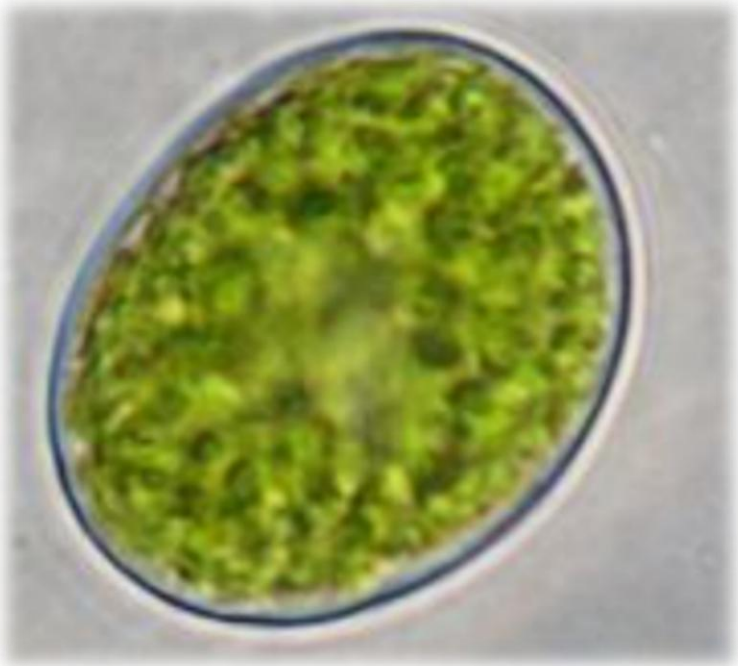
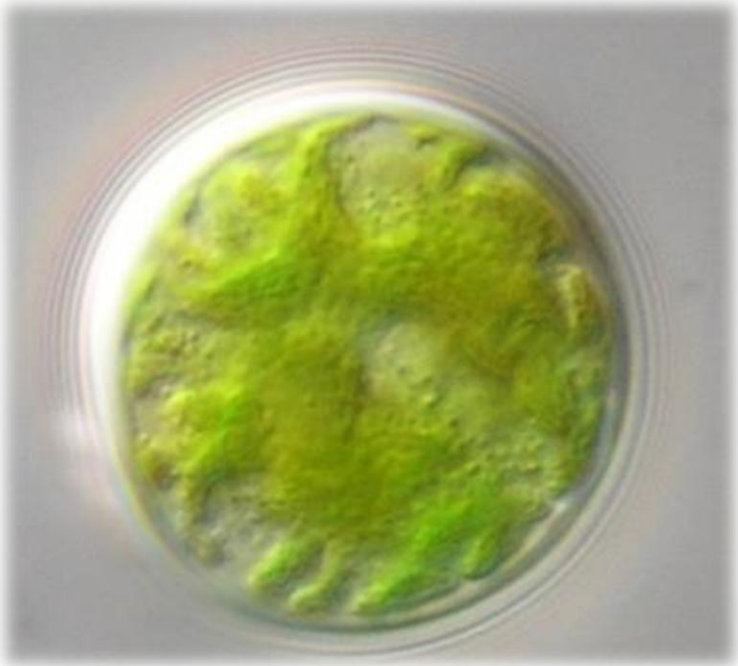
Towards uncovering the real diversity of free-living and symbiotic soil green algae

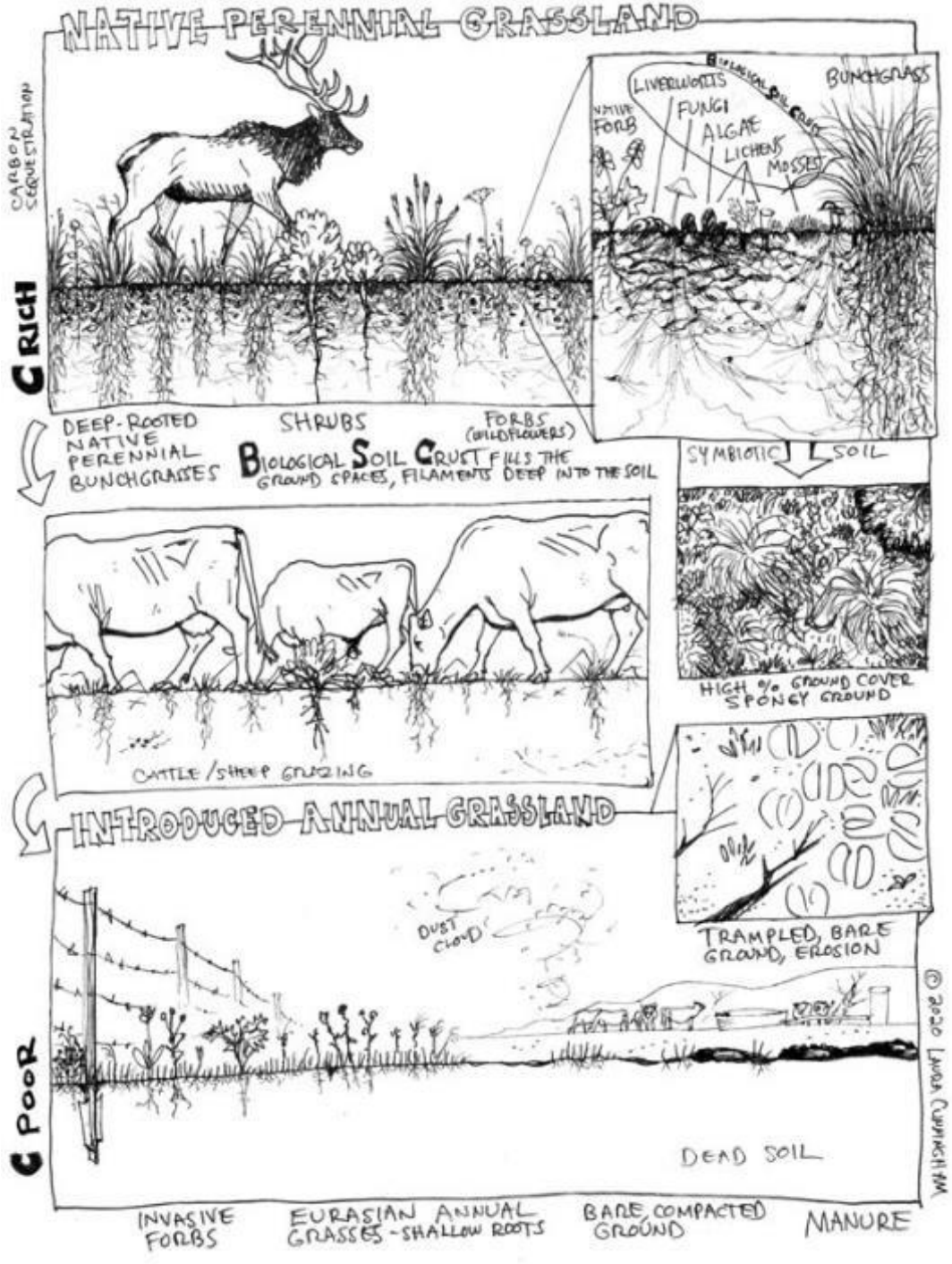




Pavel Škaloud, Veronica Malavasi, Lucie Vančurová, Zuzana Škvorová & Ivana Černajová

FACULTY OF SCIENCE
Charles University

Algal speciation & evolution lab


NATIVE PERENNIAL GRASSLAND
CARBON SEQUESTRATION
C RICH
LIVERWORTS
BUNCHGRASS
NATIVE FORB
FUNGI
ALGAE
LICHENS
MOSSES
DEEP-ROOTED NATIVE PERENNIAL BUNCHGRASSES
SHRUBS
FORBS (WILDFLOWERS)
BIOLOGICAL SOIL CRUST FILLS THE GROUND SPACES, FILAMENTS DEEP INTO THE SOIL
SYMBIOTIC SOIL
HIGH % GROUND COVER SPONGY GROUND
CATTLE / SHEEP GRAZING
INTRODUCED ANNUAL GRASSLAND
DUST CLOUD
TRAMPLED, BARE GROUND, EROSION
C POOR
DEAD SOIL
INVASIVE FORBS
EURASIAN ANNUAL GRASSES - SHALLOW ROOTS
BARE, COMPACTED GROUND
MANURE
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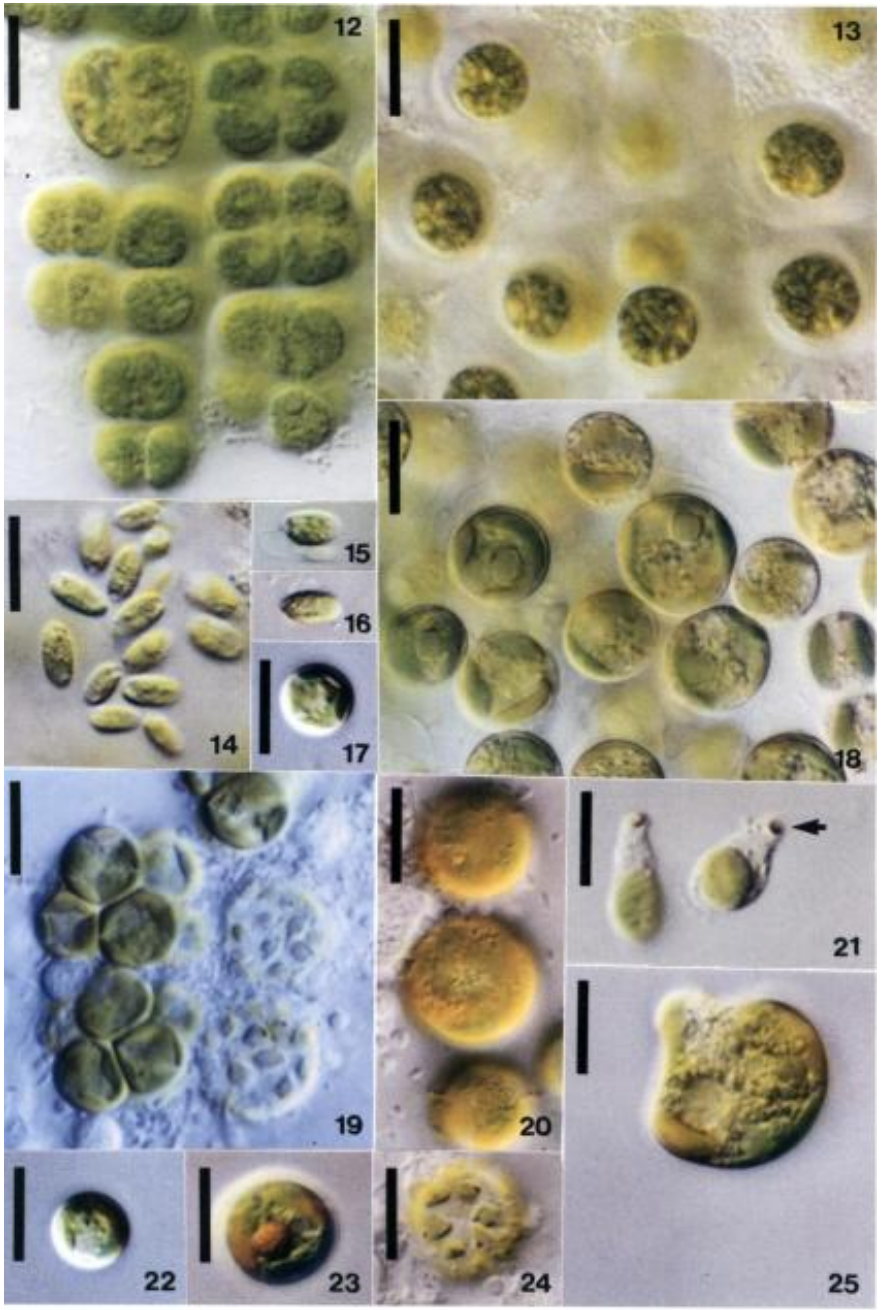
Typical studies on terrestrial green algal diversity

