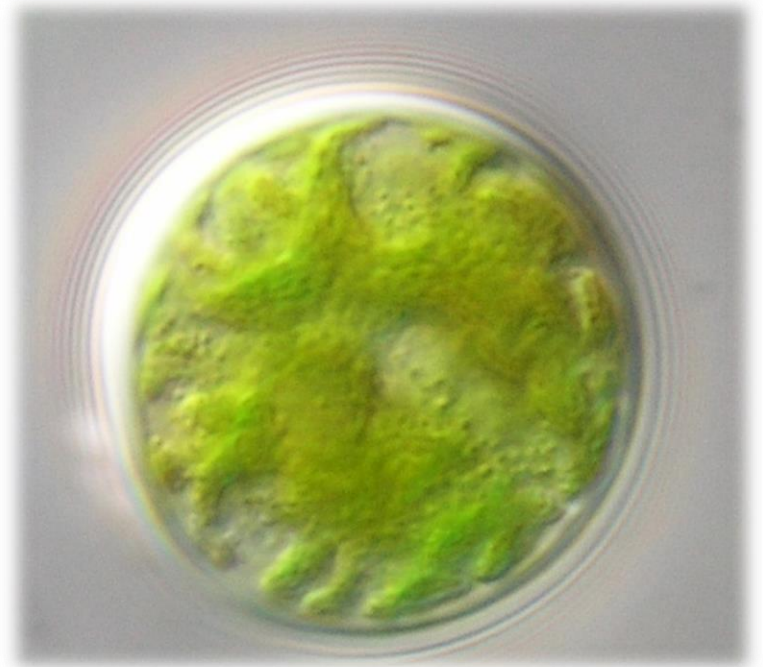
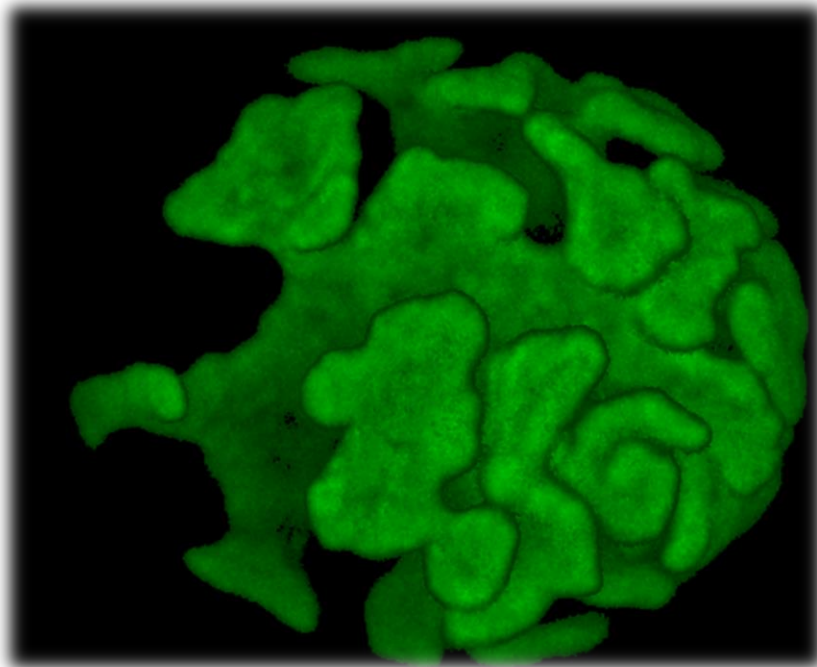


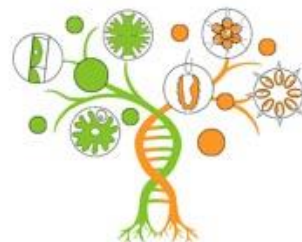
# The *Primus* project - towards understanding the nature of lichen symbiosis



Pavel Škaloud, Zuzana Vaiglová, Jana Steinová, Ivana Černajová, Patricia Moya, Helena Bestová, Ondřej Peksa



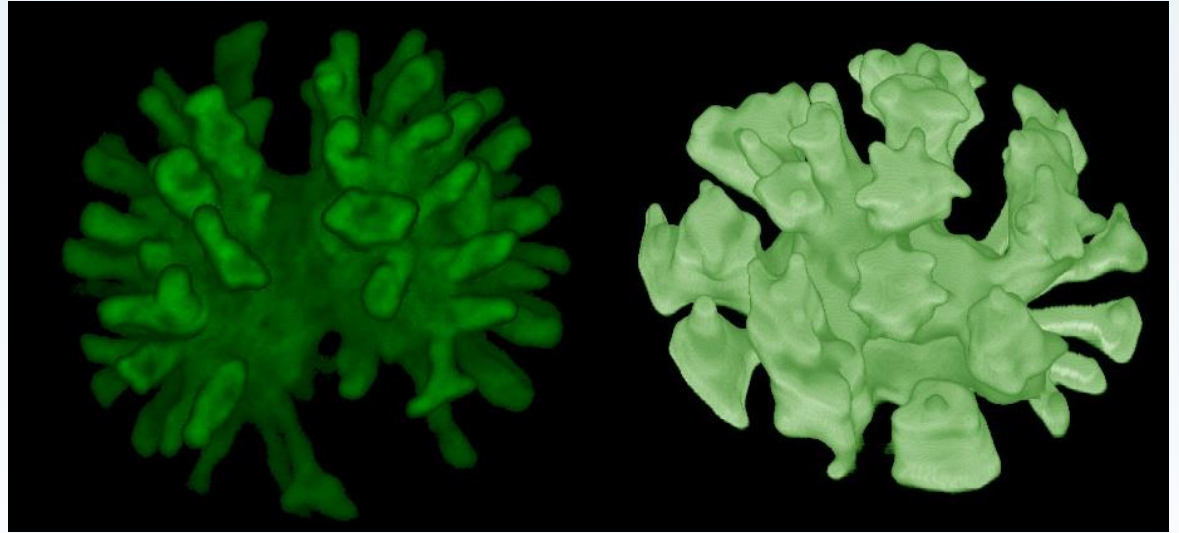
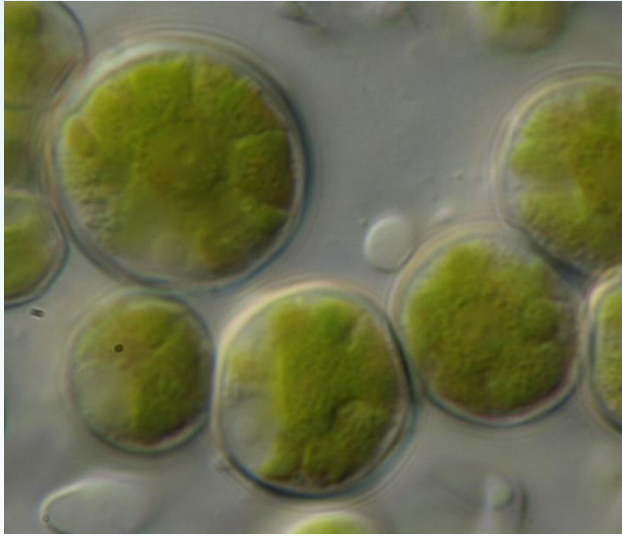
FACULTY OF SCIENCE  
Charles University



*Algal speciation & evolution lab*

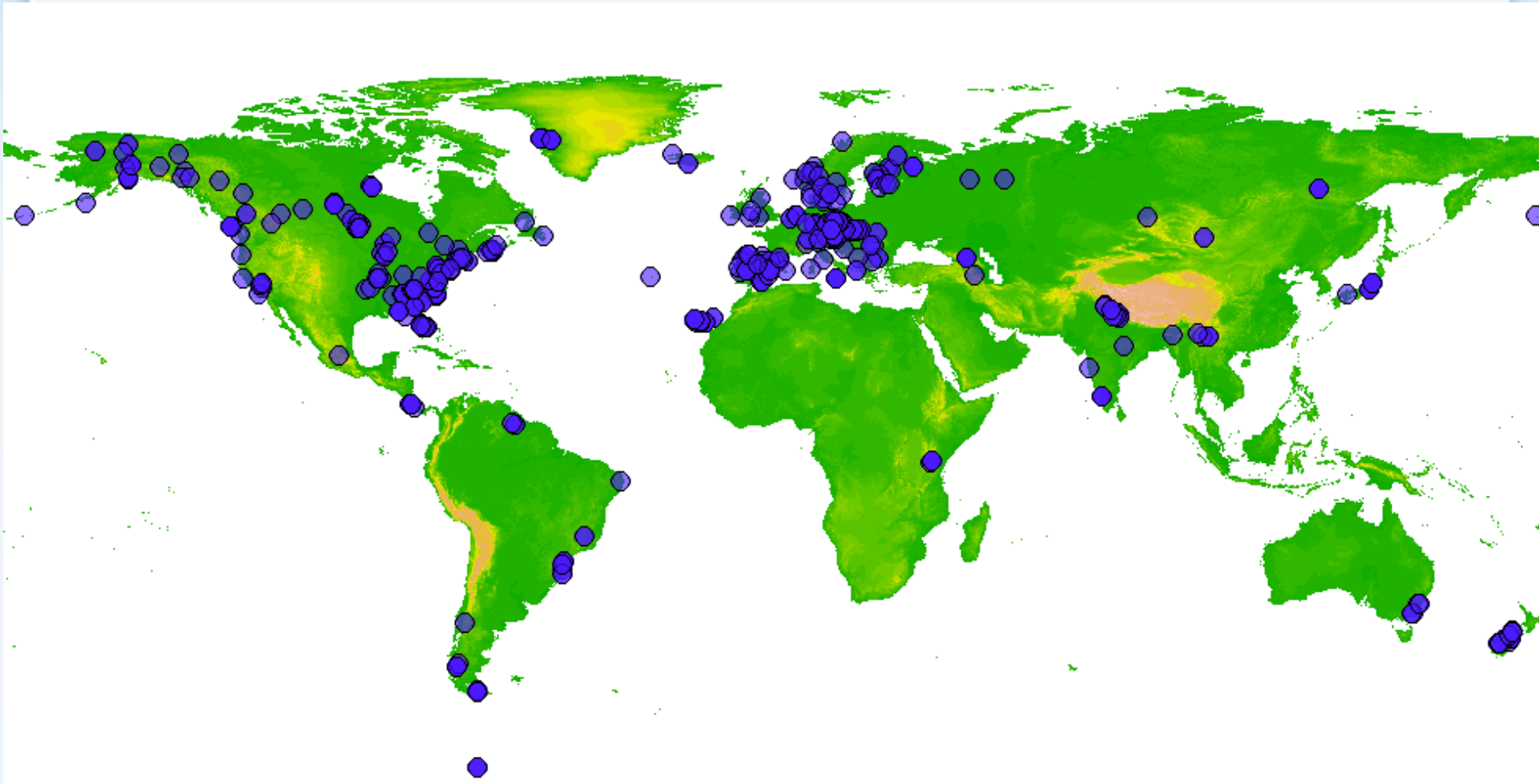
# *Asterochloris*

- *Asterochloris* – one of the most common lichen symbionts



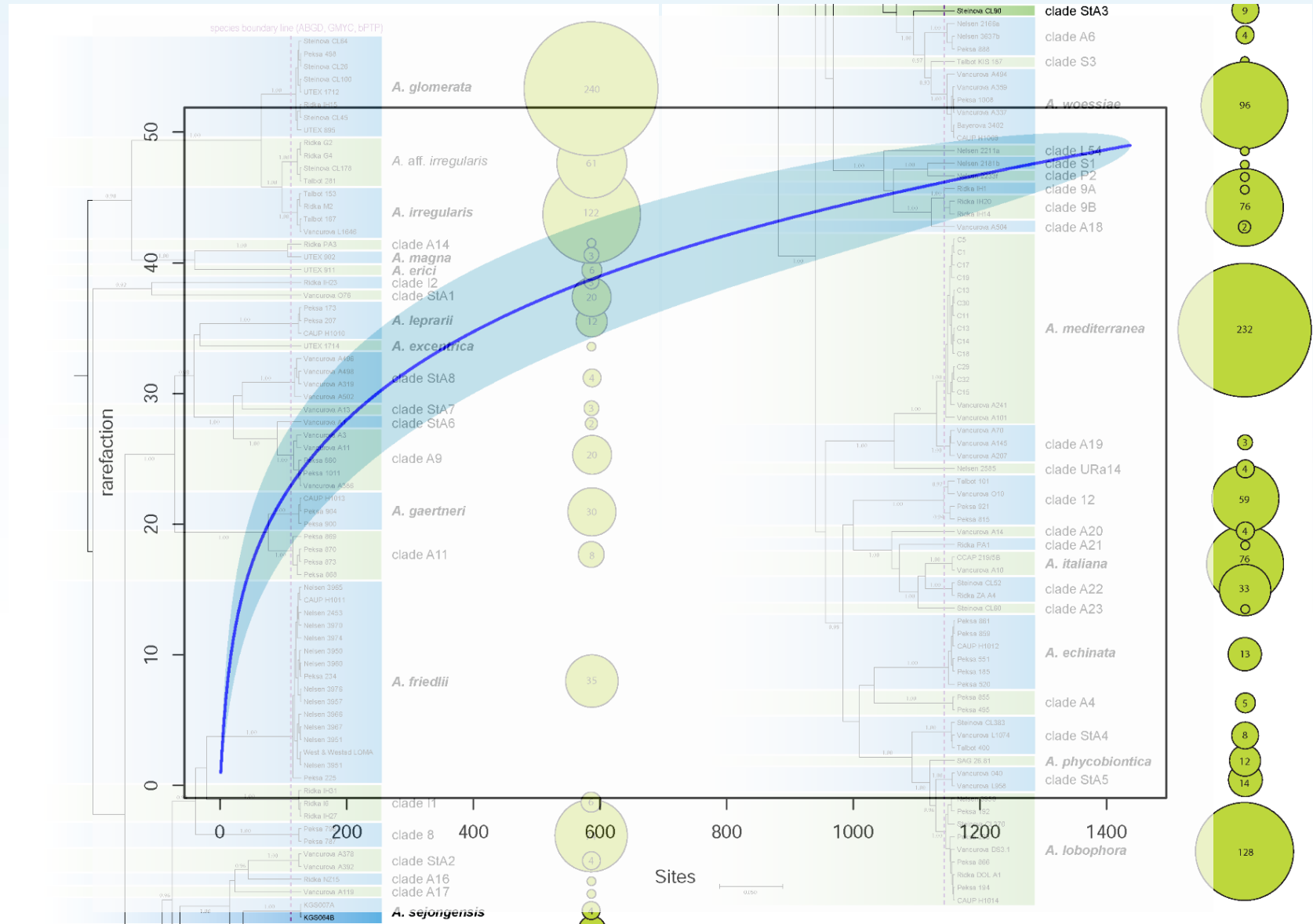
# *Asterochloris* – any patterns in diversity?

