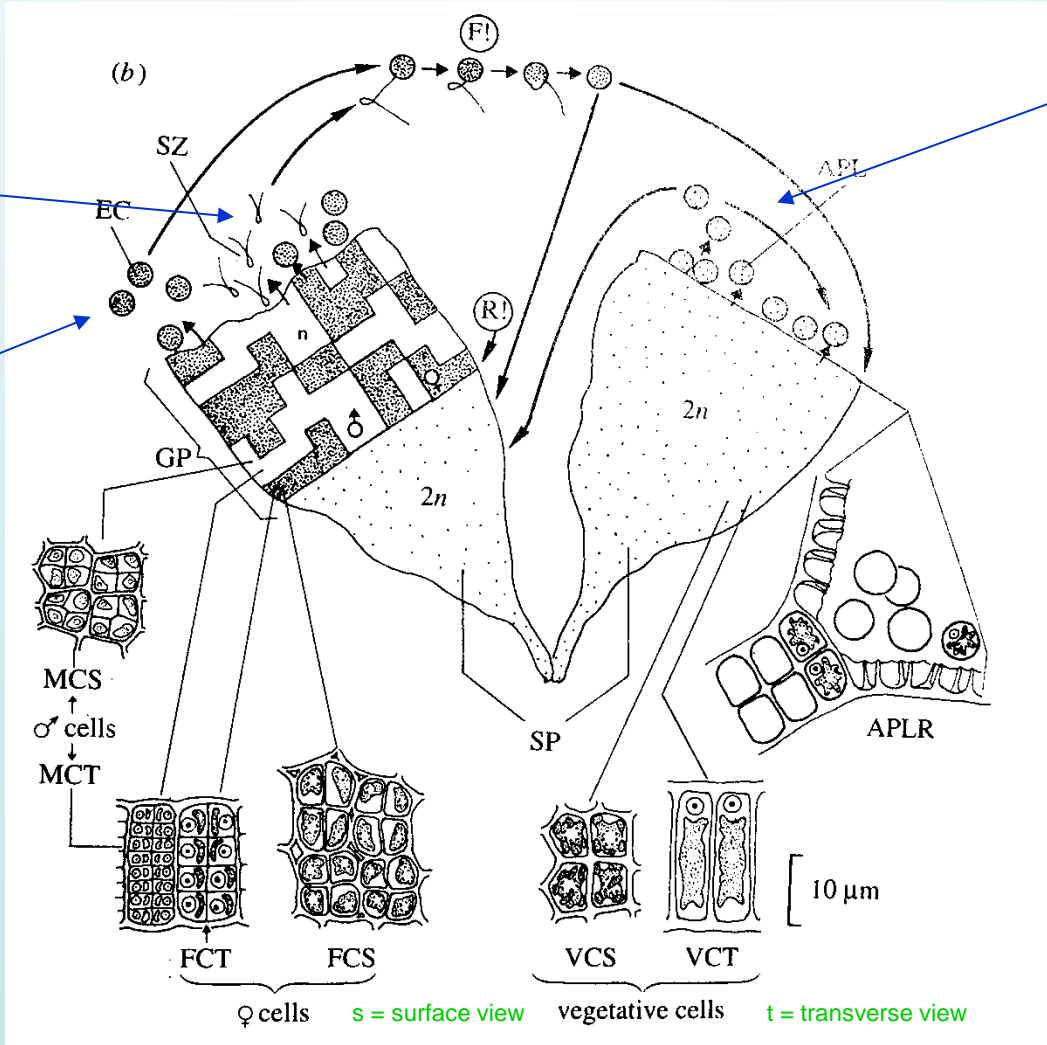
Prasiolales, *Prasiola*



Prasiolales, Prasiola

- mosaic sexual reproduction

aplanospores
(asexual reproduction)