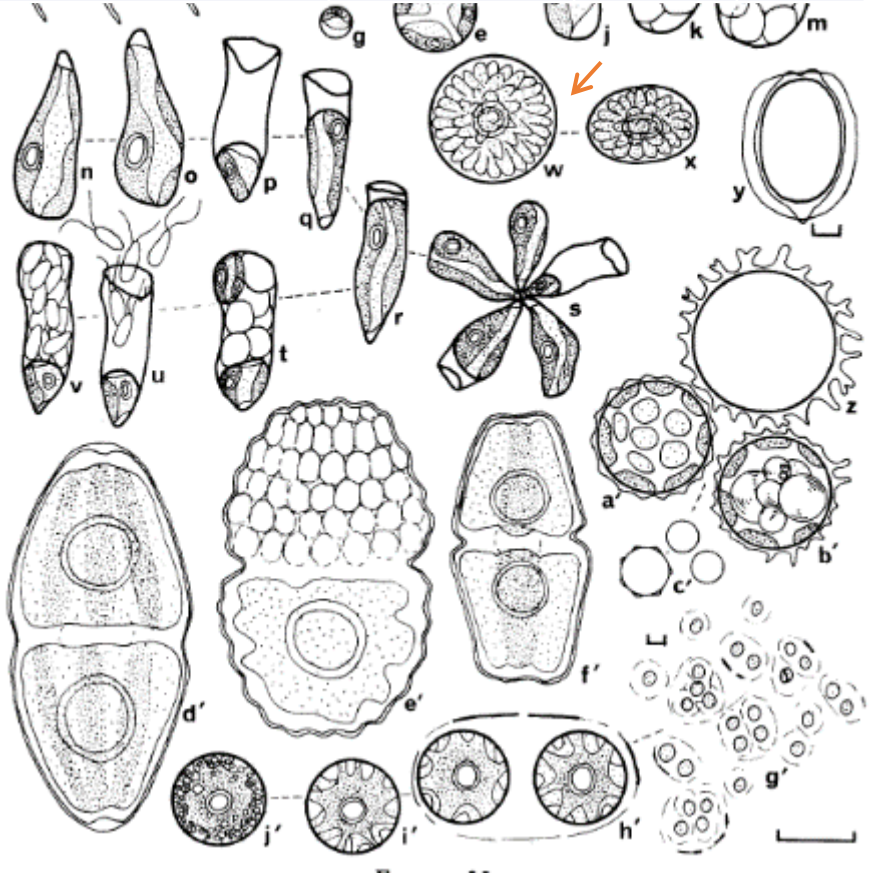


Broady 1979
Flechtner et al. 1998

Usually very few symbionts were detected. *Trebouxia* is often completely absent.

SAMPLING SITE	M1	M2	M3	O1	O2	O3	O4
Chlorolobion lunulatum Hind.	○	●	○	○			
Chloromonas aff. ***oleosa*** (cf. Mataloni and Posse 2001)							○
Chloromonas sp. zygote		●					
Desmotetra antarctica (Fritsch) Ling		●	○				
Elliptochloris cf. ***bilobata*** Tschermak-Woess	○	○	○				●
Elliptochloris subsphaerica var. ***antarctica*** (Broady) Ettl and Gärtner	○	○					
Klebsormidium dissectum (Gay) Ettl and Gärtner	○	○					
Klebsormidium dissectum (Gay) Ettl and Gärtner var. A (sensu Broady 1979)		○	○				
Klebsormidium flaccidum (Kütz.) Silva, Mattox and Blackwell		○					
Klebsormidium sp. 1		○	○				
cf. ***Klebsormidium*** sp. 2		○					
Lobosphaeropsis pyrenoidosa Reisigl	○	○					
Muriella cf. ***decolor*** Visch.		○		○			
Muriella zofingiensis (Dönz) Hind.	○	○	○	○	●		●
Oocystis sp.	○	○	○				
Prasiococcus calcarius (J. B. Petersen) Visch.				●			●
Prasiola crispa (Lightfoot) Menegh.				●	●	●	
cf. ***Pseudochlorococcum typicum*** Archib.	○	○	○	○			
Pseudococcomyxa simplex (Mainx) Fott	○	○	○	○			
Raphidonema nivale Lagerh.		●					
Rhopalocystis oleifera Schussnig	○	○	○				
Stichococcus bacillaris Näeg.	○	○	○	○	○		
cf. ***Tetracystis tetraspora*** (Arce and Bold) Brown and Bold	○						
Trebouxia sp. 1	○						
Chlorococcales sp. 1					●		
Chlorococcales sp. 2					●		
Chlorococcales sp. 3				○		●	●
Chlorococcales sp. 4	○						●
Chlorellaceae sp. 1	○						
Chlorellales				○	○		
Chlorosarcinaceae sp. 1			○				
Subtotal	**22**	**24**	**17**	**16**	**9**	**4**	**15**

	total of green algae	*Trebouxia*	symbionts
Lukešová 2001	80	**1?**	**6**
Hoffmann 2007	61	**0**	**9**
Broady 1979	52	**1**	**4**
Garraza et al. 2011	47	**1**	**3**
Schulz et al. 2015	46	**0**	**6**
Neustupa Škaloud 2004	42	**0**	**3**
Flechtner et al. 1998	37	**0**	**4**
Kaštovská et al. 2005	26	**0**	**1**
Langhans et al. 2009	19	**0**	**2**
Hawkes & Flechtner 2002	12	**0**	**1**



Broady 1979
Garraza et al. 2011

Pl. Syst Evol. 158, 243 – 247

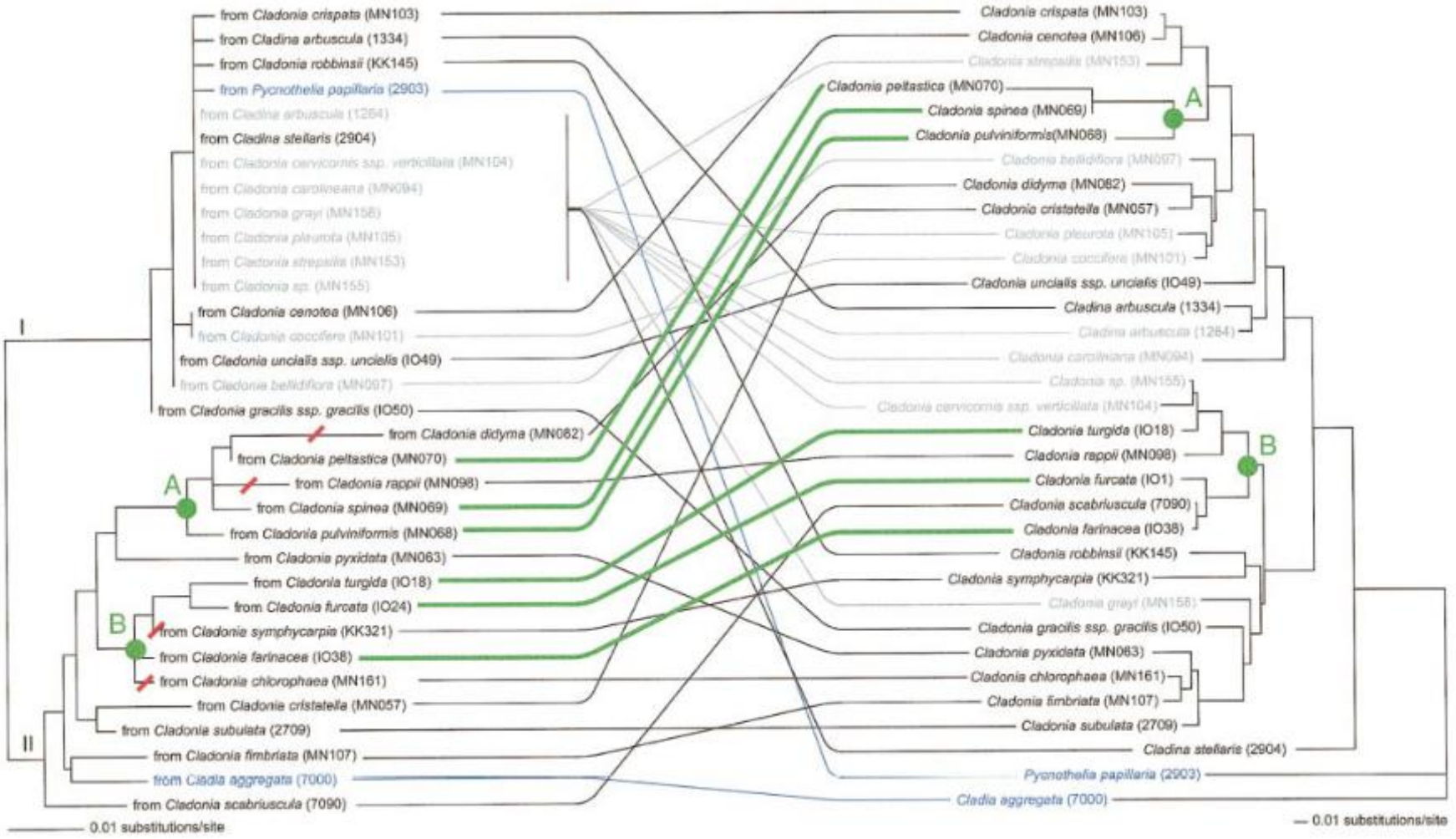
Plant Systematics and Evolution

The lichen alga *Trebouxia:* does it occur free-living?*

VERNON AHMADJIAN

Received July 9, 1987

Ahmadjian (1987): It is clear that fungi and algae have had a long coevolution that has resulted in the unique morphology of lichens, chemicals, and the clear control of algae by fungi



Piercey-Normore & DePriest (2001)

New Phytol. (1984) **97**, 455–462

OBSERVATIONS ON FREE-LIVING *TREBOUXIA* DE PUYMALY AND *PSEUDOTREBOUXIA* ARCHIBALD, AND EVIDENCE THAT BOTH SYMBIONTS FROM *XANTHORIA PARIETINA* (L.) TH. FR. CAN BE FOUND FREE-LIVING IN NATURE

BY P. BUBRICK*, MARGALITH GALUN*† AND A. FRENSDORFF†

**Department of Botany and †Department of Microbiology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978, Tel Aviv, Israel*

(*Accepted 10 February 1984*)

Lichenologist, **10**: 69–79 (1978)

MYRMECIA RETICULATA AS A PHYCOBIONT AND FREE-LIVING—FREE-LIVING *TREBOUXIA*—THE PROBLEM OF *STENOCYBE SEPTATA*

E. TSCHERMAK-WOESS*

- **Tschermak-Woess**: *Trebouxia* forms a free-living green coating on the tree. No haustoria were produced, reproduction by zoospores.