- At the moment, more than 1500 occurrence data available
  - Algal ITS rDNA + actin sequences
  - Substrate data, mycobiont identity (ITS rDNA), climatic data



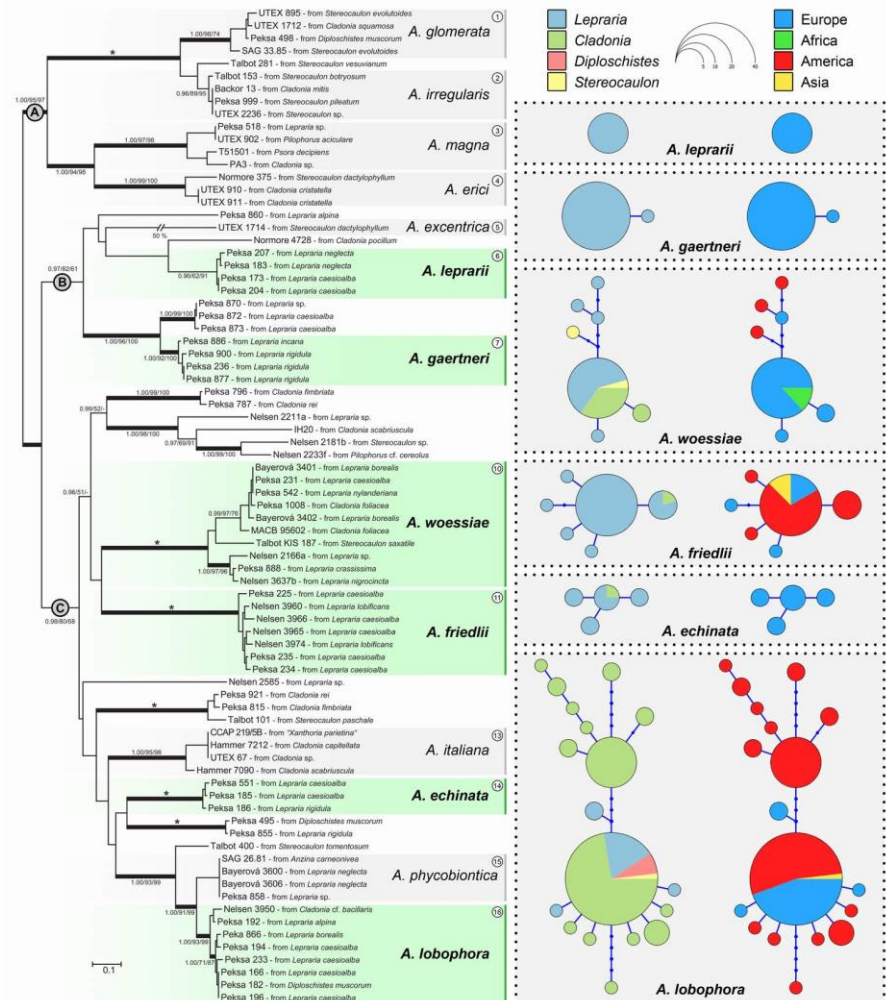
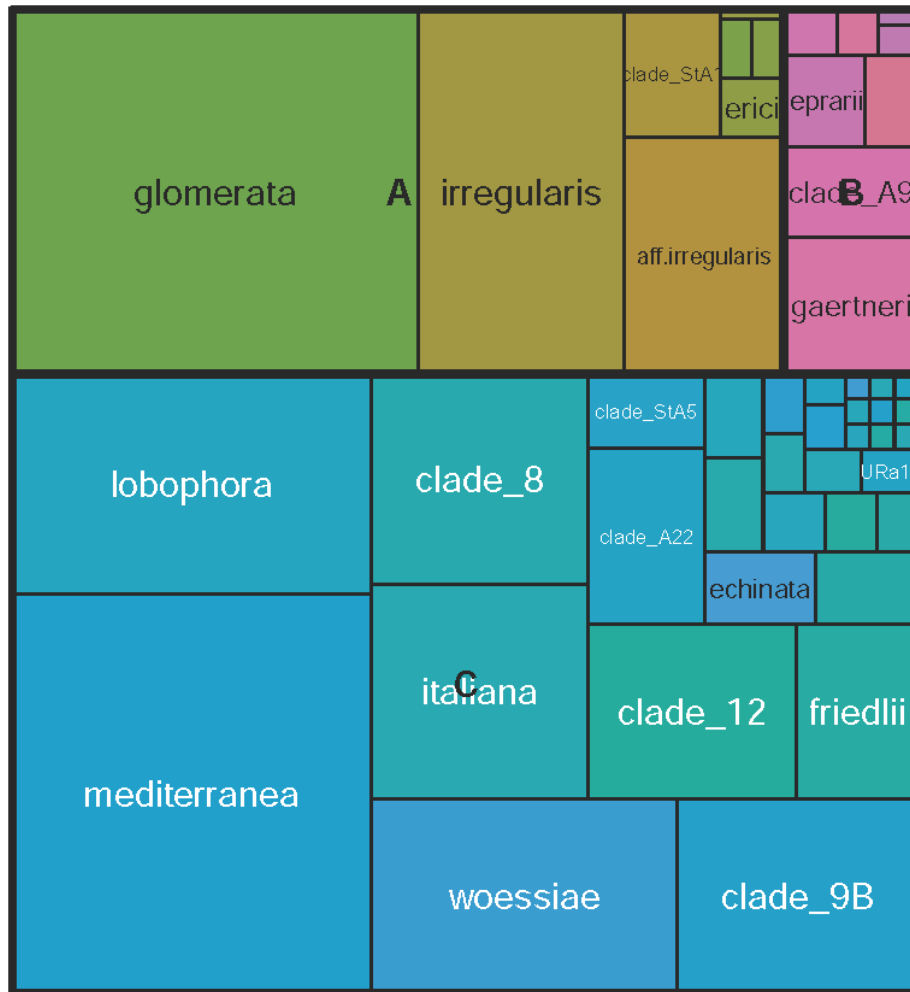
# Asterochloris diversity

- 48 species-level lineages (ABGD, GMYC, bPTP delimitation)

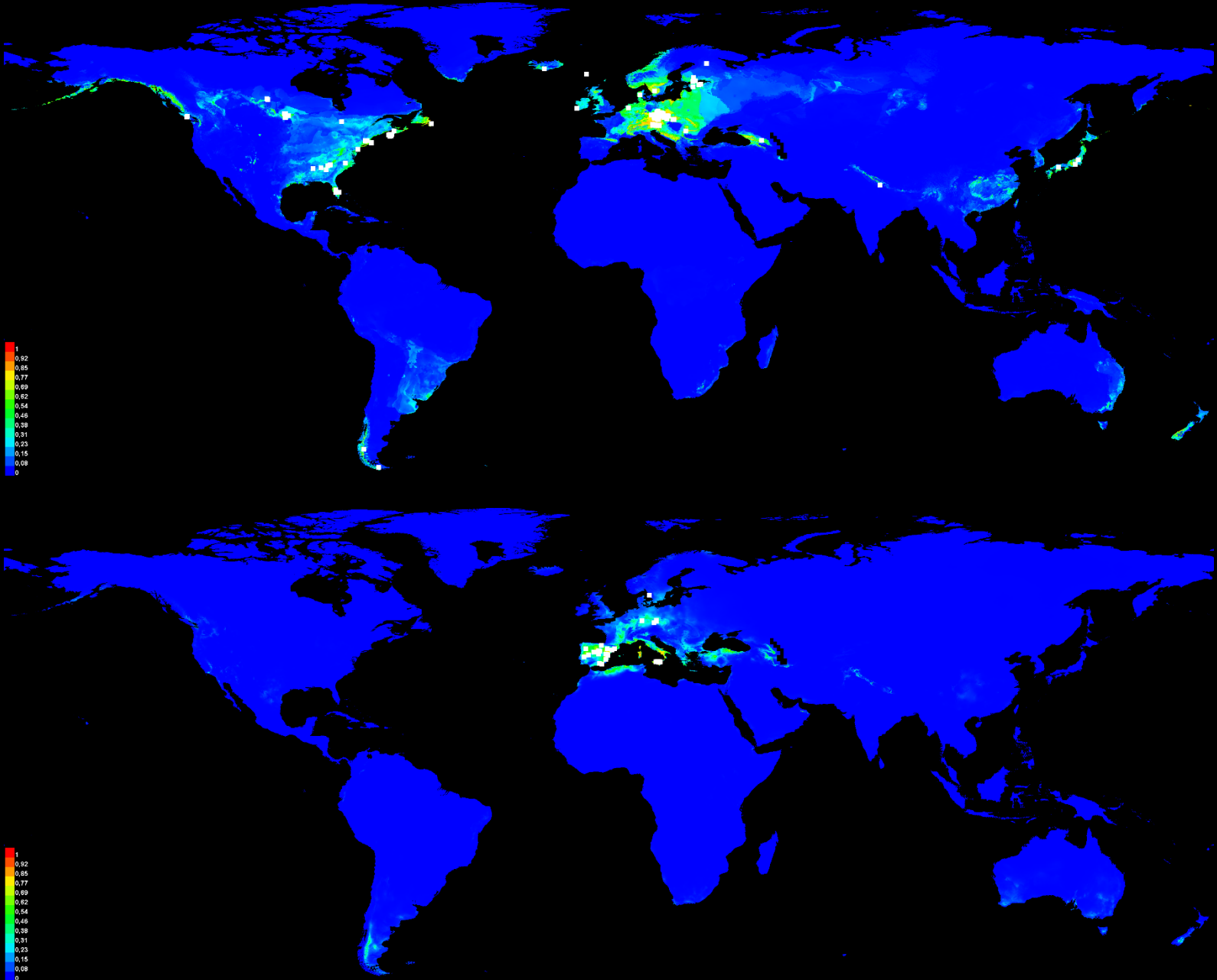


# Asterochloris diversity

- 48 species-level lineages (ABGD, GMYC, bPTP delimitation)
- 93.6 % of all sequences belong to the 19 most common species
- *A. glomerata*, *A. mediterranea* as the most frequently found photobionts

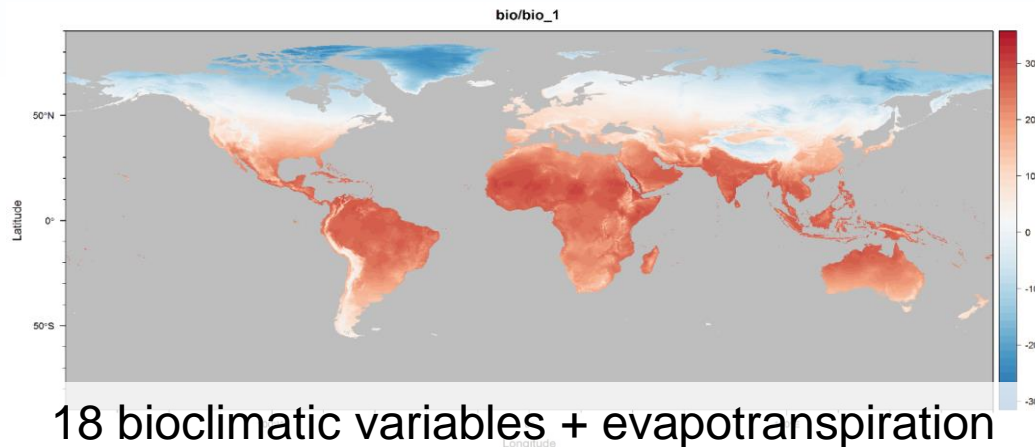
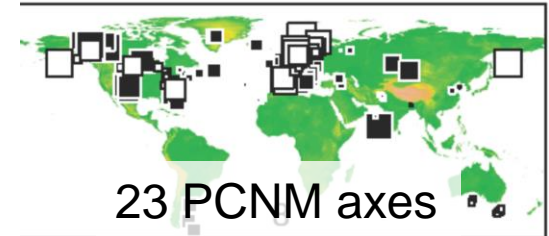
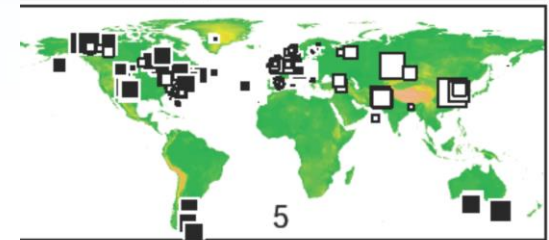
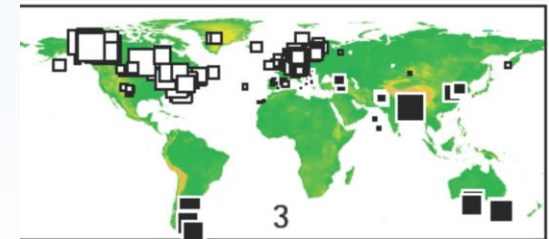
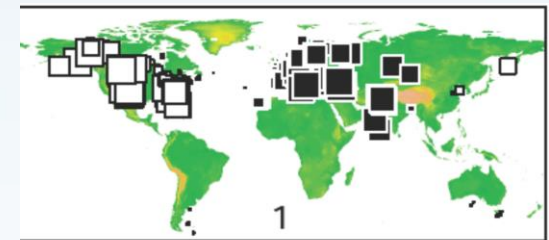
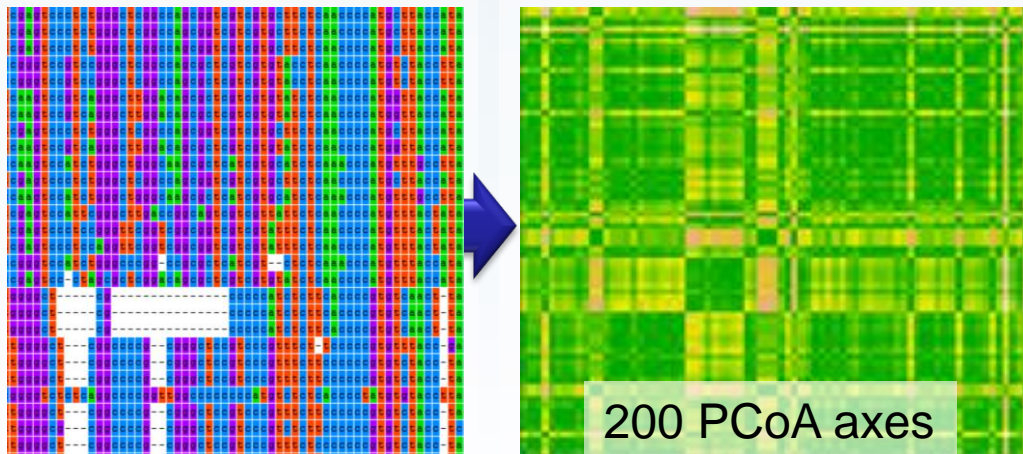


- Contrasting biogeographic patterns of *A. glomerata* and *A. mediterranea*



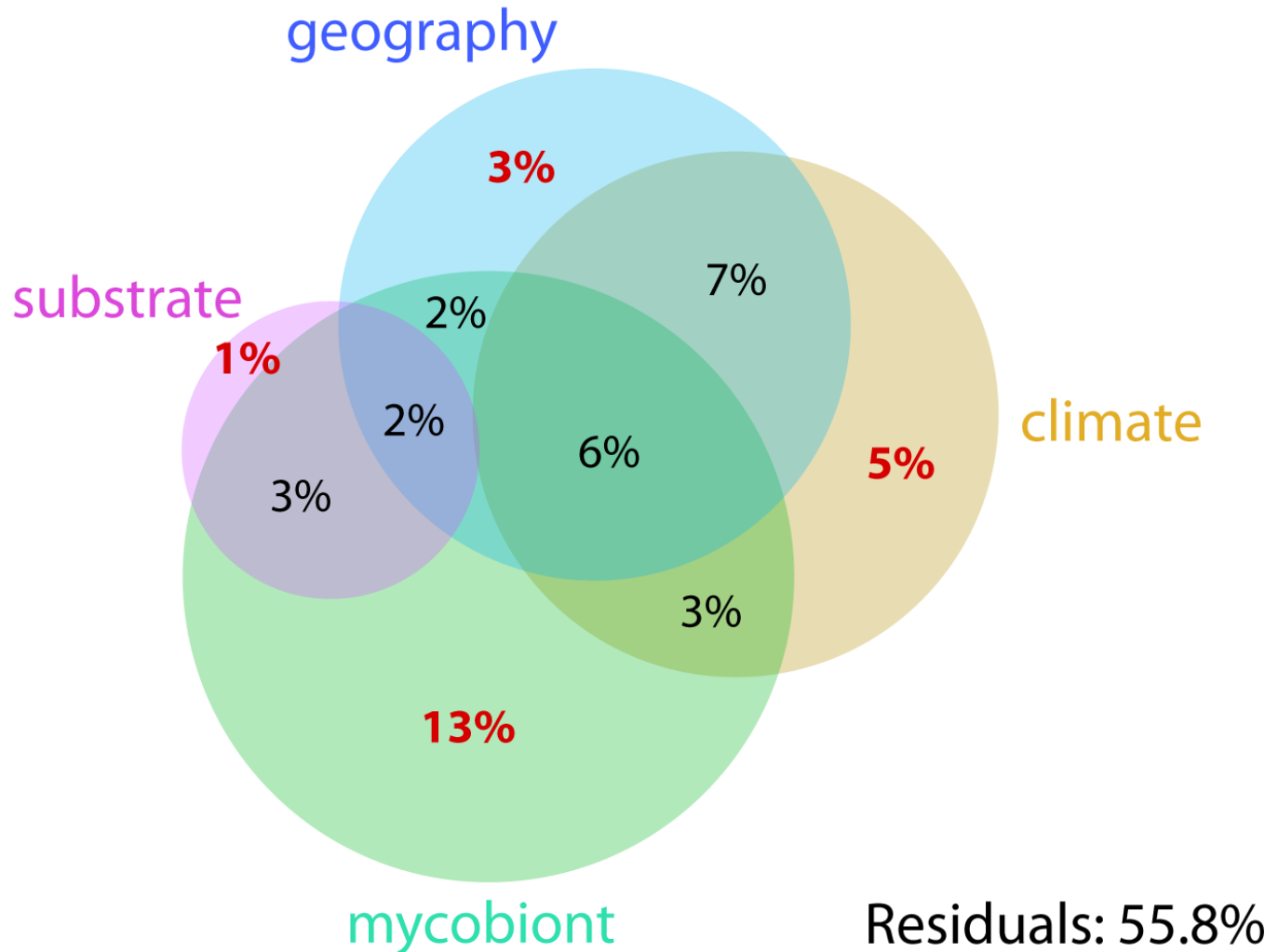
# Variation partitioning

- *Which are the main factors structuring the extant diversity?*
- Response variable: algal diversity (200 PCoA axes)
- Explanatory variables: geography, mycobiont, climate, substrate



# Variation partitioning

- Which are the main factors structuring the extant diversity?



