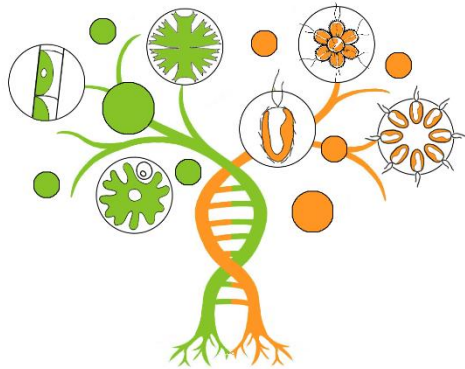


# Generating the diversity

*Uncovering the speciation mechanisms  
in eukaryotic microorganisms*

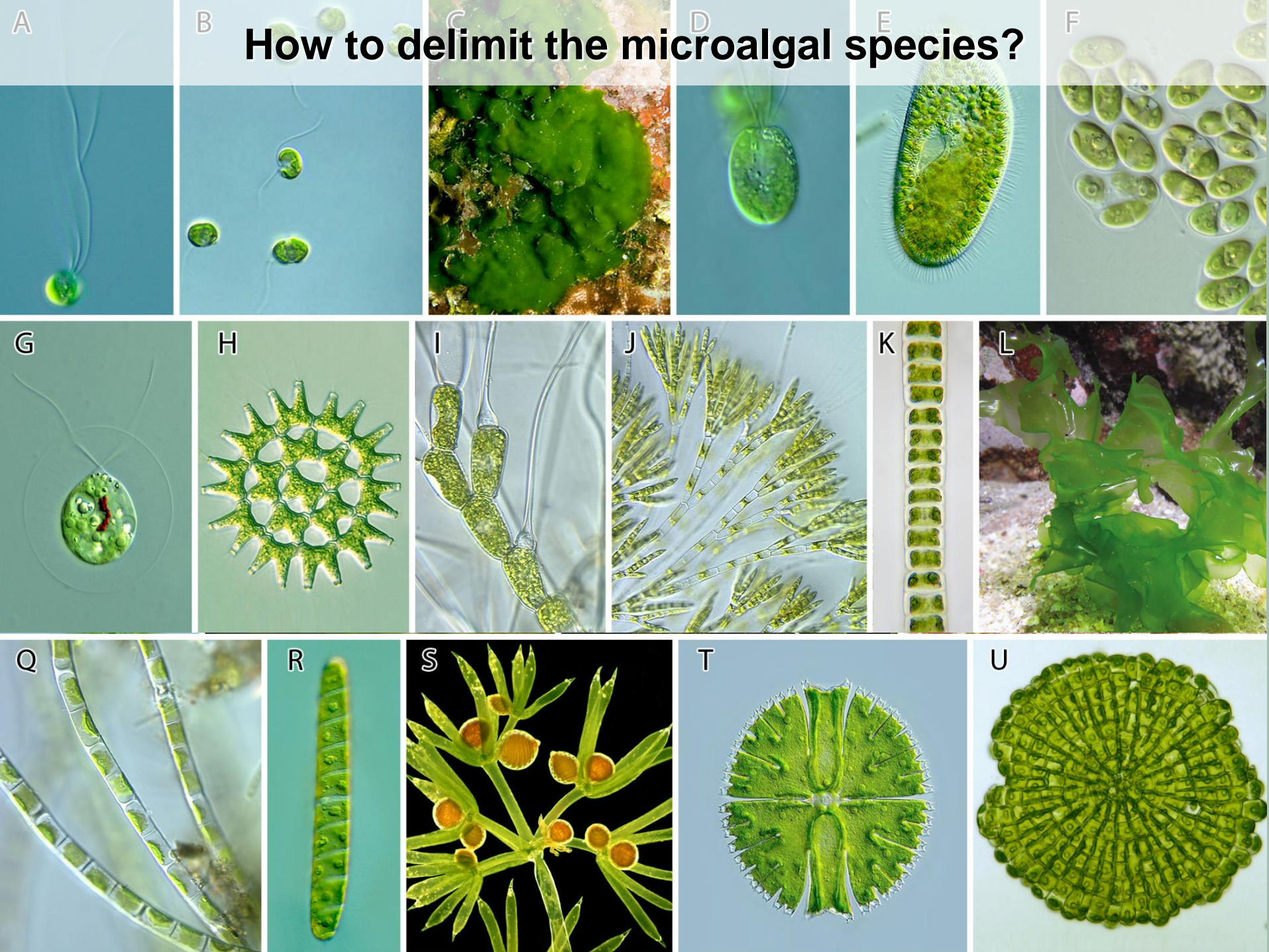


*Pavel Škaloud  
Charles University in Prague, Czech Republic  
CAUP Culture Collection of Algae*

# General outline

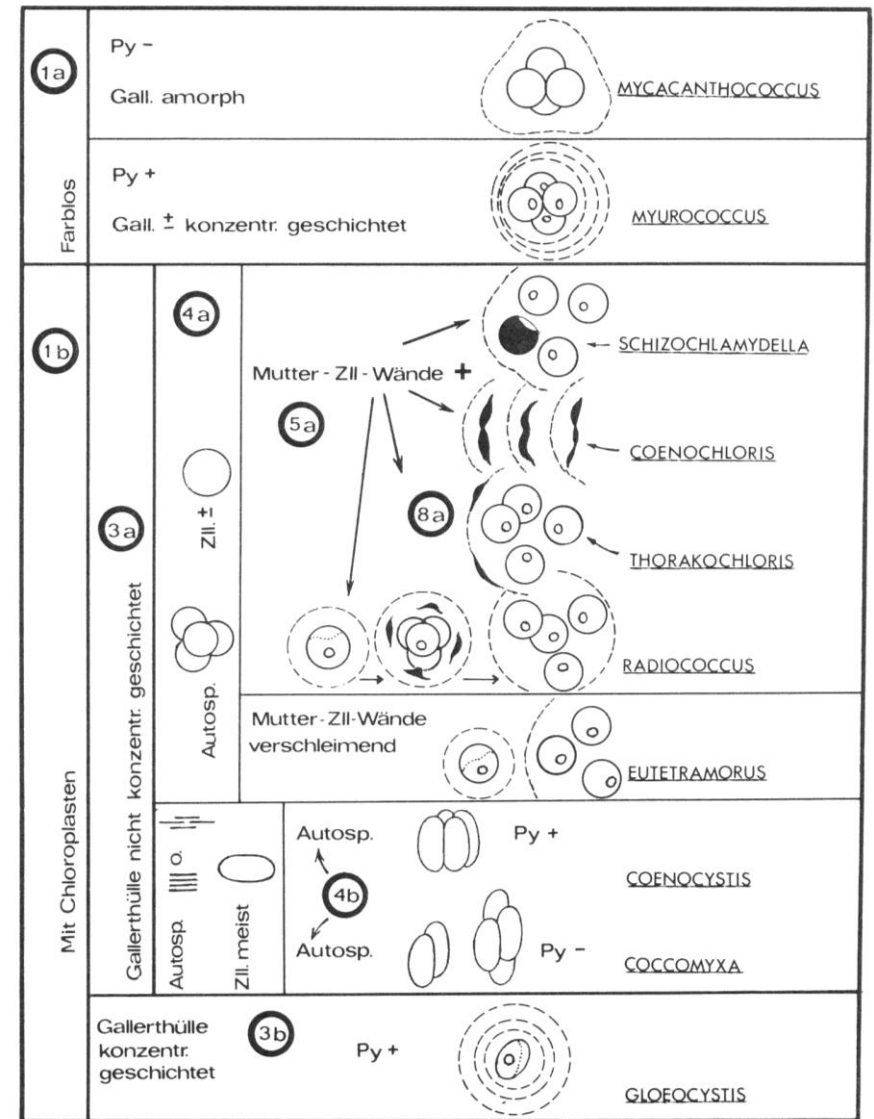
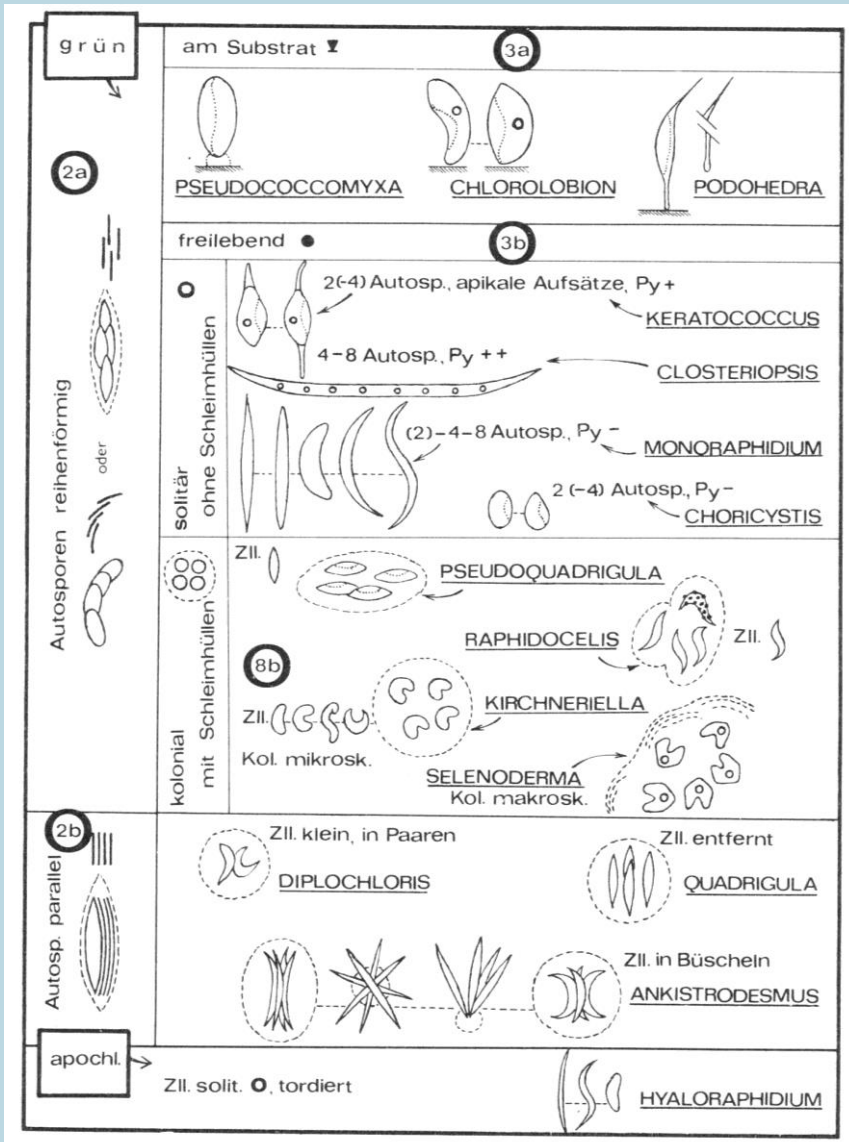
- How to delimit the microalgal species?
- What are the general causes of microalgal speciation?
- *What I do when I cannot asleep...*