- **Ahmadjian**: It's just algae that escaped from the soredia/isidia for a while, zoospores are definitely not for propagation; they are just remnants from a time when *Trebouxia* was free-living.

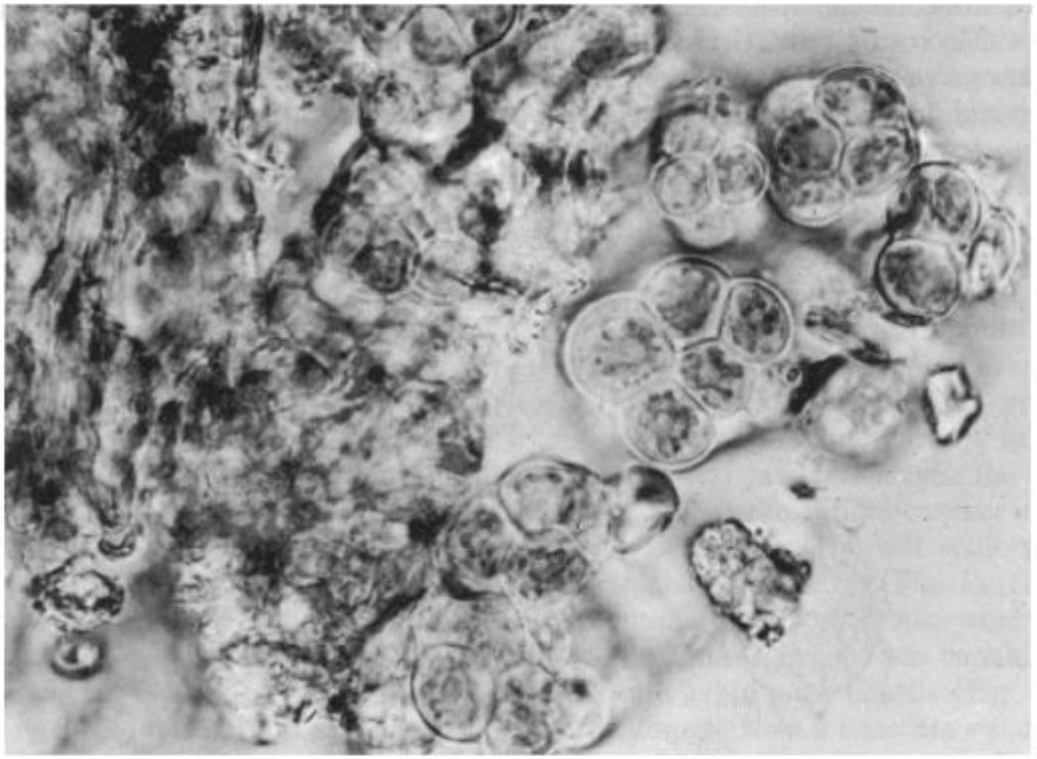
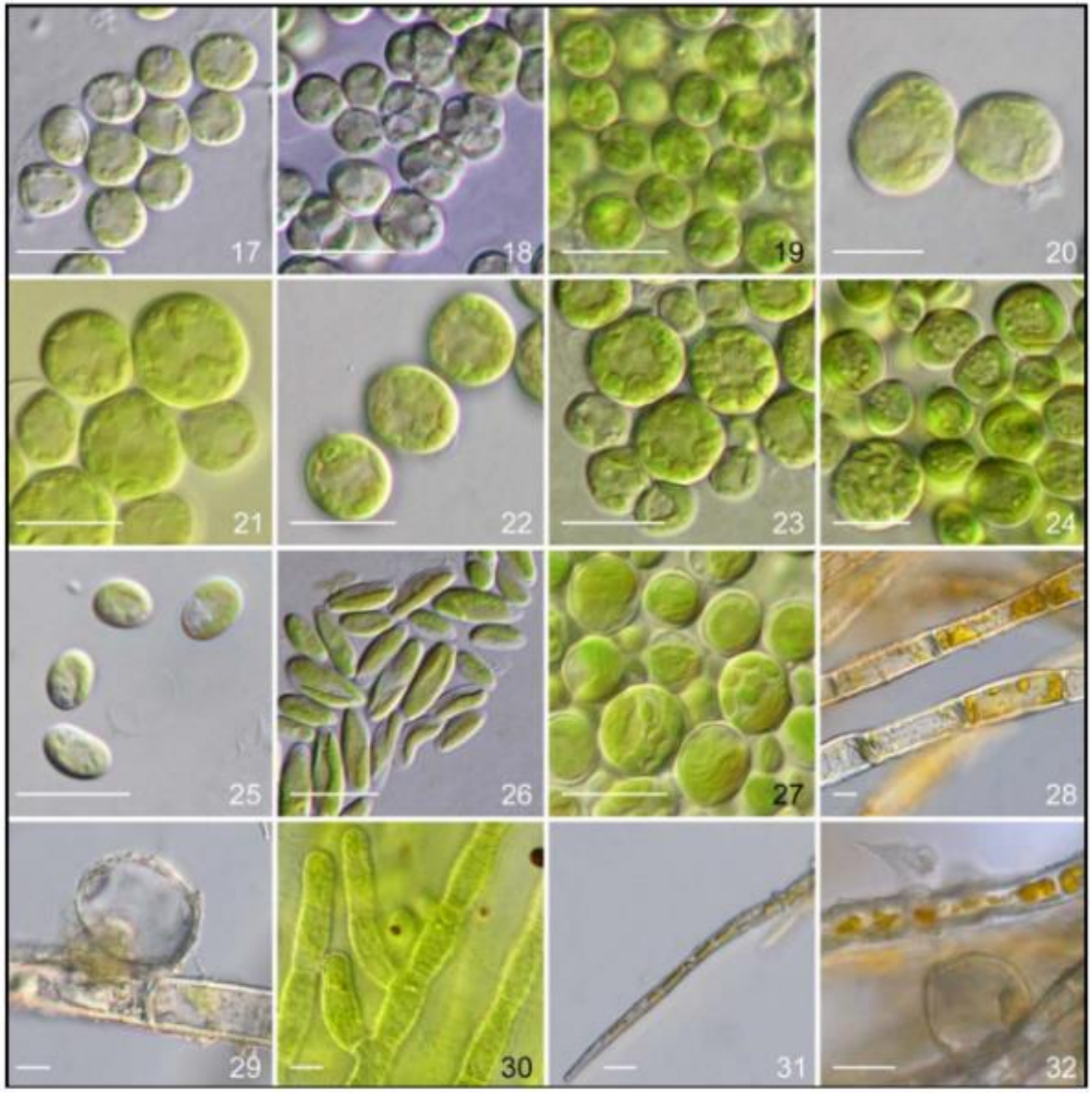


FIG. 2. *Trebouxia* sp. Cells absolutely free from fungal hyphae (on bark of *Aesculus*, Prater, Vienna). (Living specimen.) × 850.

If lichen symbionts are free-living, why are they not found in the majority of diversity studies?



Figs 17–32. 17, 18 – *Chlorella* sp. 2; 19 – *Chlorella* sp. 3; 20 – *Chlorella* sp. 4; 21–23 – *Dictyochloropsis* sp.; 24, 25 – *Elliptochloris* sp.; 26 – *Pseudococcomyxa* sp. 2; 27 – *Watanabea* sp.; 28–30 – *Printzina bossei*; 31, 32 – *P. effusa*; scale bar 10 μm.

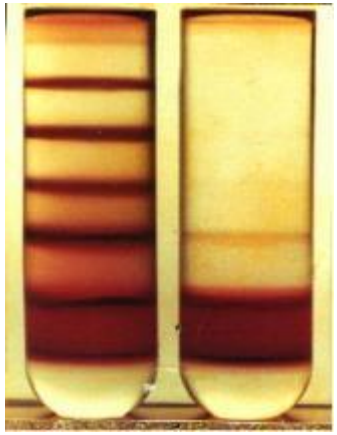
Table 2. A list of species from investigated localities. Different

Cyanobacteria
Leptolyngbya sp. 1
Leptolyngbya sp. 2
Nostoc sp. 1
Nostoc sp. 2
Nostoc cf. *entophytum* Bornet & Flahault
Nostoc cf. *punctiforme* (Kütz.) Hariot
Scytonema ocellatum Lyngbye ex Bornet & Flahault
Scytonema sp.
Chlorophyceae
Bracteacoccus sp.
Coelastrella sp.
Mychonastes homosphaera (Skuja) Kalina & Punčochářová
Scotiellopsis rubescens Vinatzer
Trebouxiophyceae
Chlorella sp. 1
Chlorella sp. 2
Chlorella sp. 3
Chlorella sp. 4
Dictyochloropsis sp.
Elliptochloris sp.
Pseudococcomyxa simplex (Mainx) Fott
Pseudococcomyxa sp.
Stichococcus bacillaris Näg.
Watanabea sp.
Ulvophyceae
Printzina bossei (De Wildeman) Thompson & Wujek
Printzina effusa (Krempelhüber) Thompson & Wujek
Printzina cf. *lagenifera* (Hildebrand) Thompson & Wujek
Trentepohlia aurea (L.) Martius
Trentepohlia monilia De Wildemann
Trentepohlia sp.