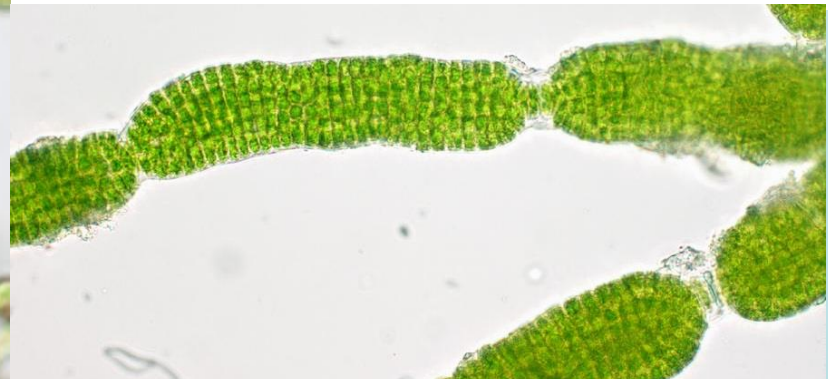
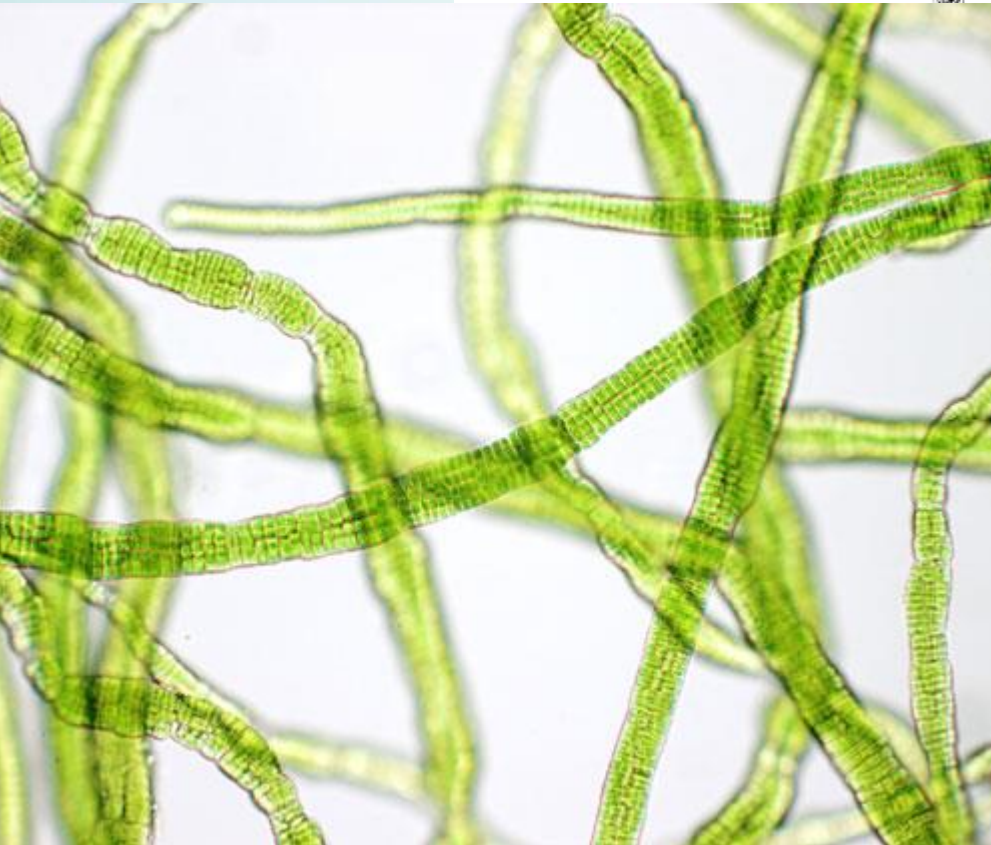
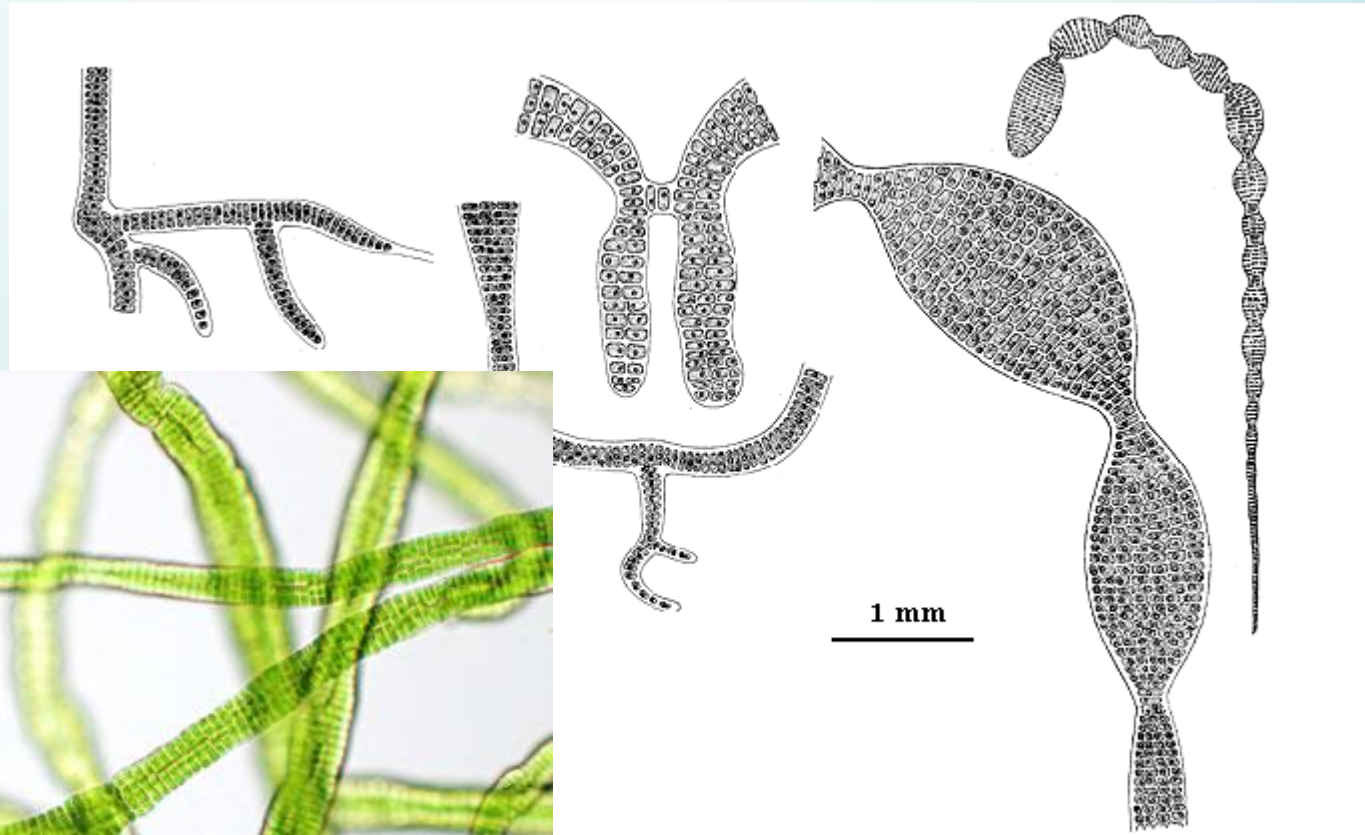
spermatozoids

egg cells

♀ cells s = surface view vegetative cells t = transverse view

Prasiolales, *Rosenvingiella*

haploid thallus,
zygotic meiosis



Prasiolales

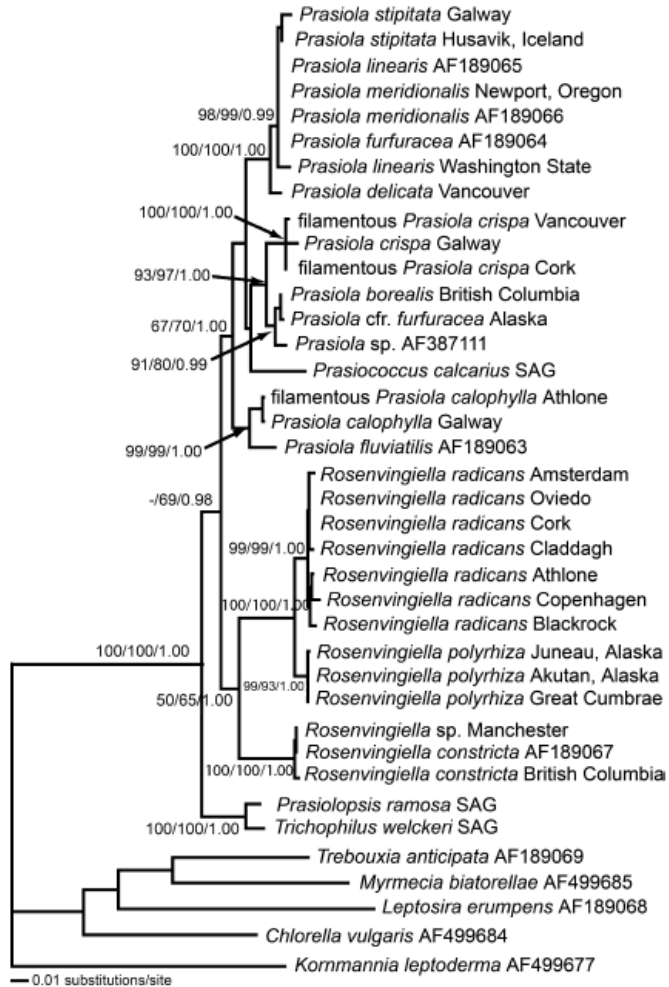


FIG. 1. Phylogenetic tree inferred from maximum-likelihood (ML) analysis of *rbcL* gene sequence data for the Prasiolales and outgroup taxa, with bootstrap support (BP) and posterior probabilities (PP) indicated at the nodes. Left number from maximum parsimony, middle from ML, and right from Bayesian analyses. The BP values lower than 50% and PP values lower than 0.95 are not shown. Branch lengths are drawn proportional to the amount of sequence change.