$P$  geography  
= **0.001**

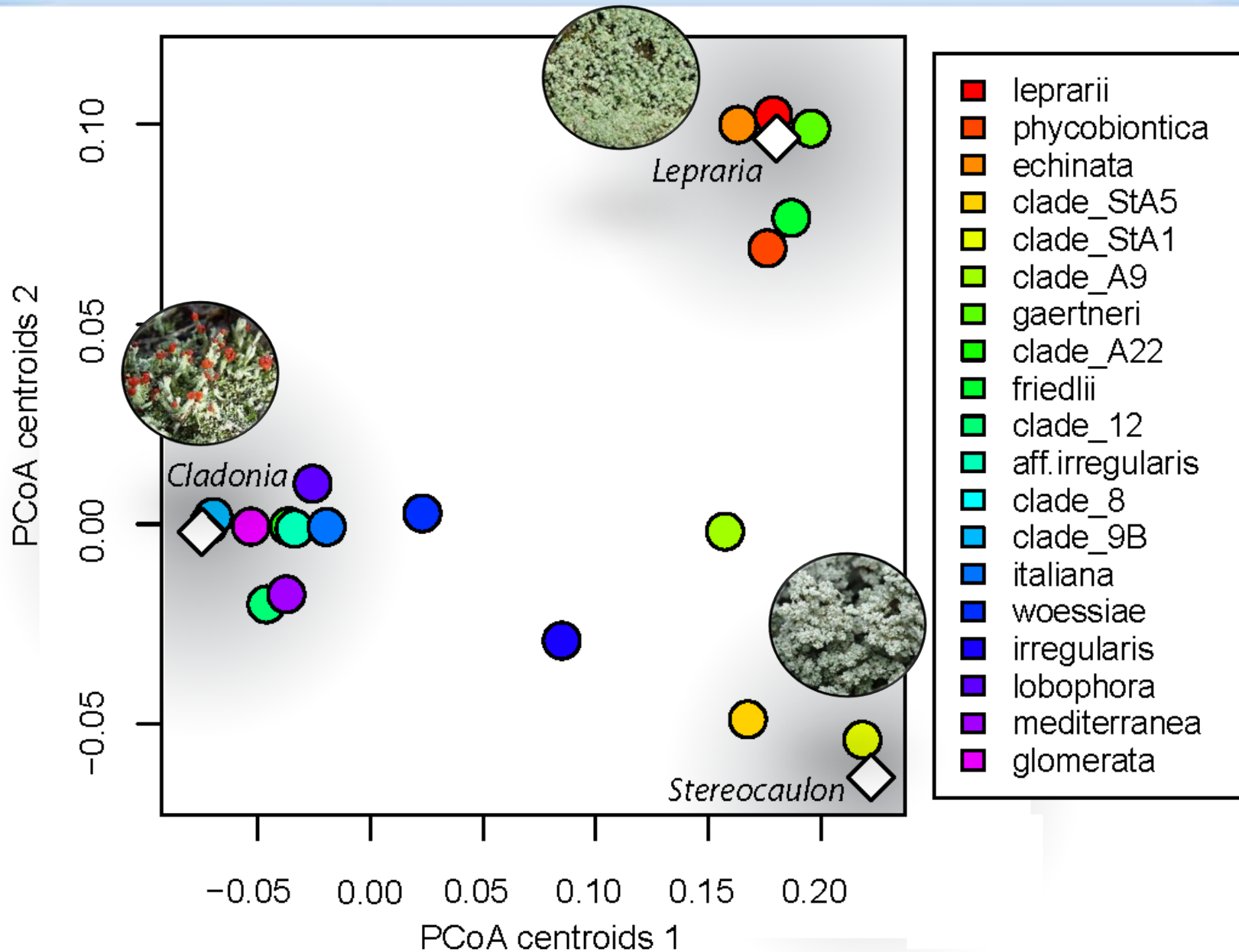
$P$  climate  
= **0.001**

$P$  mycobiont  
= **0.001**

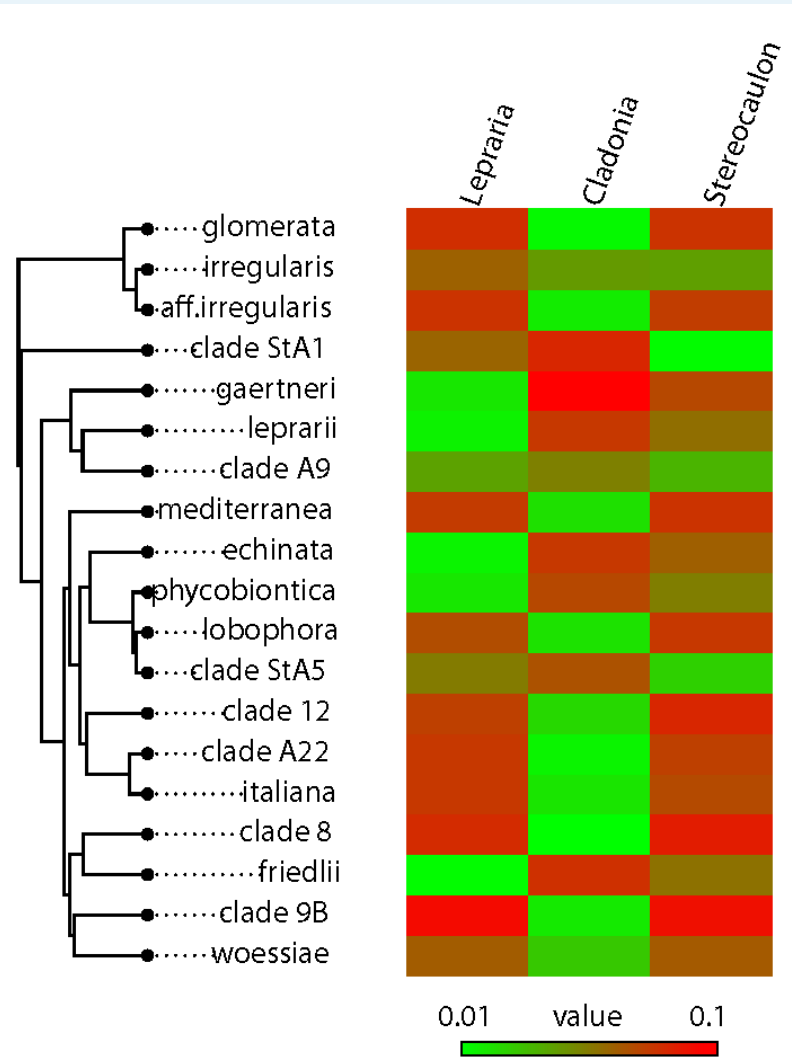
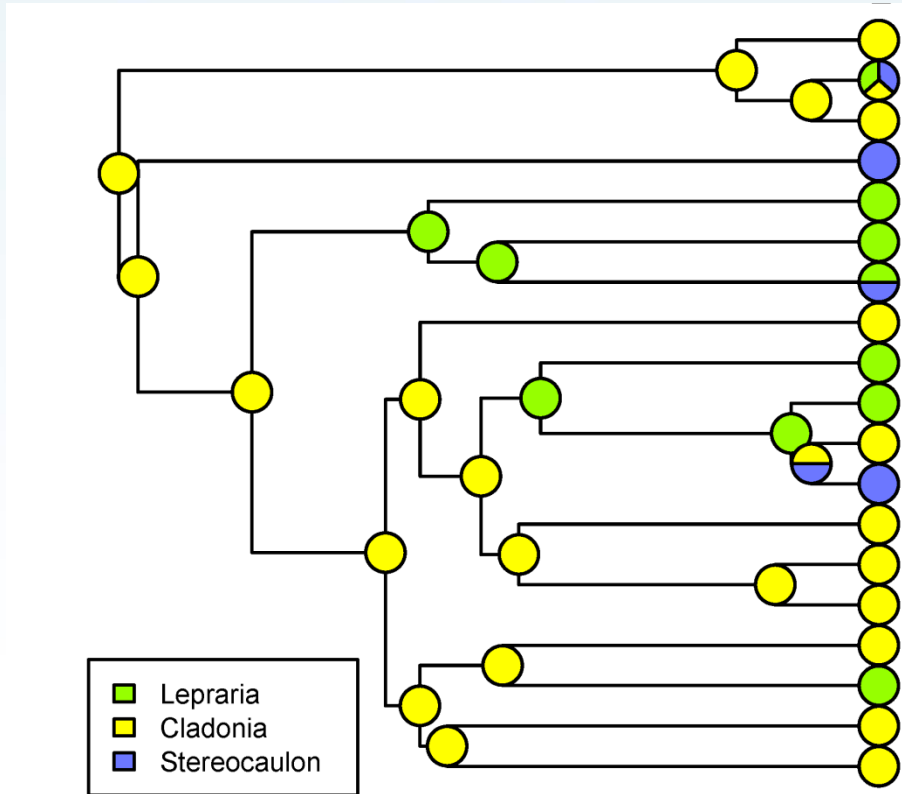
$P$  substrate  
= **0.002**