# How to delimit the microalgal species?



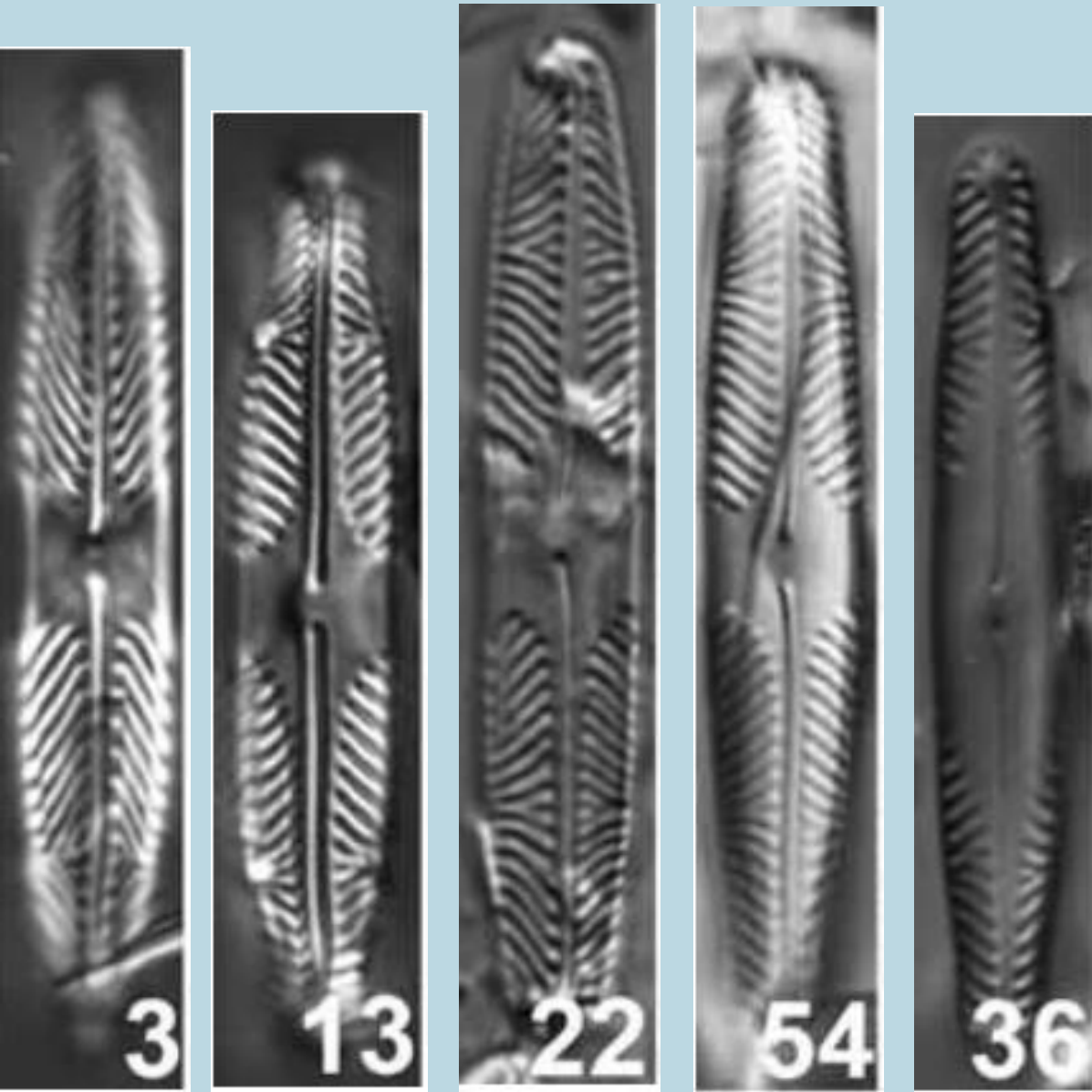
# Morphological species concept

- The traditional tool in describing the diversity



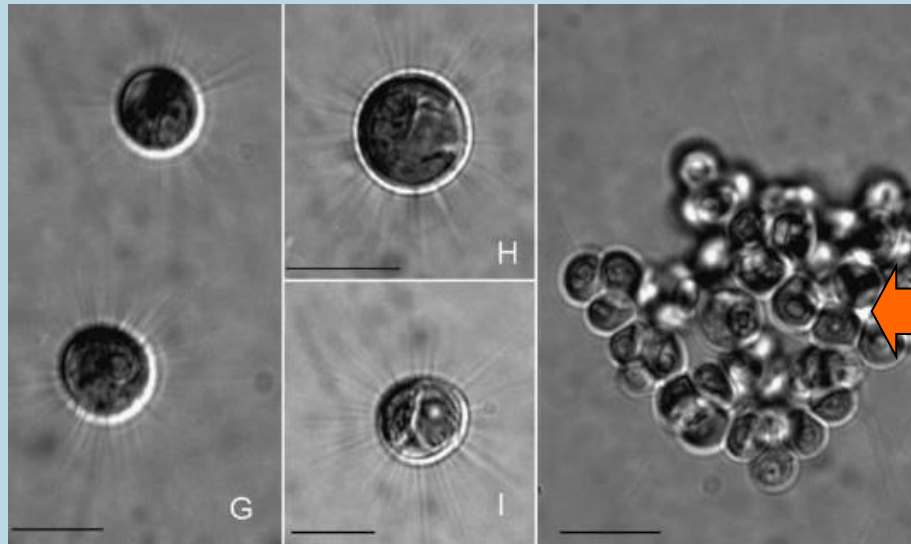
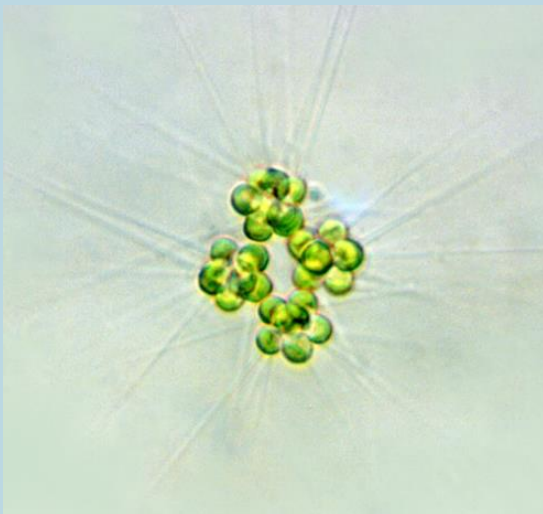
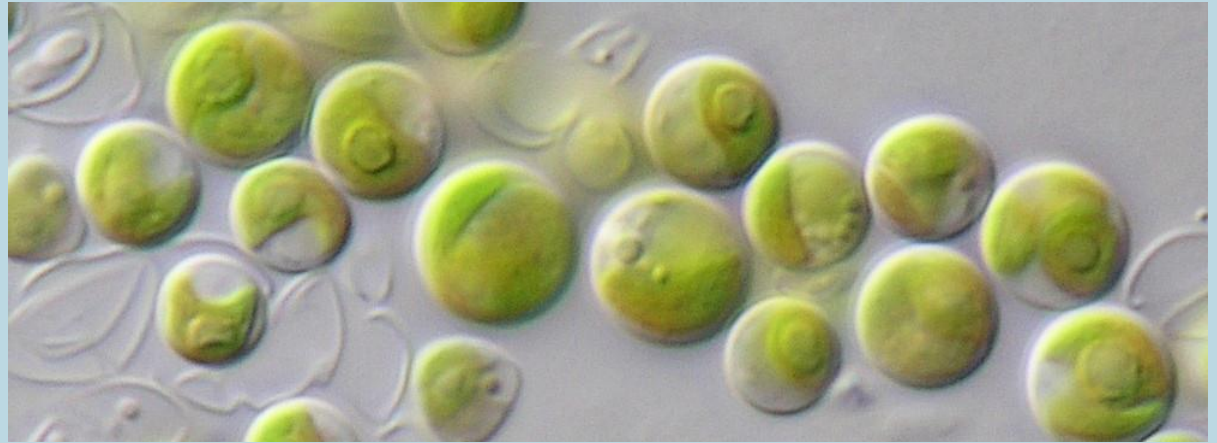
# Morphological species concept

- Still applied in some algal groups (e.g., diatoms)



# Morphological species concept

- Poor knowledge of the phenotypic plasticity
- *Micractinium*



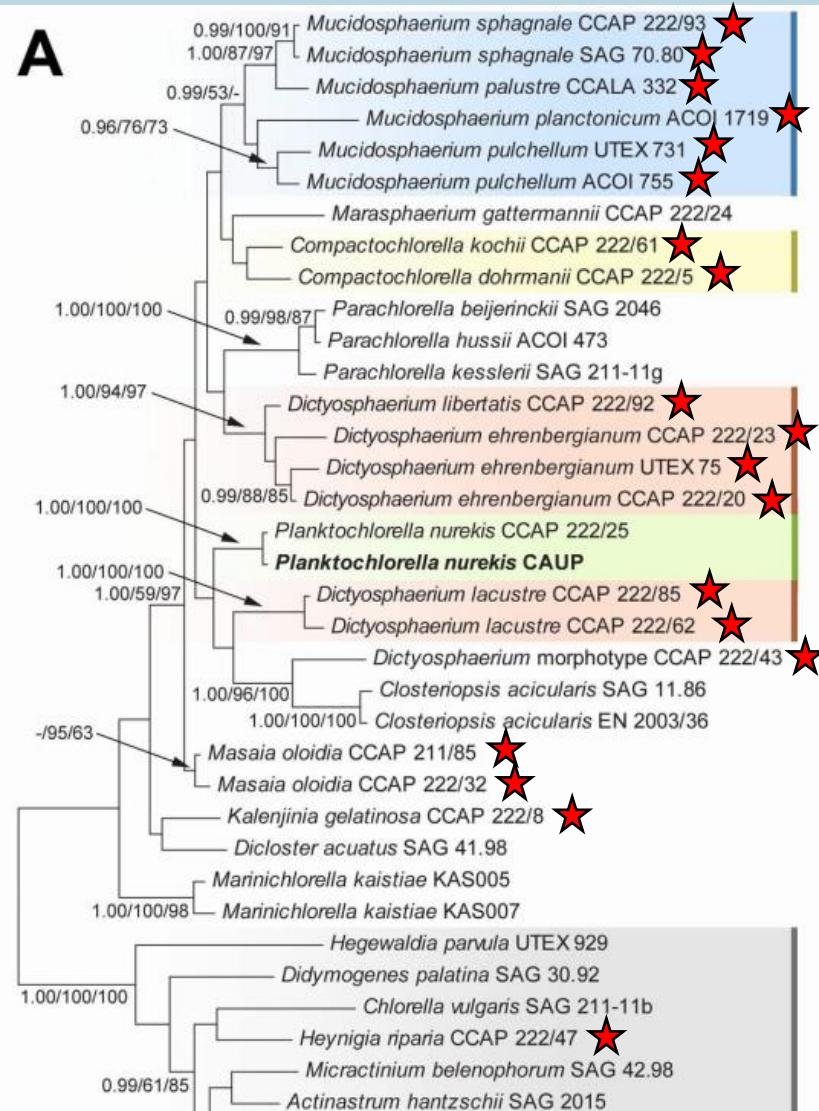
# Morphological species concept

- *Dictyosphaerium*

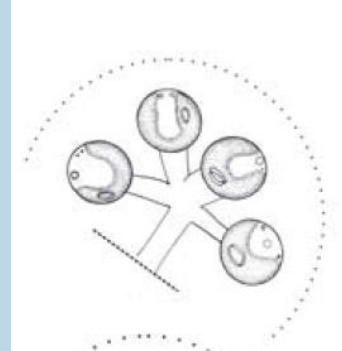


# Morphological species concept

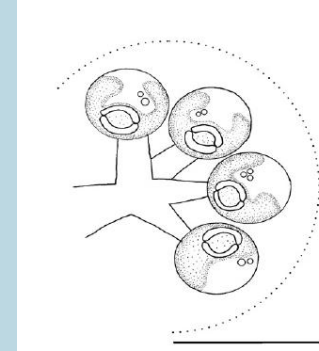
- Convergent morphological evolution = 9 cryptic genera



