

Evolving out of the water:

**Uncovering the hidden diversity
in aerophytic green algae**



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M. Eliáš¹, F. Leliaert³, T. Kalina¹, J. Neustupa¹*

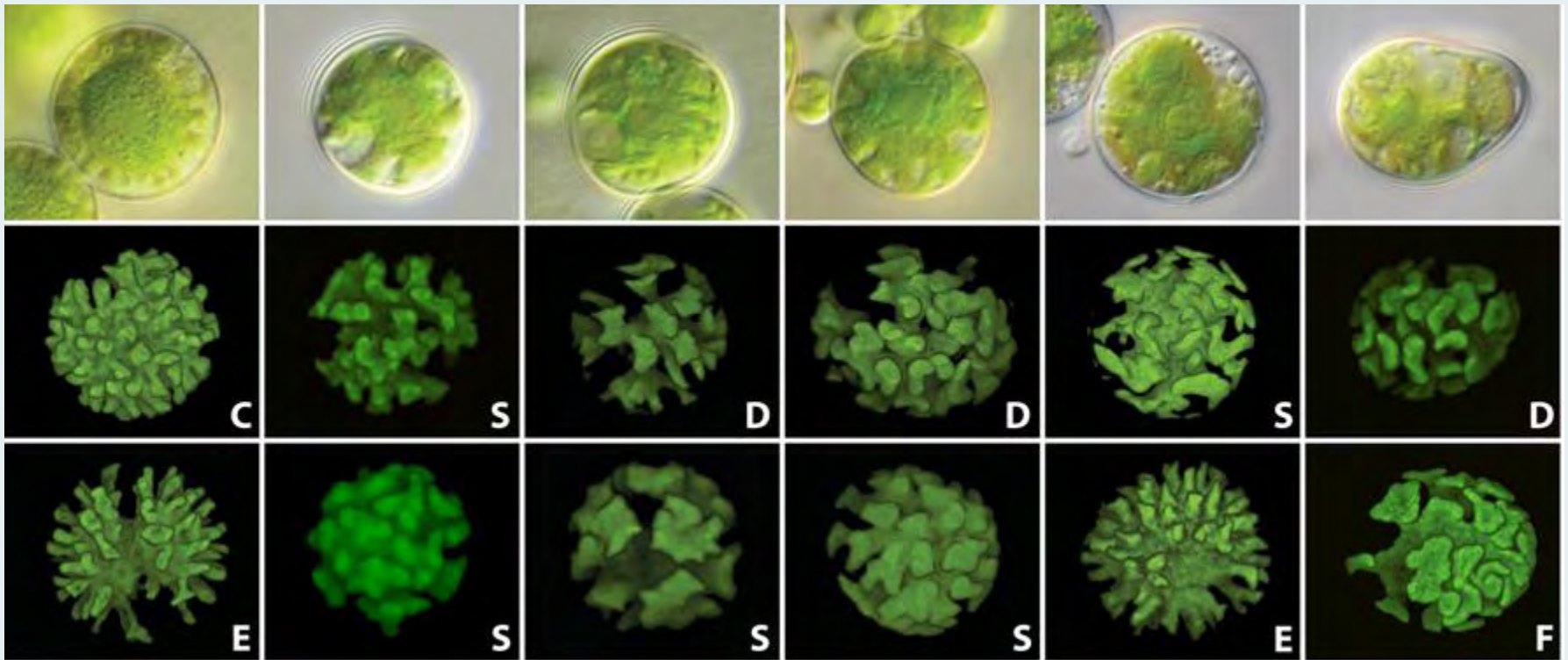
¹ Charles University in Prague, Czech Republic

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³ Ghent University, Belgium

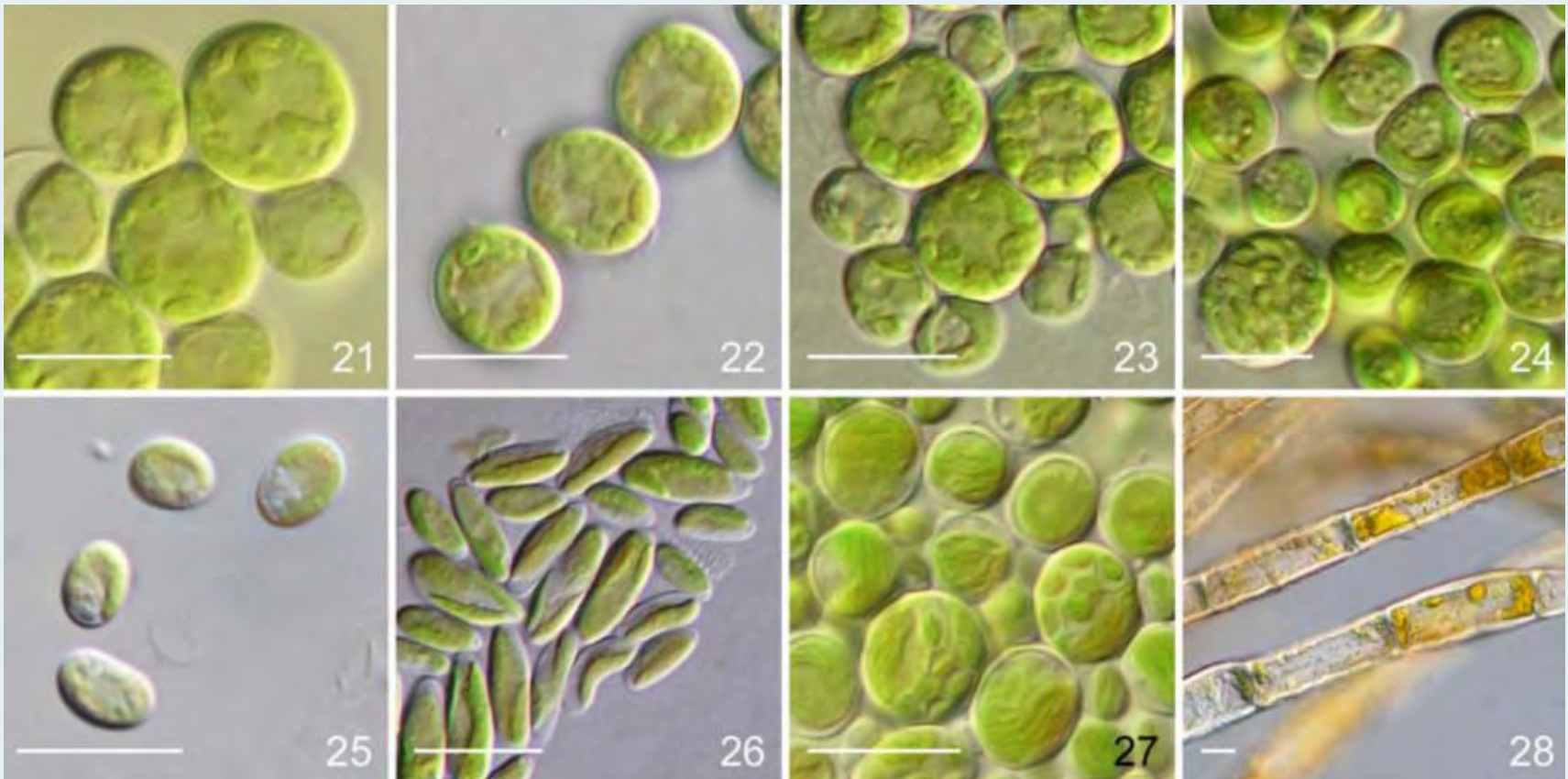
The hidden diversity in aerophytic green algae

- At the level of higher taxa (cryptic genera, new lineages)
- At the level of species



Aerophytic green algae

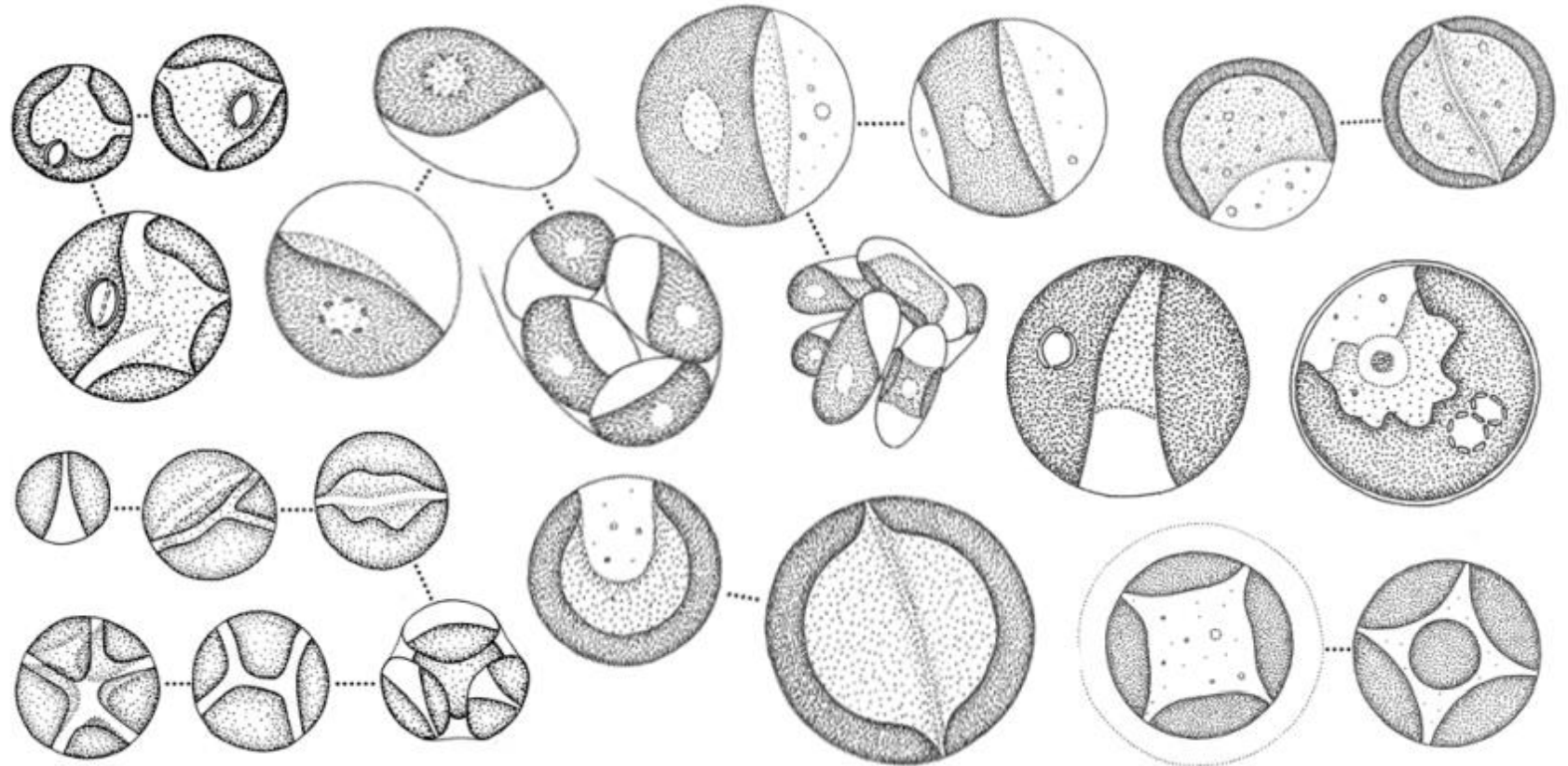
- One of the most important groups of aero-terrestrial microbial phototrophic communities, both in terms of a supposed species richness and biomass
- Very important in the colonisation of pioneer biotopes



Neustupa, J. & Škaloud P. (2008): Diversity of subaerial algae and cyanobacteria on tree bark in tropical mountain habitats. *Biologia* **63**: 806-812.