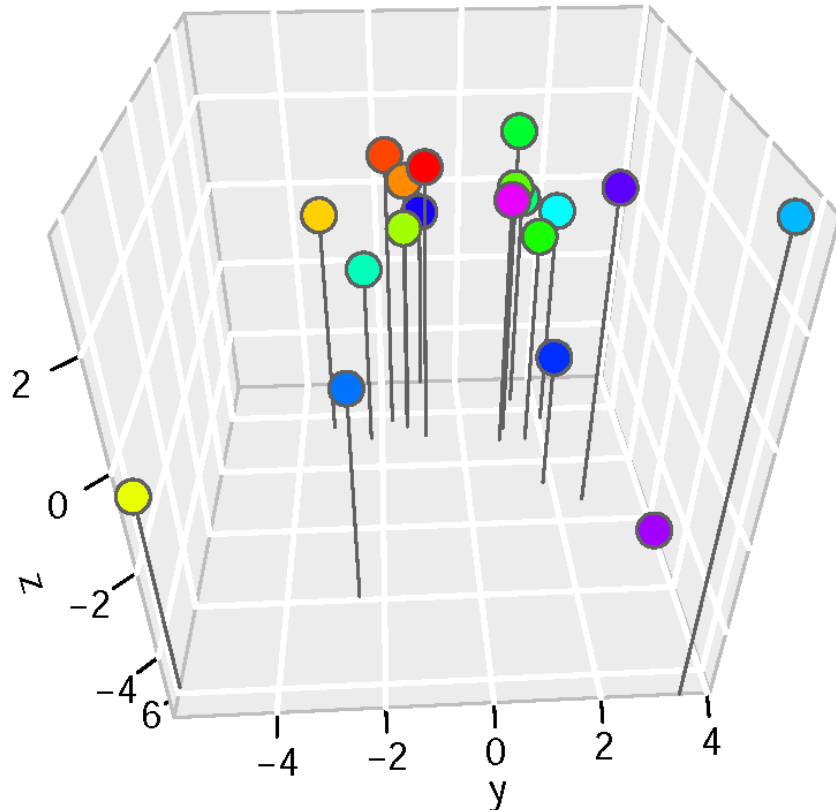
# Mycobiont selectivity



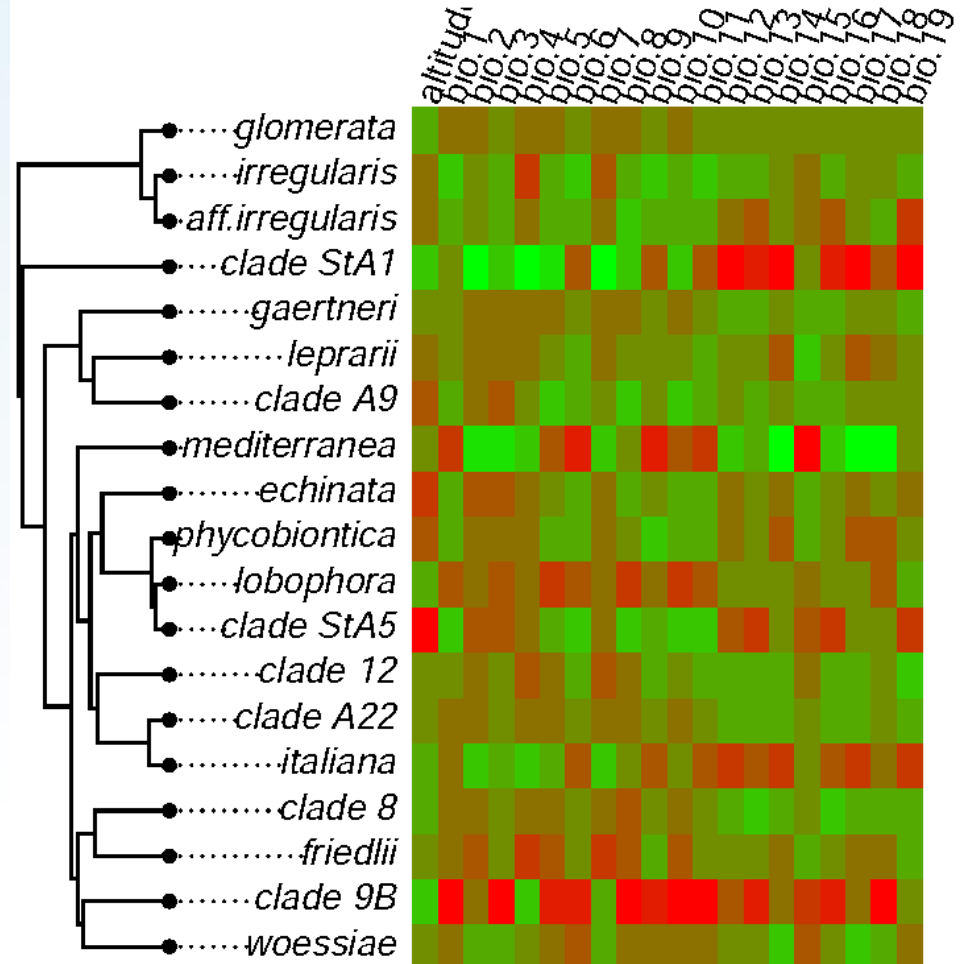
# Mycobiont selectivity



# Climate



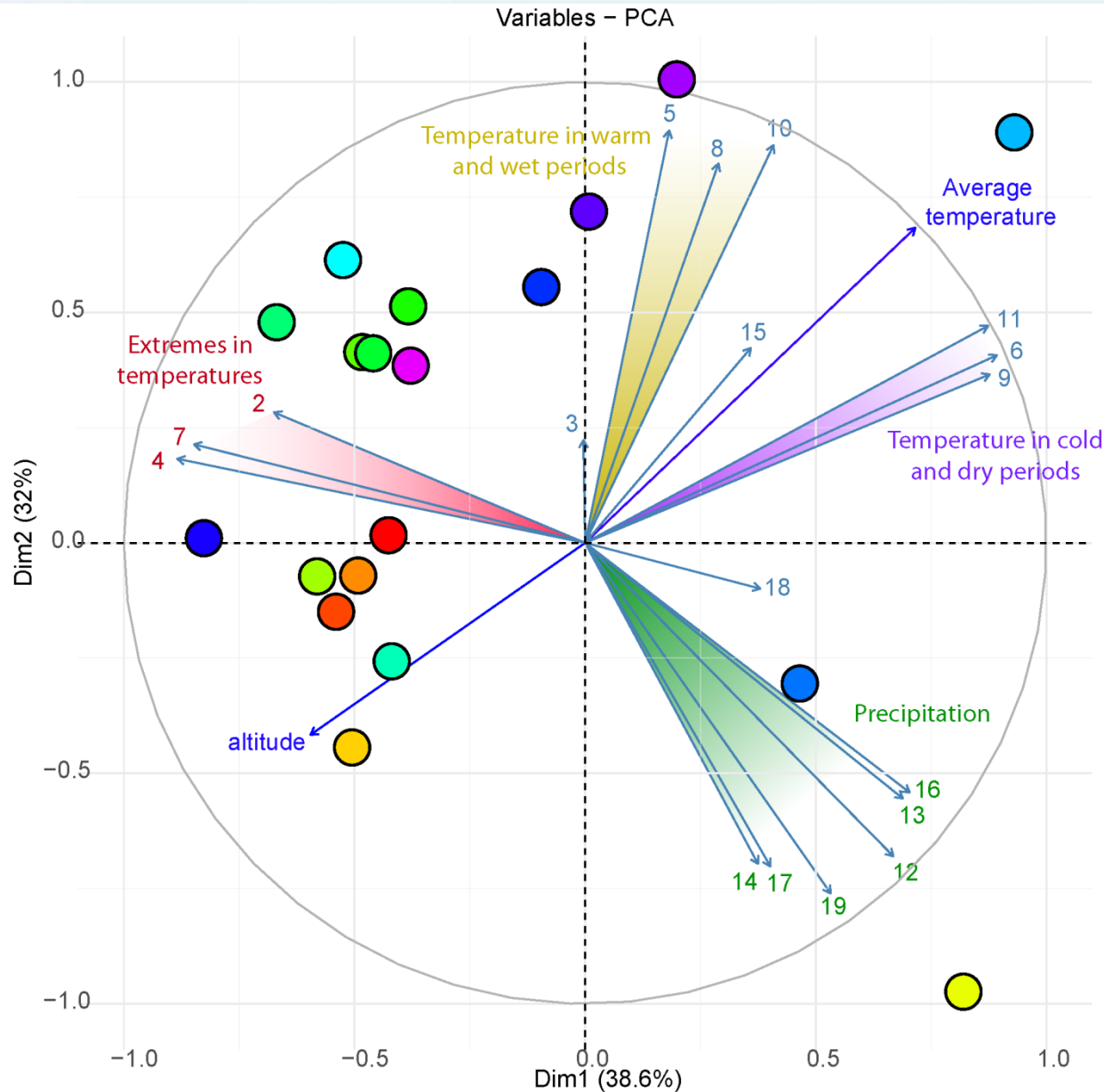
- |   |   |
|---|---|
| <span style="color: red;">■</span> leprarii           | <span style="color: cyan;">■</span> aff.irregularis |
| <span style="color: orange;">■</span> phycobiontica   | <span style="color: lightblue;">■</span> clade_8    |
| <span style="color: yellow;">■</span> echinata        | <span style="color: blue;">■</span> clade_9B        |
| <span style="color: lightyellow;">■</span> clade_StA5 | <span style="color: darkblue;">■</span> italiana    |
| <span style="color: yellowgreen;">■</span> clade_StA1 | <span style="color: purple;">■</span> woessiae      |
| <span style="color: limegreen;">■</span> clade_A9     | <span style="color: magenta;">■</span> irregularis  |
| <span style="color: green;">■</span> gaertneri        | <span style="color: darkpurple;">■</span> lobophora |
| <span style="color: brightgreen;">■</span> clade_A22  | <span style="color: purple;">■</span> mediterranea  |
| <span style="color: green;">■</span> friedlii         | <span style="color: magenta;">■</span> glomerata    |
| <span style="color: green;">■</span> clade_12         |   |



-2.73    standardized value    2.85

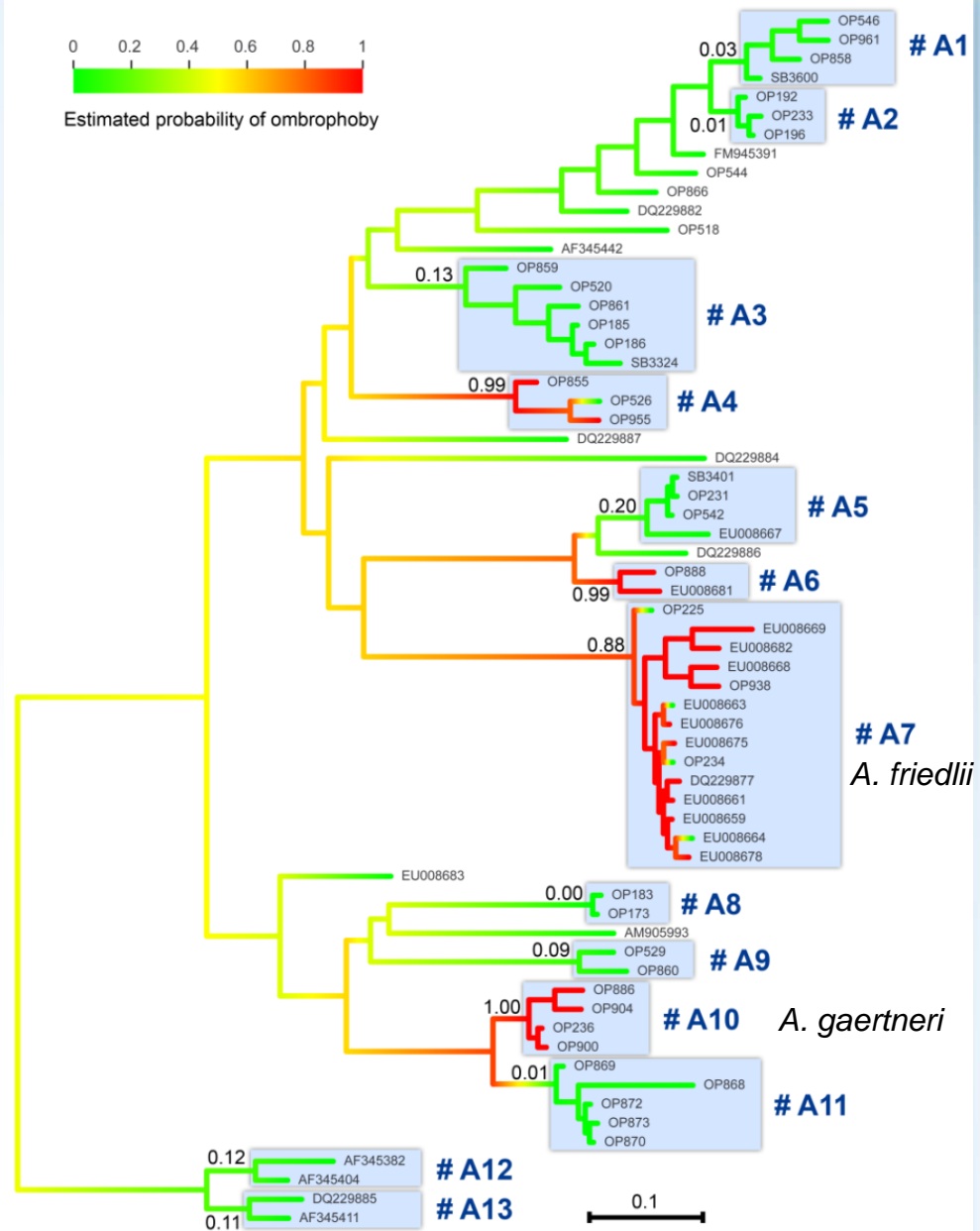
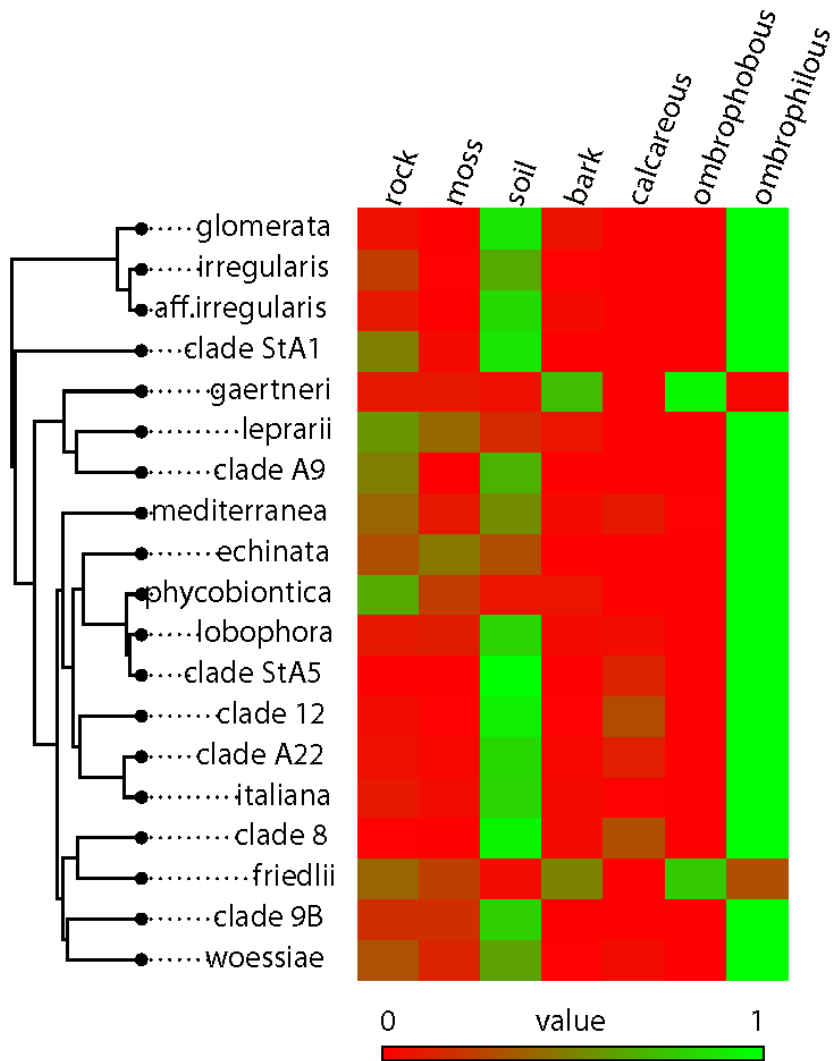
SD units

# Climate



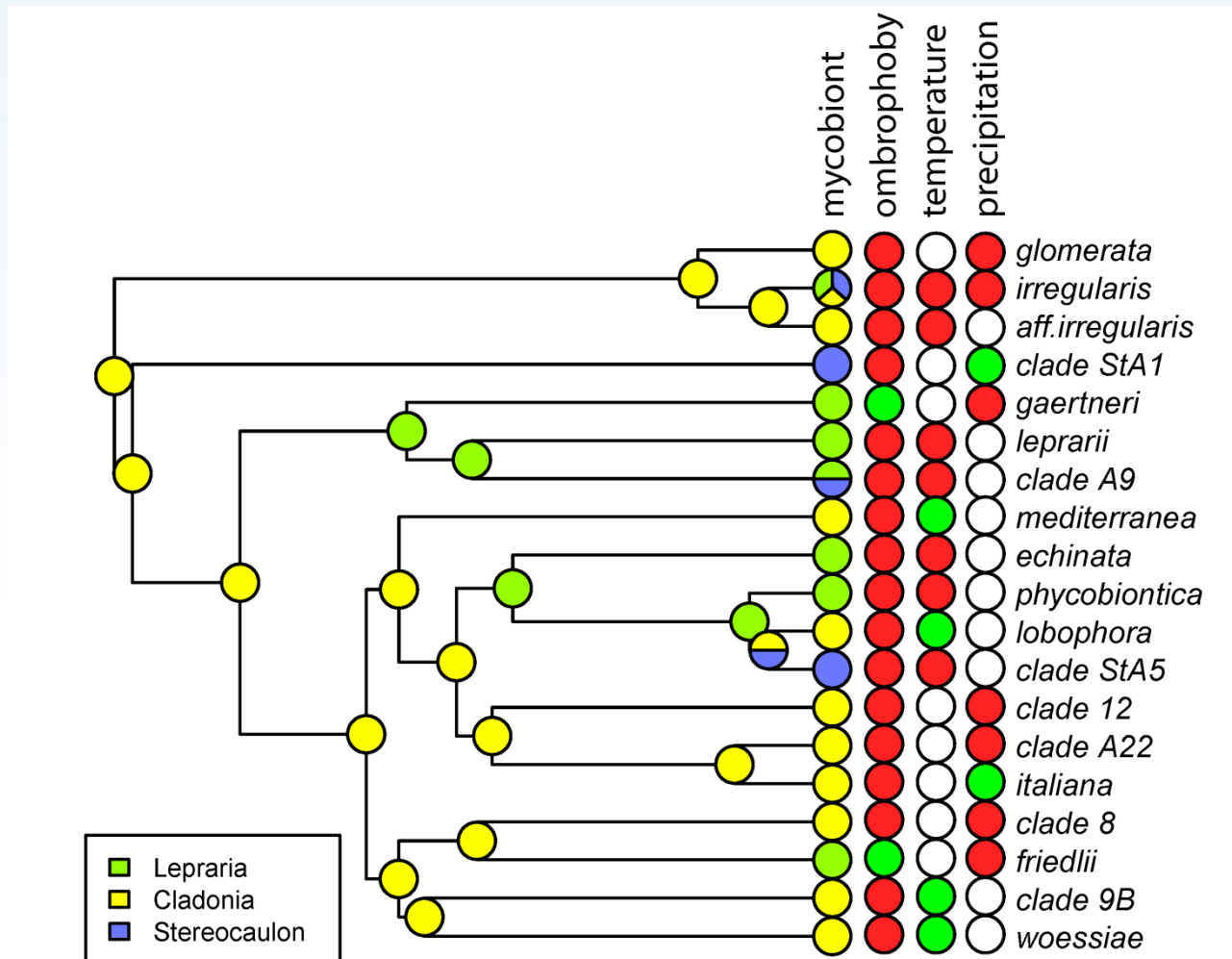
- leprarii
- phycobiontica
- echinata
- clade\_StA5
- clade\_StA1
- clade\_A9
- gaertneri
- clade\_A22
- friedlii
- clade\_12
- aff.irregularis
- clade\_8
- clade\_9B
- italiana
- woessiae
- irregularis
- lobophora
- mediterranea
- glomerata