J. Phycol. 43, 811–822 (2007)
 © 2007 Phycological Society of America
 DOI: 10.1111/j.1529-8817.2007.00372.x

MOLECULAR PHYLOGENY OF THE GREEN ALGAL ORDER PRASIOALES (TREBOUXIOPHYCEAE, CHLOROPHYTA)¹

*Fabio Rindi*²

Department of Biological Sciences, The University of Alabama, PO Box 870345, 425 Scientific Collections Bldg., Tuscaloosa, Alabama 35487-0345, USA

Lynne McIvor

Nationaal Herbarium Nederland, Universiteit Leiden Branch, Einsteinweg 2, 2300 RA, Leiden, the Netherlands

Alison R. Sherwood

Botany Department, 3190 Maile Way, University of Hawaii, Honolulu, Hawaii 96822, USA

Thomas Friedl

Albrecht-von-Haller-Institut fuer Pflanzenwissenschaften, Abteilung Experimentelle Phykologie und Sammlung von Algenkulturen, Universität Göttingen, Untere Karspuele 2, 37073 Göttingen, Germany

Michael D. Guiry

Martin Ryan Institute, National University of Ireland, Galway, Ireland

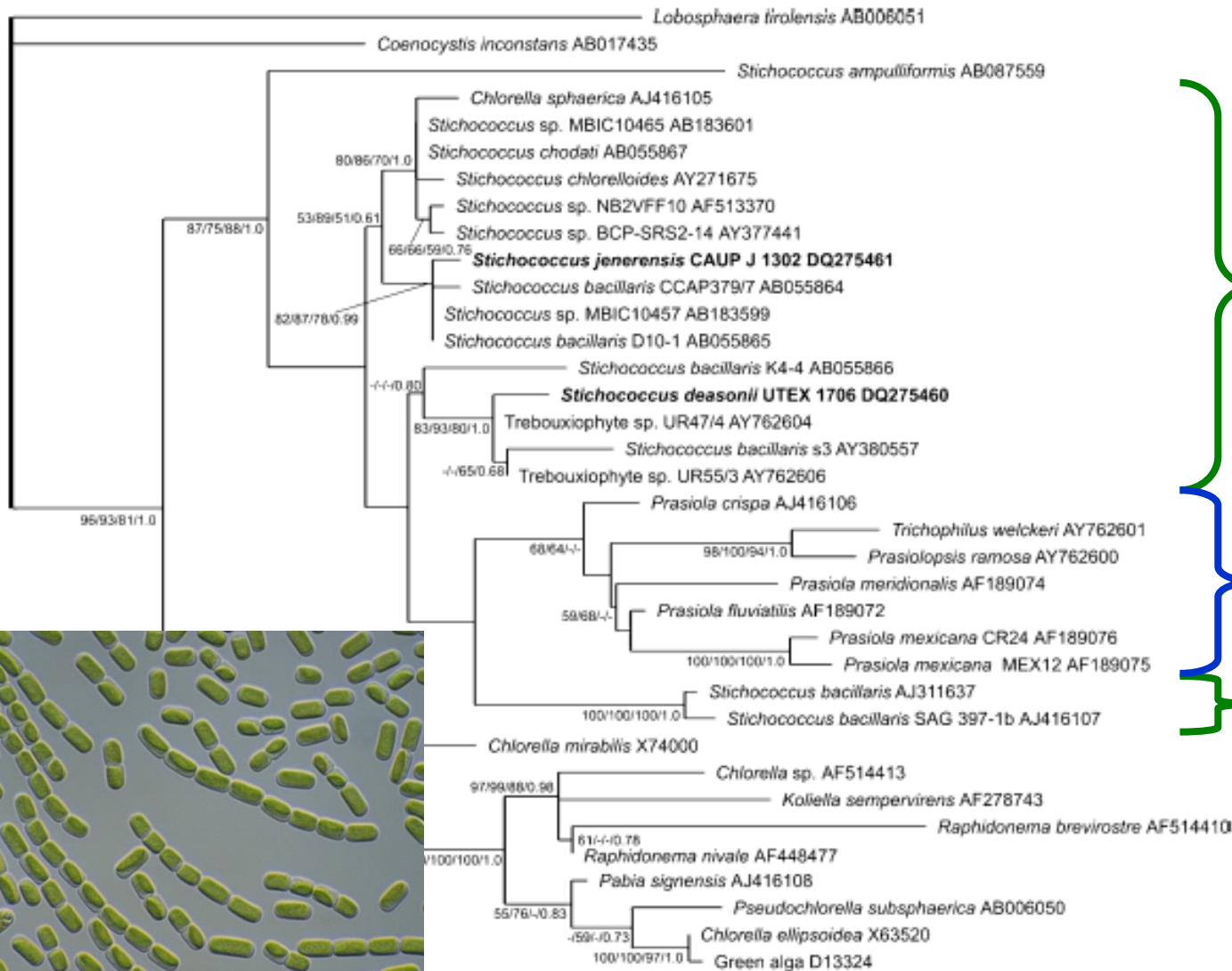
and *Robert G. Sheath*

Department of Biological Sciences, California State University San Marcos, San Marcos, California 92096, USA



Prasiolales, *Stichococcus*

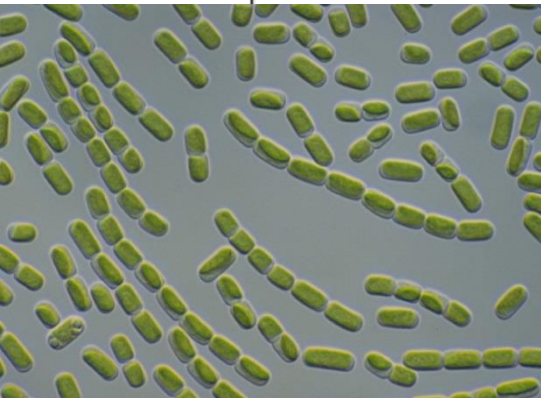
- *Prasiola* – crown group in *Stichococcus* lineage