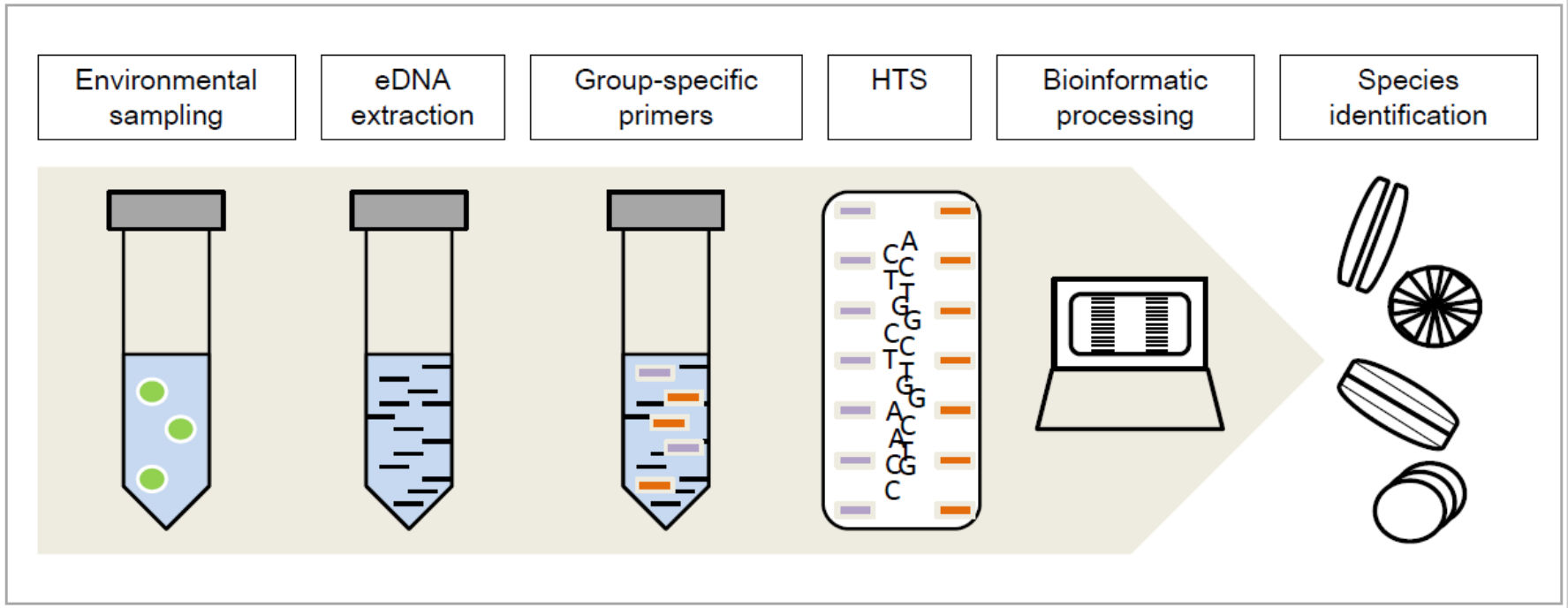
Neustupa & Škaloud (2008)

Aims

- What is the real diversity of soil green algae?
- Why are there so few lichen photobionts found in studies on soil algae?
- If present in soil, would we be able to find all lichen symbionts detected at a given site?



- Cultivation on agar plates
- DNA metabarcoding
- Single-cell PCR (Percoll separation method)




CAUP

Study site

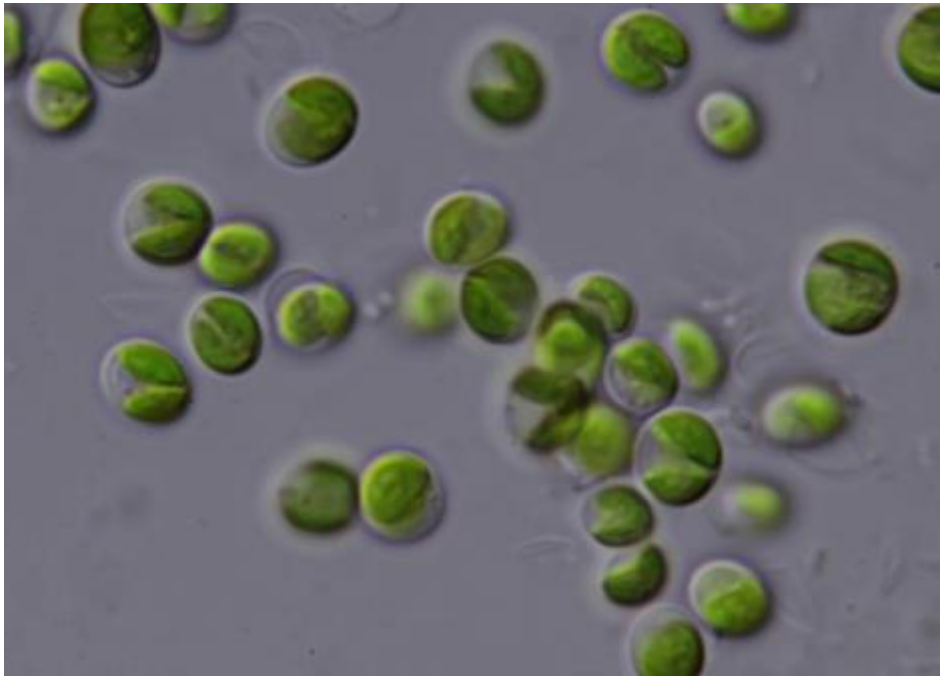
- Vinařická hora, Czech Republic
  - a temperate locality
  - A total of 39 lichen species

- Sampling
  - 2020-2021
  - 5 collection times (11/20, 2/21, 5/21, 8/21, 11/21)
  - 2 sampling sites (VIN1, VIN2)
  - 2 mixed samples (soil, rock surfaces)

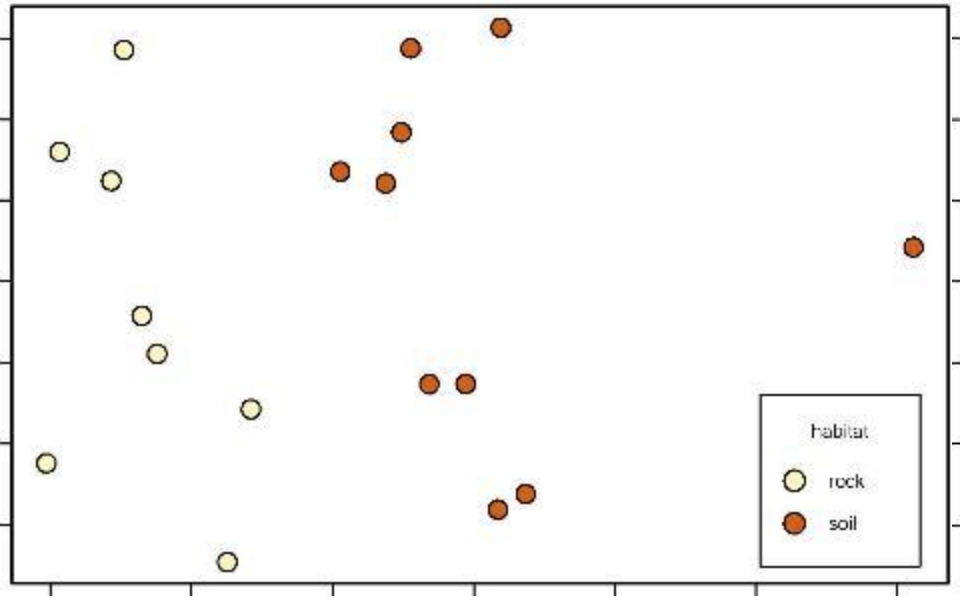
Results

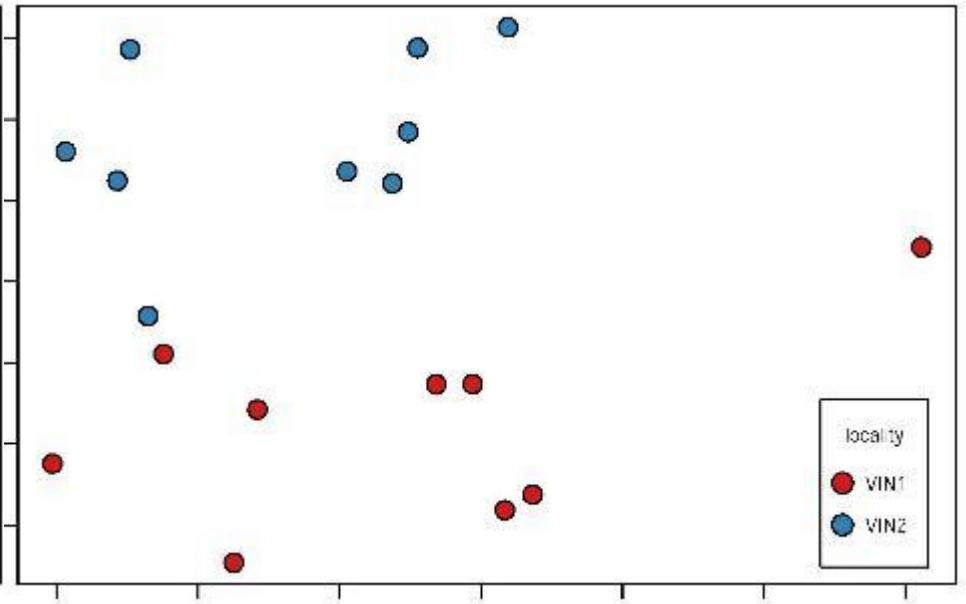
- cultivation: 162 cultures
- single cell PCR: 48 sequences
- DNA metabarcoding: 1,320,270 reads

- cultivation: 49 species
- single cell PCR: 20 species
- DNA metabarcoding: 147 species