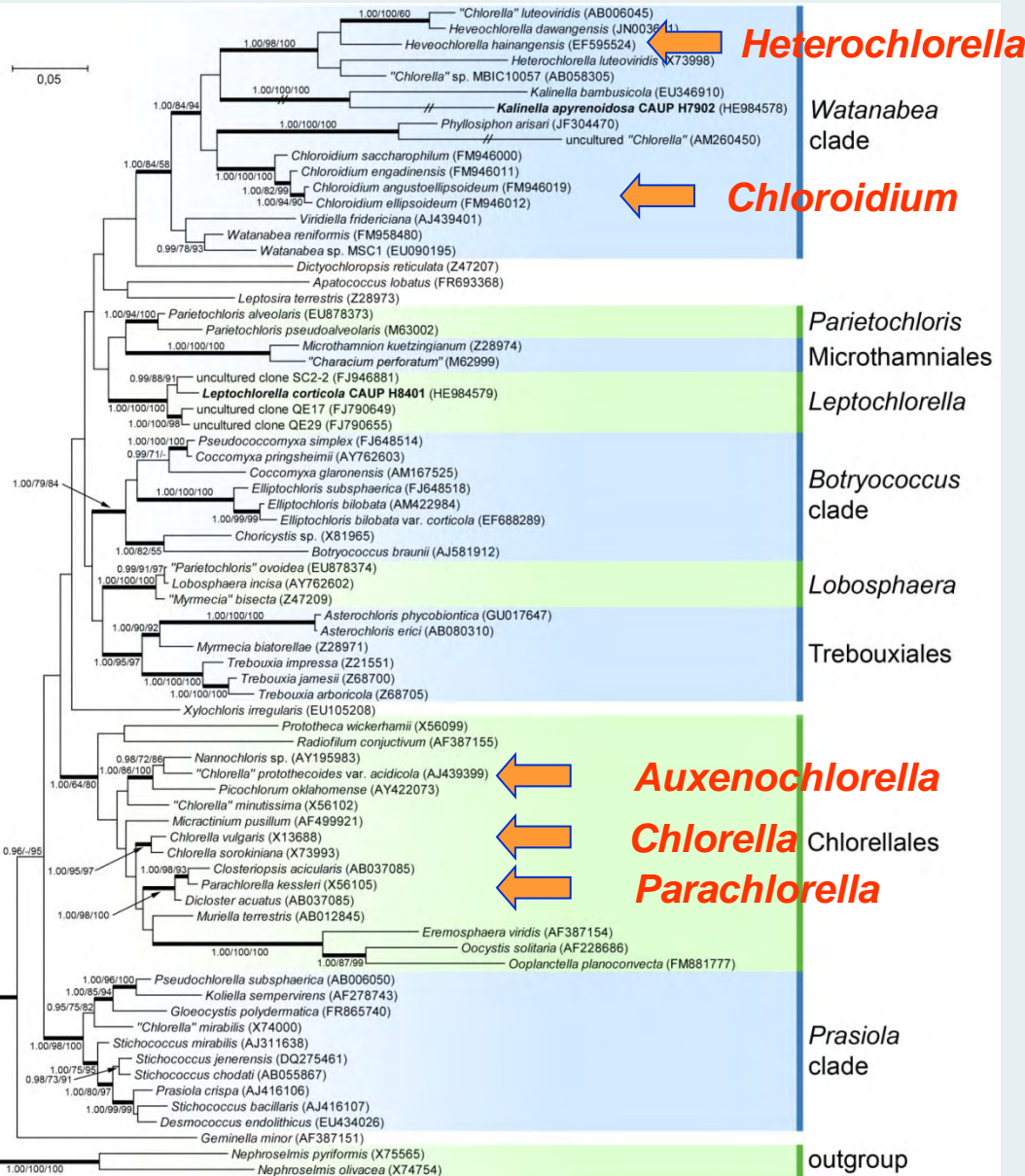
Chlorella-like green algae

- Coccoid unicellular green algae with globular to oval cells that reproduce entirely by autospores
- Probably the most abundant and diversified group of aerophytic algae



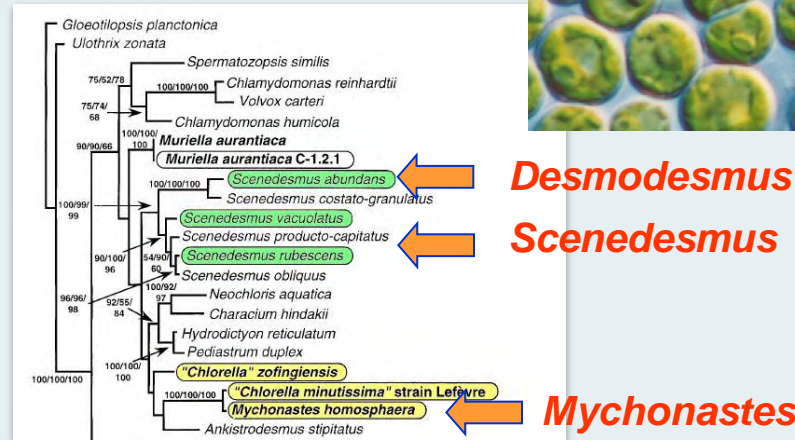
Chlorella-like green algae

Trebouxiophyceae:



- In subaerial conditions, the drought stress drives the selection towards the globular forms with low surface-to-volume ratio
- Phylogenetic differentiation of *Chlorella*-like coccoid green algae considerably exceeds their morphological diversity.

Chlorophyceae:

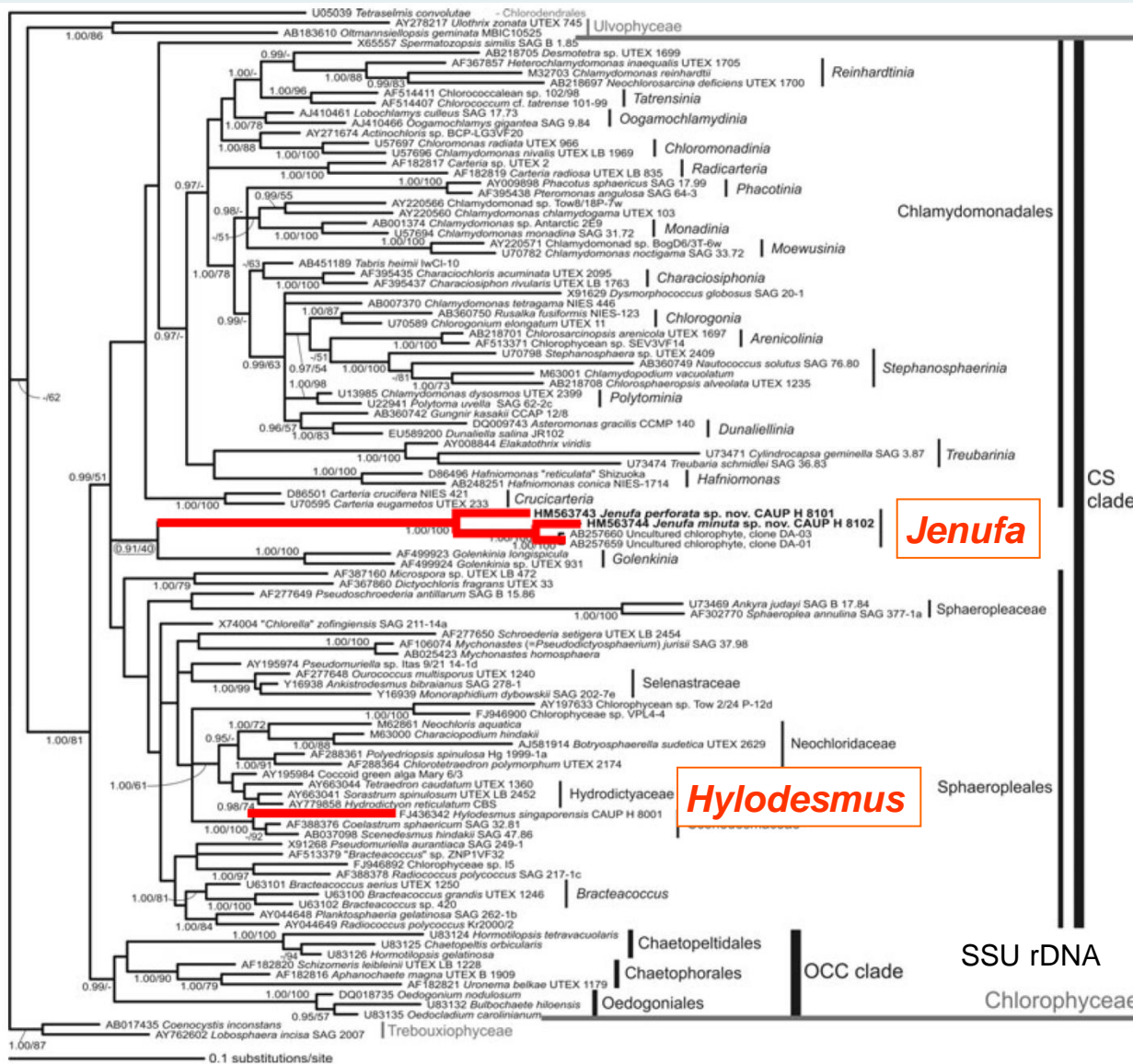


Aims I.

- To investigate the diversity of aerophytic, *Chlorella*-like green algae in (sub-)tropical environments
- To characterize novel lineages
- To assess the biogeographic patterns of aerophytic algae



New lineages of *Chlorella*-like green algae



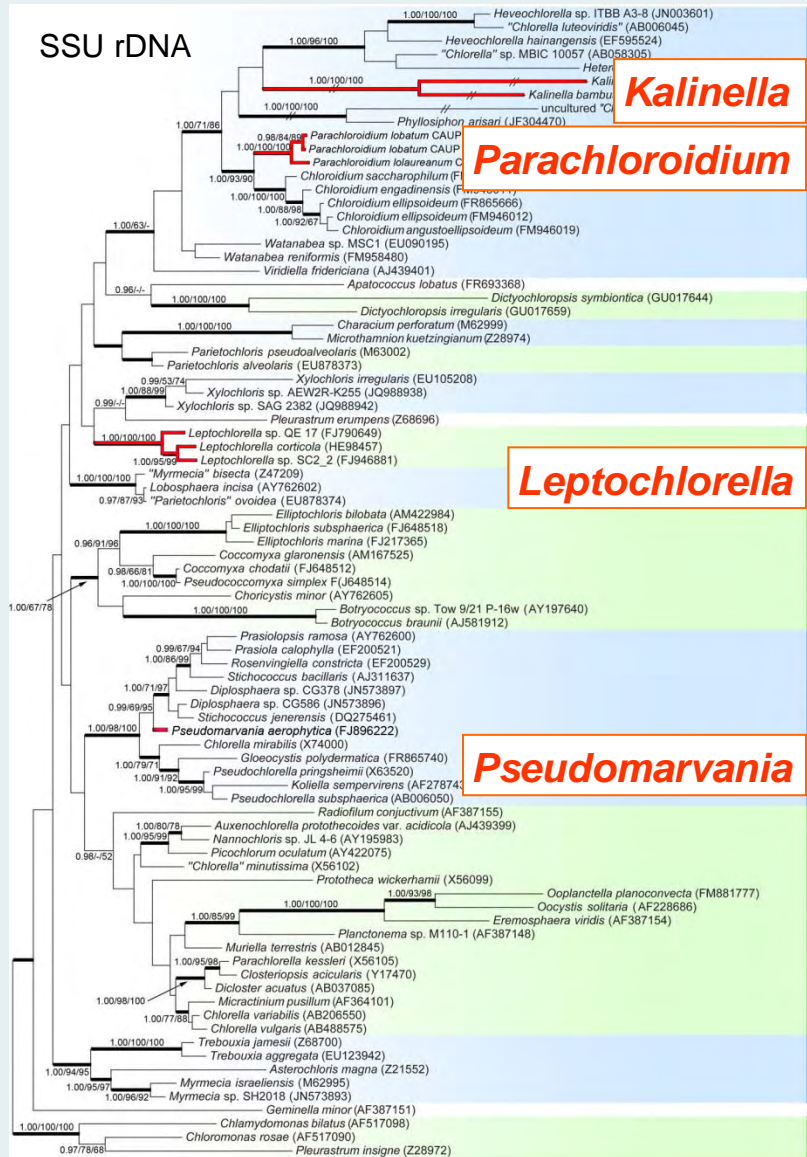
Chlorophyceae:



Němcová, Y., Eliáš, M., Škaloud, P., Hodač, L. & Neustupa, J. (2011): *Jenufa*, gen. nov.: a new genus of coccoid green algae (Chlorophyceae, incertae sedis) previously recorded by environmental sequencing. *J. Phycol.* **47**: 928-938.