Stichococcus

Prasiola

Stichococcus



Prasiolales, *Diplosphaera*

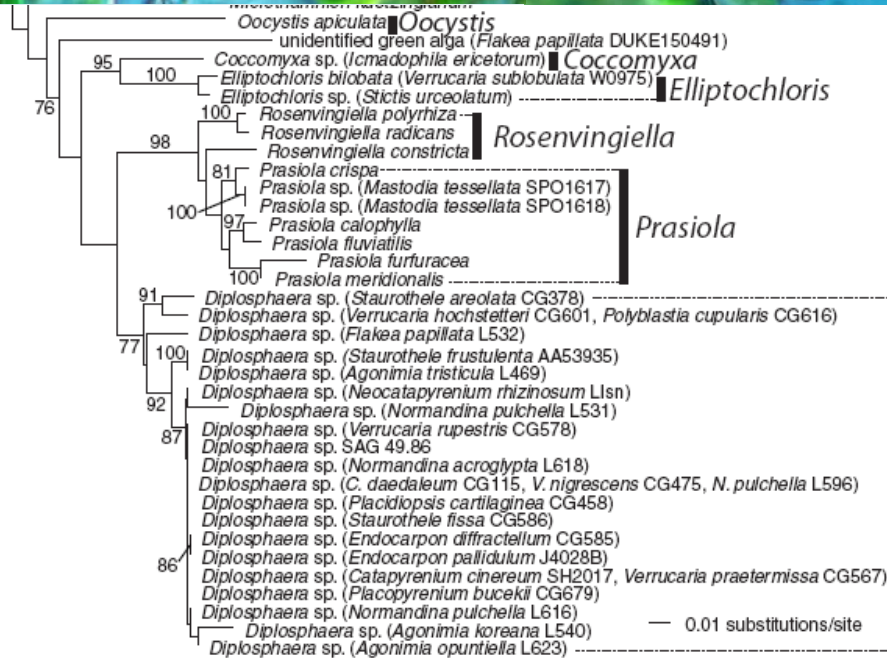
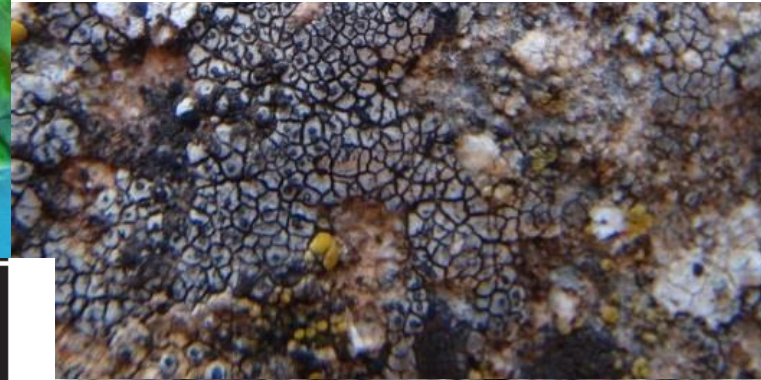
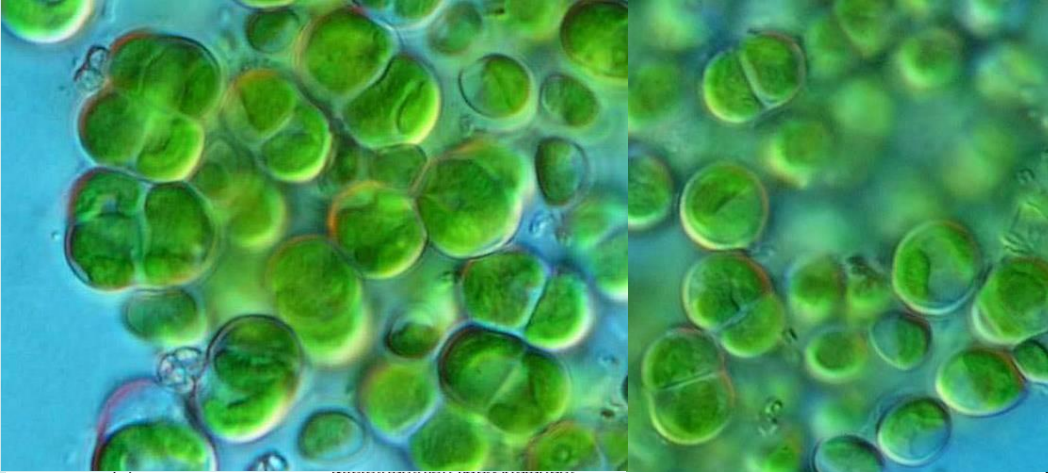
- free living, lichen photobiont

Eur. J. Phycol., (2011), 46(4): 399–415



Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota)

HOLGER THÜS¹, LUCIA MUGGIA², SERGIO PÉREZ-ORTEGA³, SERGIO E. FAVERO-LONGO⁴, SUZANNE JONESON⁵, HEATH O'BRIEN⁶, MATTHEW P. NELSEN^{7,8}, RHINAIXA DUQUE-THÜS¹, MARTIN GRUBE², THOMAS FRIEDL⁹, JULIET BRODIE¹, CARRIE J. ANDREW¹⁰, ROBERT LÜCKING⁷, FRANÇOIS LUTZONI¹¹ AND CÉCILE GUIDAN¹



Diplosphaera

Prasiola-group

Monophyly of Trebouxiophyceae

J. Phycol. **45**, 1206–1212 (2009)

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DOI: 10.1111/j.1529-8817.2009.00731.x

ANALYSIS OF A PLASTID MULTIGENE DATA SET AND THE PHYLOGENETIC POSITION OF THE MARINE MACROALGA *CAULERPA FILIFORMIS* (CHLOROPHYTA)¹

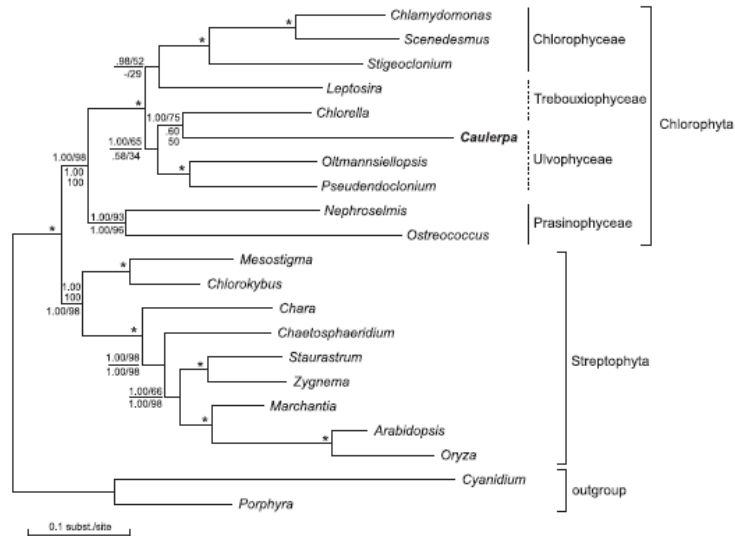
*G. C. Zuccarello*², *Natalie Price*

School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

Heroen Verbruggen and Frederik Leliaert

Phycology Research Group and Center for Molecular Phylogenetics and Evolution, Ghent University, Krijgslaan 281 (S8), B-9000 Gent, Belgium

FIG. 2. Phylogenetic tree resulting from Bayesian inference (BI) of 23 chloroplast genes at the amino acid level, showing the position of *Caulerpa filiformis* among the Ulvophyceae+Trebouxiophyceae+Chlorophyceae (UTC) taxa. Values above branches indicate BI posterior probabilities and maximum-likelihood (ML) bootstrap support from the analyses of protein data under a CpREV+G8 model; values below branches indicate BI posterior probabilities and ML bootstrap support from the analyses of nucleotide data (1st and 2nd codon position) under a GTR+G8 model. The nodes that received full support in BI and ML analyses of both protein and nucleotide data are denoted by asterisks. Solid brackets on the right indicate monophyletic taxa; dashed brackets indicate nonmonophyletic taxa. GTR, general time reversible.



Monophyly of Trebouxiophyceae

The Chloroplast Genomes of the Green Algae *Pedinomonas minor*, *Parachlorella kessleri*, and *Oocystis solitaria* Reveal a Shared Ancestry between the Pedinomonadales and Chlorellales

Monique Turmel, Christian Otis, and Claude Lemieux

Département de Biochimie et de Microbiologie, Université Laval, Québec (Québec) Canada