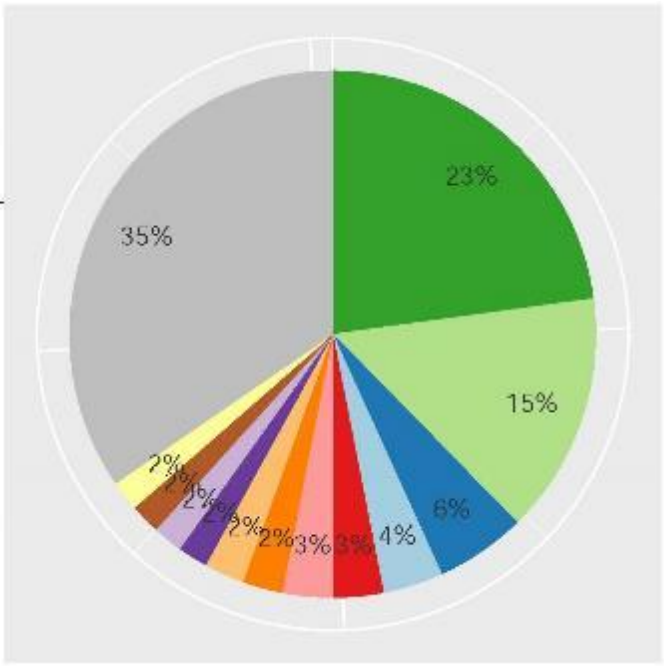


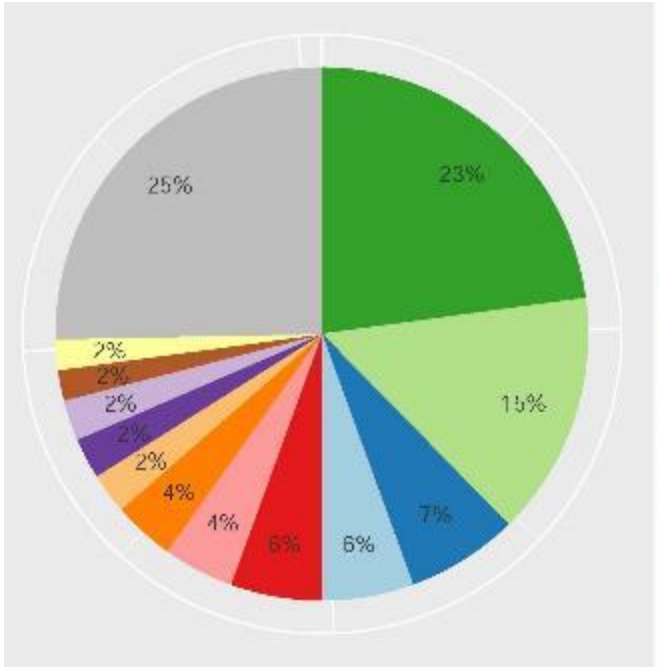
NMDS



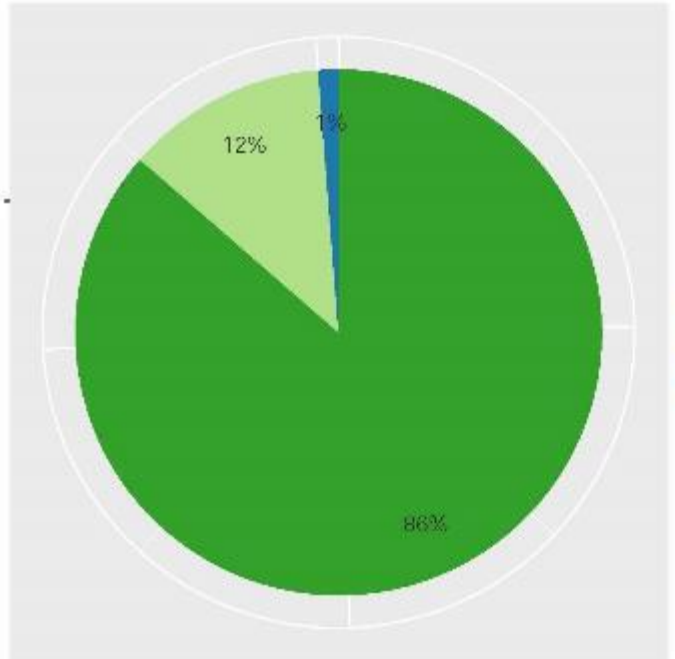


RESULTS - cultivation





Just three *Trebouxia* cultures



- cultivation: 49 species
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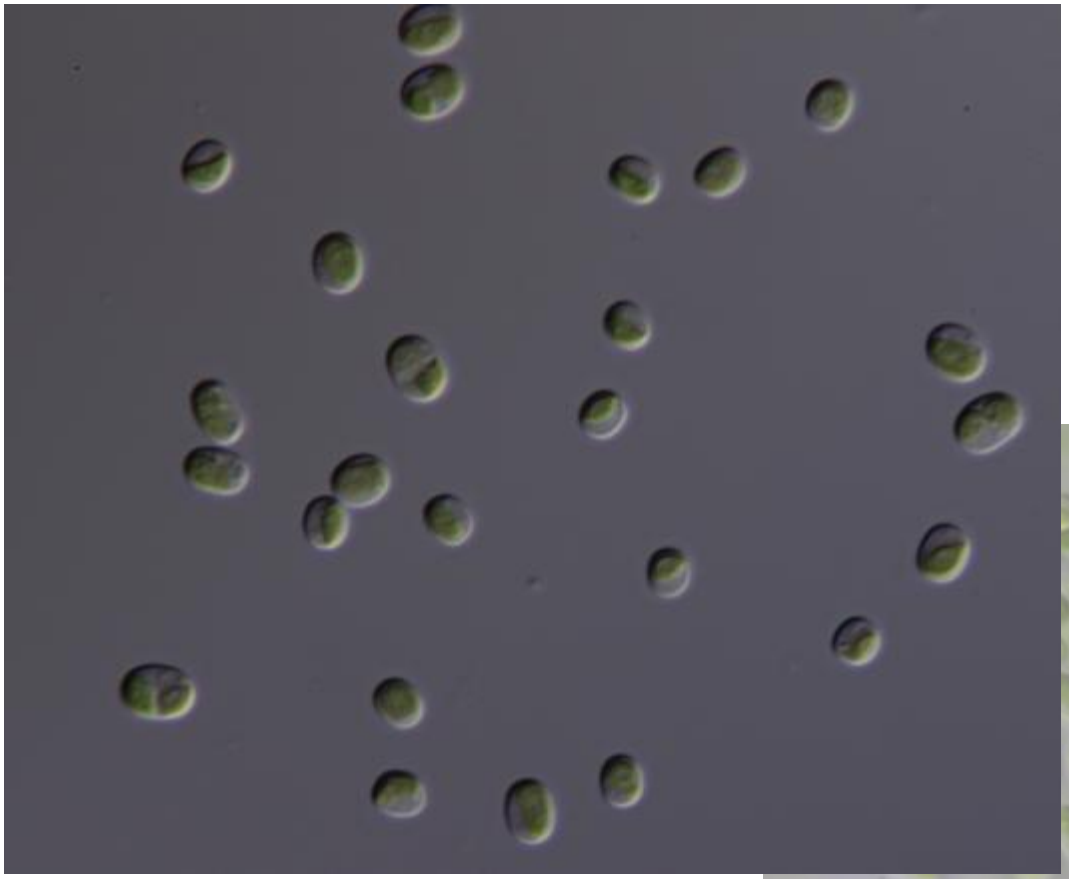
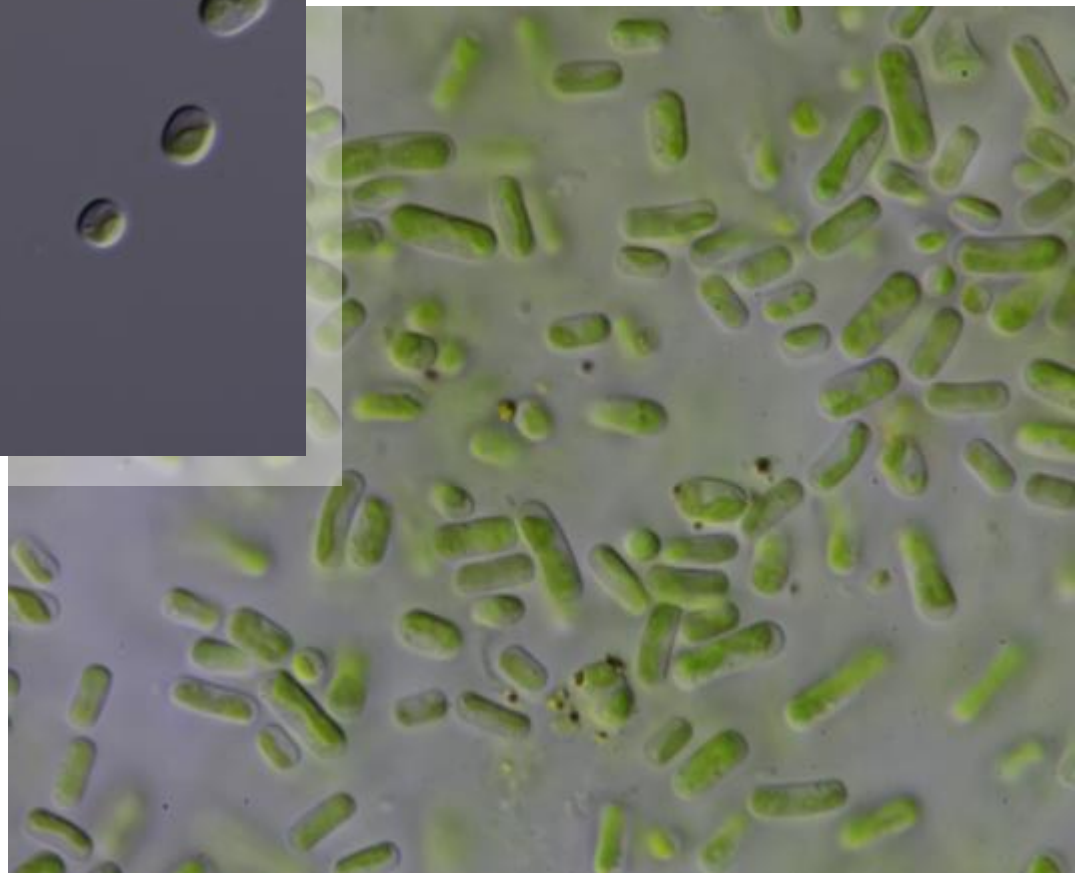
RESULTS - cultivation