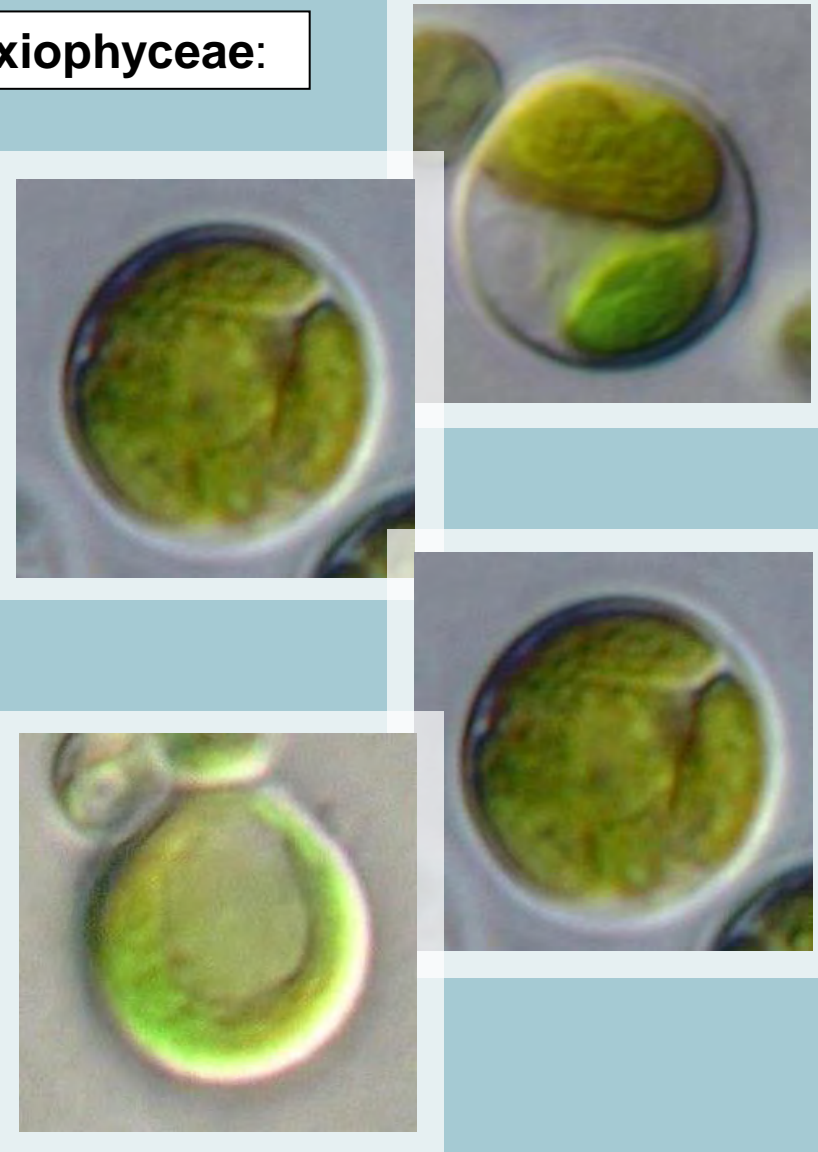
New lineages of *Chlorella*-like green algae

SSU rDNA



Trebouxiophyceae:

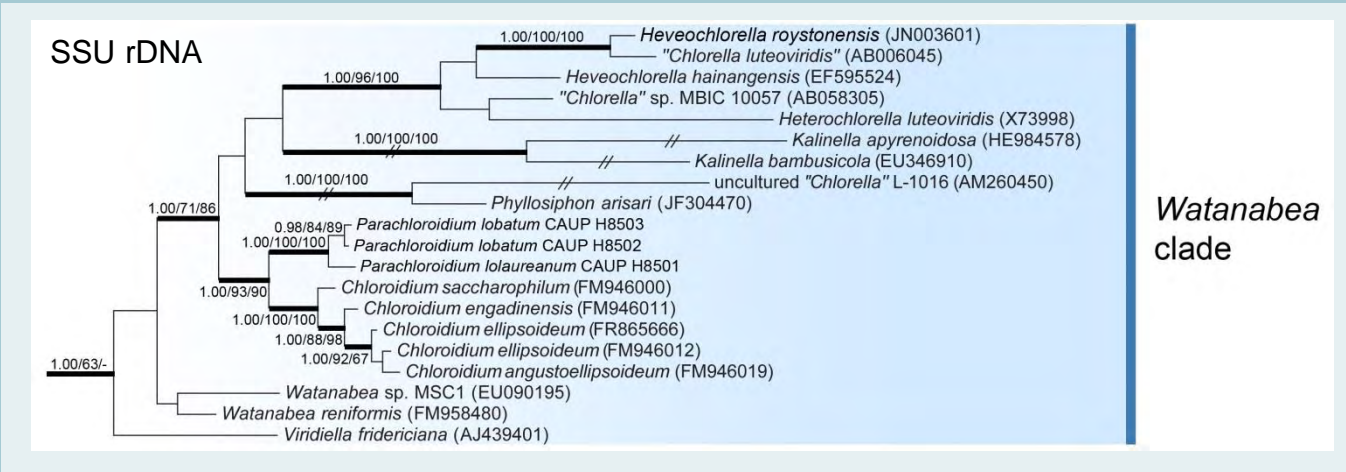
- Watanabea clade
- Dictyochloropsis
- Microthamniales
- Parietochloris
- Xylochloris
- Leptochlorella
- Lobosphaera
- Botryococcus clade
- Prasiola clade
- Chlorellales
- Trebouxiales
- outgroup



Neustupa, J., Němcová, Y., Veselá, J., Steinová, J. & Škaloud, P. (2013): *Parachloroidium* gen. nov. (Trebouxiophyceae, Chlorophyta), a novel genus of coccoid green algae from subaerial corticolous biofilms. *Phycologia* (in press).

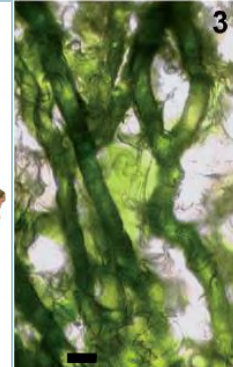
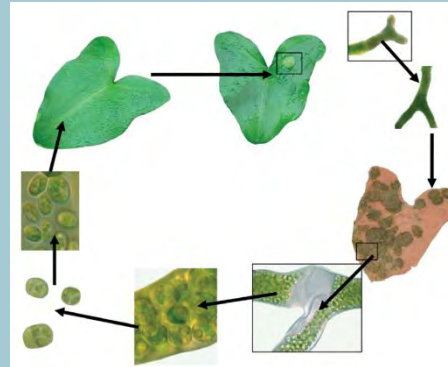
Trebouxiophyceae, the *Watanabea*-lineage

- A diverse, yet very little known lineage of aerophytic algae
- Enhanced genetic and morphological variability
- A unique amino acid insertion (**Lys**) in the *rbcl* gene sequence of *Chloroidium* and *Parachloroidium* species



rbcl

EDG	-	CY	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G	
EDG	-	CY	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G	
EDG	-	CY	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G	
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G
EDG	K	O	F	I	A	V	V	A	P	L	D	L	F	E	R	G	S	V	I	N	L	F	S	I	V	G

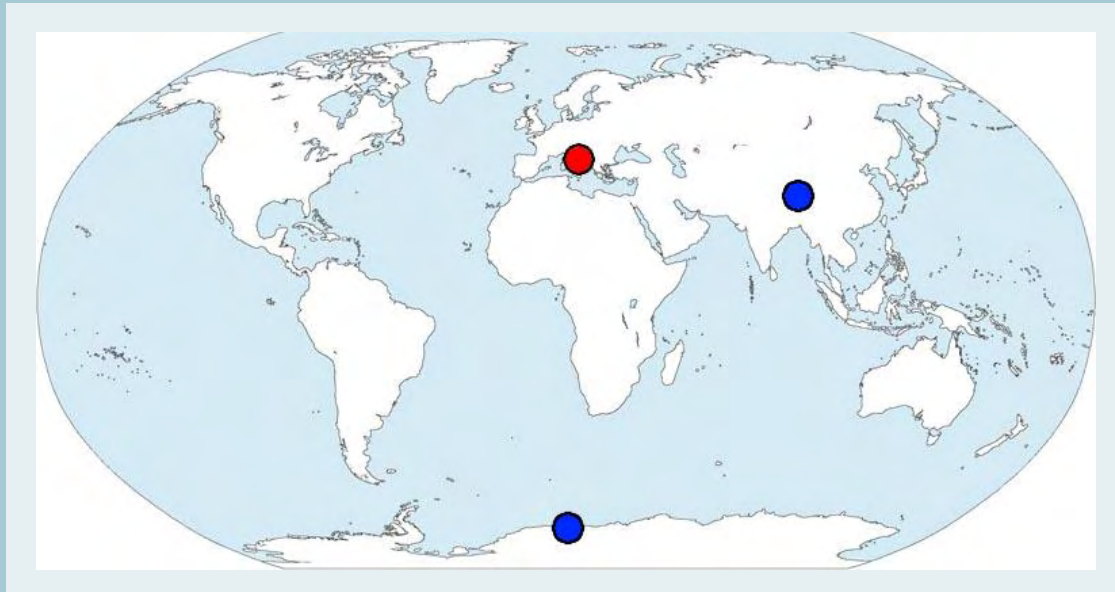


Phyllosiphon
(Aboal &
Werner 2011)

Neustupa, J., Němcová, Y., Veselá, J., Steinová, J. & Škaloud, P. (2013): *Parachloroidium* gen. nov. (Trebouxiophyceae, Chlorophyta), a novel genus of coccoid green algae from subaerial corticolous biofilms. *Phycologia* (in press).

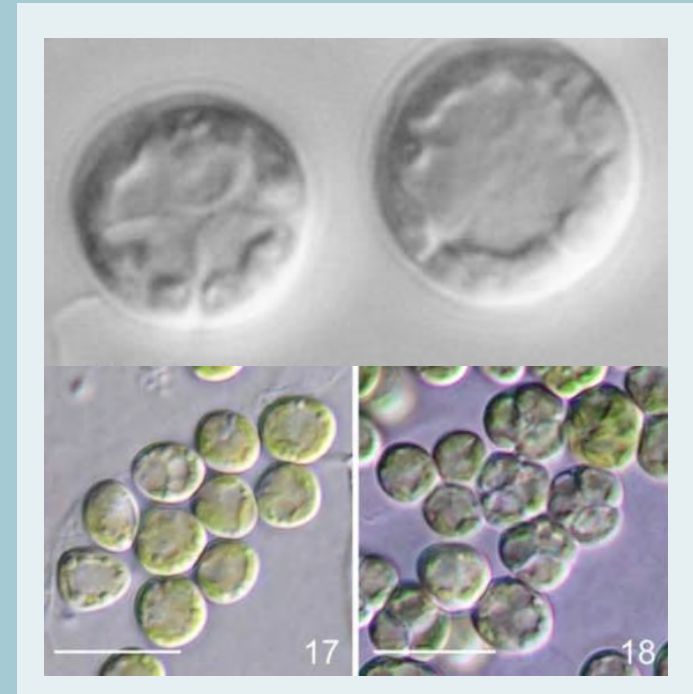
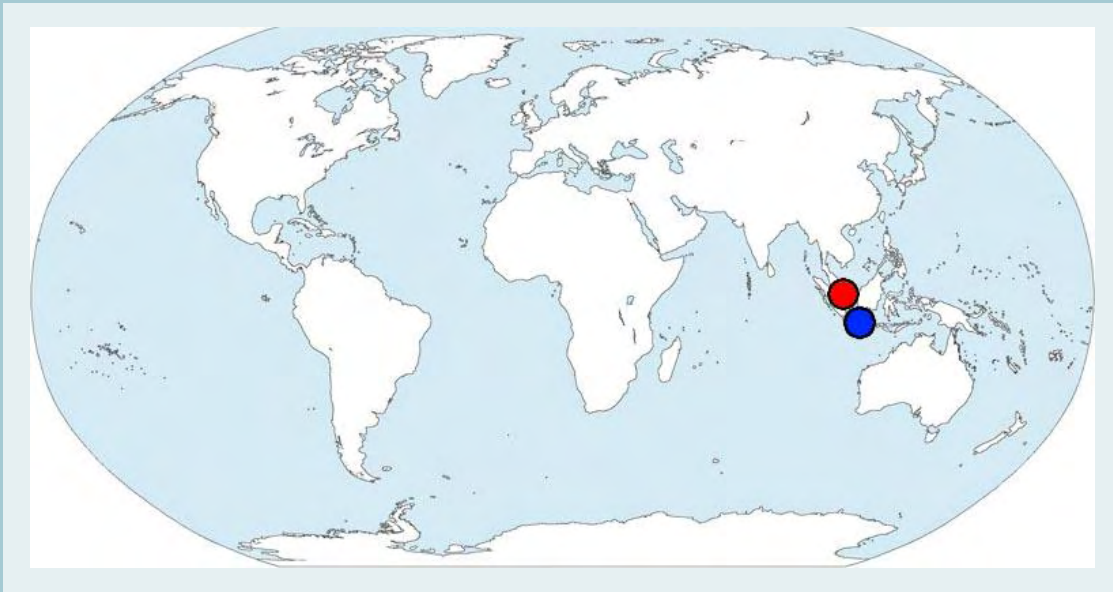
Diversity and biogeography of new genera