# Substrate



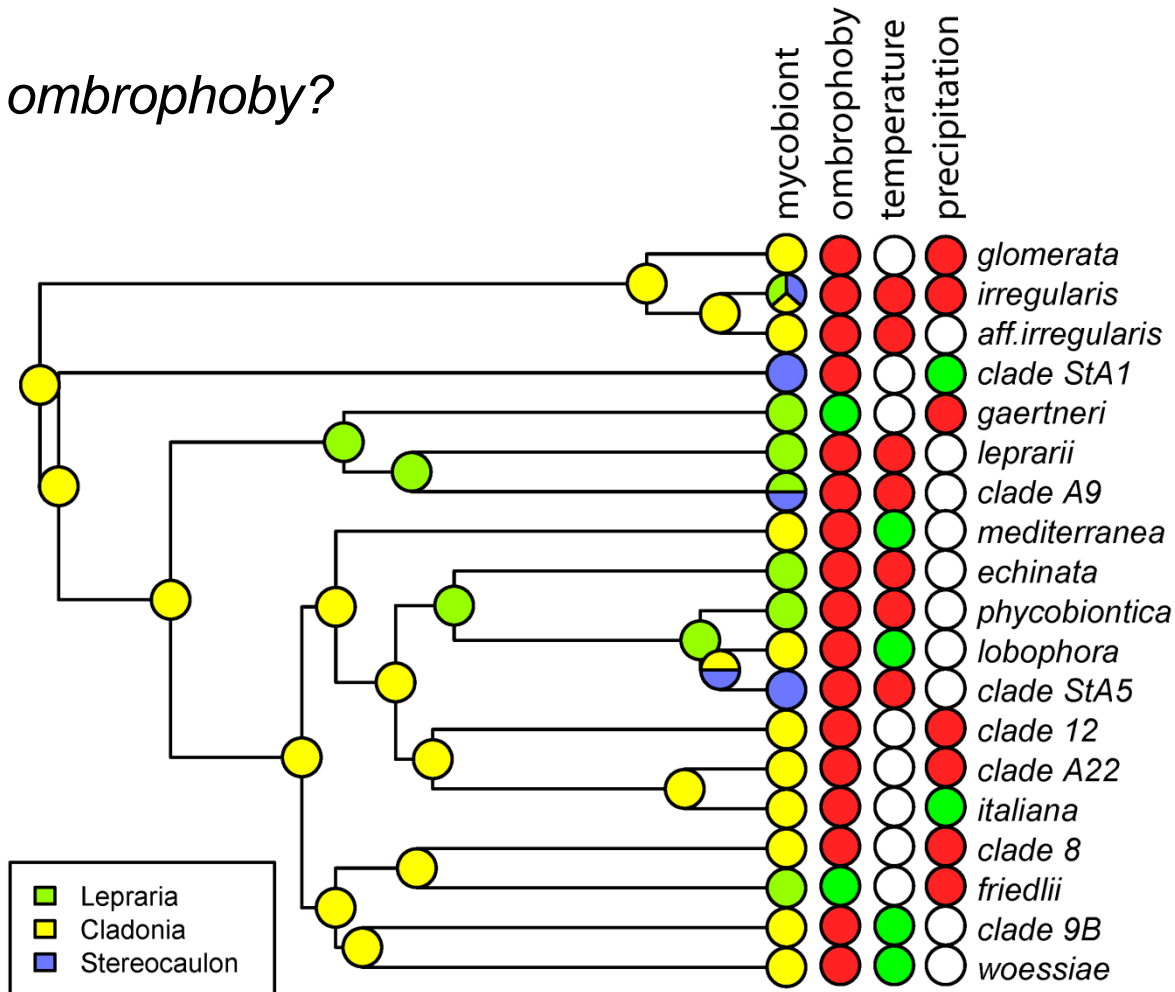
# Species differences

- Distinct differences among the species
- *Cladonia* was the ancestral mycobiont of the genus *Asterochloris*



# Species differences

- *Why so strong fungal selectivity? Is it problematic to cooperate with a number of different mycobionts?*
- *Are algal habitat/climatic preferences mirrored in fungal ones?*
- *How does the composition of photobiont pools reflect the habitat and climate?*
- *Why the ombrophoby?*
- *pH?*



# The Primus project

## Objective 1: Distribution and ecological differentiation of symbiotic partners

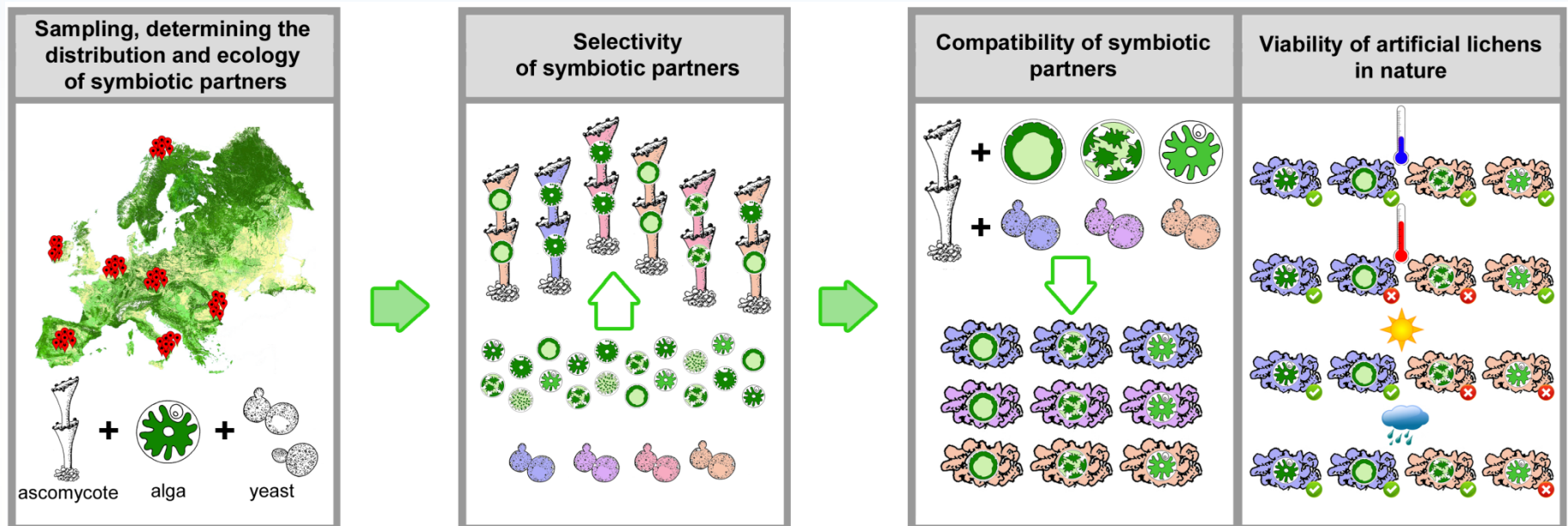
- Which abiotic and ecological factors influence the distribution of symbiotic partners?
- Do the coexisting partners exhibit comparable distribution patterns?

## Objective 2: Selection towards the pool of available partners

- How is the available pool of symbionts mirrored in the realized diversity of symbiotic partners?
- How do the distribution ranges of lichen species correspond to the available pools of algae?

## Objective 3: Compatibility and viability of symbiotic partners

- Is it possible to artificially join the ecologically distinct partners into viable lichen organisms?
- Are the compatible partners able to live in conditions suitable for only one of the symbionts?



Objective 1

Objective 2

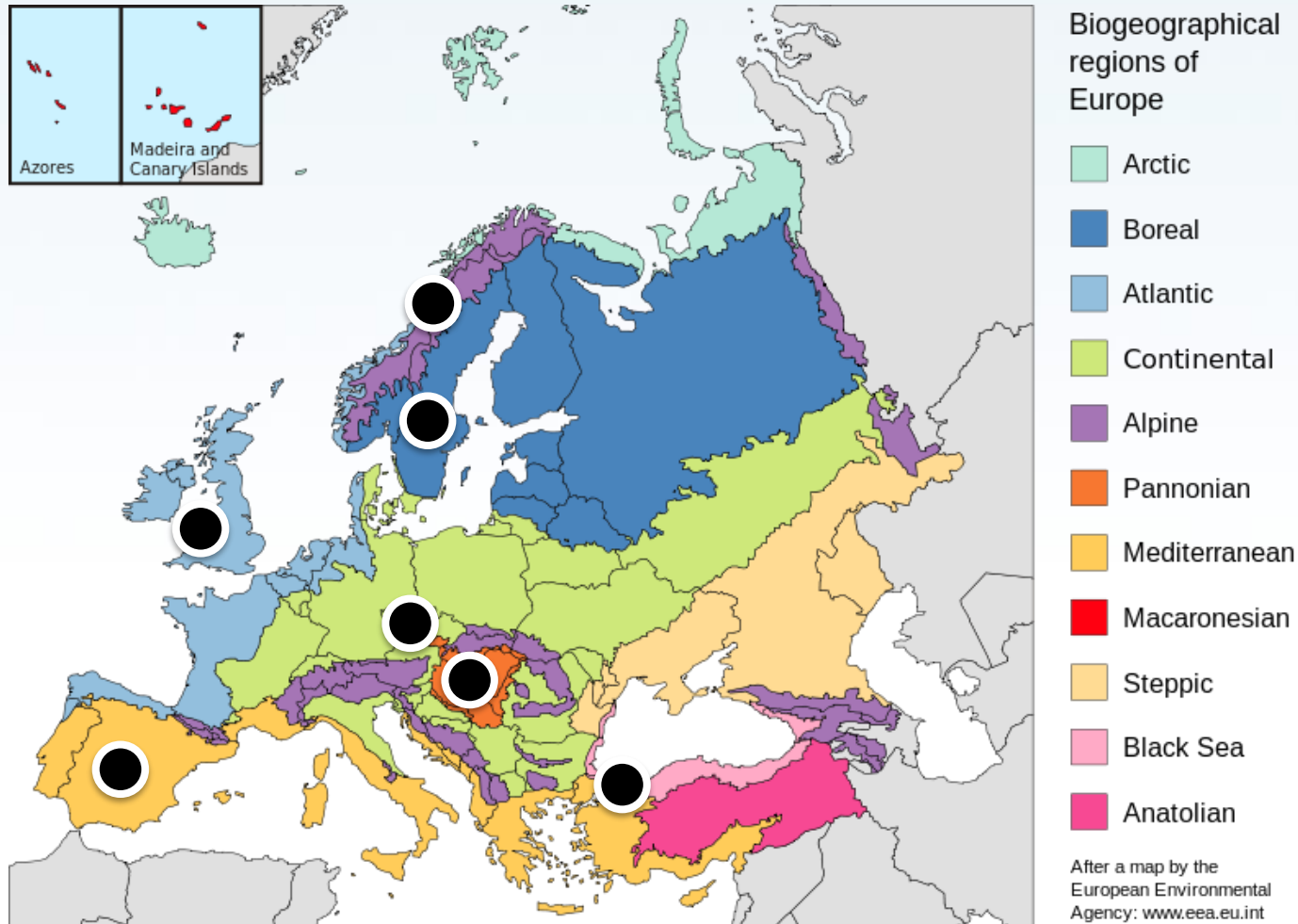
Objective 3



# 1. Distribution and ecological differentiation of symbiotic partners

- 7 biogeographic regions in Europe
  - 8 sampling sites along the pH gradient
    - 20 *Cladonia* specimens
    - 1 composite soil and lichen sample