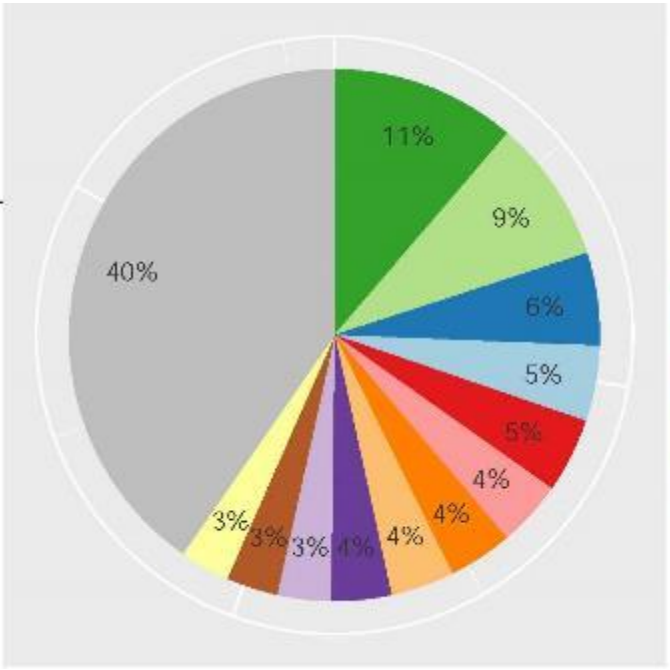


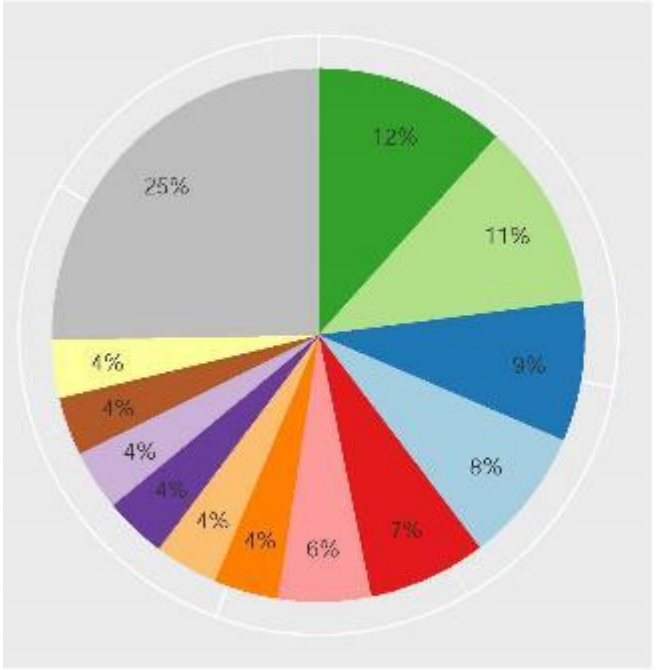
Diplosphaera

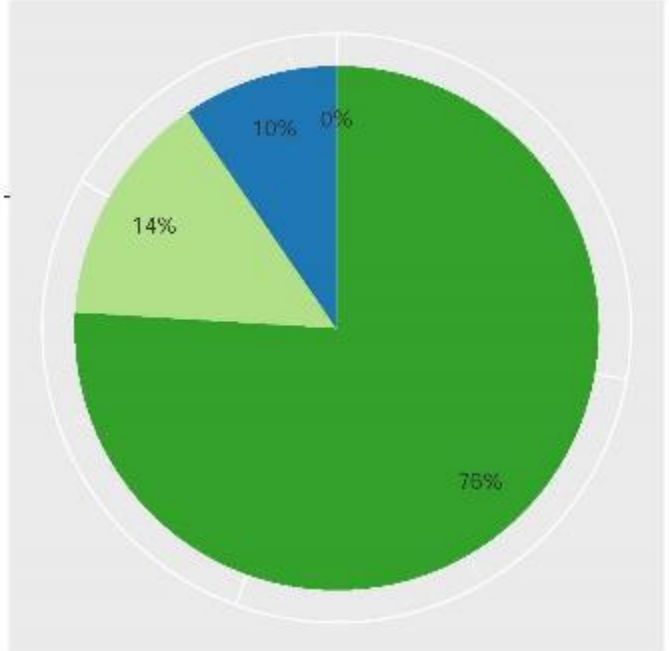
Deuterostichococcus



RESULTS – DNA metabarcoding







- cultivation: 49 species
- single cell PCR: 20 species
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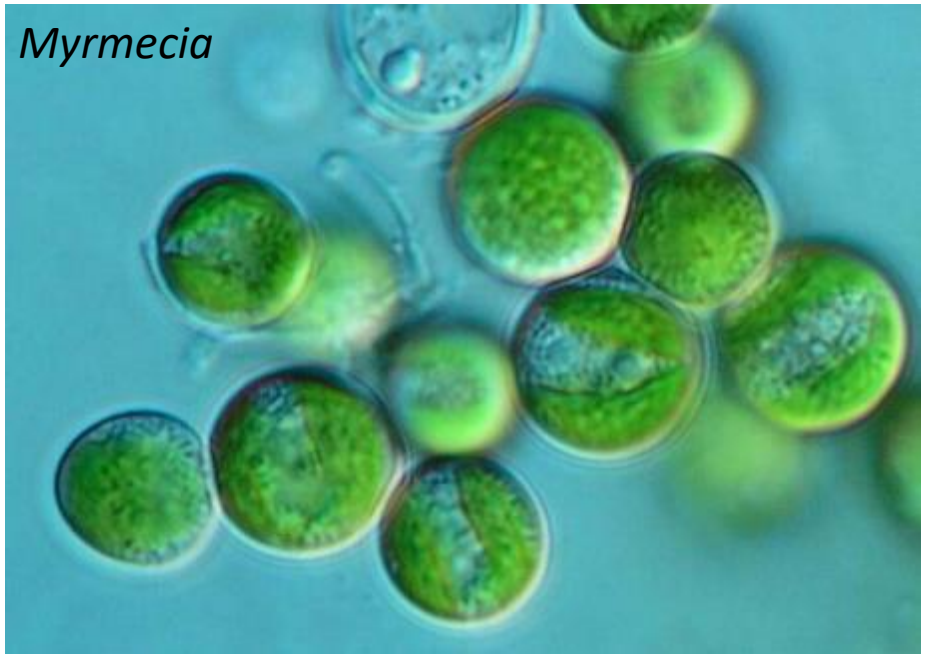
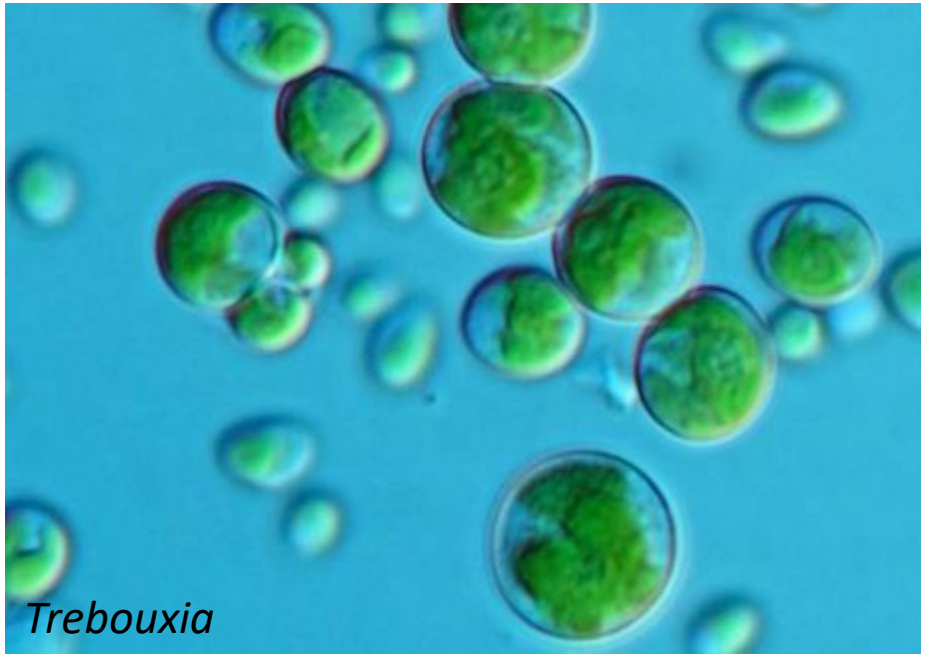
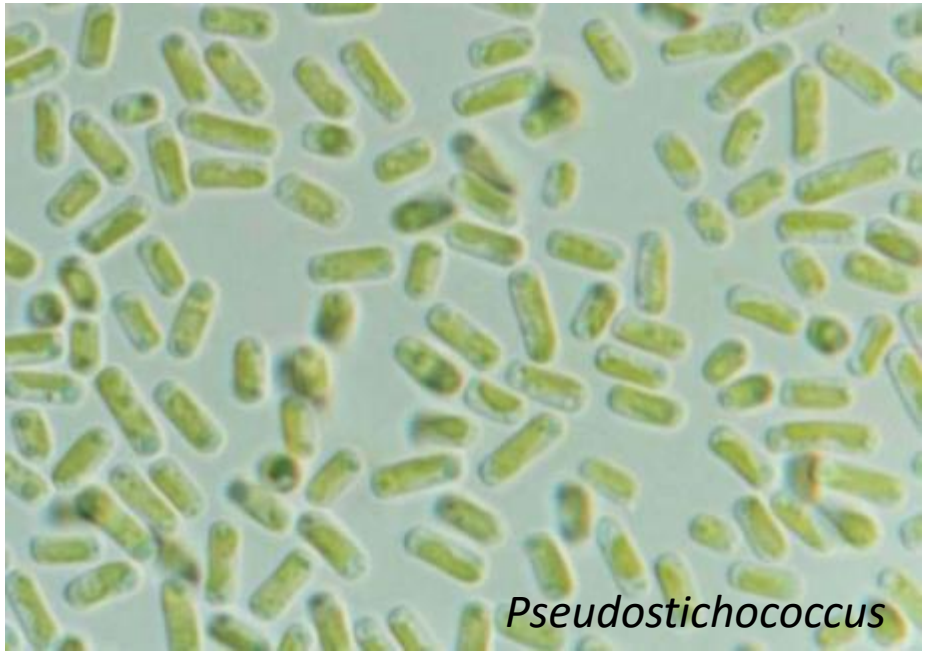
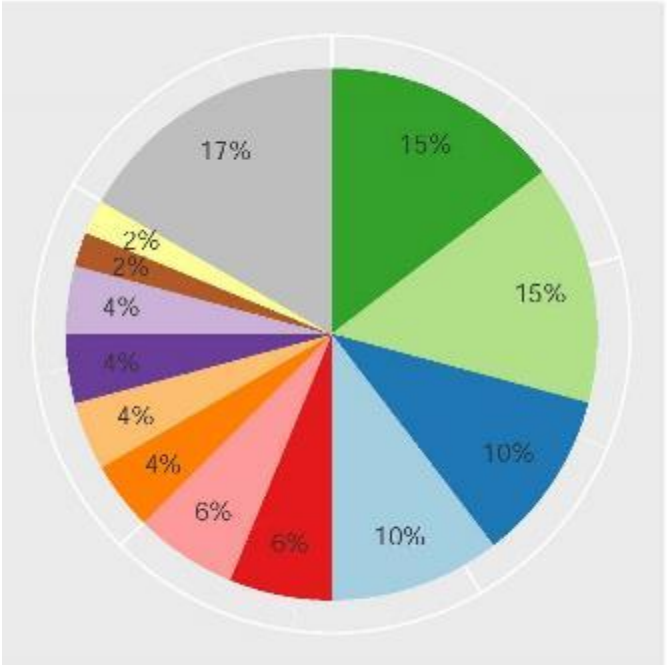
RESULTS – DNA metabarcoding