- Rare taxa or ecologically restricted in their distribution?
- ***Leptochlorella*** – molecular diversity studies detected this genus in two profoundly different habitats:
 - Phytobenthos of a lake in Antarctica (De Wever et al., 2009)
 - Quartz pavement in central Tibet (Wong et al., 2010)



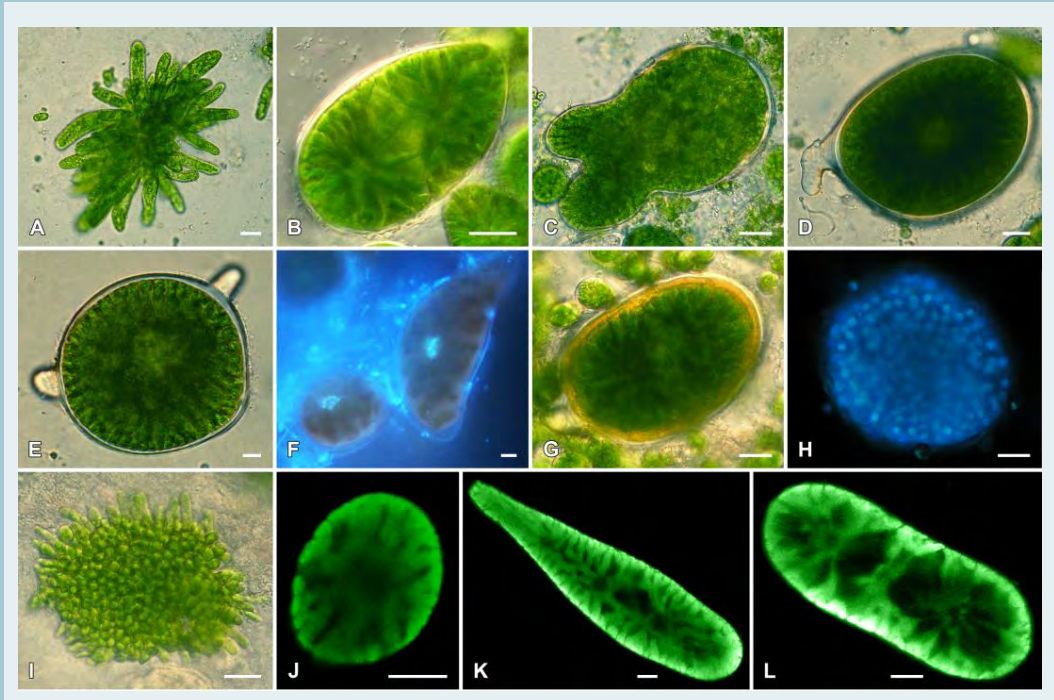
Diversity and biogeography of new genera

- Rare taxa or ecologically restricted in their distribution?
- ***Jenufa*** – a frequently occurring, and occasionally even dominant, species of microalgal growths on the tree bark in humid tropical habitats:
 - Tropical forest, West Java, Indonesia (Neustupa & Škaloud, 2008)
 - Lowland tropical forest, Singapore (Neustupa & Škaloud, 2010)



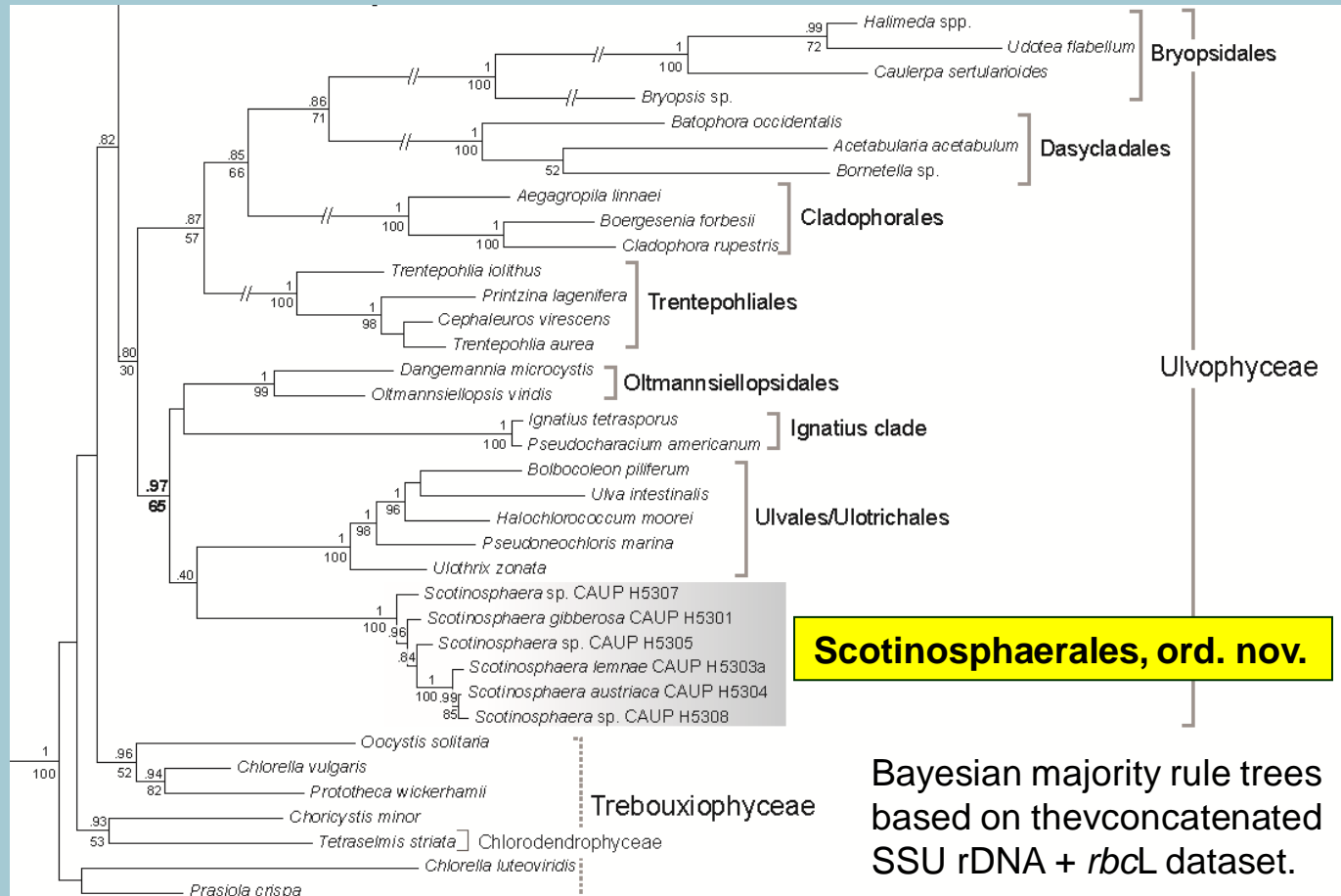
Scotinosphaera – hidden molecular diversity

- *Scotinosphaera*: although known for over a century (Klebs, 1881), its correct taxonomic placement was unclear (considered as a member of the Chlorophyceae)
- A strain isolated from the soil sample in the Czech Republic



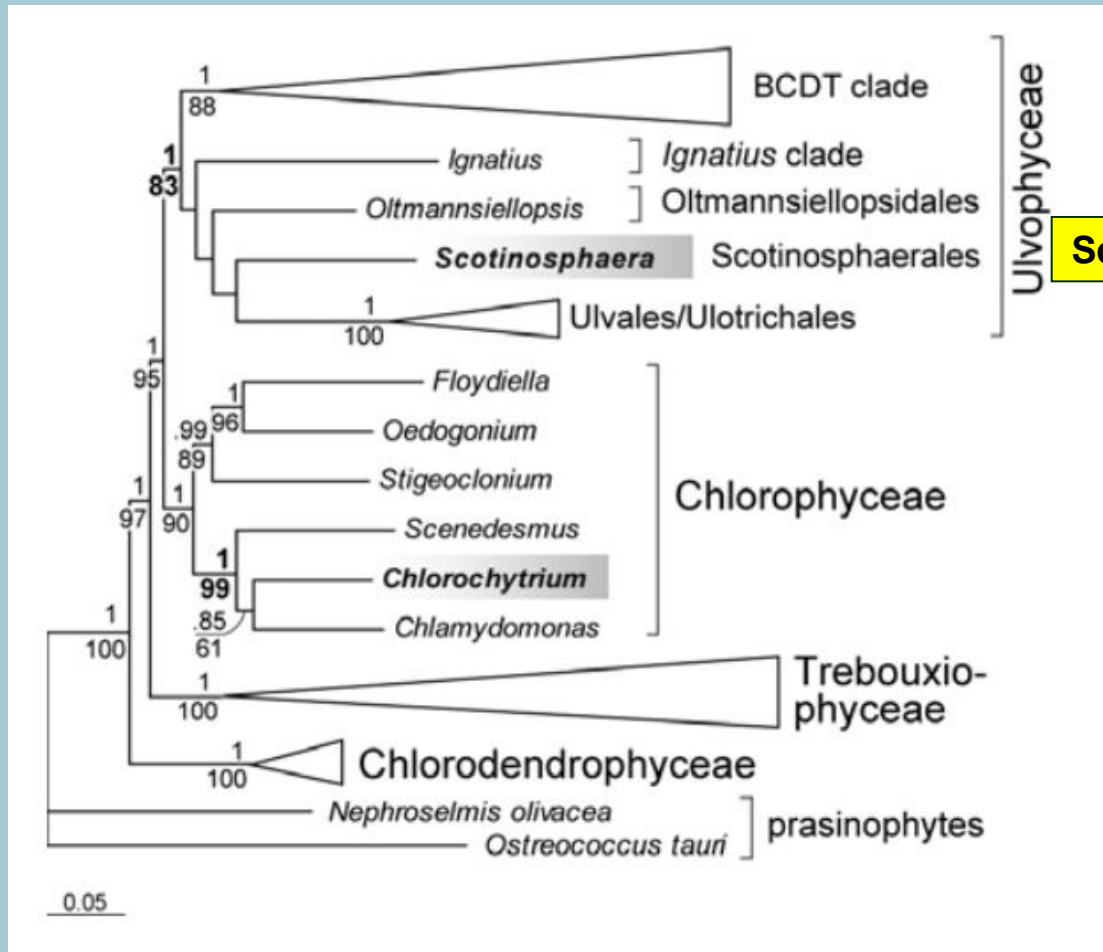
Scotinosphaera – hidden molecular diversity