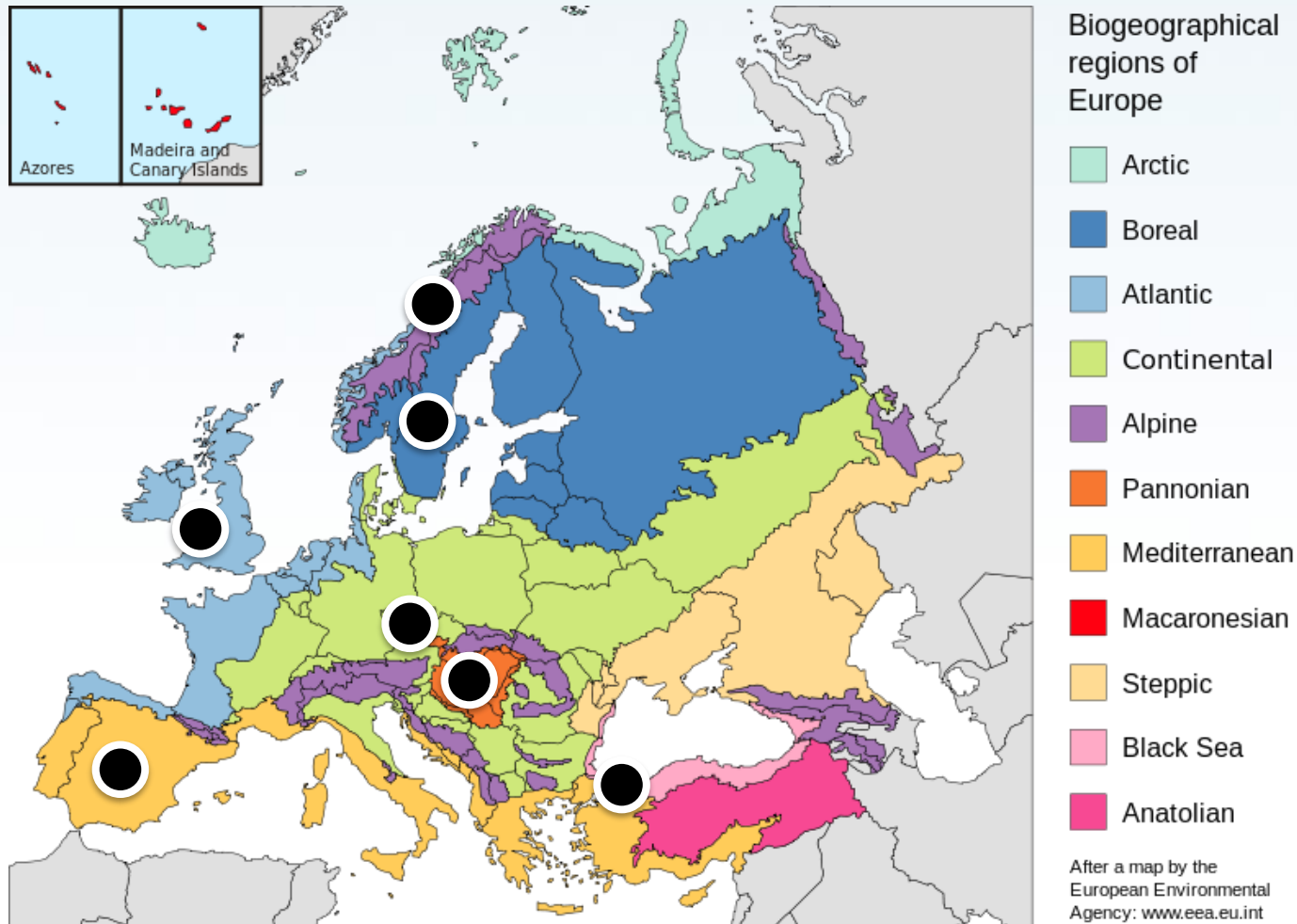
**1,120 lichens investigated**





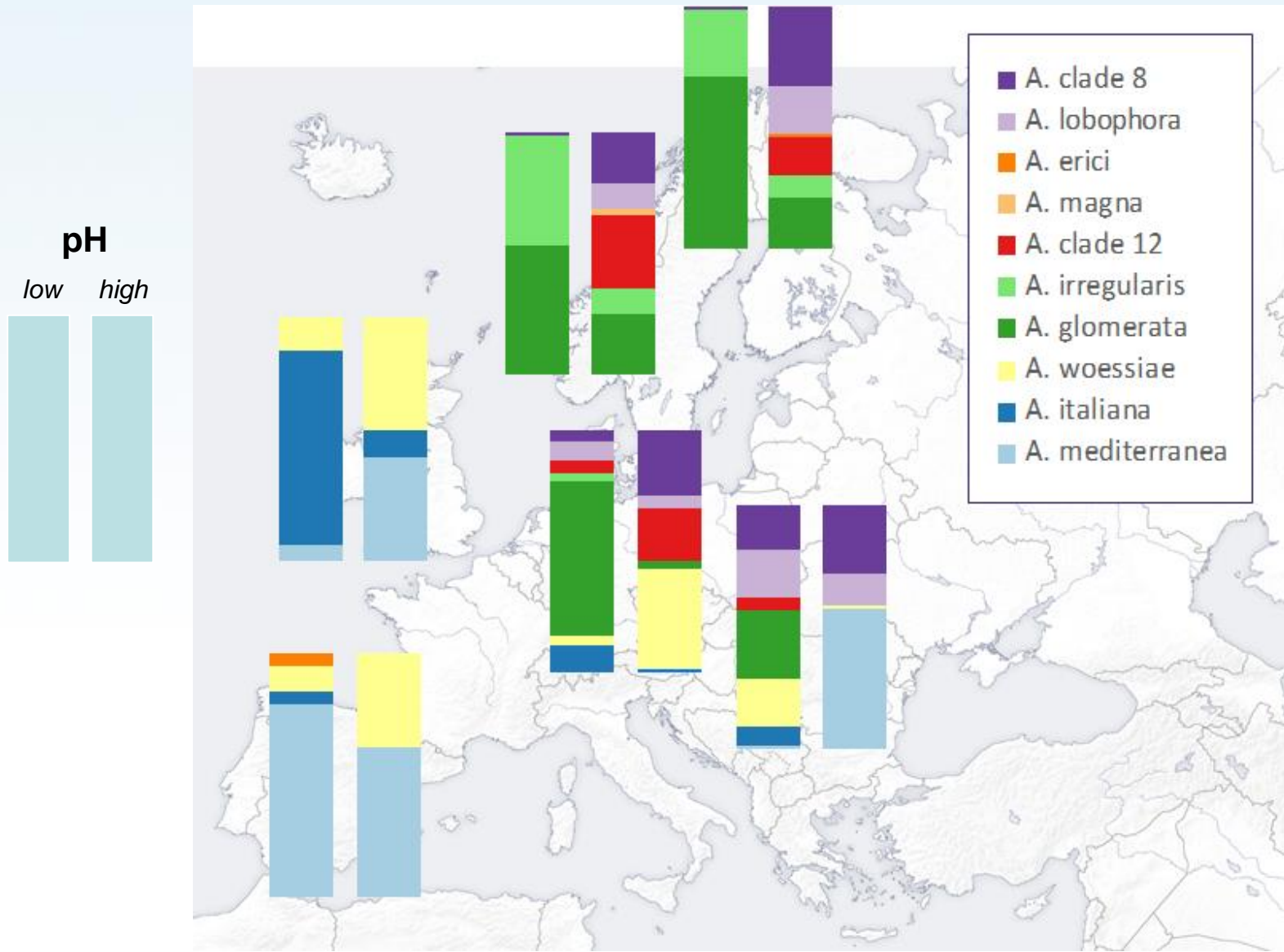
# 1. Distribution and ecological differentiation of symbiotic partners

- 1,120 lichen thalli
  - Sanger sequencing of algae, fungi, and yeasts
- 56 composite soil and lichen samples
  - Illumina meta-barcoding of symbionts, soil chemical analyses



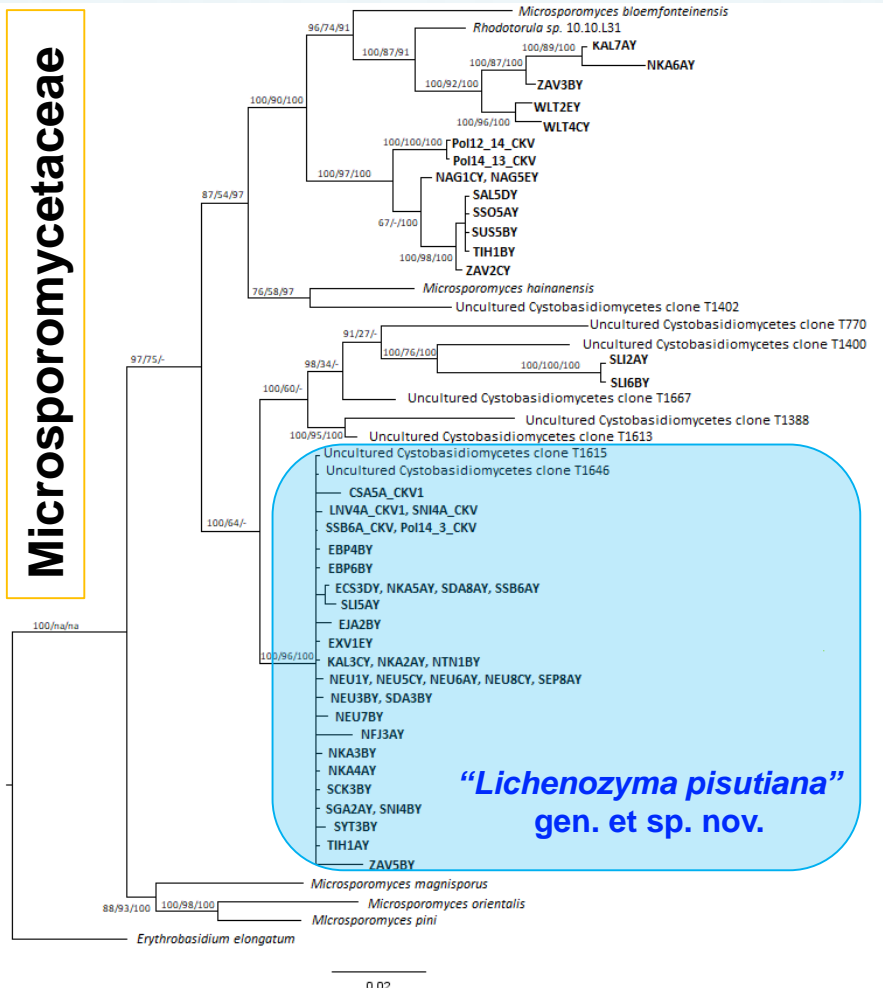
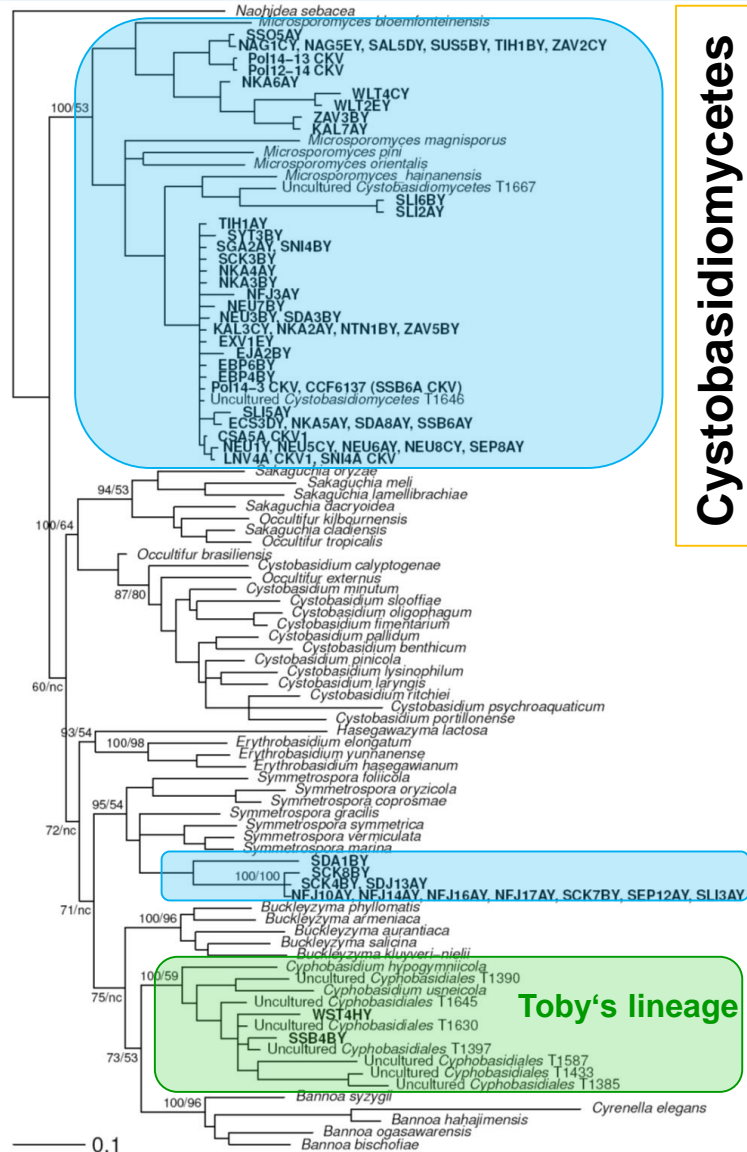
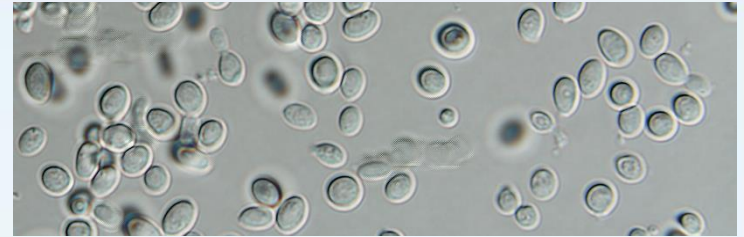
# 1. Distribution and ecological differentiation of symbiotic partners

- Photobionts: both geographical and ecological patterns are obvious



# 1. Distribution and ecological differentiation of symbiotic partners

- Lichen yeasts: diversity still barely known



# 1. Distribution and ecological differentiation of symbiotic partners

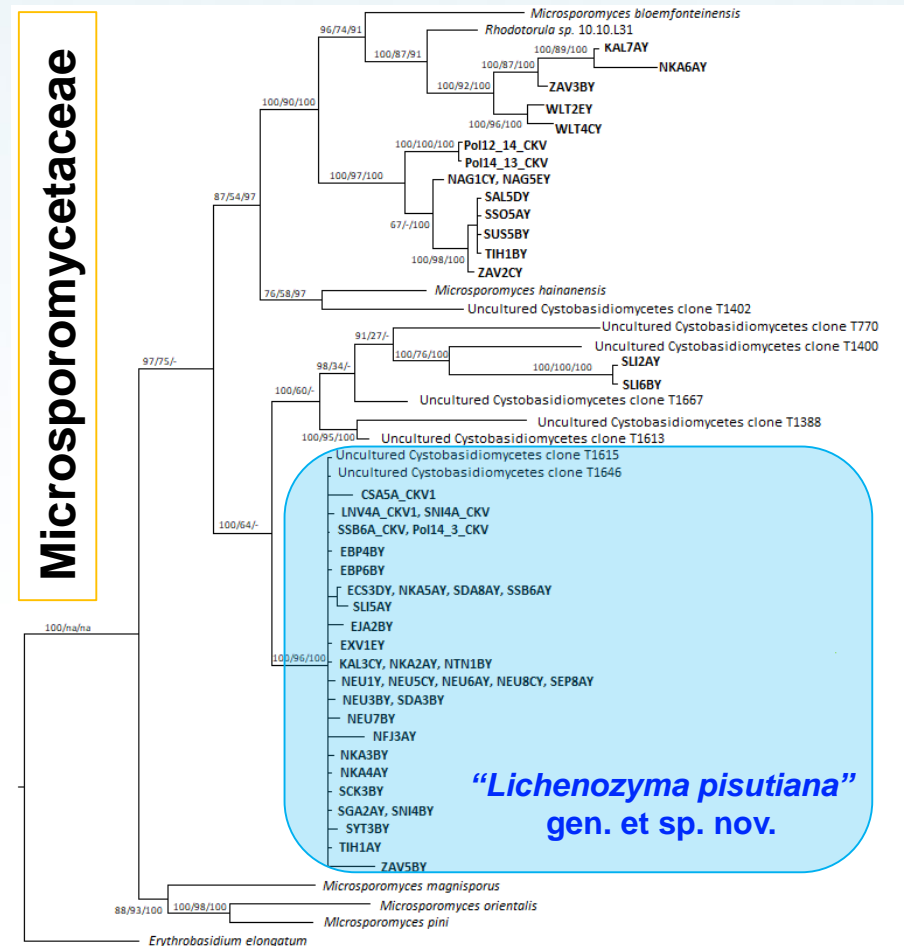
- Lichen yeasts: detected in both corticate and ecorticate species
  - No effect to lichen morphology as previously hypothesized



*Cladonia pocillum*



*Cladonia rangiferina*

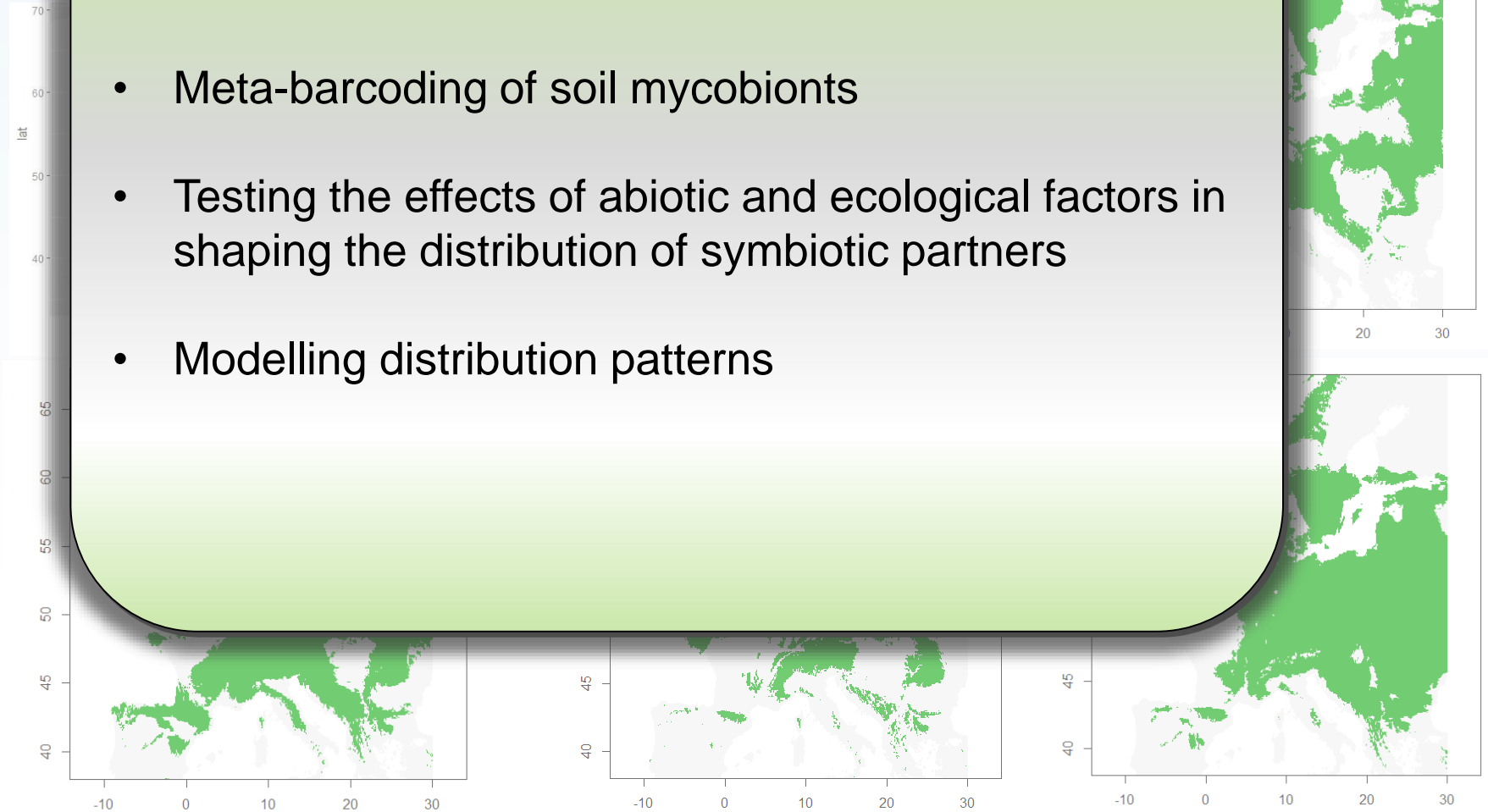


# 1. Distribution and ecological differentiation of symbiotic partners

- Modelling present and future distribution patterns

## Future plans

- Meta-barcoding of soil mycobionts
- Testing the effects of abiotic and ecological factors in shaping the distribution of symbiotic partners
- Modelling distribution patterns