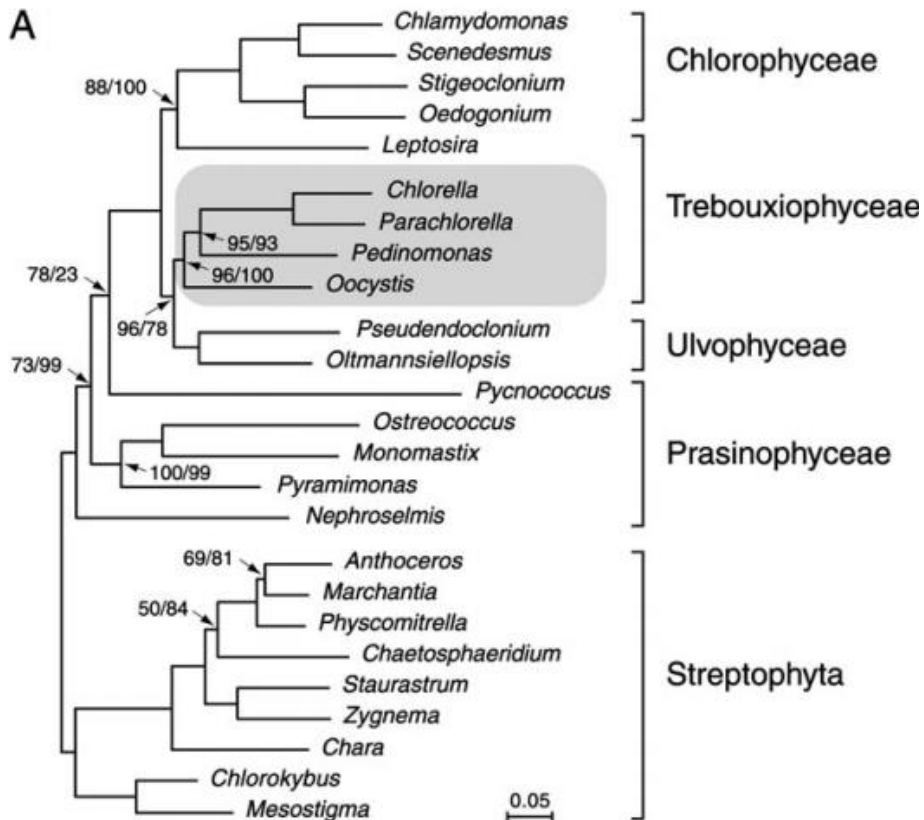
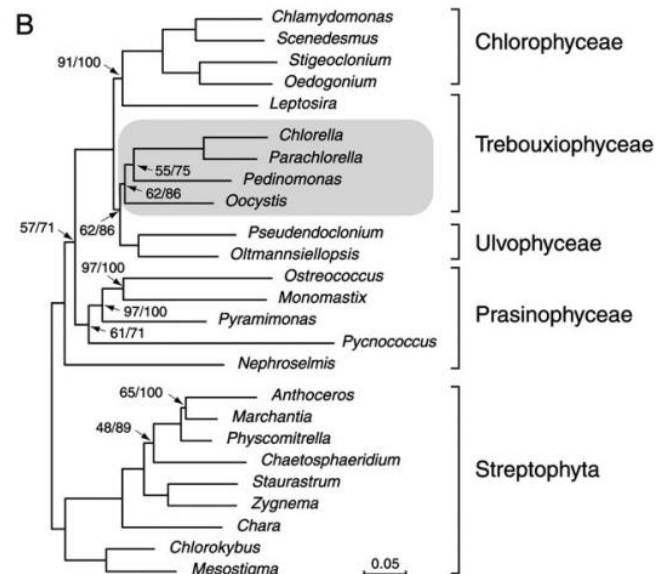


FIG. 4.—Phylogenies inferred from 71 concatenated chloroplast protein-coding genes (first two codon positions) and their deduced amino acid sequences. (A) Best ML tree inferred from the amino acid data set. (B) Best ML tree inferred from the nucleotide data set. Support values indicate ML bootstrap values/Bayesian posterior probabilities. When both values were 100%, they are not shown. The clade containing *Chlorella* and the three taxa newly investigated in the present study is highlighted in gray.



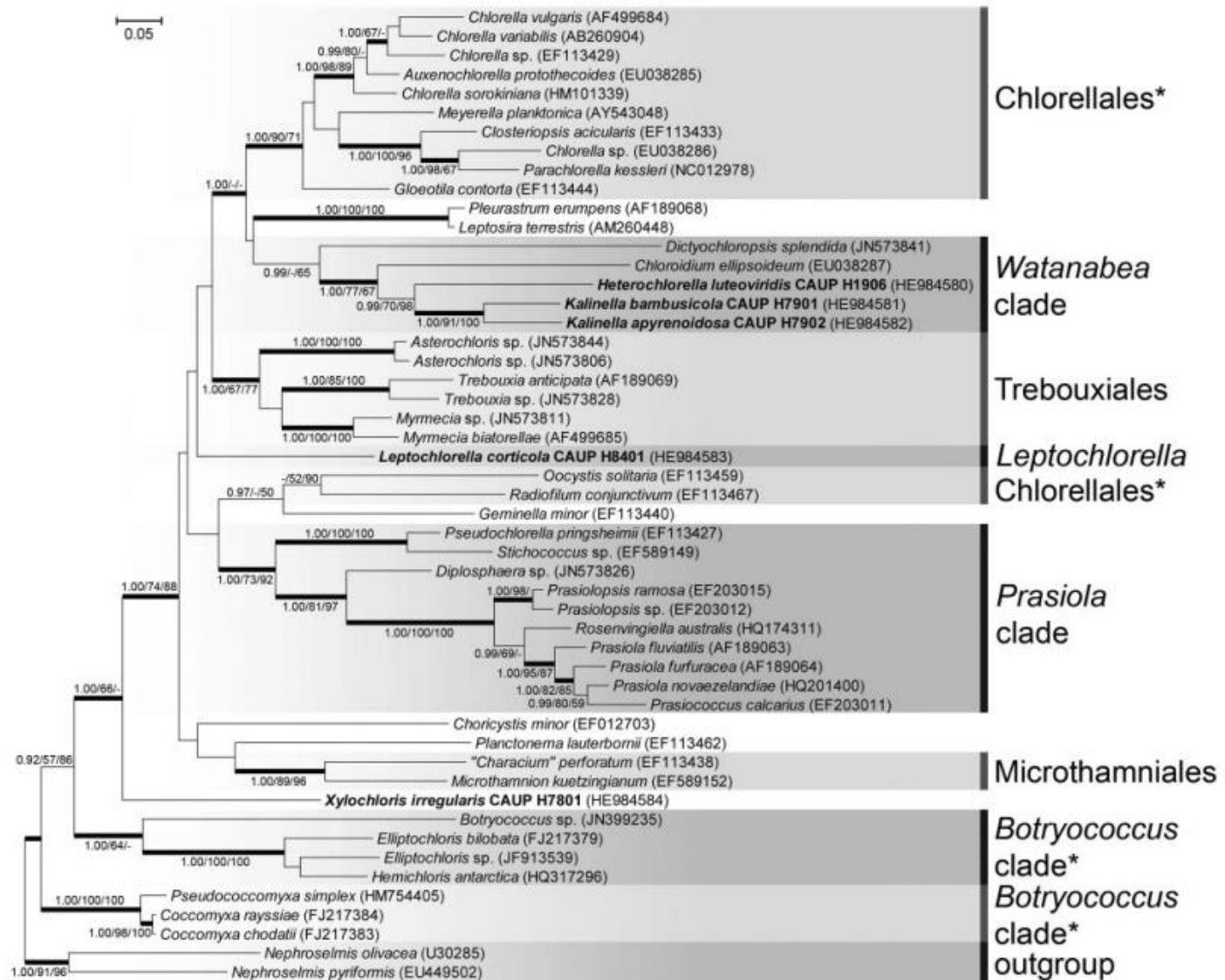


Fig. 4. Phylogenetic position of the investigated microalgae within the class Trebouxiophyceae (Chlorophyta) based on *rbcL* sequences. The tree was inferred using MrBayes. Three partitions were analysed separately using the GTR + G + I model for the 1st and 3rd codon positions, and the IC + G + I model for the 2nd codon position. The tree was arbitrarily rooted with strains of