- A new lineage within Ulvophyceae: the **Scotinosphaerales**



Scotinosphaera – hidden molecular diversity

- A new lineage within Ulvophyceae: the **Scotinosphaerales**

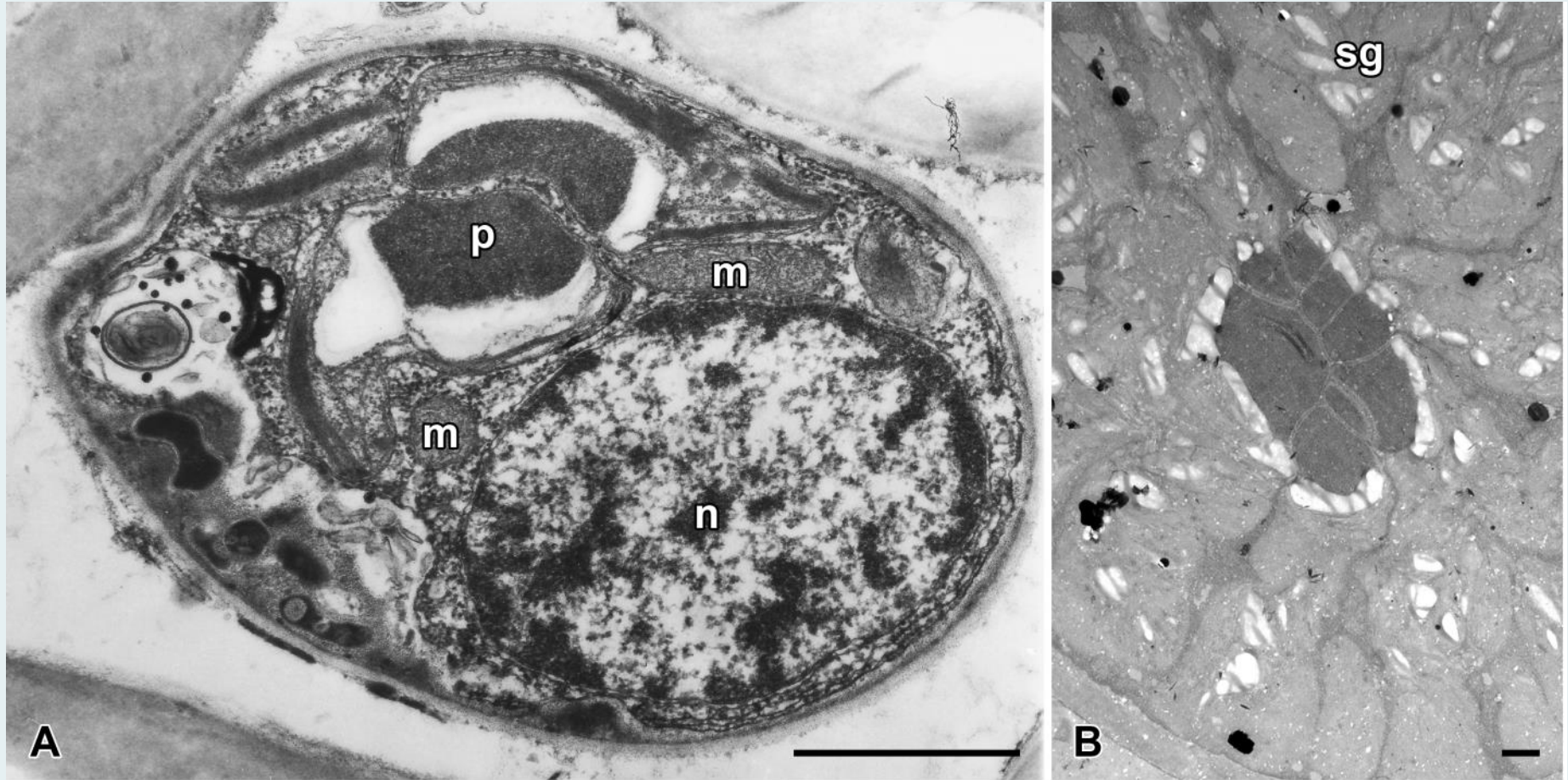


Scotinosphaerales, ord. nov.

Bayesian majority rule trees based on a 10-gene dataset.

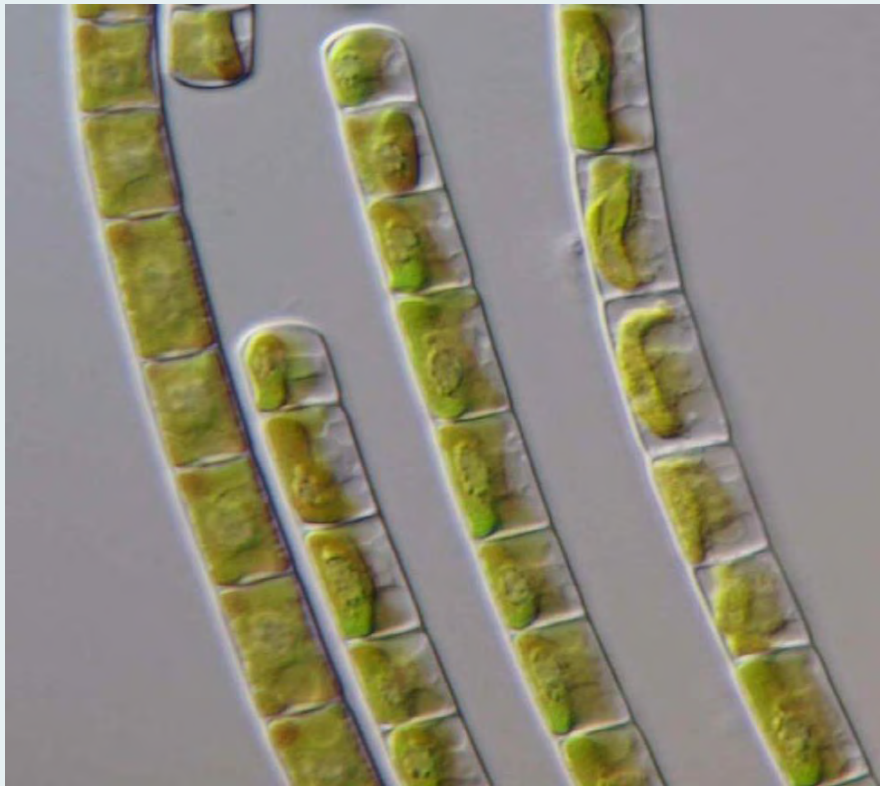
Scotinosphaera – hidden molecular diversity

- Unique ultrastructural feature: the pyrenoid is not penetrated by thylakoid membranes, but invaginated by cytoplasmic channels.

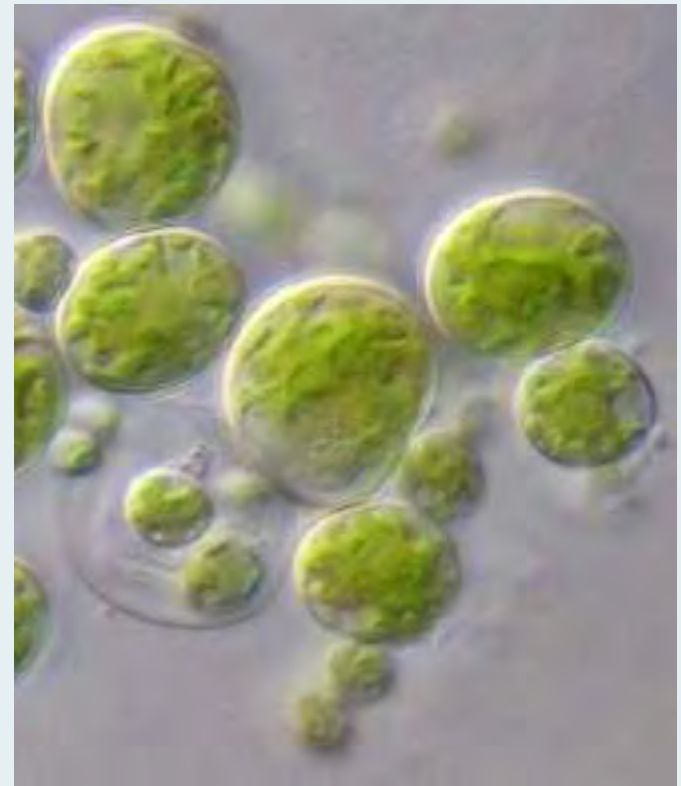


Aims II.

- To investigate the real species diversity within traditionally defined taxa of aerophytic green algae
- To assess potential ecological differentiation of the hidden, morphologically uniform taxa



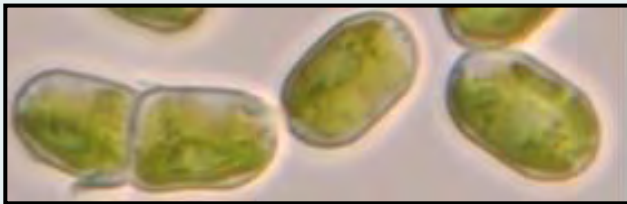
Klebsormidium – ubiquitous, common free-living green alga (Streptophyta)



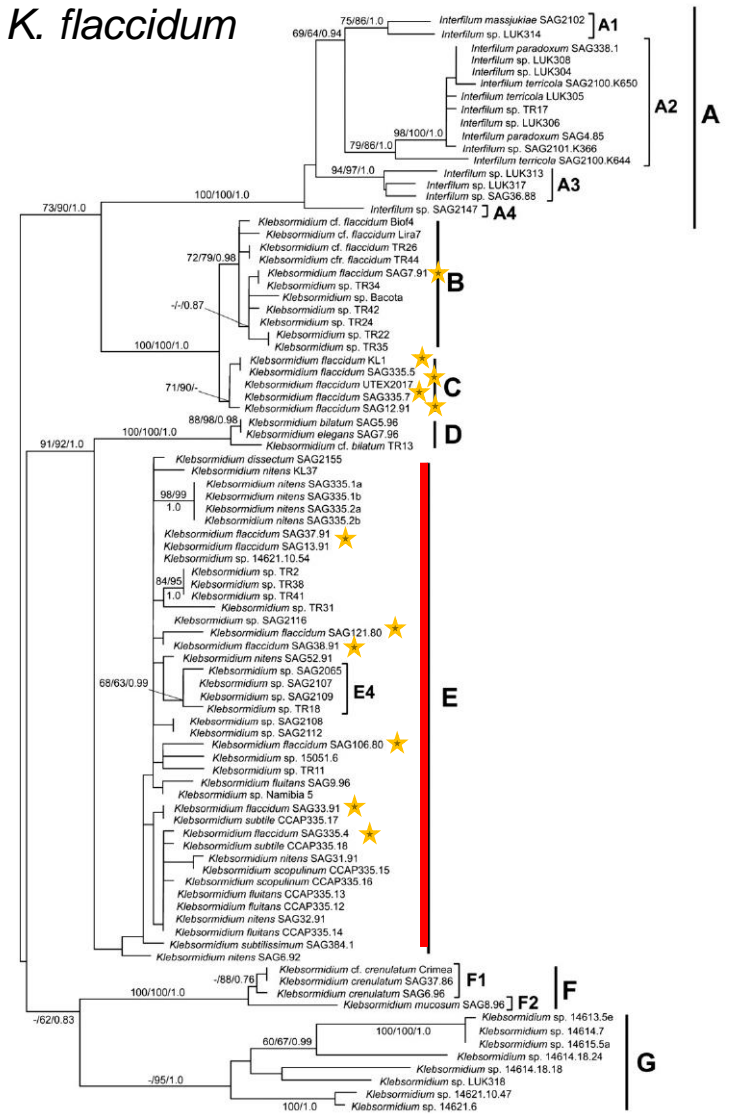
Asterochloris – photobiont of all Cladoniineae lichens (Chlorophyta)

Hidden diversity in *Klebsormidium*

- *Klebsormidium* – the identical morphology evolved multiple times in separate clades, which makes it very hard to characterize it unambiguously
- *Clade E* – a high number of morphologically similar lineages (probably cryptic species)