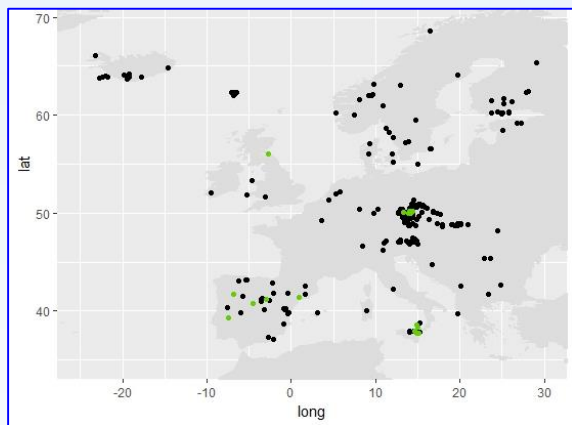
identifying overlaps and „holes“



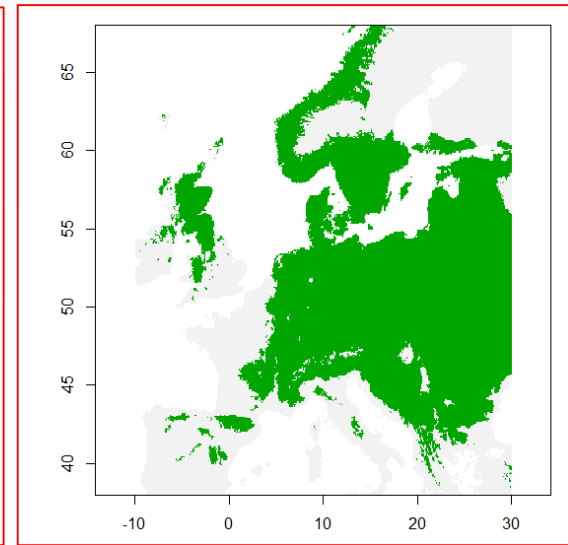
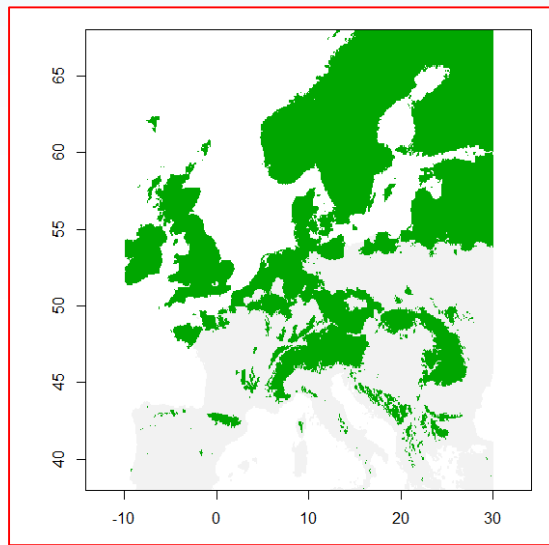
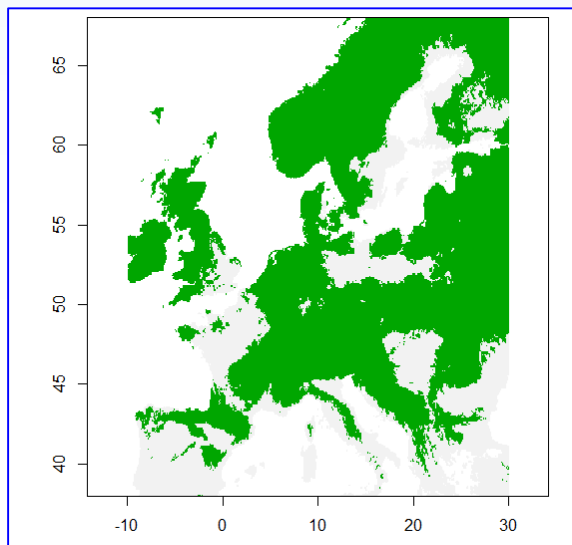
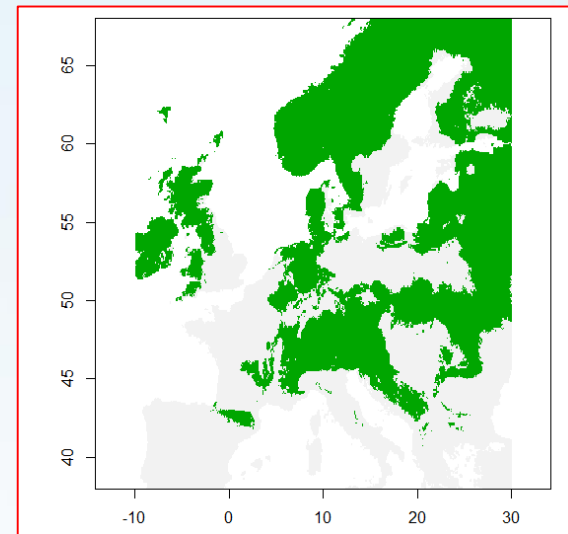
FUTURE CLIMATIC PREDICTIONS

# Modelling recent and future distribution patterns

mycobiont



and its  
photobionts



identifying overlaps and „holes“



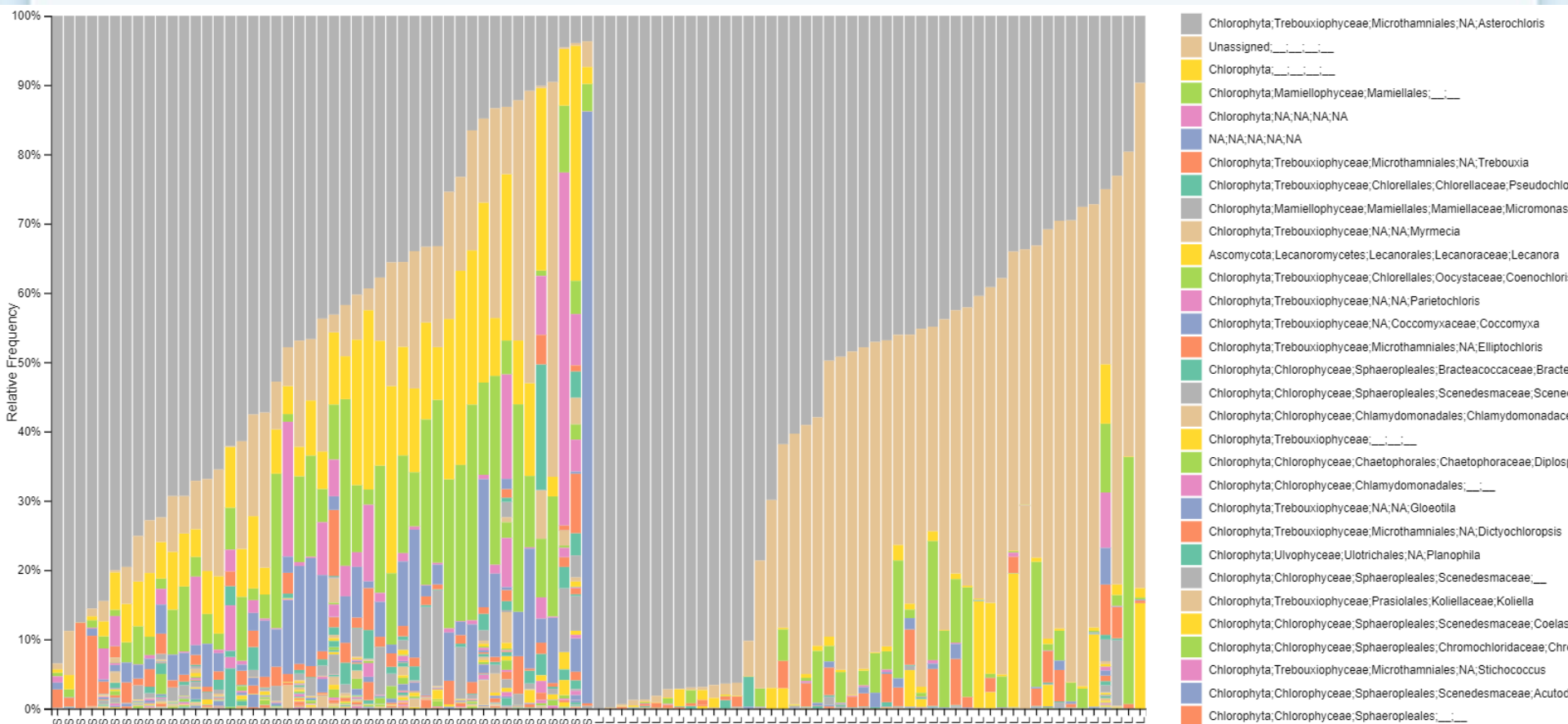
FUTURE CLIMATIC PREDICTIONS





## 2. Selection towards the pool of available partners

- Illumina meta-barcoding of algae occurring in both soil and composite lichen samples
- 6.6 mil algal sequences after filtering
- *Asterochloris* as the most dominant genus

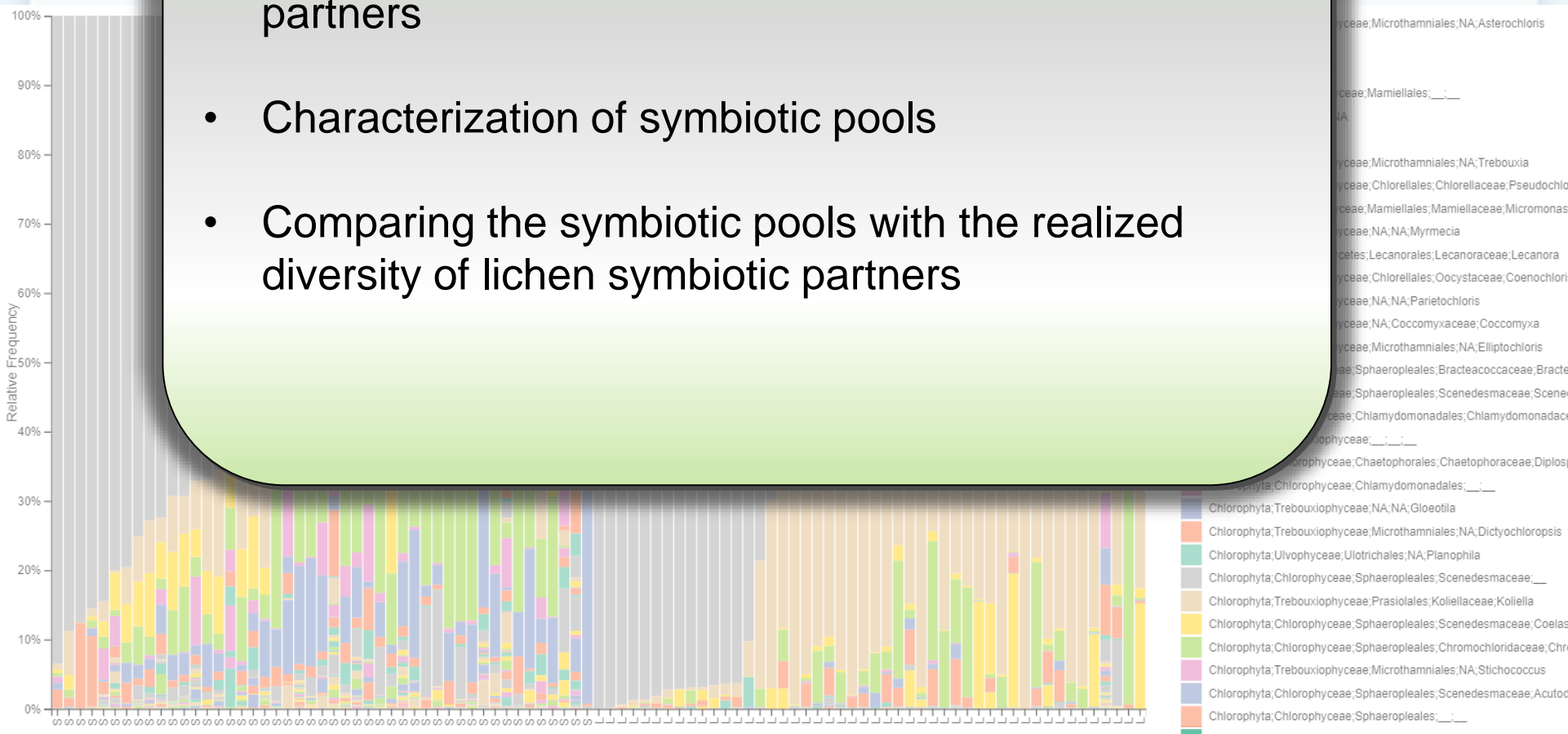


## 2. Selection towards the pool of available partners

- Illumina meta-barcoding of symbionts in both soil and composite lichen samples
- 6.6
- As

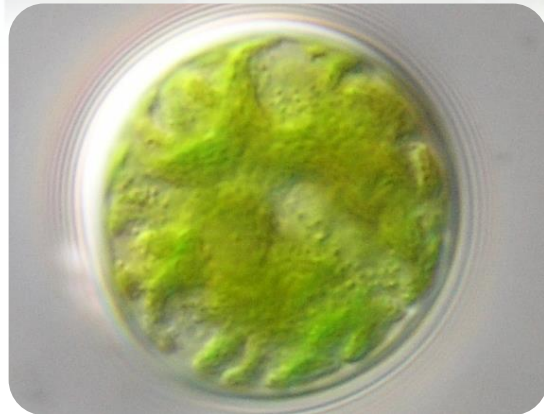
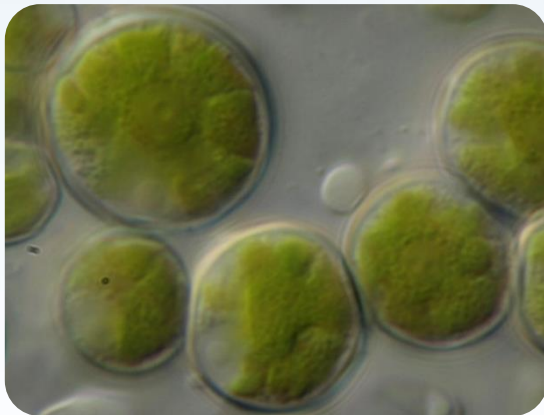
### Future plans

- Genetic characterization of all algal and fungal partners
- Characterization of symbiotic pools
- Comparing the symbiotic pools with the realized diversity of lichen symbiotic partners