Monophyly of Trebouxiophyceae



New phylogenetic hypotheses for the core Chlorophyta based on chloroplast sequence data

Karolina Fučíková¹, Frederik Leliaert^{2,3}, Endymion D. Cooper⁴, Pavel Škaloud⁵, Sofie D'Hondt², Olivier De Clerck², Carlos F. D. Gurgel⁶, Louise A. Lewis¹, Paul O. Lewis¹, Juan M. Lopez-Bautista³, Charles F. Delwiche⁴ and Heroen Verbruggen^{7*}

¹ Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA

² Phycology Research Group, Biology Department, Ghent University, Ghent, Belgium

³ Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA

⁴ Department of Cell Biology and Molecular Genetics and the Maryland Agricultural Experiment Station, University of Maryland, College Park, MD, USA

⁵ Department of Botany, Faculty of Science, Charles University in Prague, Prague, Czech Republic

⁶ School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA, Australia

⁷ School of Botany, University of Melbourne, Melbourne, VIC, Australia

Edited by:

Debashish Bhattacharya, Rutgers,
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USA

Reviewed by:

Jinling Huang, East Carolina
University, USA

Cheong Xin Chan, The University of
Queensland, Australia

***Correspondence:**

Heroen Verbruggen, School of
Botany, University of Melbourne,
Melbourne, 3010 VIC, Australia
e-mail: heroen.verbruggen@
unimelb.edu.au

Phylogenetic relationships in the green algal phylum Chlorophyta have long been subject to debate, especially at higher taxonomic ranks (order, class). The relationships among three traditionally defined and well-studied classes, Chlorophyceae, Trebouxiophyceae, and Ulvophyceae are of particular interest, as these groups are species-rich and ecologically important worldwide. Different phylogenetic hypotheses have been proposed over the past two decades and the monophyly of the individual classes has been disputed on occasion. Our study seeks to test these hypotheses by combining high throughput sequencing data from the chloroplast genome with increased taxon sampling. Our results suggest that while many of the deep relationships are still problematic to resolve, the classes Trebouxiophyceae and Ulvophyceae are likely not monophyletic as currently defined. Our results also support relationships among several trebouxiophycean taxa that were previously unresolved. Finally, we propose that the common term for the grouping of the three classes, "UTC clade," be replaced with the term "core Chlorophyta" for the well-supported clade containing Chlorophyceae, taxa belonging to Ulvophyceae and Trebouxiophyceae, and the classes Chlorodendrophyceae and Pedinophyceae.

Keywords: chloroplast DNA, Chlorophyta, fast site removal, green algae, multi-gene phylogeny, molecular systematic, phylogenomics, Viridiplantae

Monophyly of Trebouxiophyceae

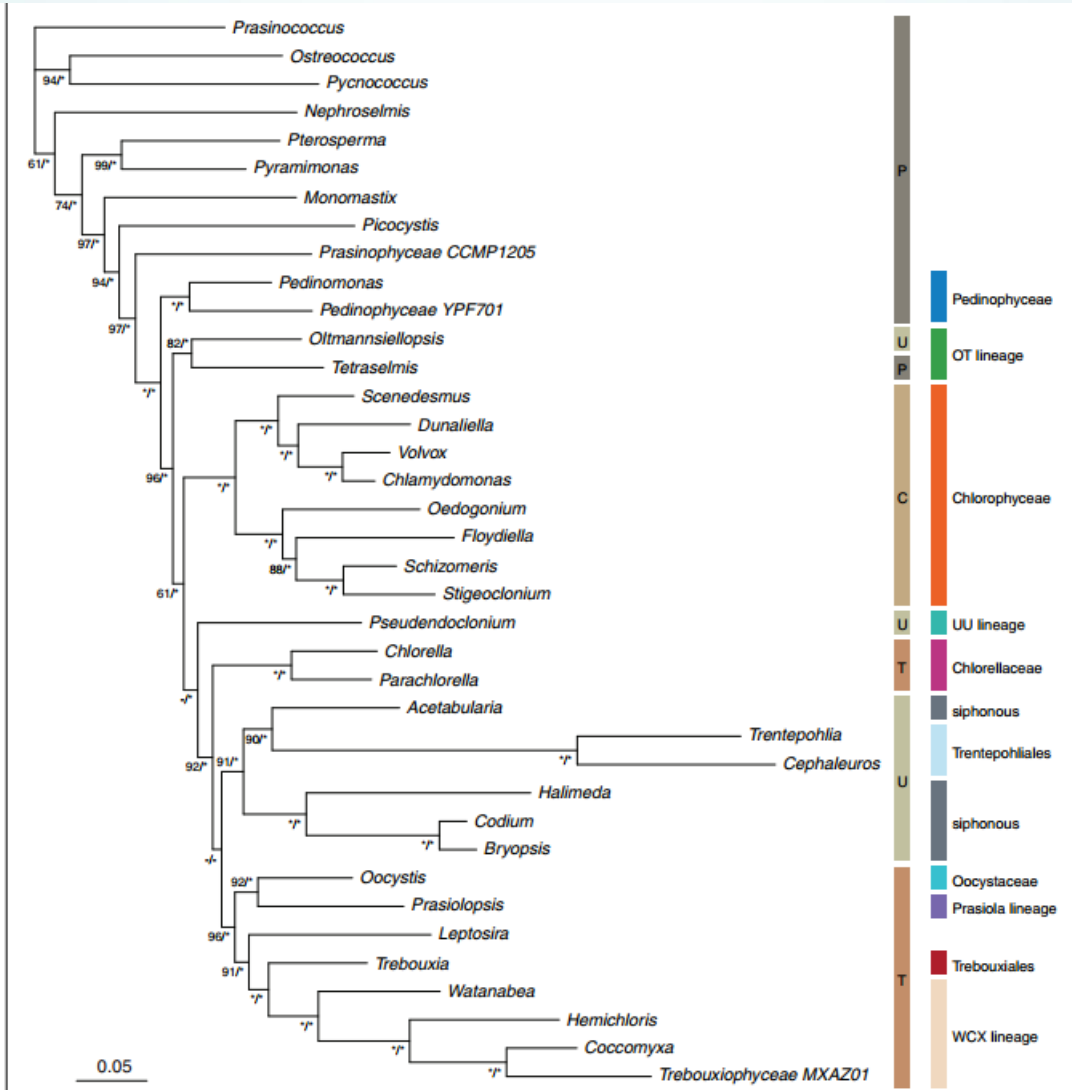


FIGURE 3 | Maximum likelihood phylogeny inferred from the genome-scale alignment (80% slowest sites). Bootstrap percentages and Bayesian posterior probabilities (MrBayes) are indicated along branches. The classical classification into Prasinophyceae (P), Chlorophyceae (C),

Ulvoophyceae (U) and Trebouxiophyceae (T) as well as the lineage-based classification discussed in the present paper are indicated to the right of the phylogeny. Values under 60 BS and 0.90 BPP are indicated as -, values of 100 BS and 1.00 BPP as *.