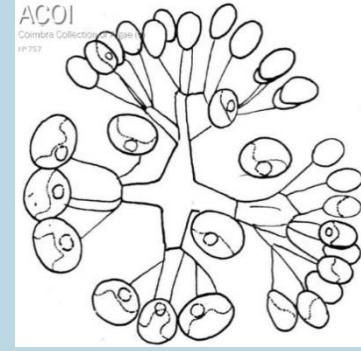
*Chlorella*



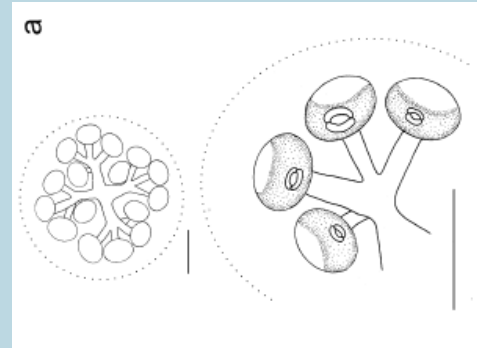
*Heynigia*



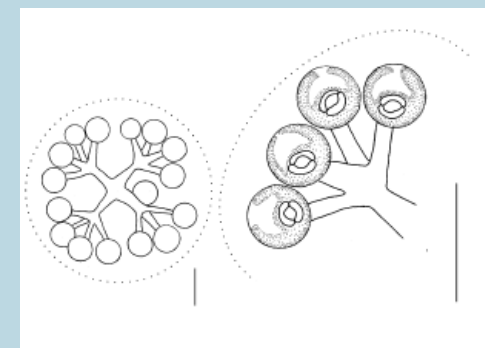
*Hindakia*



*Dictyosphaerium*



*Mucidosphaerium*

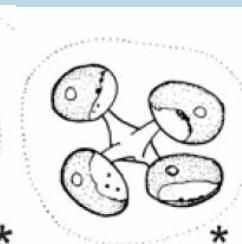
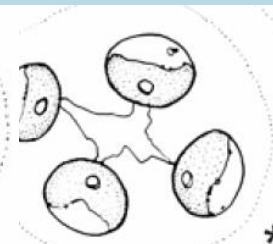
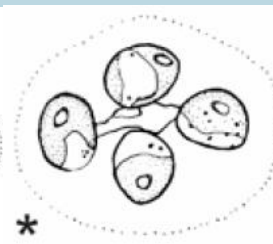
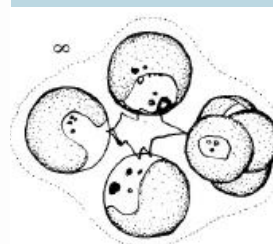


*Mychonastes*

*Compactochlorella*

*Kalenjinia*

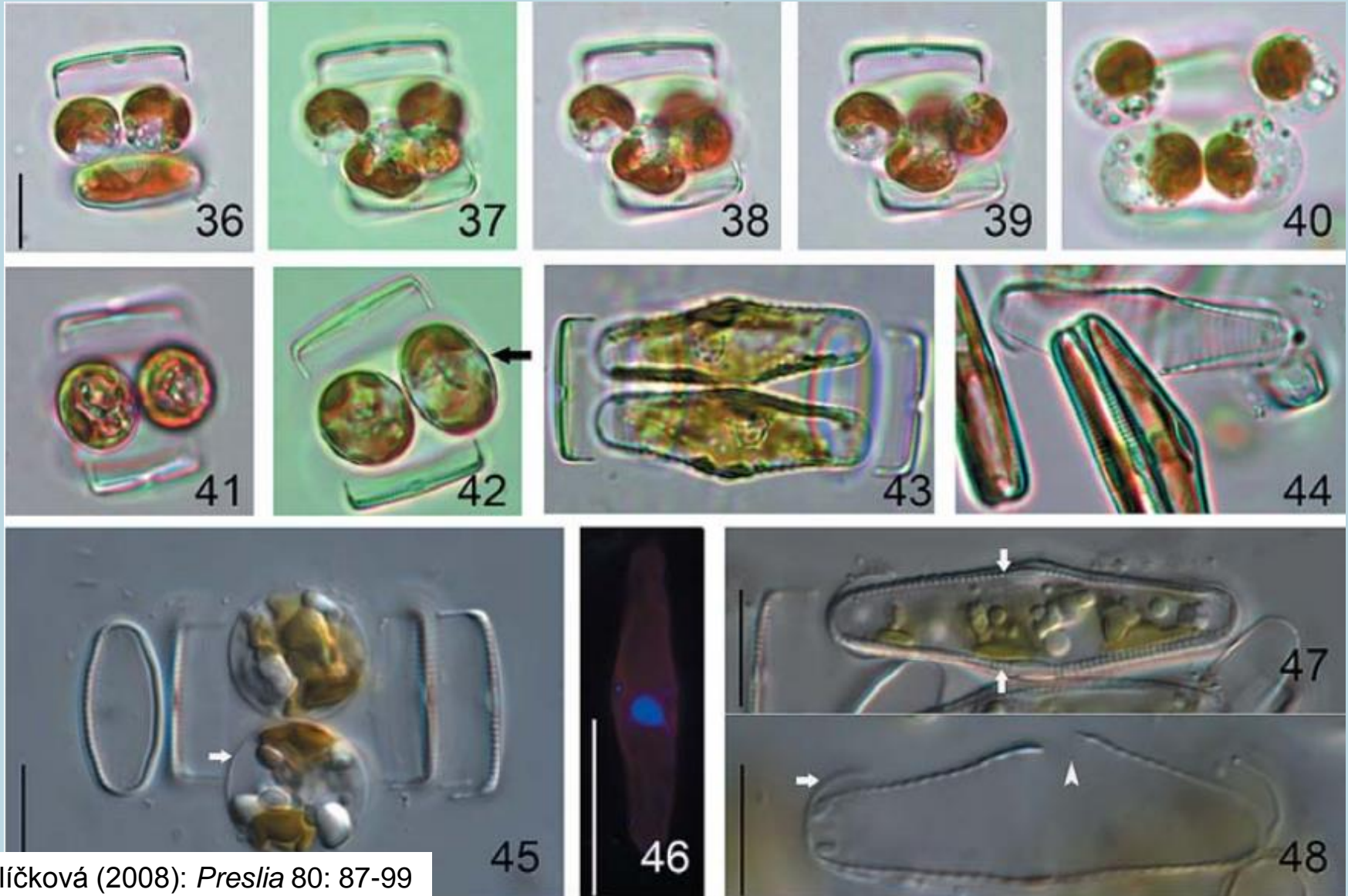
*Masaia*





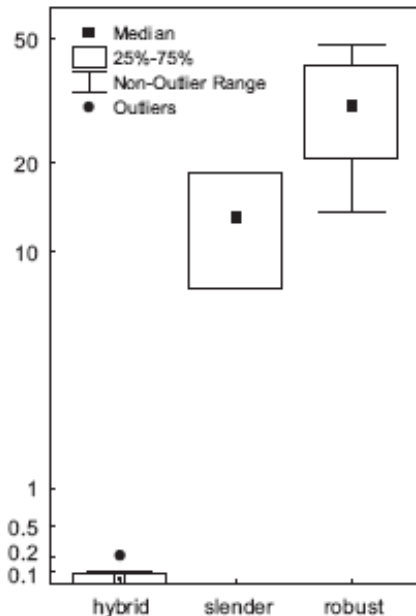
# Biological species concept

- Applied for sexually reproducing organisms only

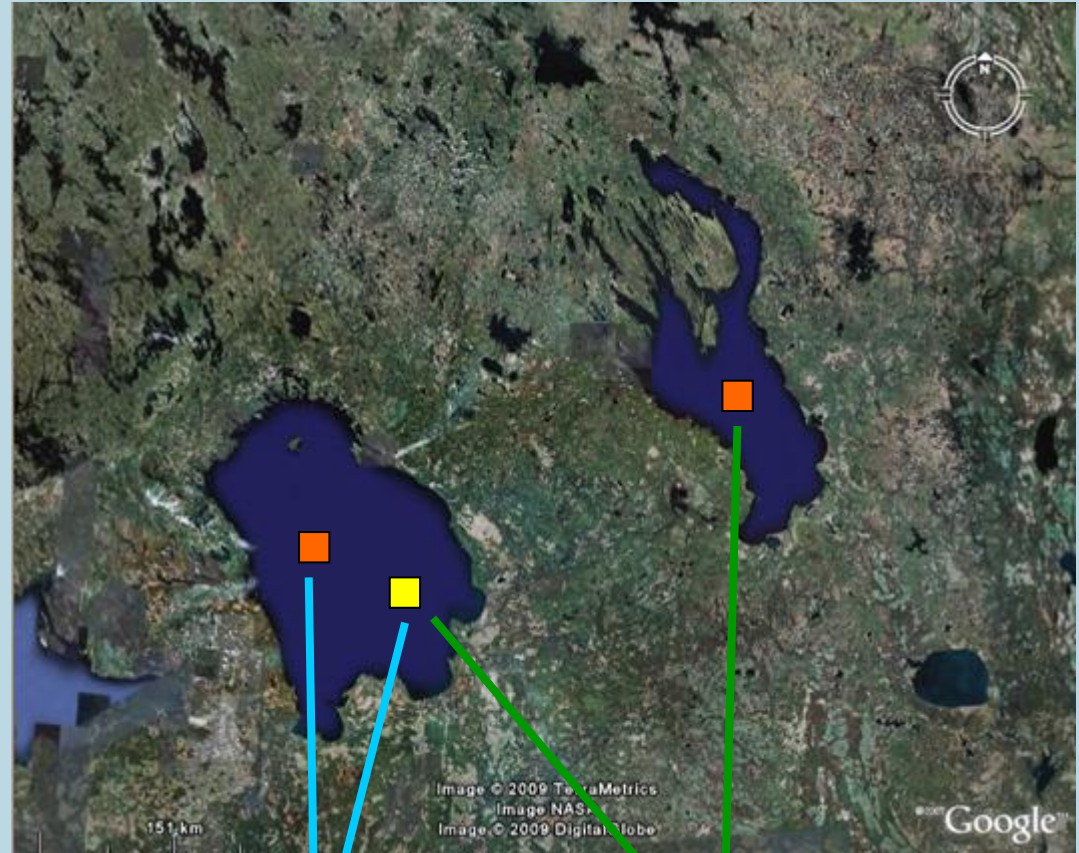


# Biological species concept

- Laboratory vs nature
  - mating of allopatric populations



**Figure 5.** Boxplots of the average percentage of cells involved in sexual reproduction in crosses with *Eunotia bilunaris* strains of the same ('slender' and 'robust') and different groups ('hybrid'). A  $\log(x+1)$  scale was used because of the large differences between the different categories. For crosses within the group 'slender' three different combinations of strains (four different strains) were used, within 'robust' five combinations (seven strains) and between slender and robust ten (four 'slender' and six 'robust' strains).

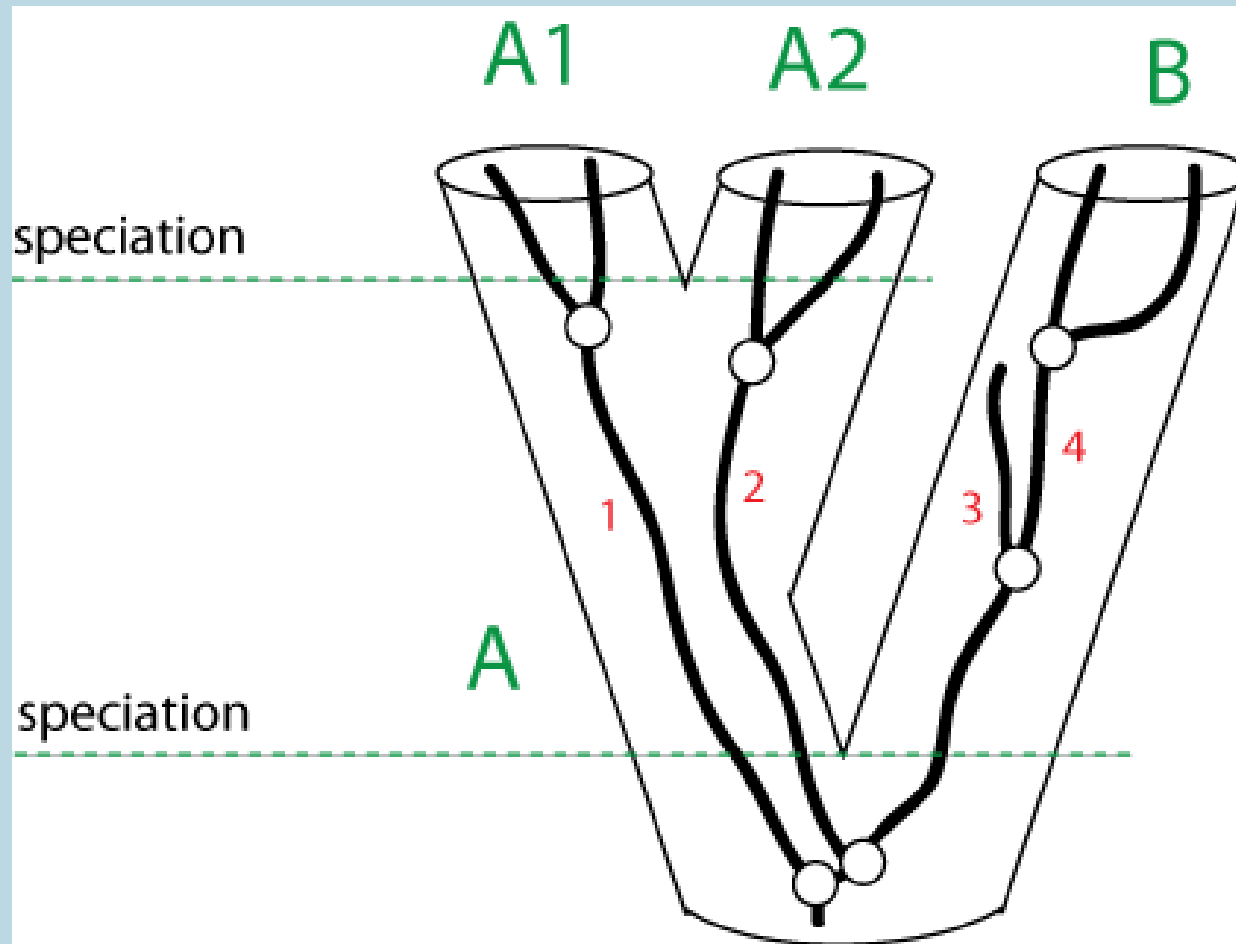


X

OK

# Phylogenetic species concept

- Based on a tree topology



# Phylogenetic species concept

- Where are the species barriers?