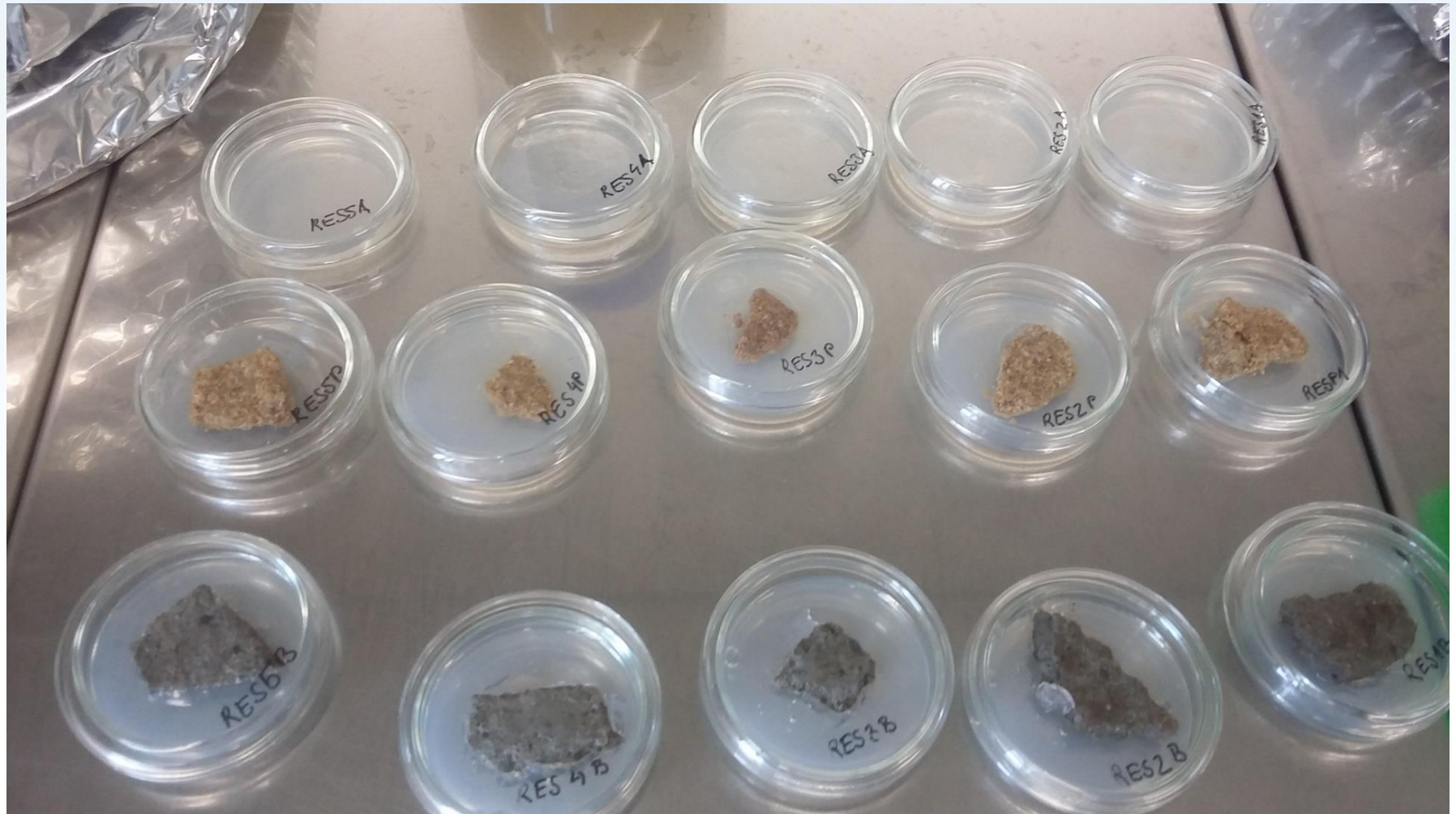
### 3. Compatibility and viability of symbiotic partners

- Several cultures established:
  - 7 *Asterochloris* species
  - 14 *Cladonia* species
  - 7 Cystobasidiomycetes genotypes



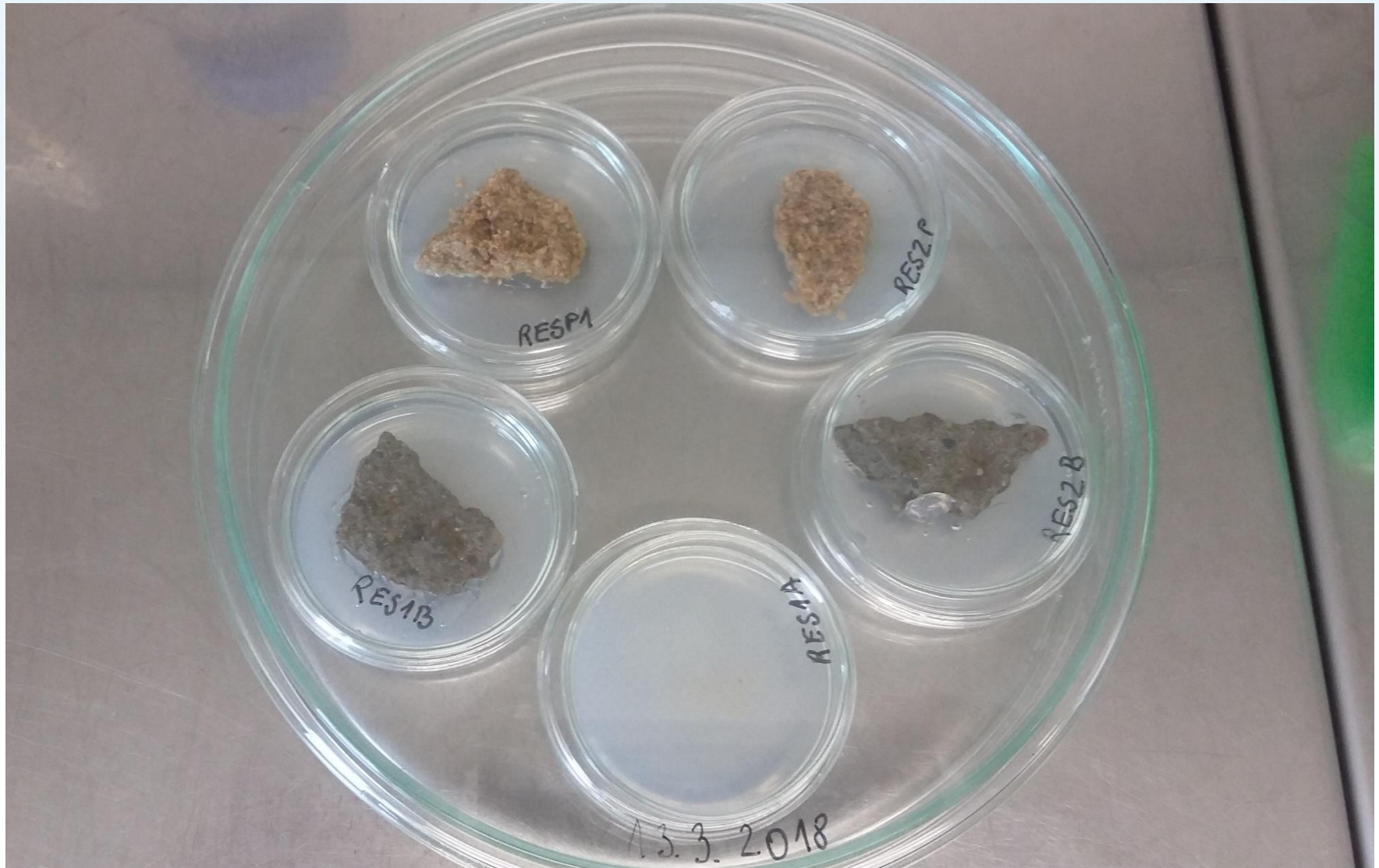
### 3. Compatibility and viability of symbiotic partners

- Relichenisation experiments



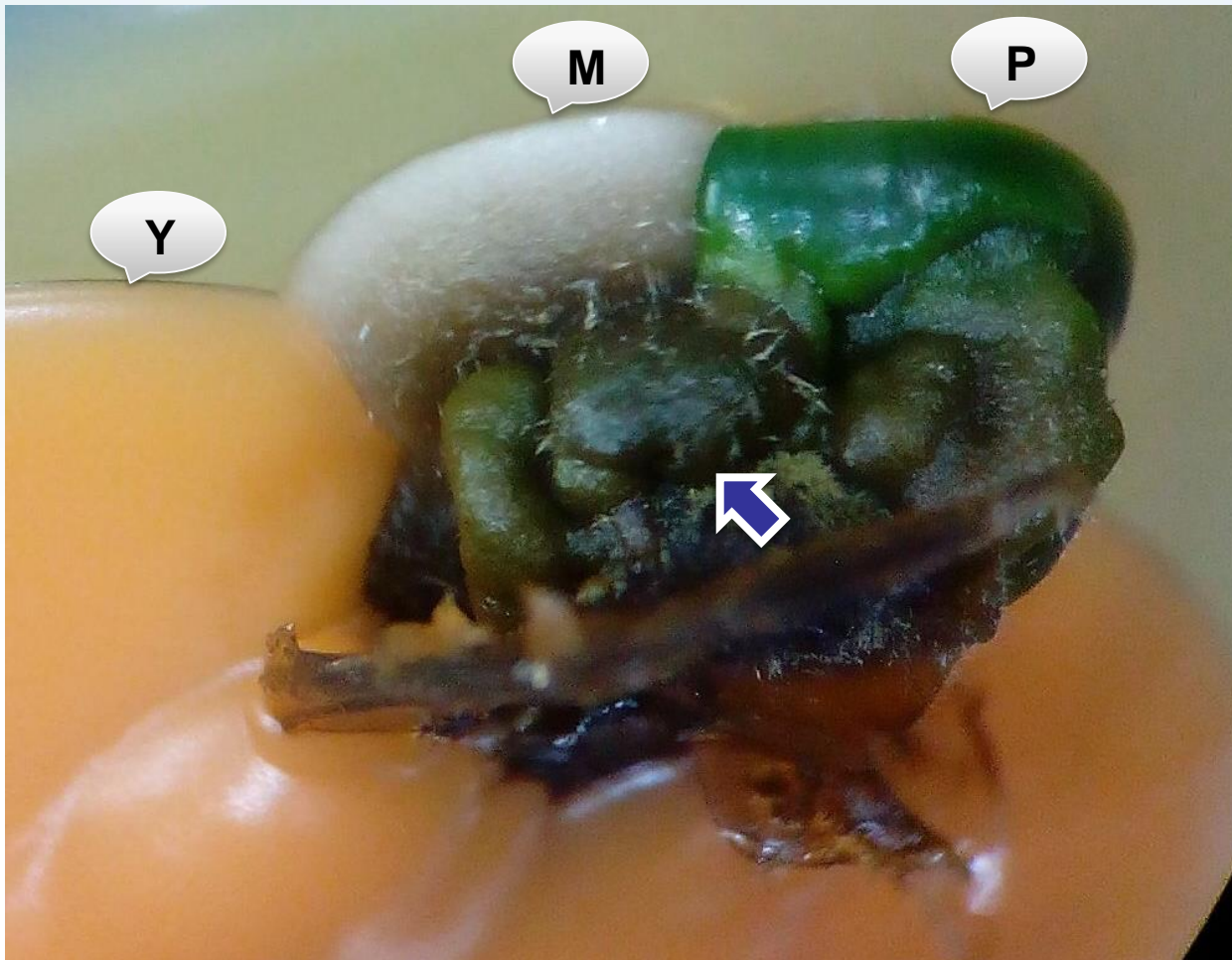
### 3. Compatibility and viability of symbiotic partners

- Relichenisation experiments



### 3. Compatibility and viability of symbiotic partners

- Relichenisation experiments
- The yeast profits on the symbiosis, but probably does not involve the thallus formation

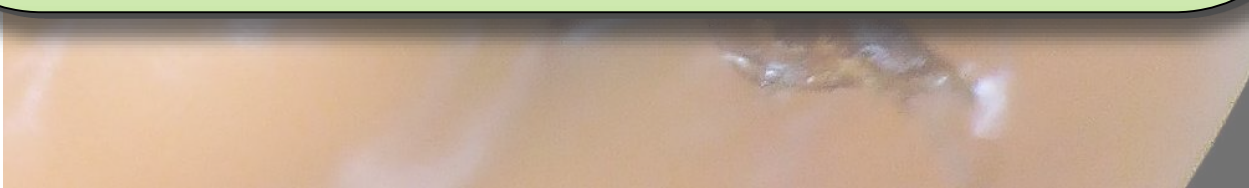


### 3. Compatibility and viability of symbiotic partners

- Relichenisation experiments

#### **Future plans**

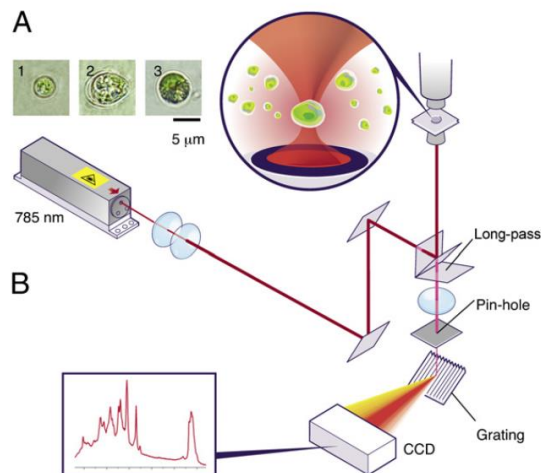
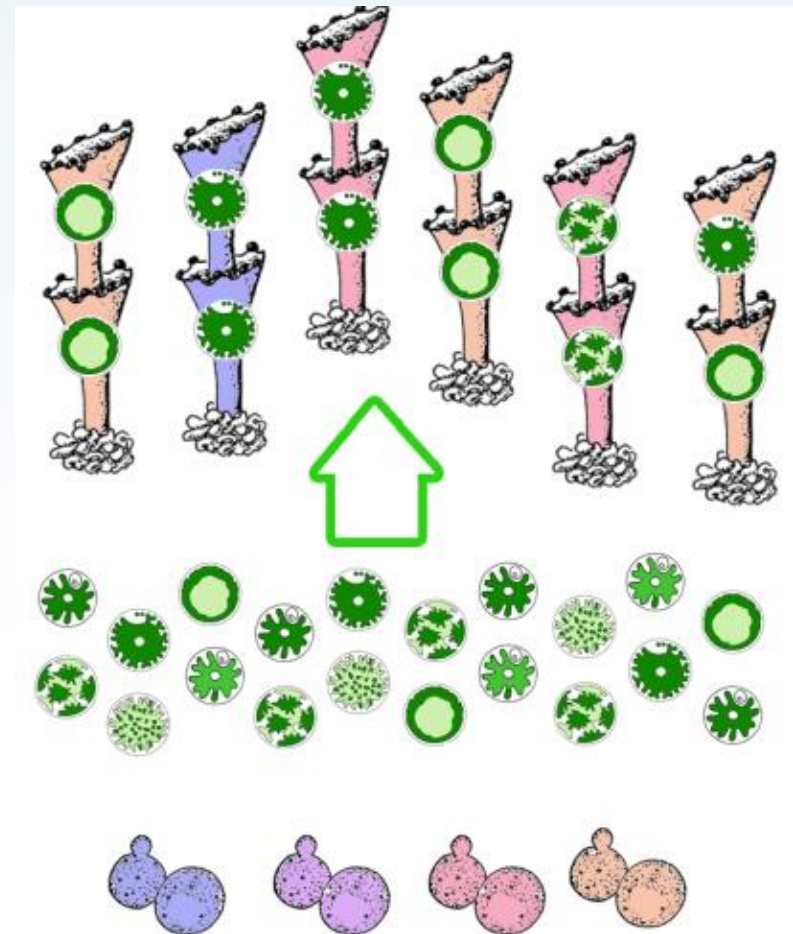
- Optimization of the relichenisation methodology
- Detection of the extent of symbiosis
- Artificial formation of several symbiotic combinations, evaluation of their fitness





# Many open questions still remain to be addressed

- How to differentiate between the real algal symbionts and epiphytes?
- Are the fungi genetically uniform?
- What is the function of yeasts in the lichen thallus? Are they real symbionts or only endophytes?
- How to evaluate the extent of symbiosis in artificial lichens? Raman micro-spectroscopy?





**Thank you for your attention**



**CHARLES UNIVERSITY**

**Primus Research  
Programme**