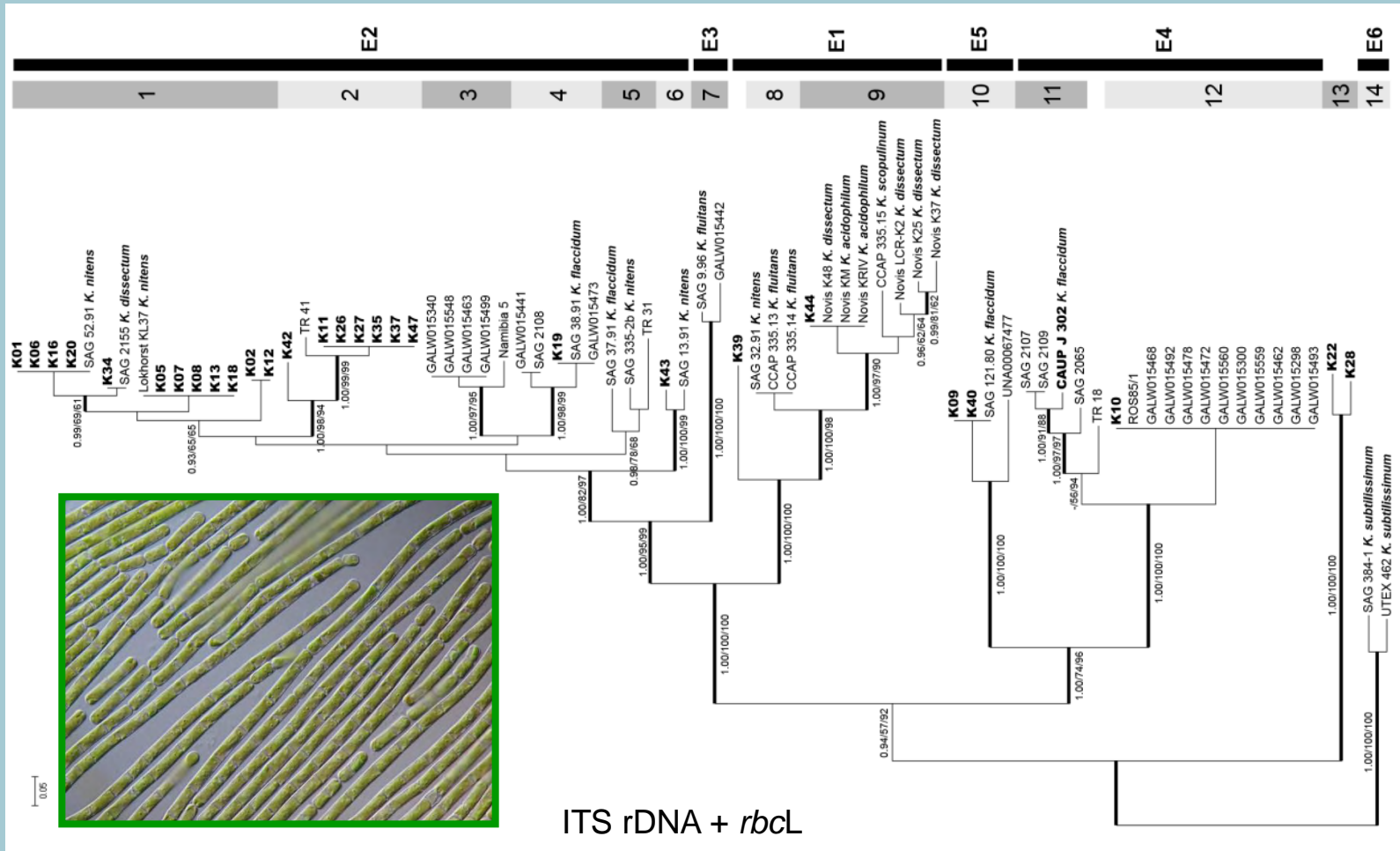


★ *K. flaccidum*



Hidden diversity in *Klebsormidium*

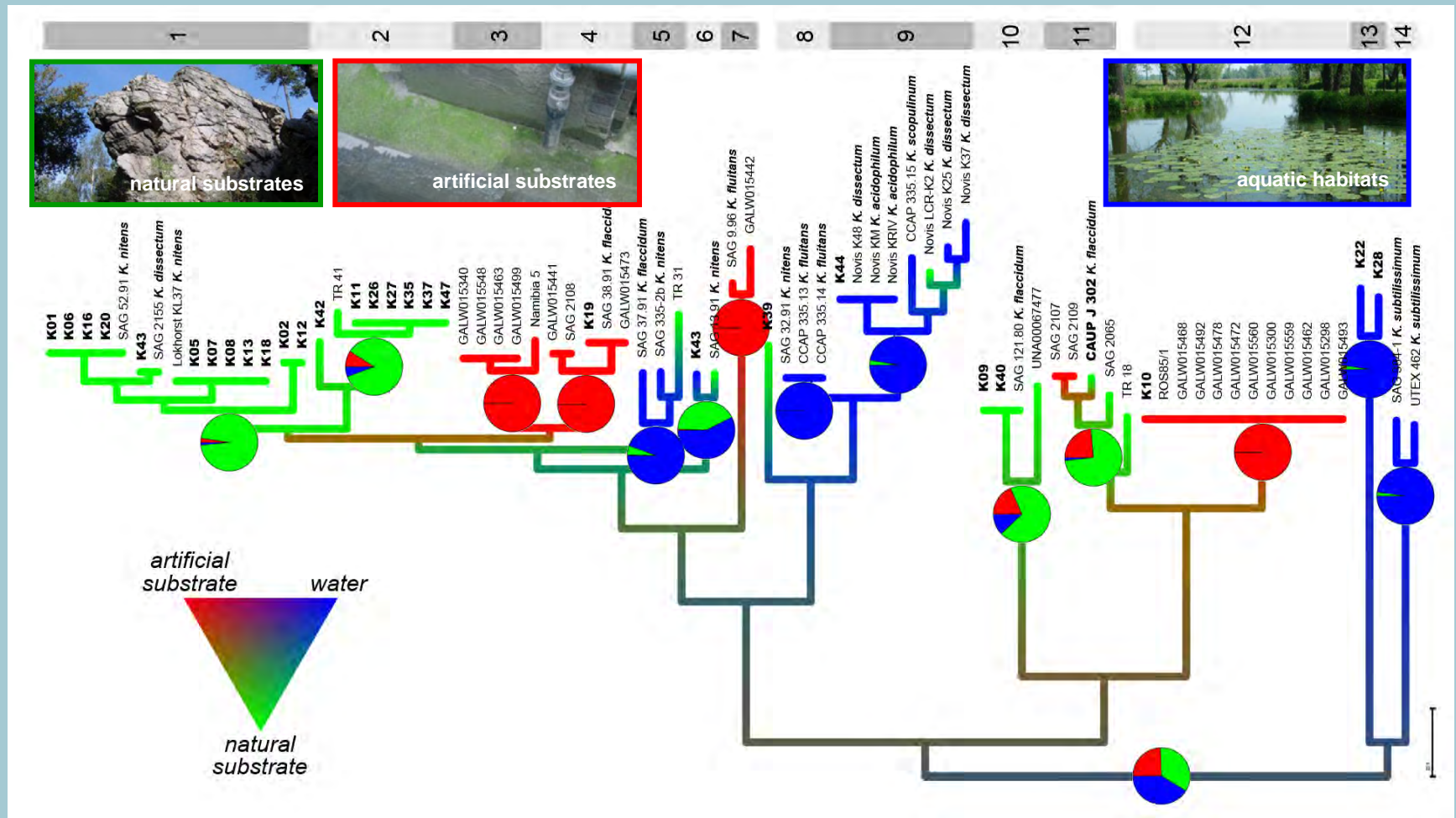
- Klebsormidium*, Clade E – 14 well-resolved lineages within a single morphospecies



Škaloud P. & Rindi, F. (2013): Ecological differentiation of cryptic species within an asexual protist morphospecies: a case study of filamentous green alga *Klebsormidium* (Streptophyta) *J. Euk. Microbiol.* **60**: 350-362.

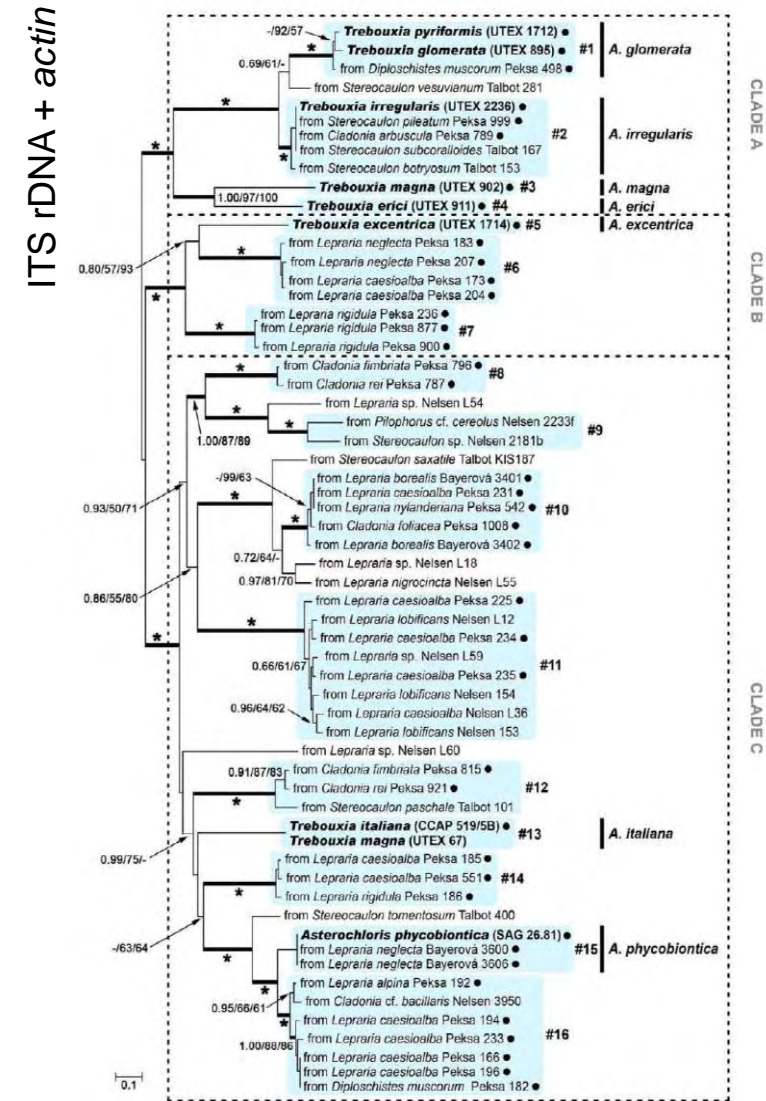
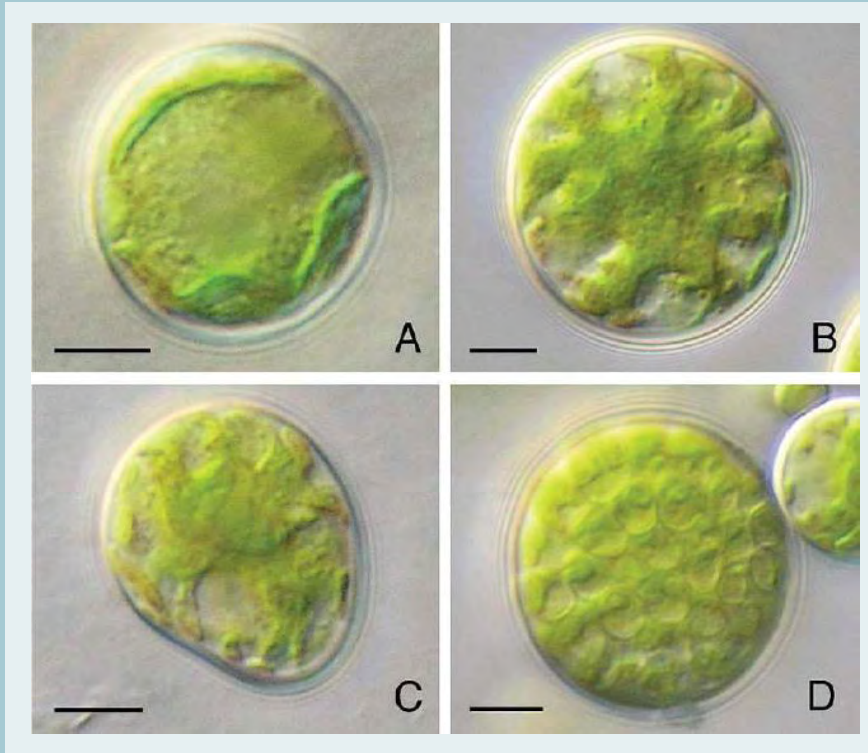
Hidden diversity in *Klebsormidium*

- Klebsormidium*, Clade E – Strong ecological preferences of the lineages to one of three habitat types