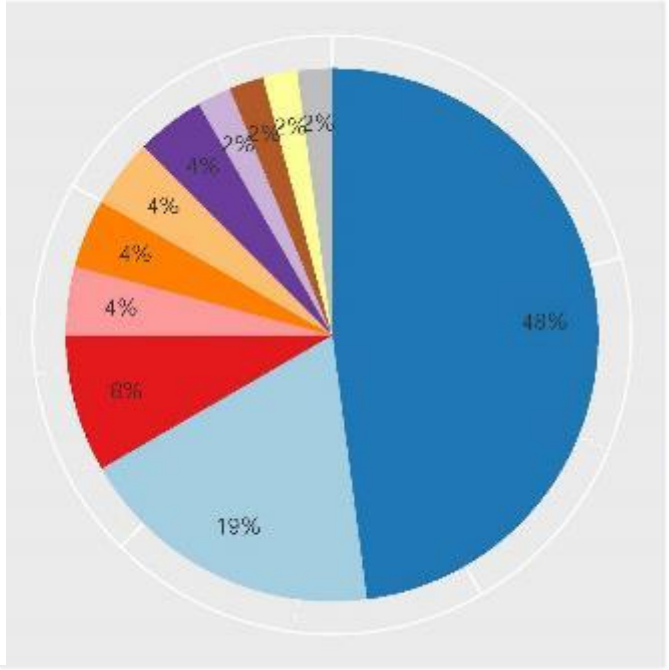


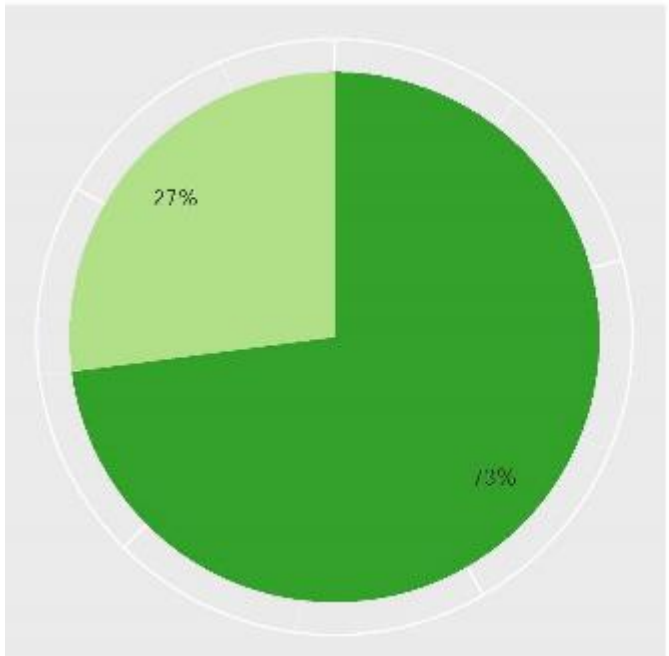




RESULTS – single-cell PCR







- cultivation: 49 species
- single cell PCR: 20 species
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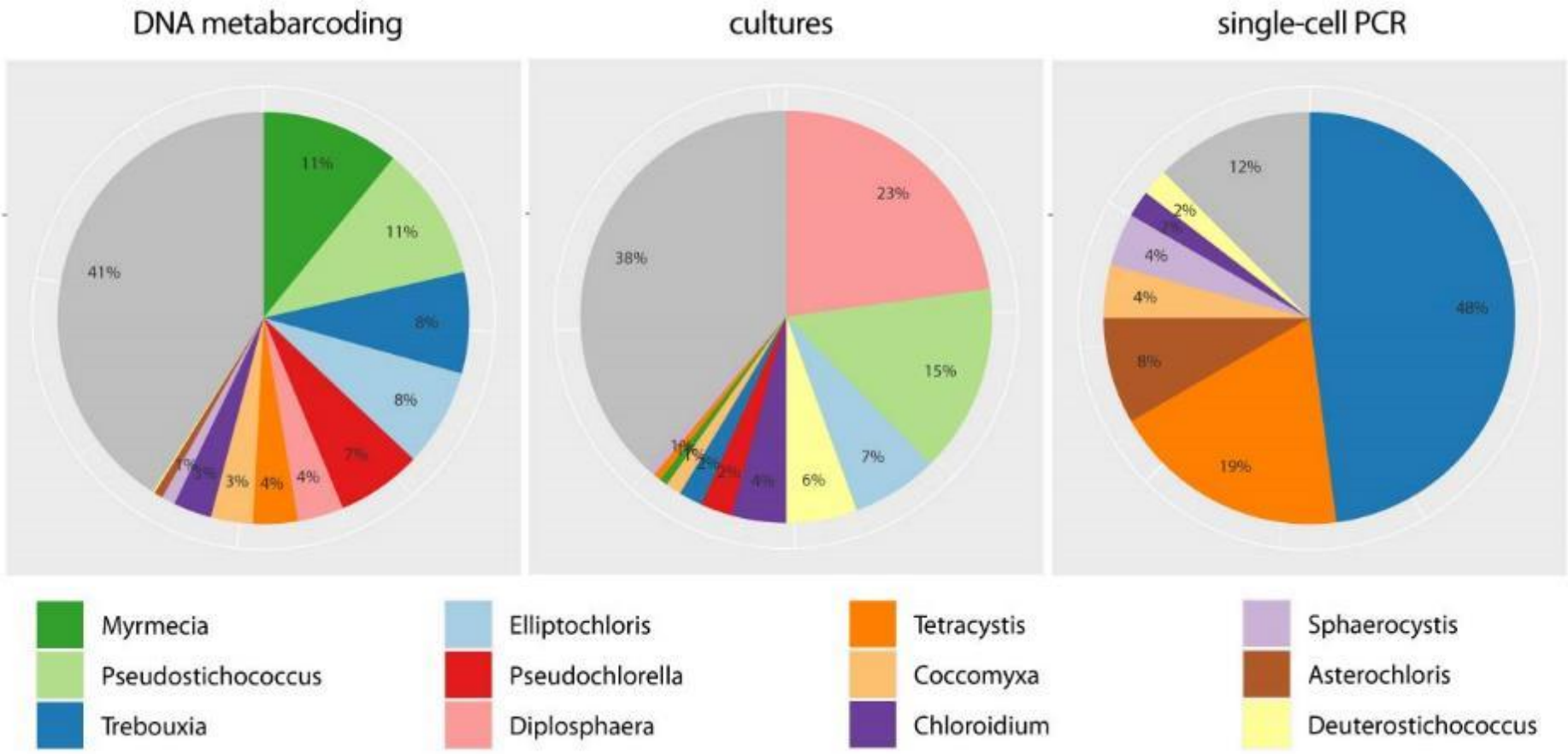
COMPARISON OF METHODS



- cultivation: 49 species
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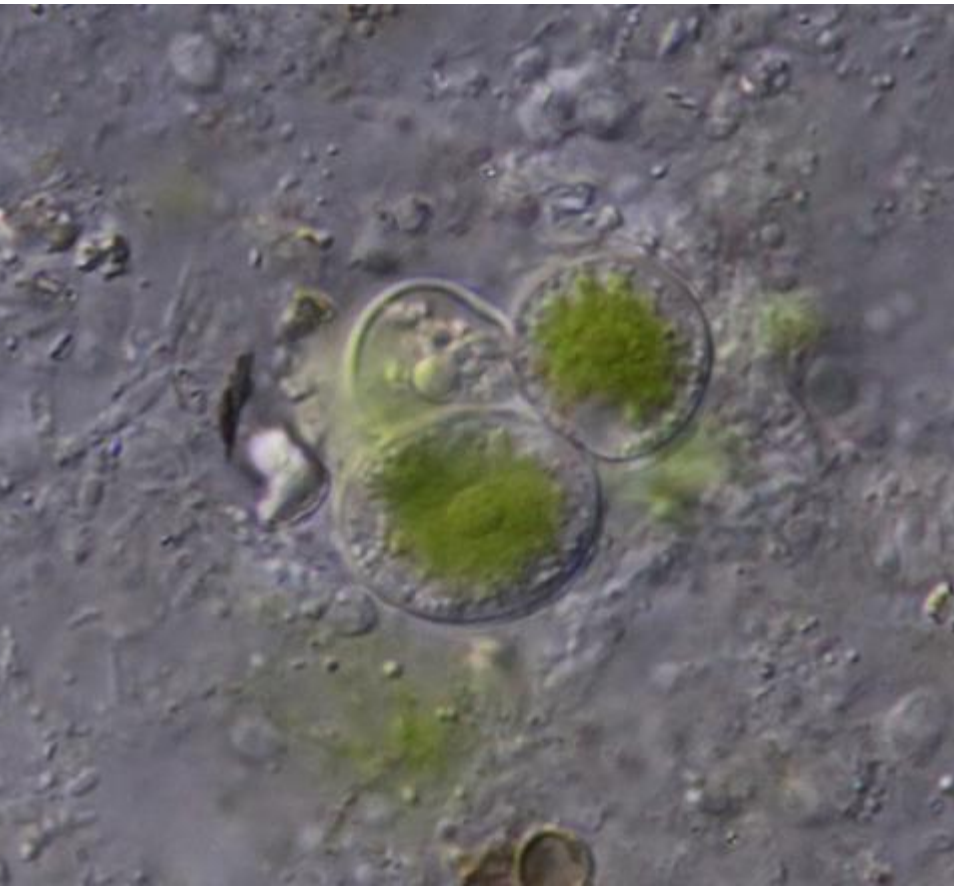
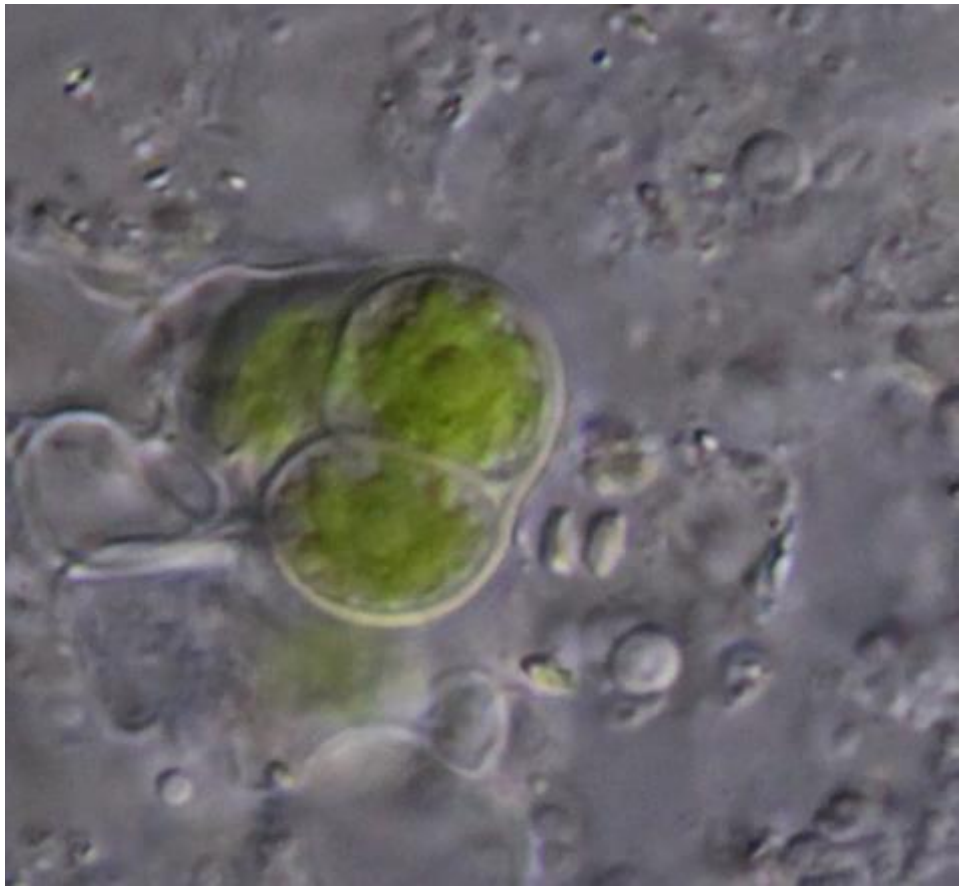
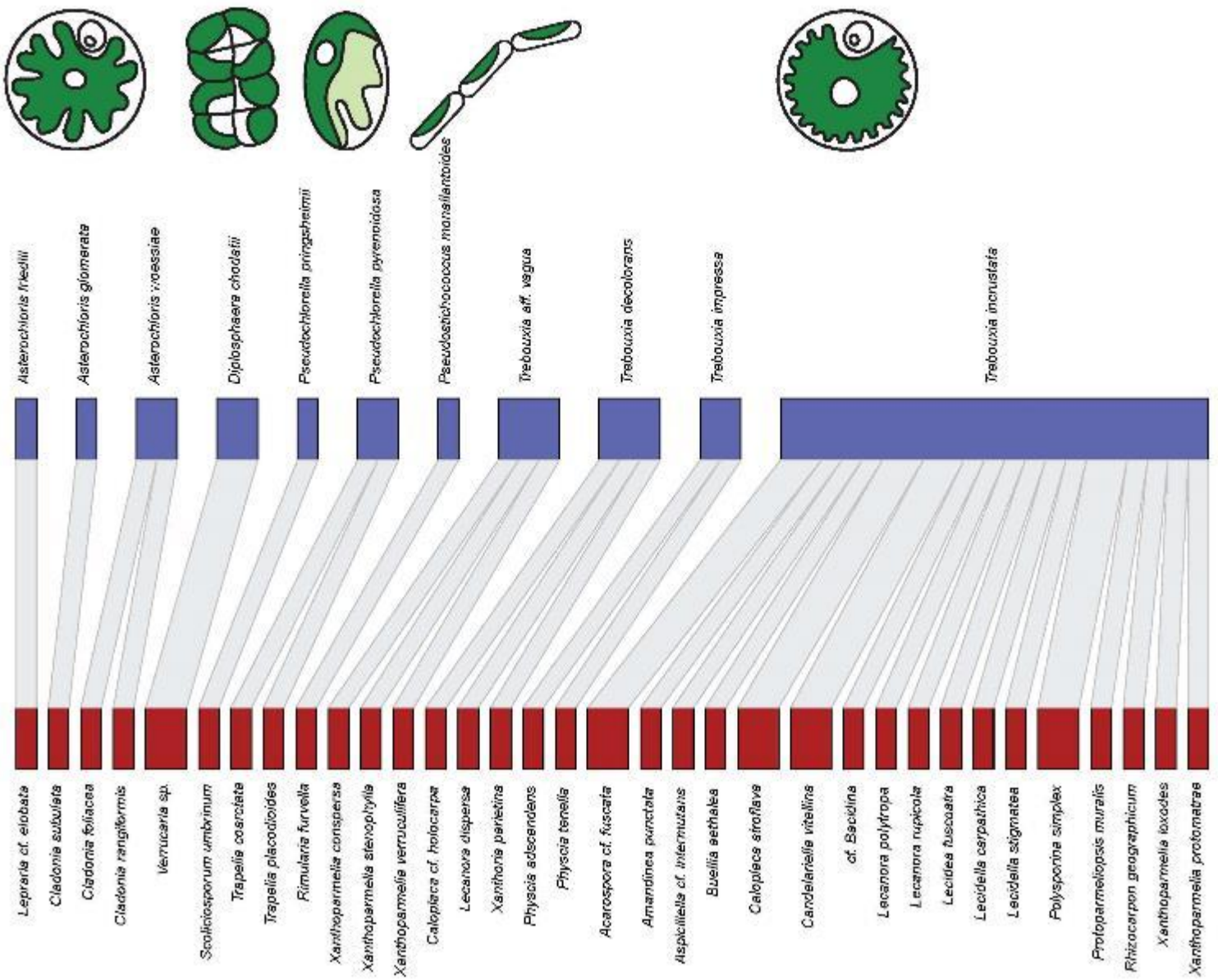
COMPARISON OF METHODS