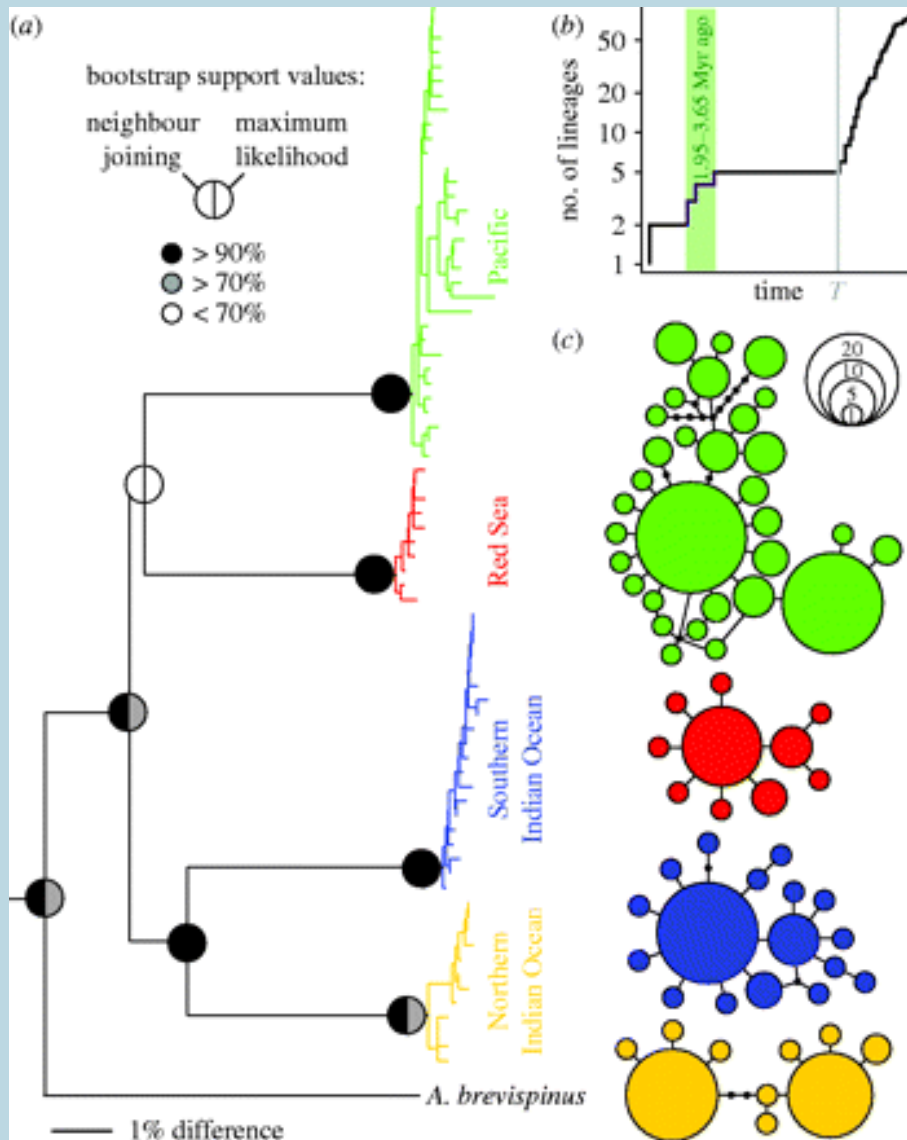
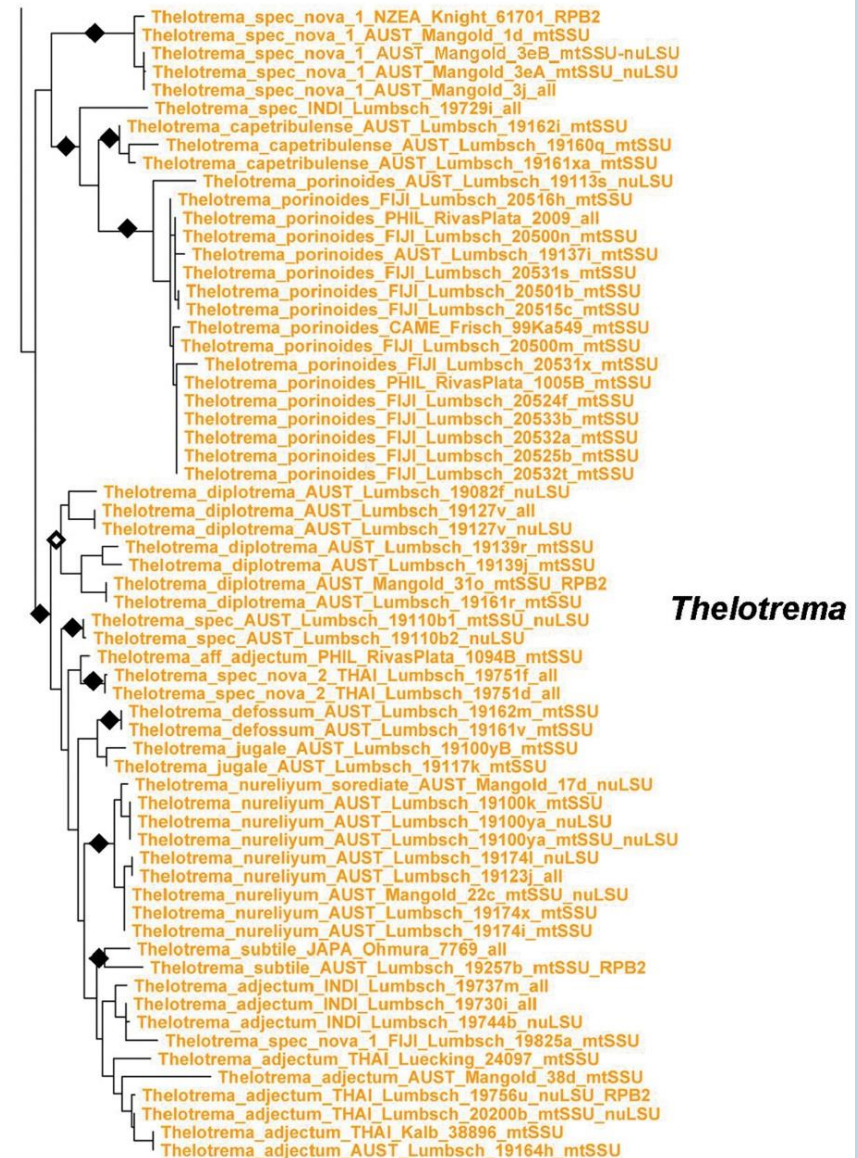
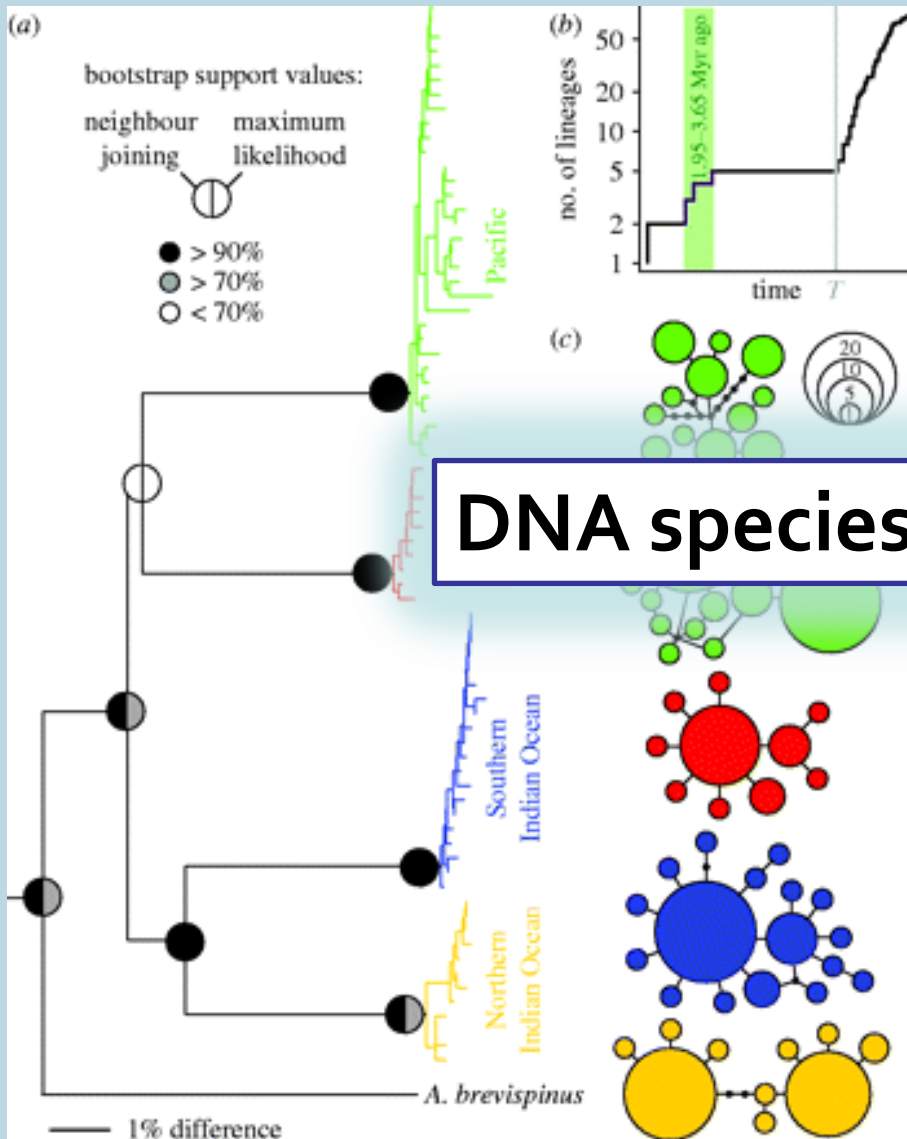


Fig. 8



# Phylogenetic species concept

- Where are the species barriers?