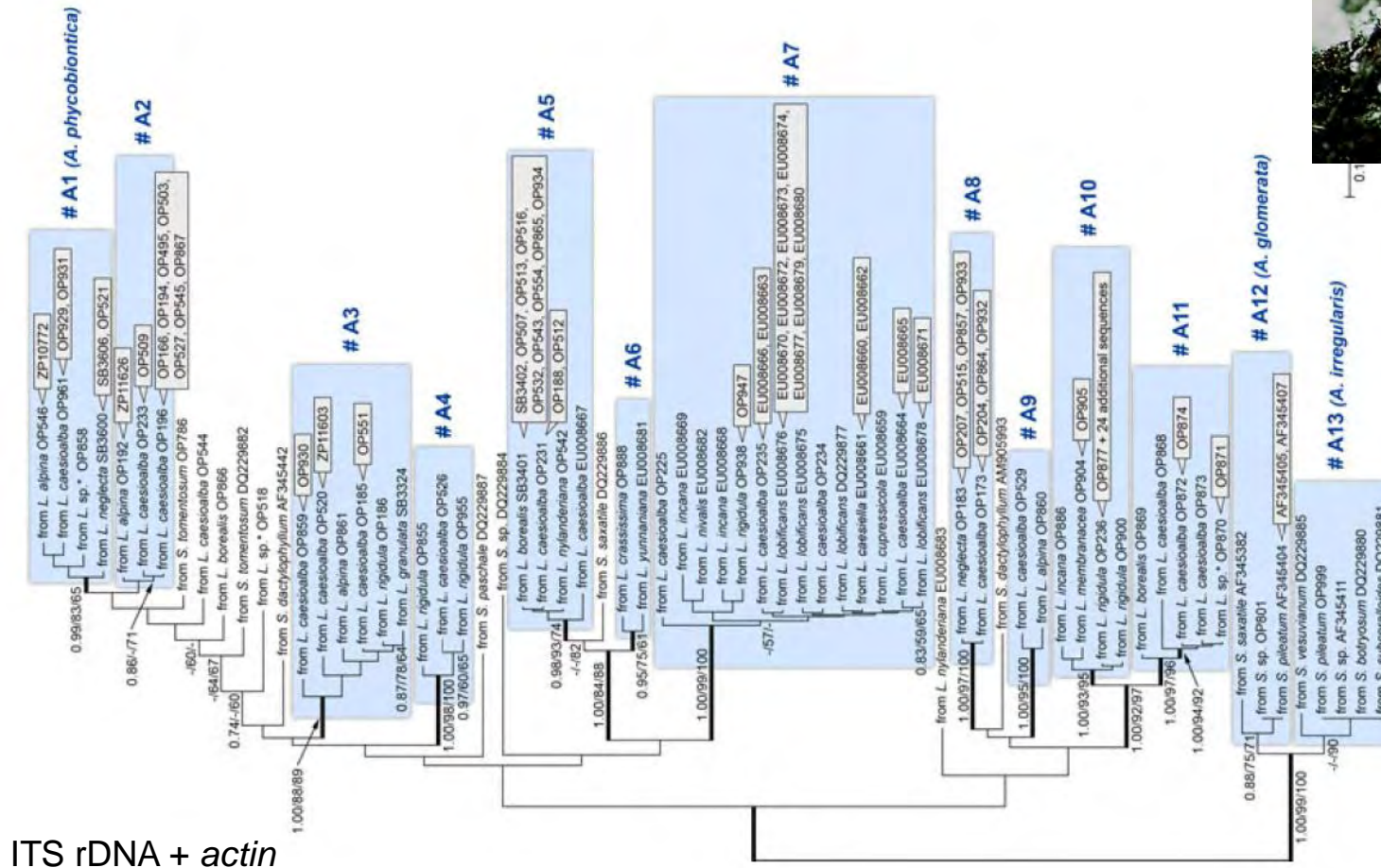
Hidden diversity in *Asterochloris*

- Large cryptic diversity within the genus
- Only 15% of isolated photobionts could be assigned with certainty to previously described species.



Hidden diversity in *Asterochloris*

- Lepraria* symbionts: Bayesian phylogeny of 107 thalli

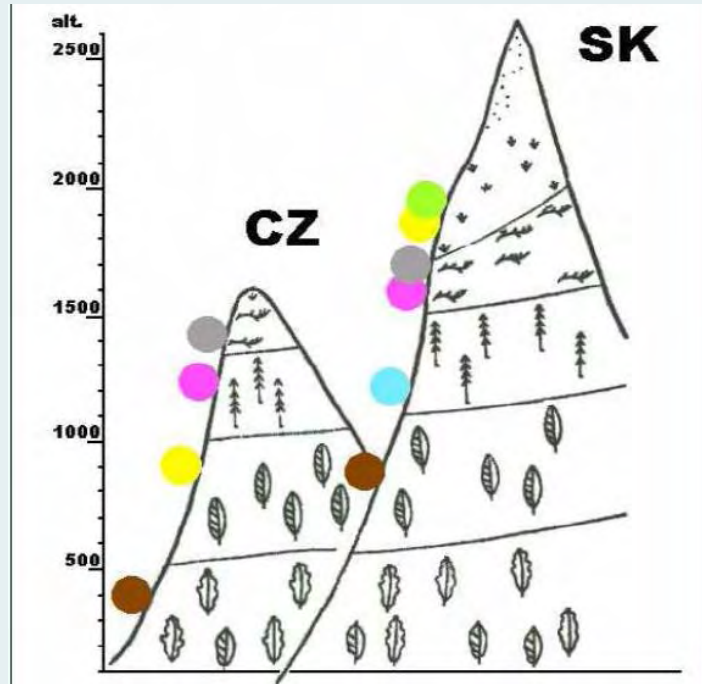
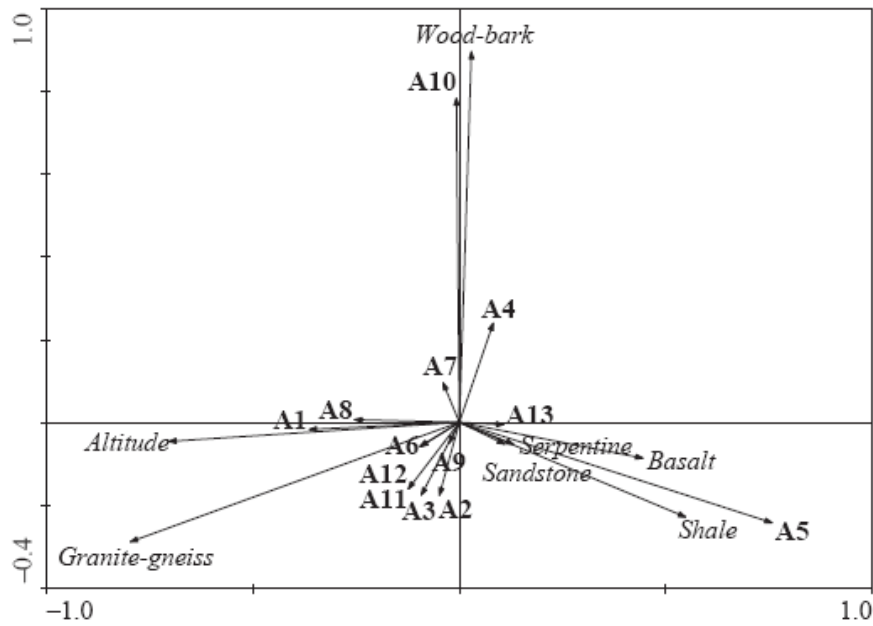


Peksa, O. & Škaloud, P. (2011): Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Mol. Ecol.* **20**: 3936-3948.

Hidden diversity in *Asterochloris*

- Phylogenetic signal: Randomization tests showed significant ecological similarity of related strains for all tested traits:

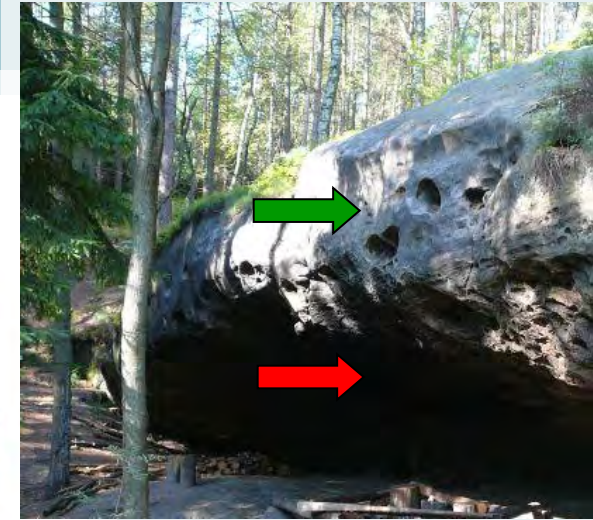
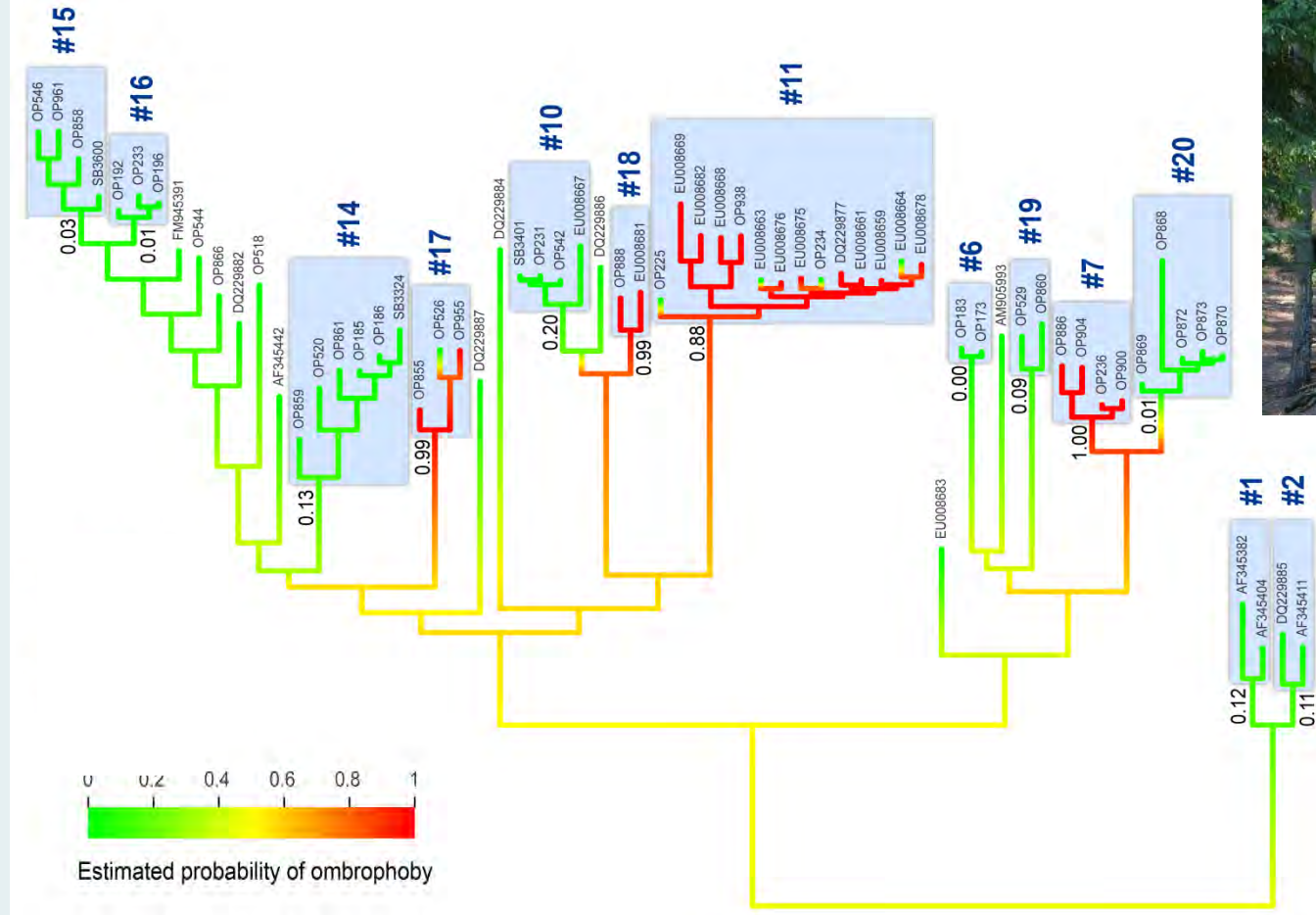
Trait	Pagel's λ			K statistics		Ecological similarity
	λ	Likelihood ratio	P-value	K value	P-value	P-value
Exposure to rain	0.946	1.53	<0.0001	0.2126	0.001	<0.0001
Altitude	0.045	1.01	<0.0001	0.0832	0.005	<0.0001
Substrate type	0.652	1.05	0.0011	0.1168	0.002	<0.0001



Peksa, O. & Škaloud, P. (2011): Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Mol. Ecol.* **20**: 3936-3948.