- Lichen photobionts grow very slowly on inorganic media, where they have no chance to compete with fast growing algae such as *Diplosphaera* or *Stichococcus*.
- However, photobiont genera in fact represent the dominant component of soil green algae.
- Lichen photobionts are free-living.

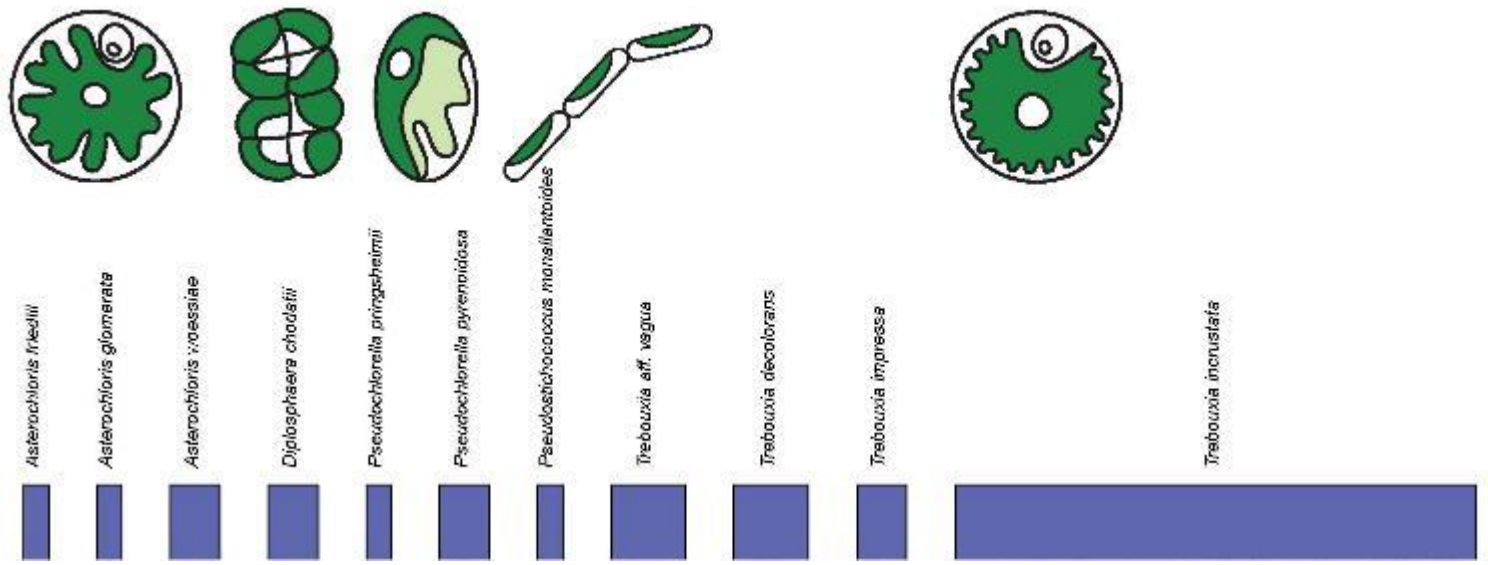




Symbiotic interactions



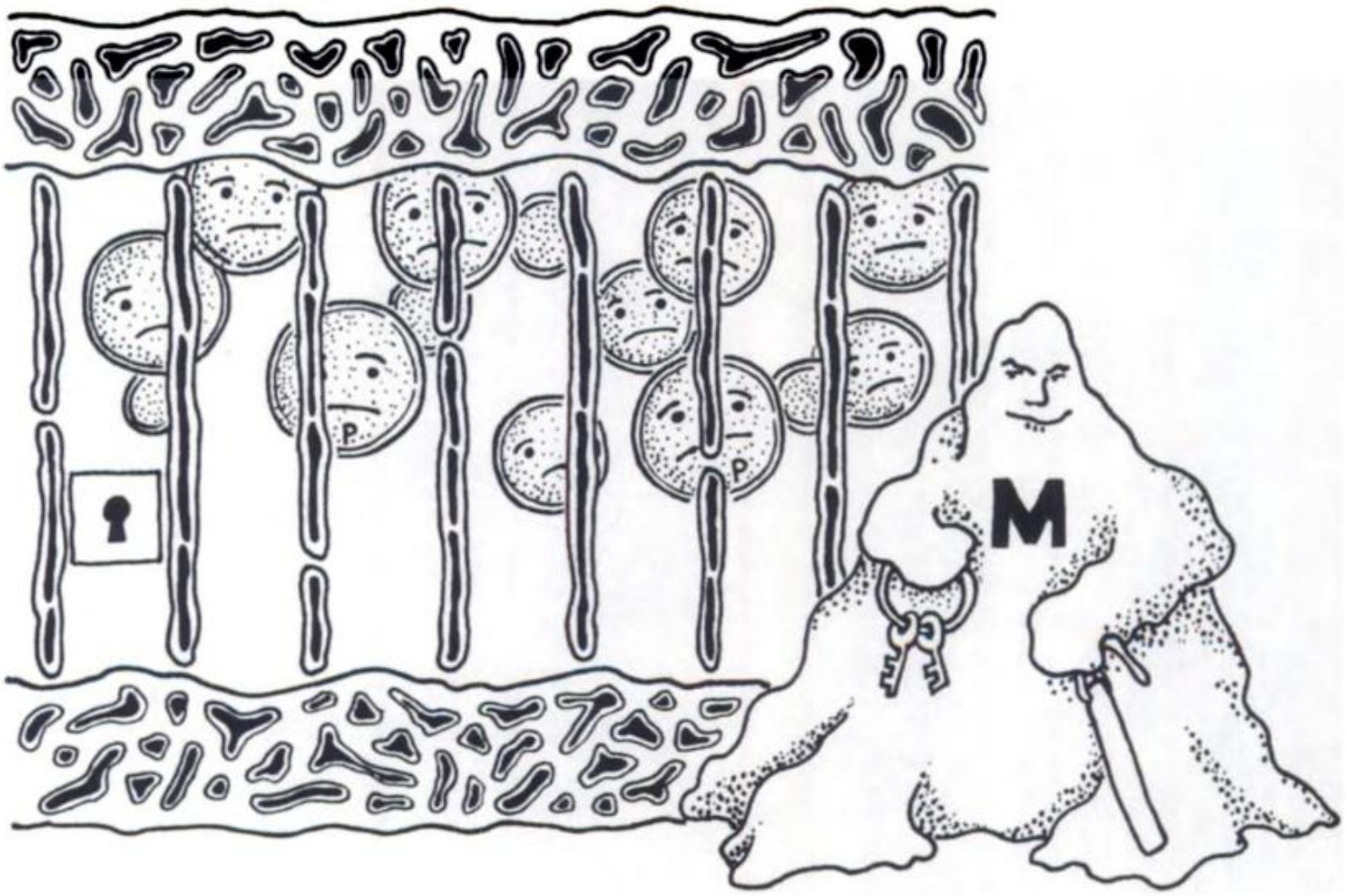
Symbiotic interactions



species	endosymbionts	DNA metabarcoding	cultures	single-cell PCR
Trebouxia incrustata	**21**	23199		7
Trebouxia decolorans	**3**	1875		1
Trebouxia aff. vagua	**3**	2319		
Diplosphaera chodatii	**2**	21849	37	
Peudochlorella pyrenoidosa	**2**	22991	3	
Trebouxia impressa	**2**	2839		5
Asterochloris woessiae	**2**			
Asterochloris friedlii	**1**			
Peudochlorella pringsheimii	**1**	12507	1	
Pseudostichococcus monallantoides	**1**	102457	24	
Asterochloris glomerata	**1**	1676		

The lichen alga *Trebouxia:* does it occur free-living?*

VERNON AHMADJIAN



Ahmadjian (1993)

CHARLES UNIVERSITY

Primus Research Programme