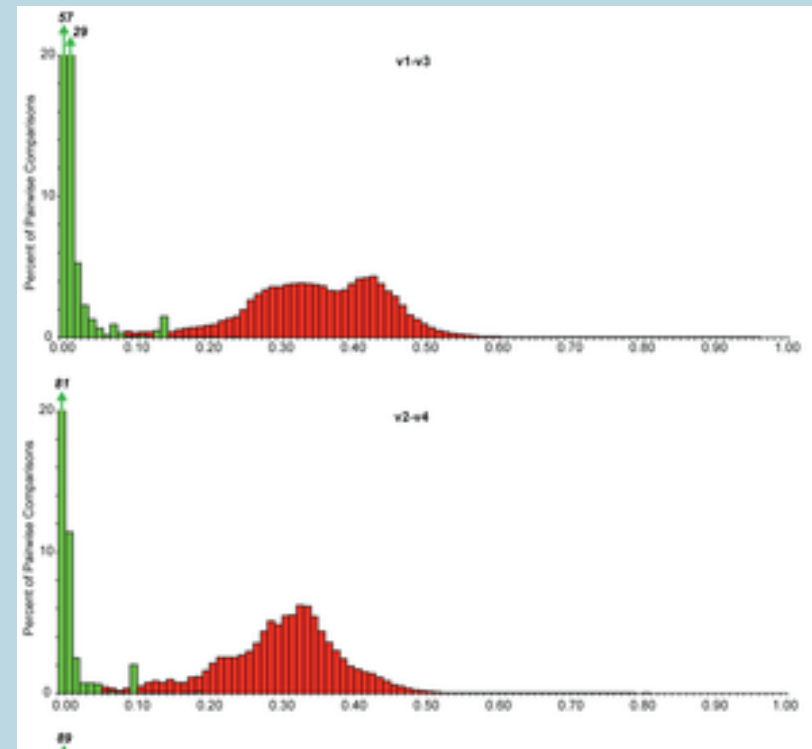
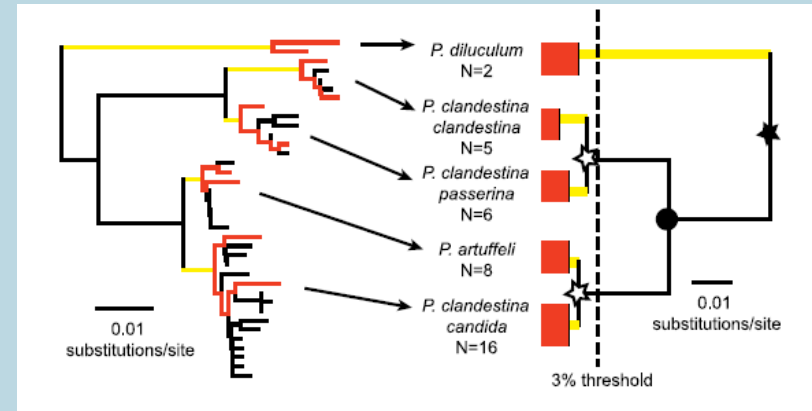
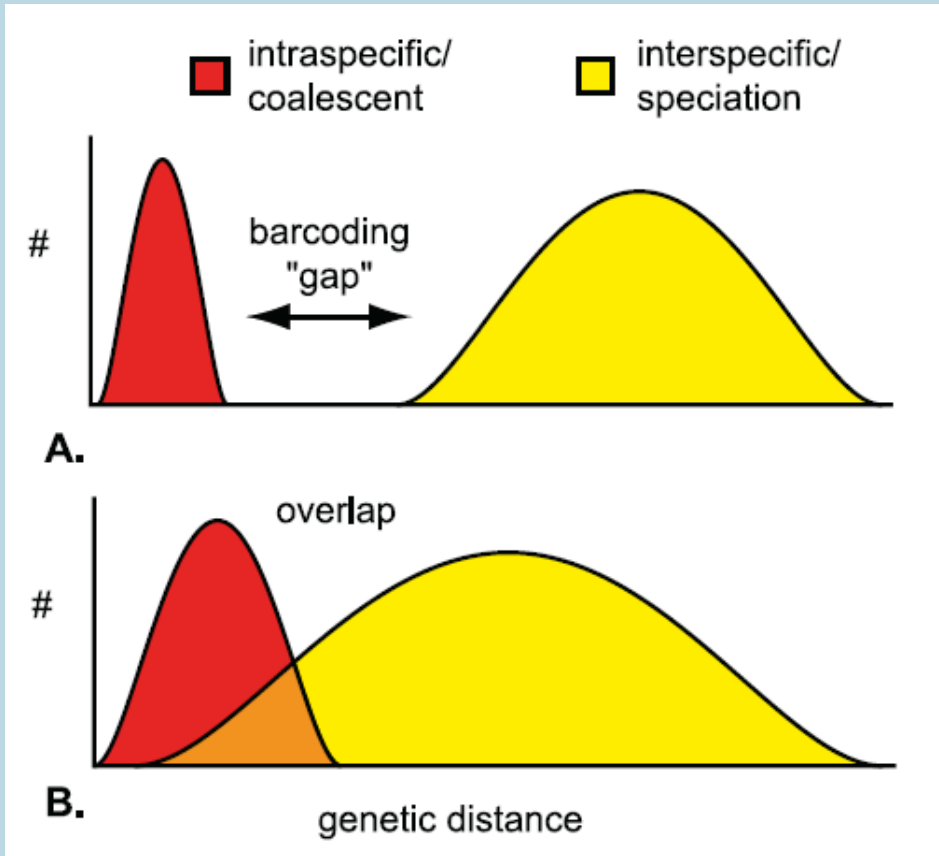
## DNA species delimitation

Fig. 8



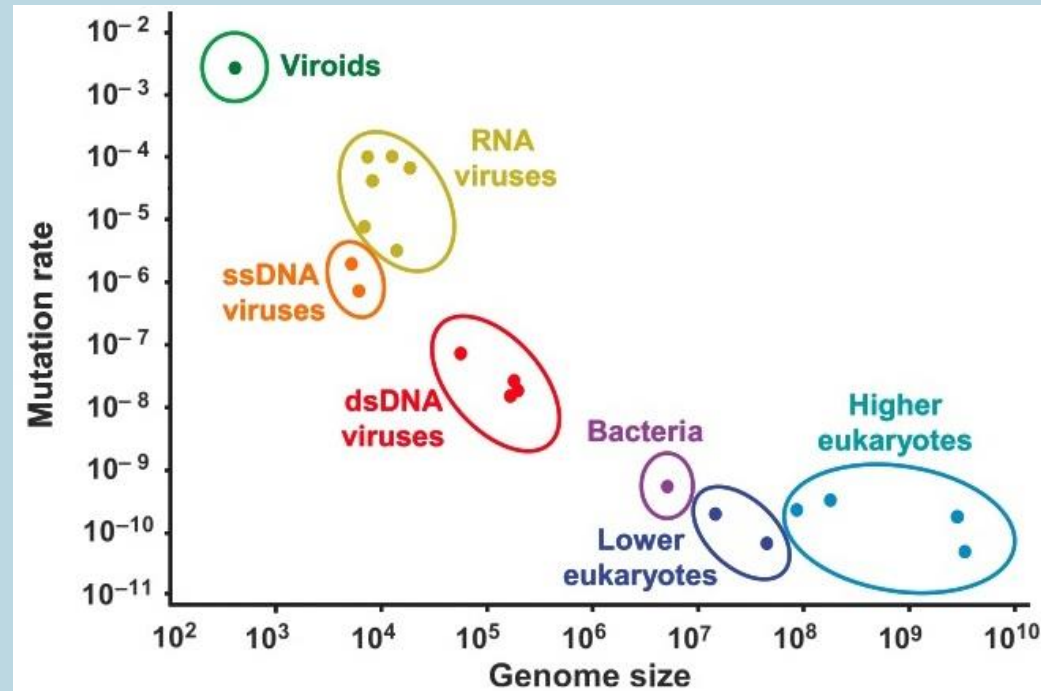
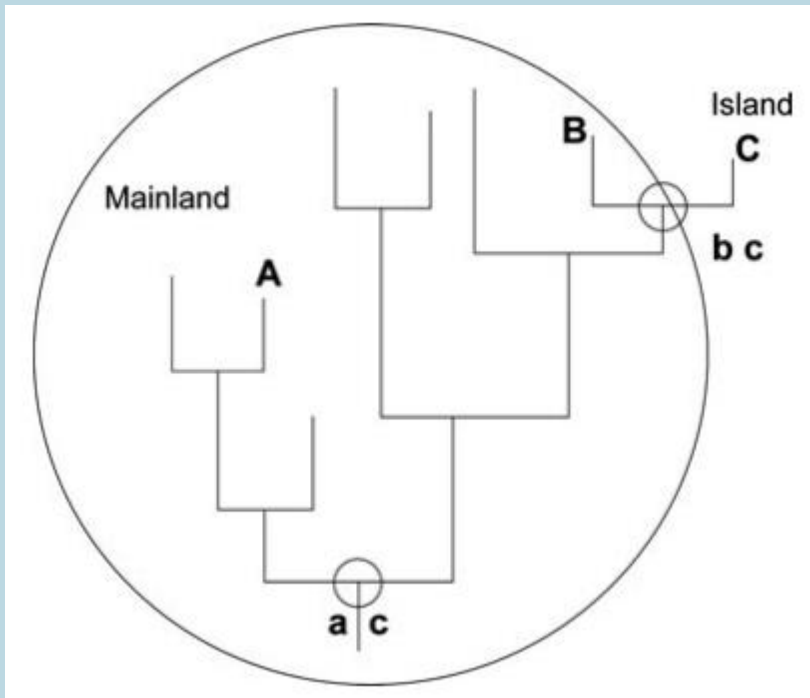
# DNA species delimitation

- DNA barcoding



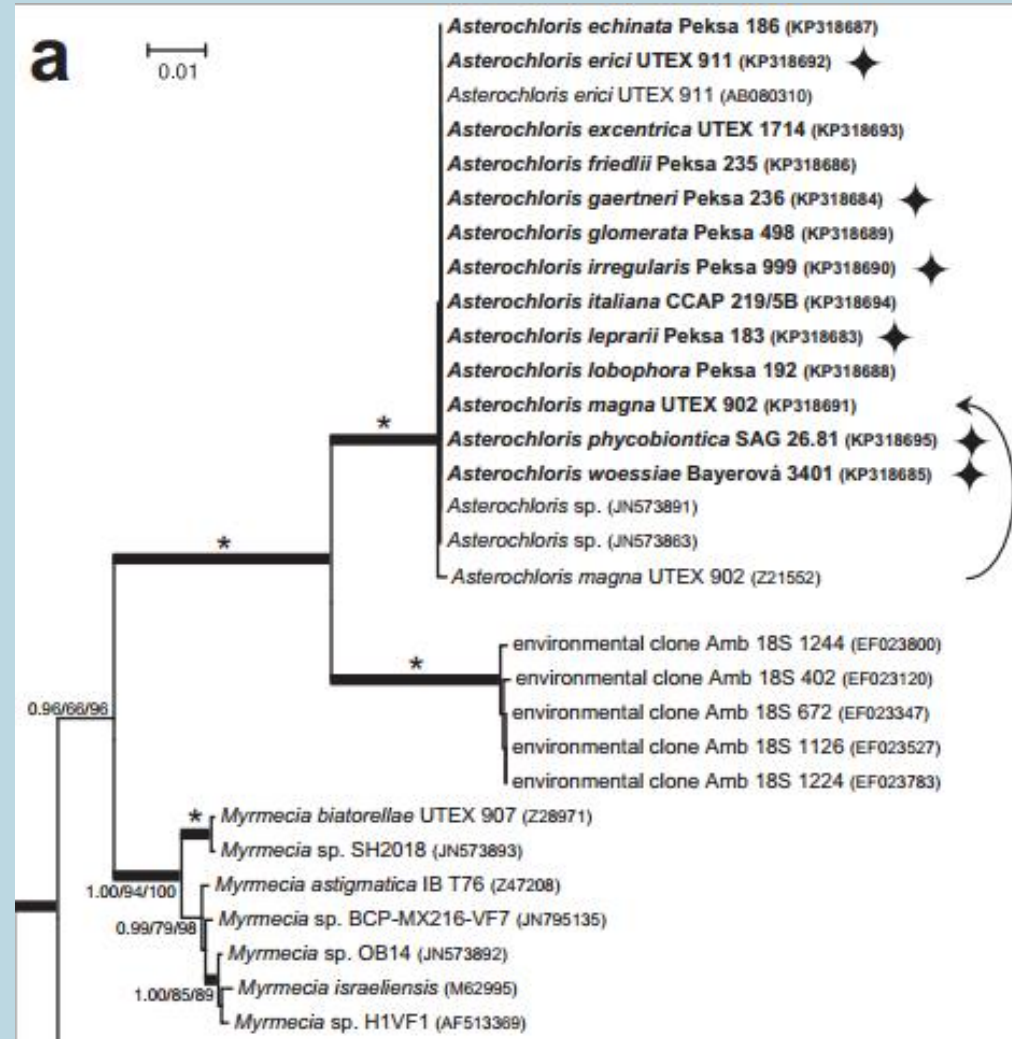
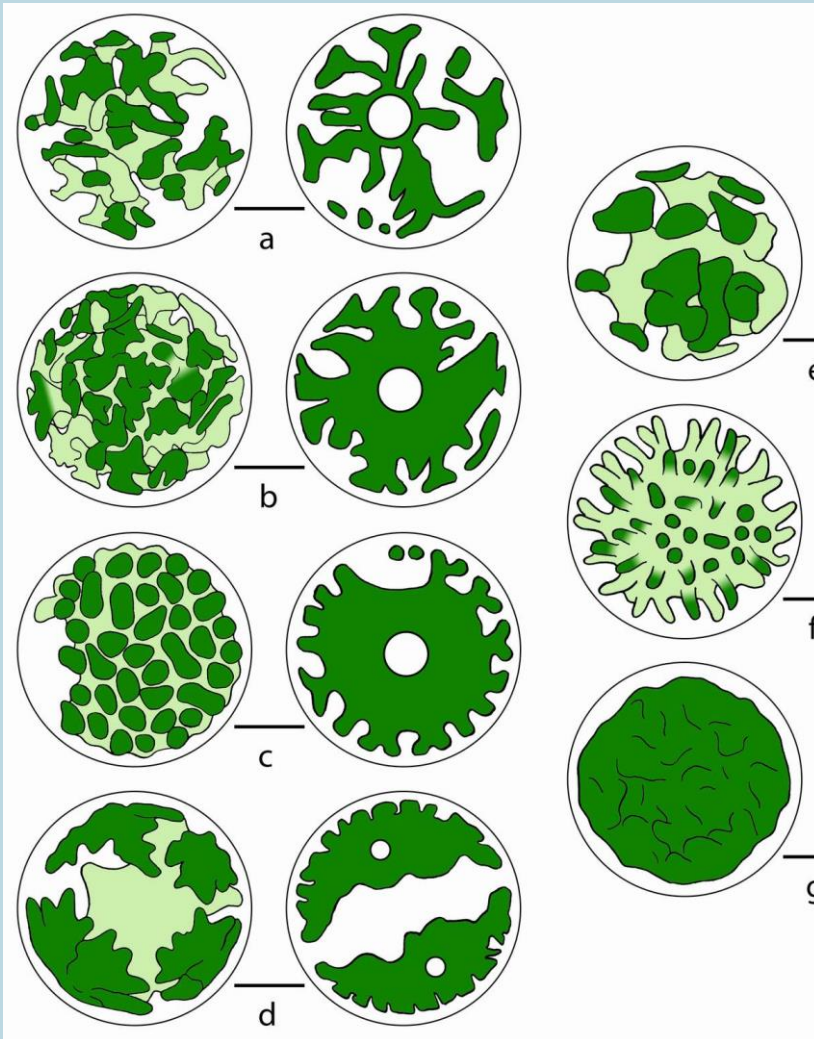
# DNA species delimitation