Monophyly of Trebouxiophyceae

Lemieux et al. *BMC Evolutionary Biology* 2014, **14**:211
<http://www.biomedcentral.com/1471-2148/14/211>



RESEARCH ARTICLE

Open Access

Chloroplast phylogenomic analysis resolves deep-level relationships within the green algal class Trebouxiophyceae

Claude Lemieux*, Christian Otis and Monique Turmel

Abstract

Background: The green algae represent one of the most successful groups of photosynthetic eukaryotes, but compared to their land plant relatives, surprisingly little is known about their evolutionary history. This is in great part due to the difficulty of recognizing species diversity behind morphologically similar organisms. The Trebouxiophyceae is a species-rich class of the Chlorophyta that includes symbionts (e.g. lichenized algae) as well as free-living green algae. Members of this group display remarkable ecological variation, occurring in aquatic, terrestrial and aeroterrestrial environments. Because a reliable backbone phylogeny is essential to understand the evolutionary history of the Trebouxiophyceae, we sought to identify the relationships among the major trebouxiophycean lineages that have been previously recognized in nuclear-encoded 18S rRNA phylogenies. To this end, we used a chloroplast phylogenomic approach.

Results: We determined the sequences of 29 chlorophyte chloroplast genomes and assembled amino acid and nucleotide data sets derived from 79 chloroplast genes of 61 chlorophytes, including 35 trebouxiophyceans. The amino acid- and nucleotide-based phylogenies inferred using maximum likelihood and Bayesian methods and various models of sequence evolution revealed essentially the same relationships for the trebouxiophyceans. Two major groups were identified: a strongly supported clade of 29 taxa (core trebouxiophyceans) that is sister to the Chlorophyceae + Ulvophyceae and a clade comprising the Chlorellales and Pedinophyceae that represents a basal divergence relative to the former group. The core trebouxiophyceans form a grade of strongly supported clades that include a novel lineage represented by the desert crust alga *Pleurostrosarcina brevispinosa*. The assemblage composed of the *Oocystis* and *Geminella* clades is the deepest divergence of the core trebouxiophyceans. Like most of the chlorellaleans, early-diverging core trebouxiophyceans are predominantly planktonic species, whereas core trebouxiophyceans occupying more derived lineages are mostly terrestrial or aeroterrestrial algae.

Conclusions: Our phylogenomic study provides a solid foundation for addressing fundamental questions related to the biology and ecology of the Trebouxiophyceae. The inferred trees reveal that this class is not monophyletic; they offer new insights not only into the internal structure of the class but also into the lifestyle of its founding members and subsequent adaptations to changing environments.

Keywords: Chlorophyta, Trebouxiophyceae, Plastid genome, Phylogenomics

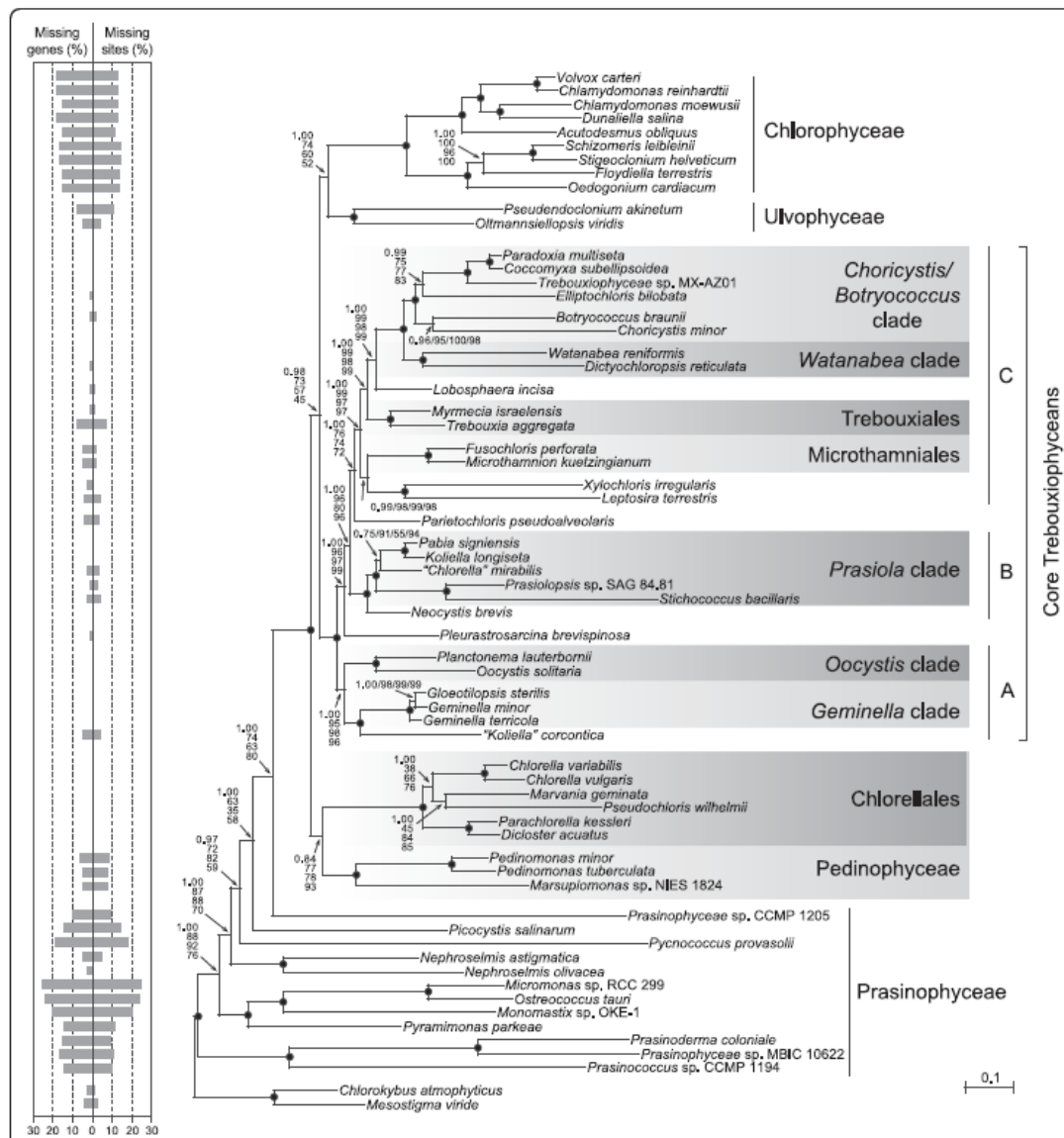


Figure 1 Phylogeny of 61 chlorophytes inferred using a data set of 15,549 positions assembled from 79 cpDNA-encoded proteins. The tree presented here is the best-scoring ML tree inferred under the GTR + Γ 4 model. Support values are reported on the nodes: from top to bottom, or from left to right, are shown the posterior probability (PP) values for the PhyloBayes CATGTR + Γ 4 analyses and the bootstrap support (BS) values for the RAxML GTR + Γ 4, LG4X and gcpREV + Γ 4 analyses. Black dots indicate that the corresponding branches received BS and PP values of 100% in all four analyses. Shaded areas identify the clades that are well supported in 18S rDNA phylogenies. The histograms on the left indicate the proportion of missing data for each taxon. The scale bar denotes the estimated number of amino acid substitutions per site.