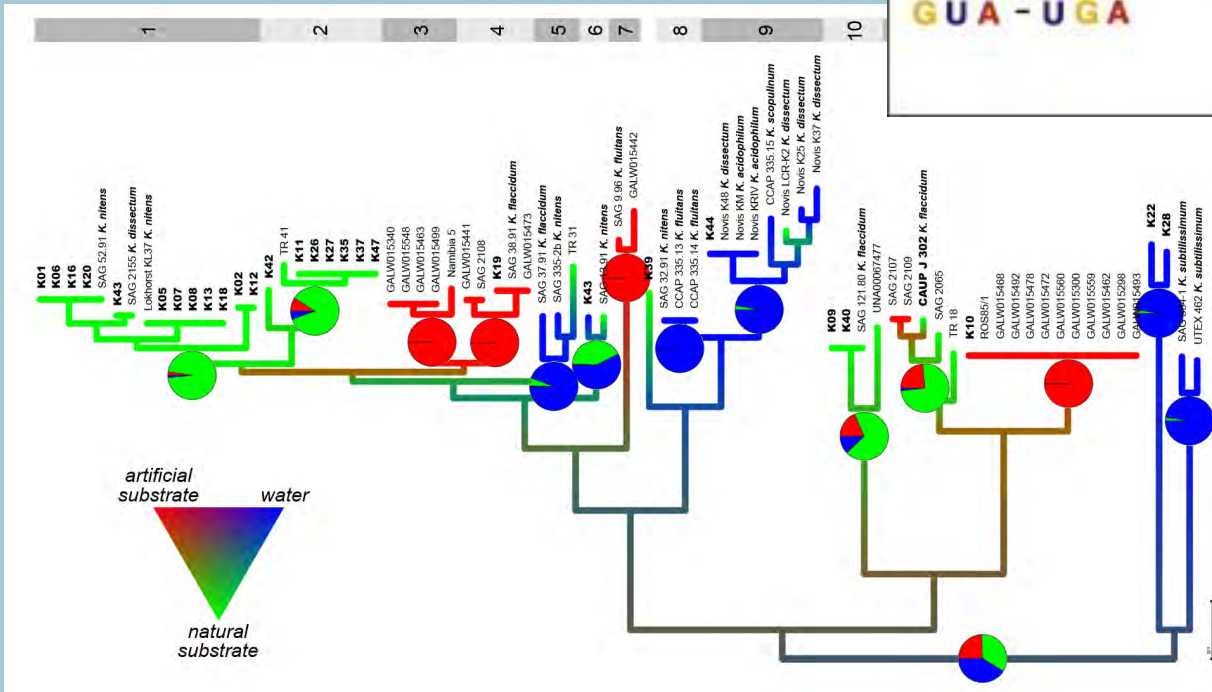
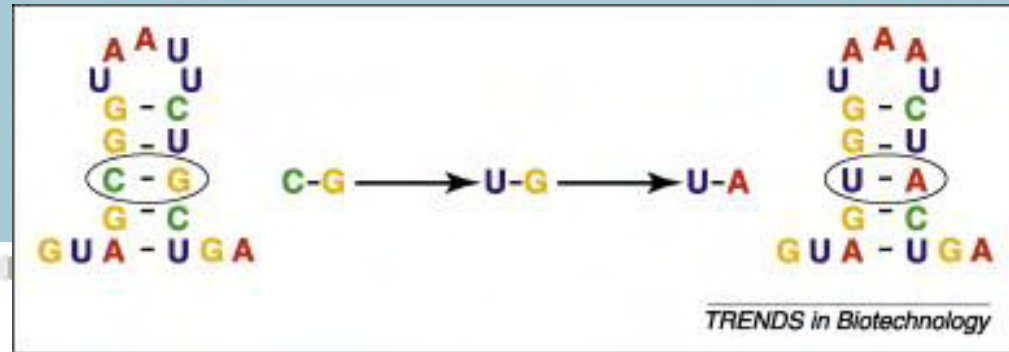
Hidden diversity in *Asterochloris*

- Strong ecological differentiation of particular species in their relation to liquid water (precipitation)



Species concept

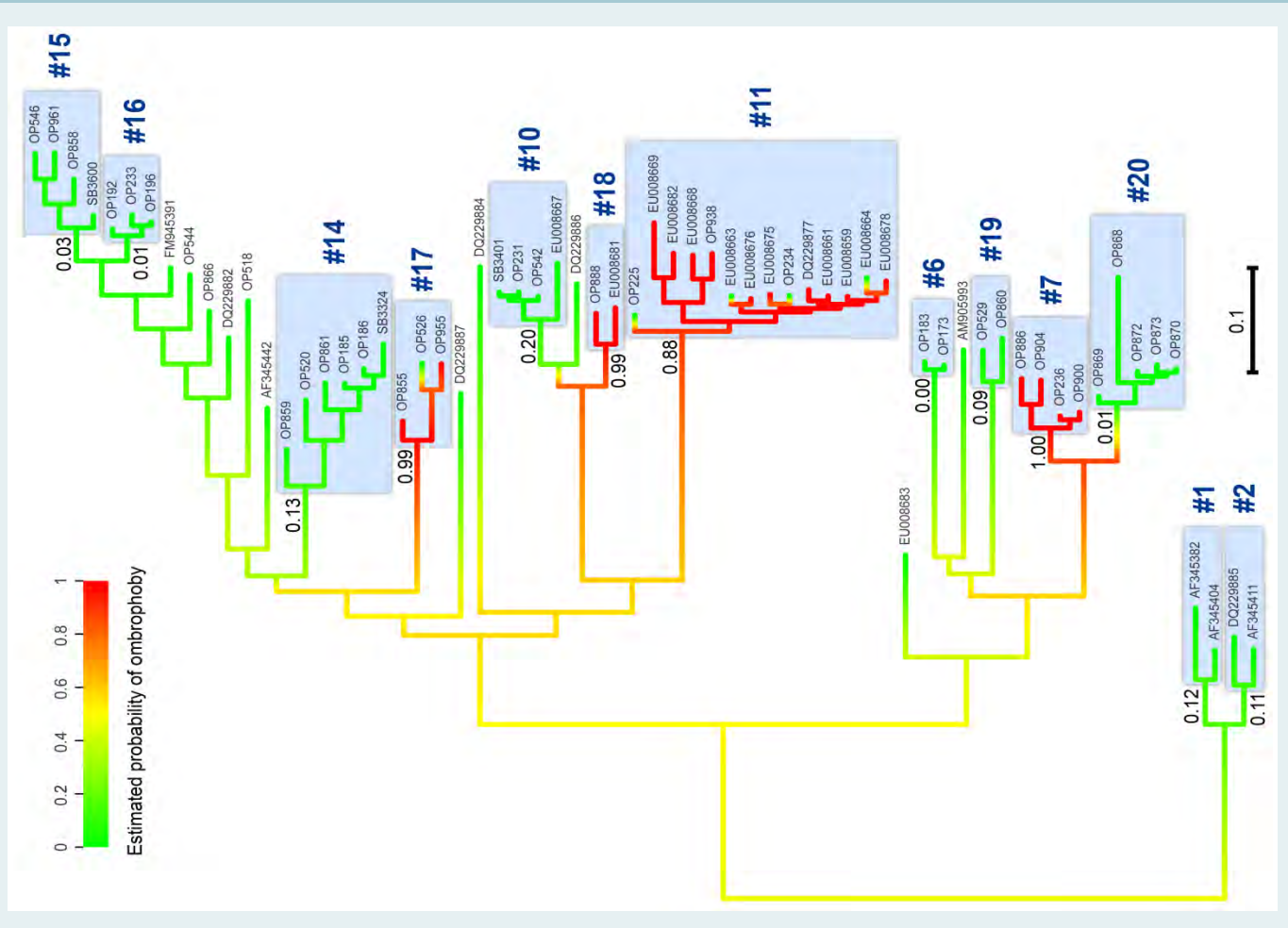
- rbcL and actin data provided higher resolution than ITS rDNA
- Both CBC (compensatory base changes) and ITS-2 barcoding species concepts fail to recognize ecologically differentiated species in both *Klebsormidium* and *Asterochloris*



Škaloud P. & Rindi, F. (2013): Ecological differentiation of cryptic species within an asexual protist morphospecies: a case study of filamentous green alga *Klebsormidium* (Streptophyta) *J. Euk. Microbiol.* **60**: 350-362.

Species concept

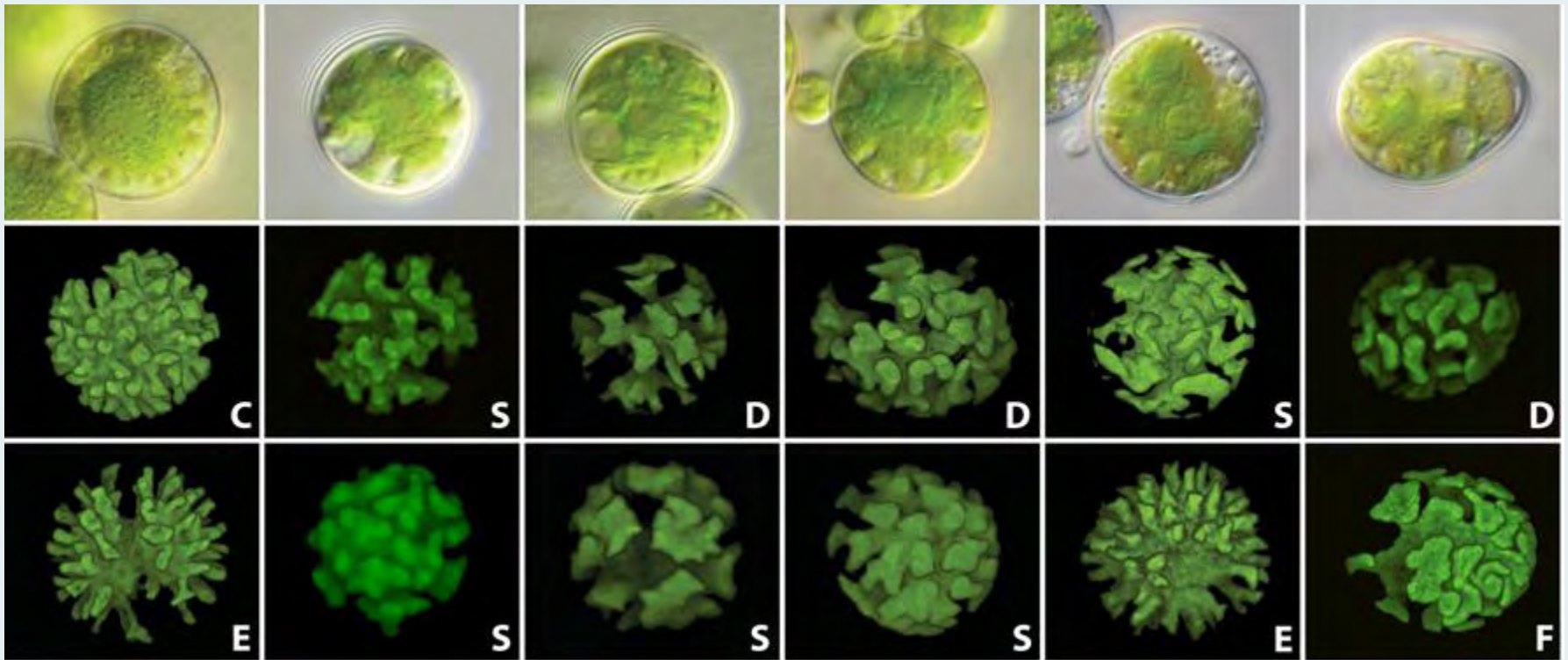
- Asterochloris* species are uniform in their SSU rDNA sequences, but different in their various ecological preferences and distribution



Peksa, O. & Škaloud, P. (2011): Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Mol. Ecol.* **20**: 3936-3948.

Species concept

- Species barriers should be determined very carefully, using a combination of several independent attributes (incl. ecological data)
- Species should help us to organize the diversity in a meaningful fashion



Conclusions

- Coccoid green aerophytic algae actually represent one of the richest sources of novel phylogenetic diversity
- Molecular investigation of “boring green balls” could in fact considerably improve our understanding of green algal evolution
- Morphologically defined species of aerophytic green algae could in fact consist of a large number of cryptic, yet ecologically well differentiated species
- Species barriers should be determined very carefully, and specifically for each of the group of aerophytic algae

The background of the slide is a microscopic image showing numerous small, green, spherical cells, likely yeast or bacteria, scattered across a light blue background. The cells are in various stages of focus, with some appearing sharp and others blurred.

Thank you for your attention

- The research has been supported by the grant no. P506/12/0955 of the Czech Science Foundation.