- Uneven mutation rates
  - tropical vs temperate zone
  - islands vs continents
  - free-living vs symbionts
  - ...



# DNA species delimitation

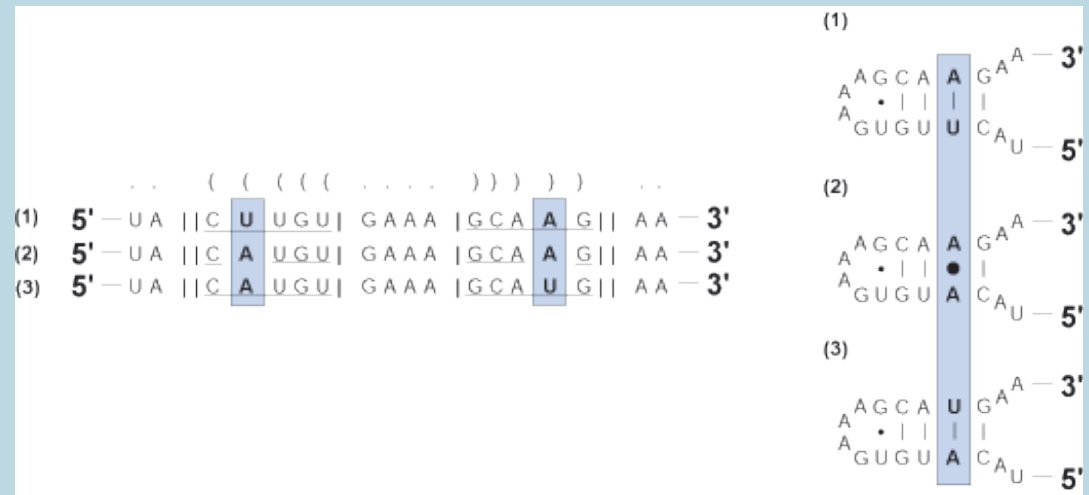
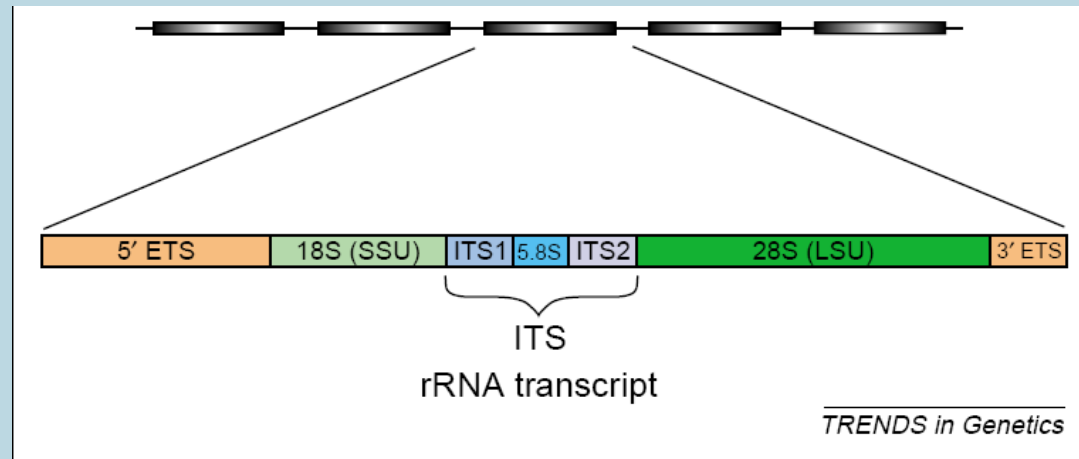
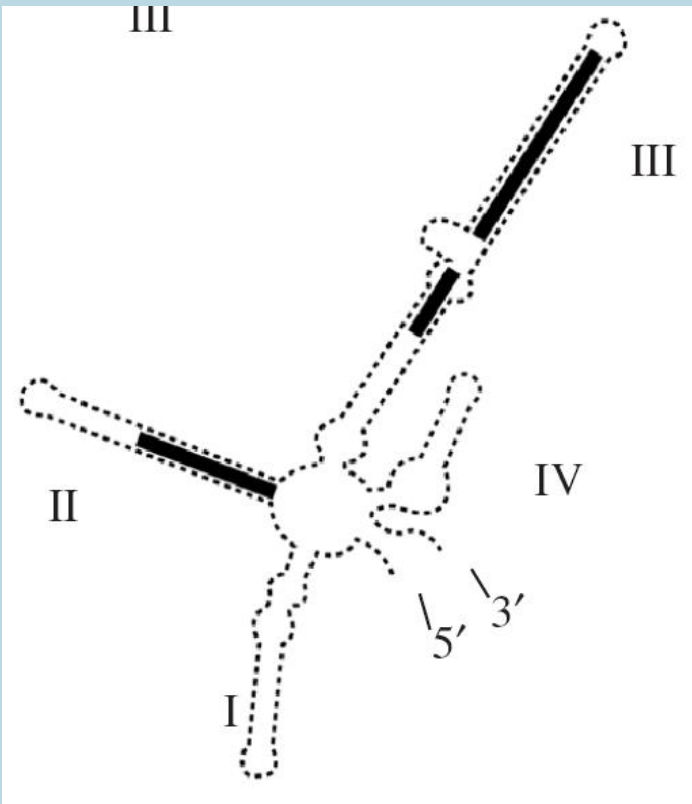
- Uneven mutation rates





# DNA species delimitation

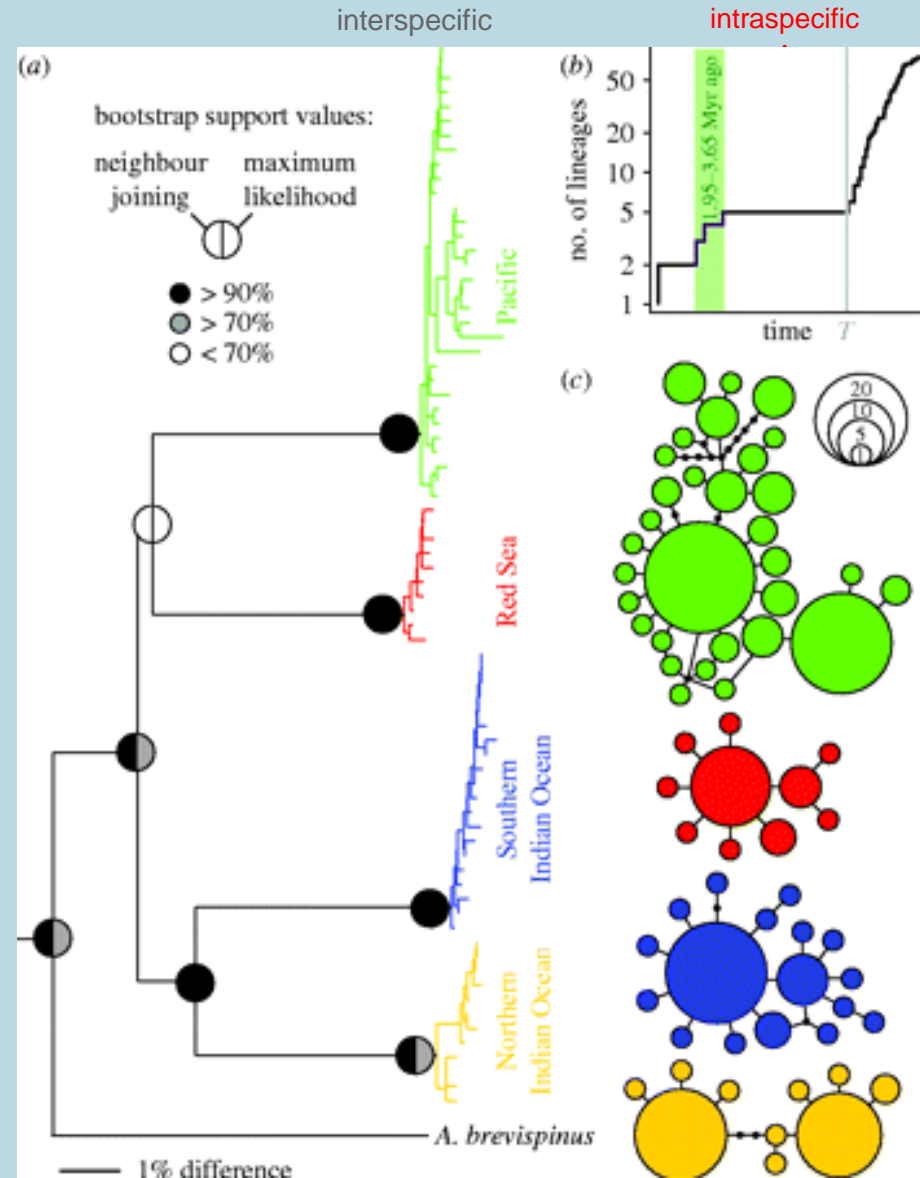
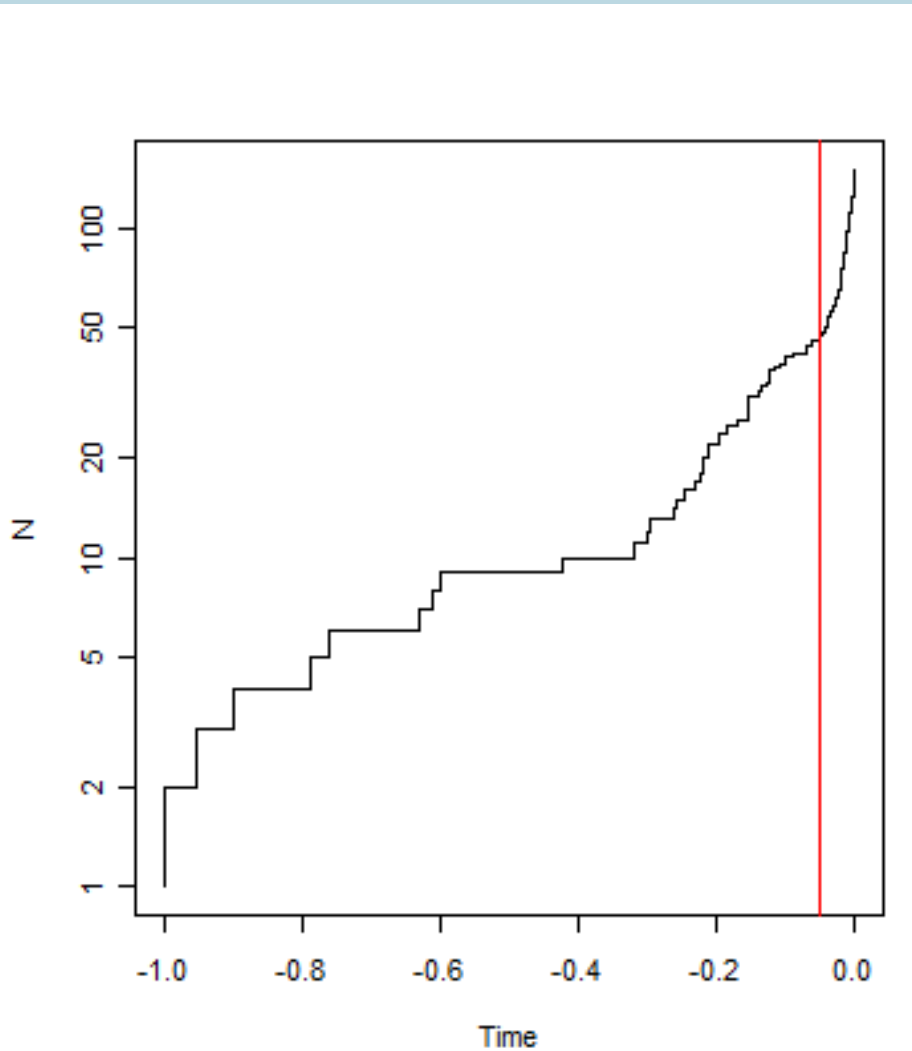
- CBC species concept