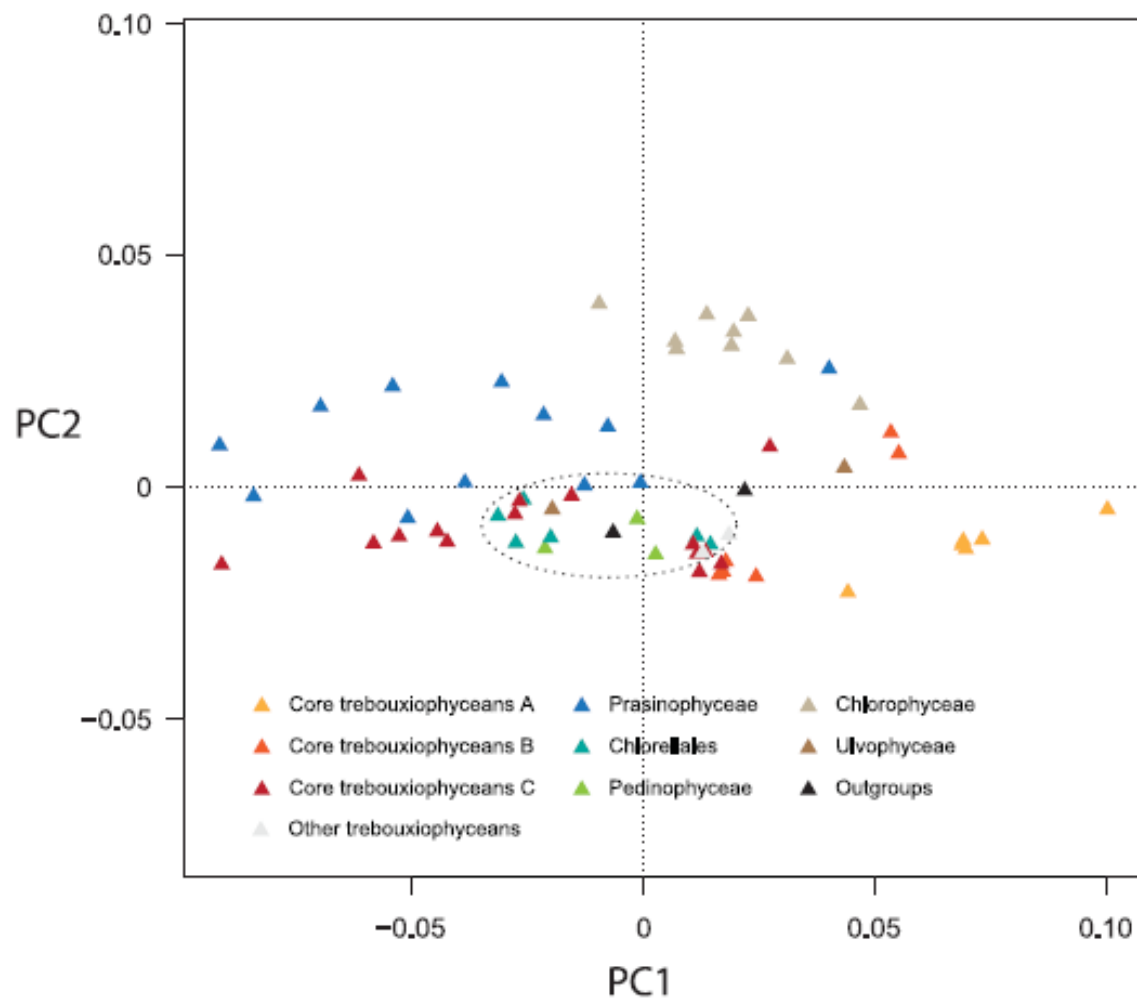


Figure 2 Correspondence analysis of amino acid usage in the data set of 15,549 positions. The members of the Chlorellales and Pedinophyceae are found within the circled area.

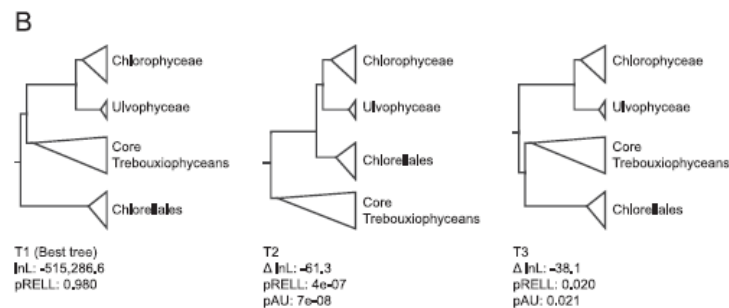
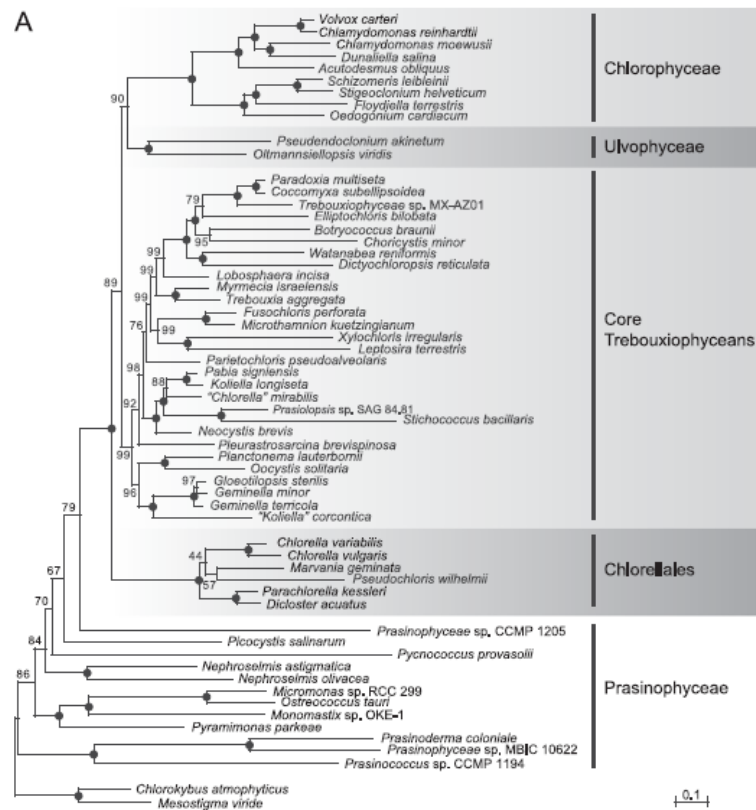


Figure 3 Influence of the Pedinophyceae on the placement of the Chlorellales. (A) Phylogeny of chlorophytes inferred under the GTR+ Γ 4 model using the amino acid data set of 15,549 positions after exclusion of the Pedinophyceae. The best-scoring RAxML tree is presented and support values are reported on the nodes, with black dots indicating 100% BS values. The scale bar denotes the estimated number of amino acid substitutions per site. (B) Confidence assessment of the three possible topologies (T1, T2 and T3) for the placement of the Chlorellales under the GTR+ Γ 4 model. The Δ InL value indicates the difference in log likelihood relative to the best tree (T1). Local bootstrap probabilities were estimated by resampling of the estimated log-likelihood (RELL). pAU, *P* value for the approximately unbiased (AU) test [60] as implemented in CONSEL 0.20 [61].