# DNA species delimitation

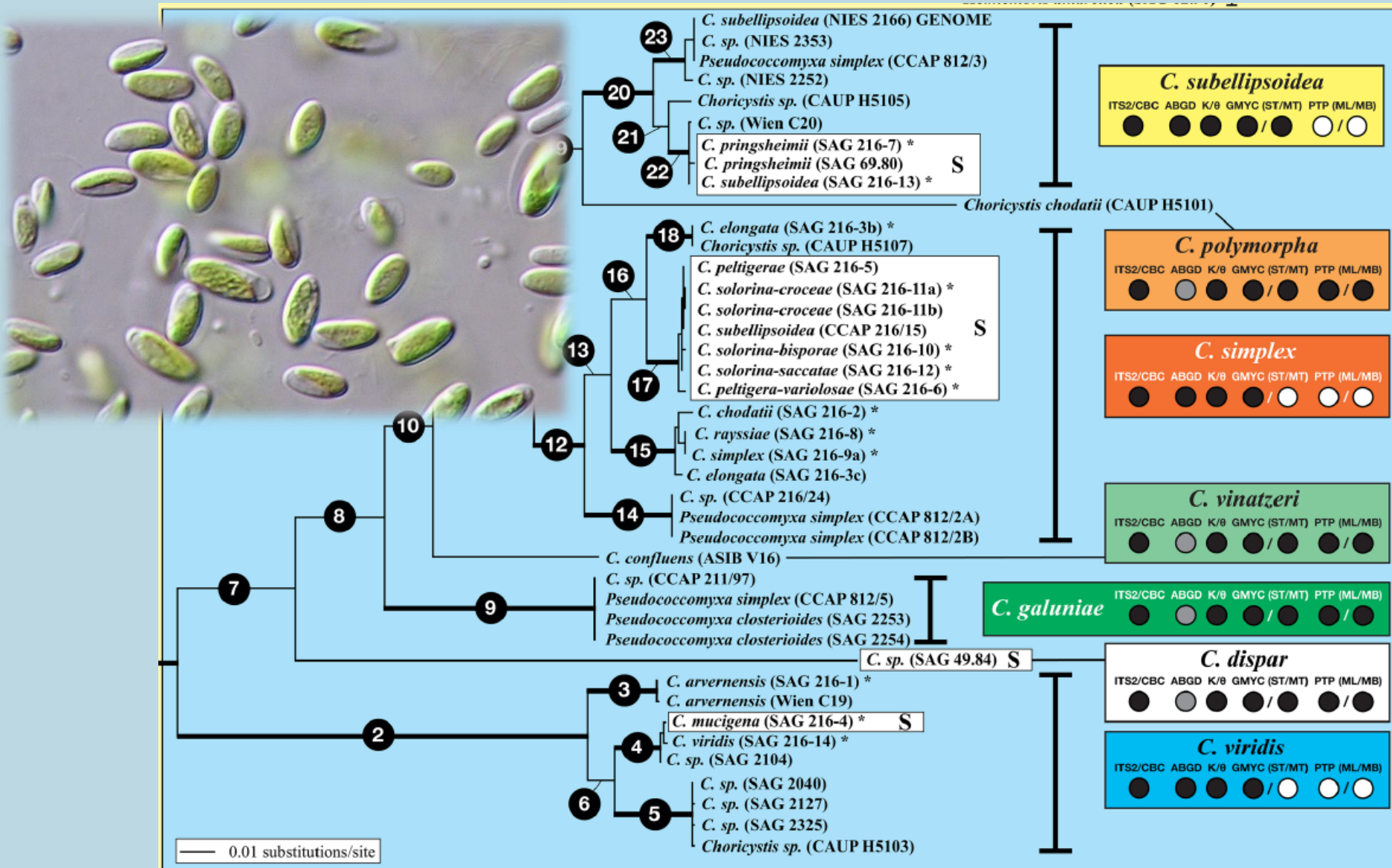
- Coalescence-based species concepts
- GMYC, BP&P, bPTP, .....



# DNA species delimitation

- Coccomyxa*

Darienko et al. (2015): *Plos ONE* 10: e0127838



# DNA species delimitation

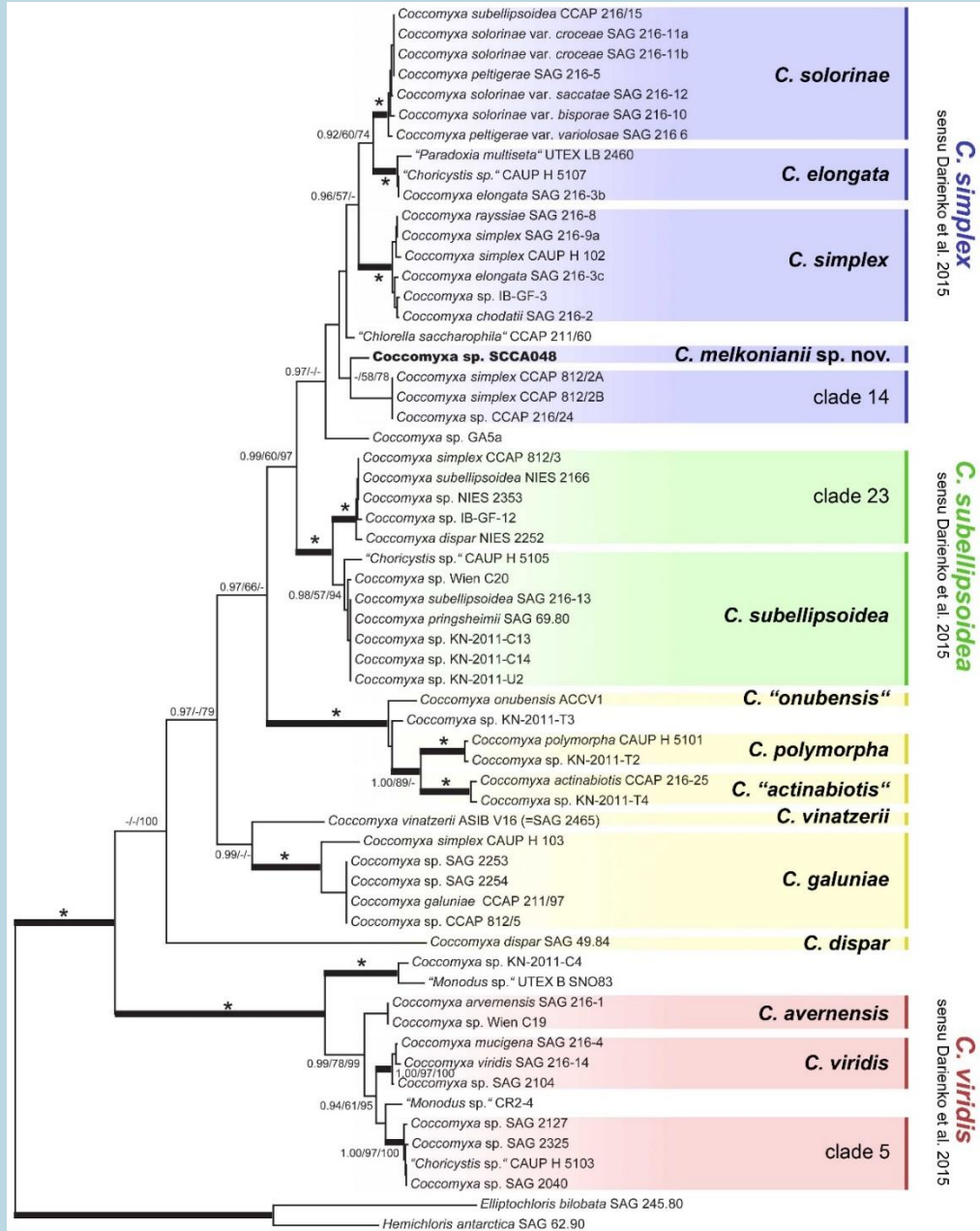
- Coccomyxa*

original dataset:

- 43 sequences

new dataset

- 61 sequences



# DNA species delimitation

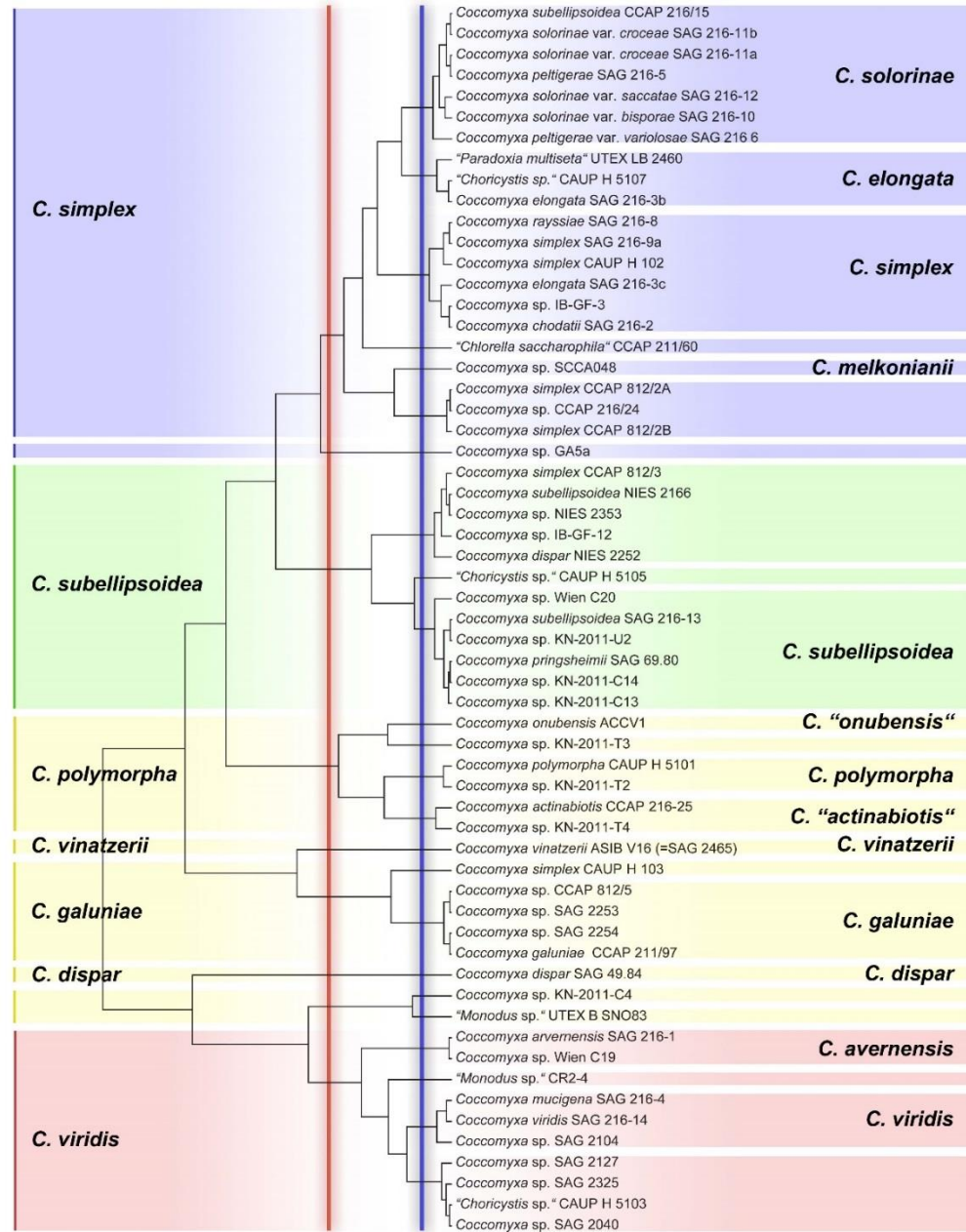
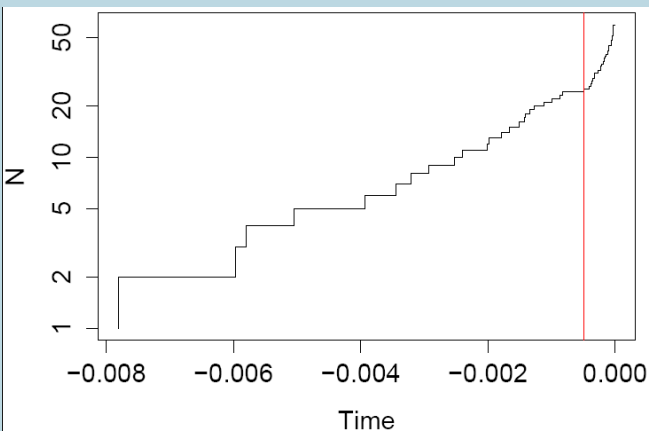
- Coccomyxa*

original GMYC:

➤ 9 species

new GMYC

➤ 24 species

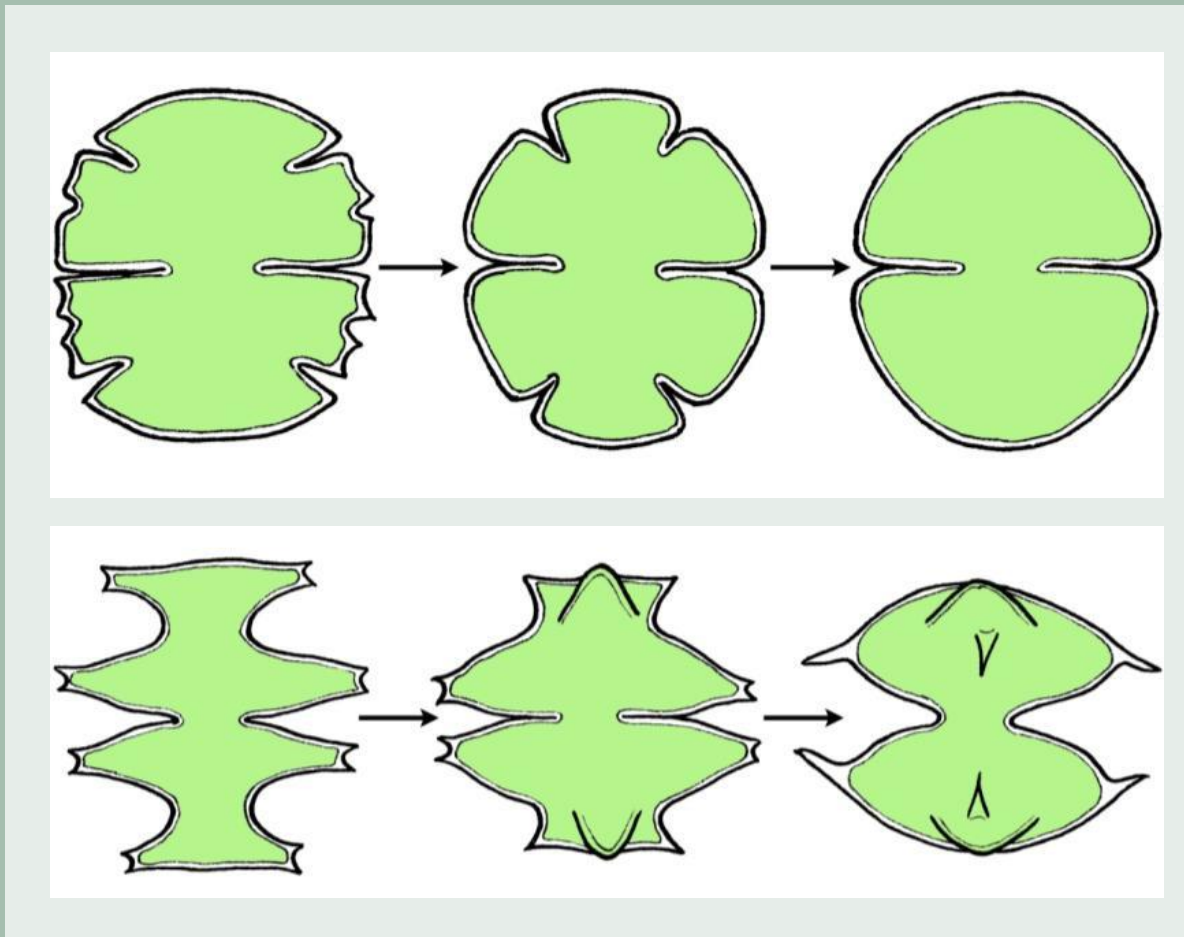


# How to delimit the microalgal species?

- No one species concept is universally applicable
- Polyphasic approach?
- Better understanding of speciation mechanisms?

# What are the general causes of microalgal speciation?

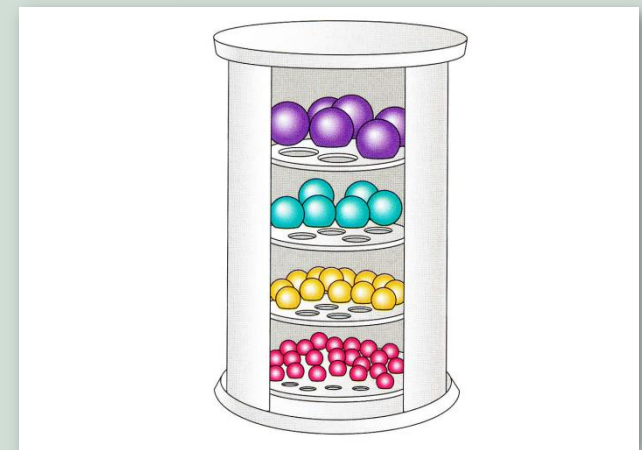
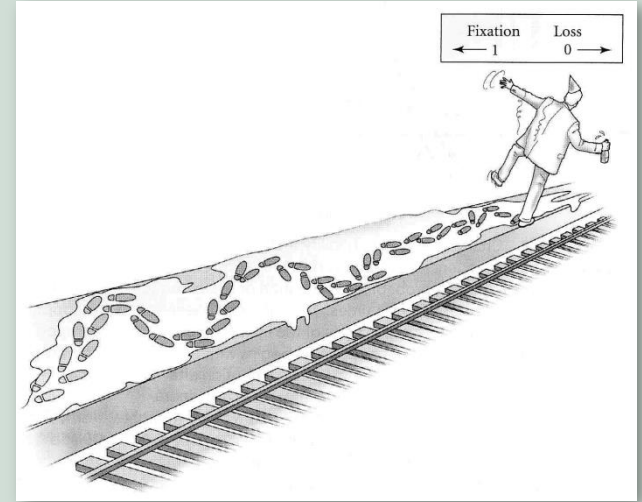
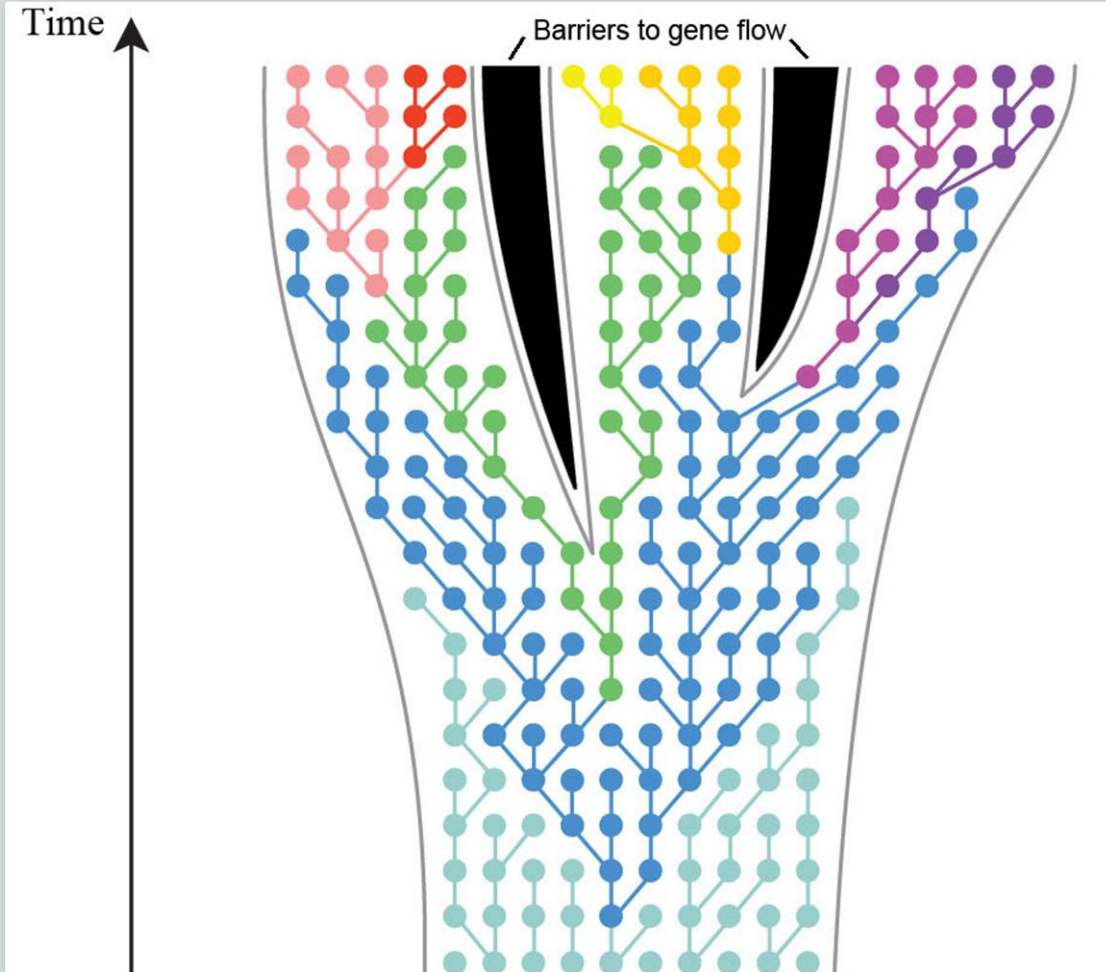
1. Allopatric / sympatric speciation models
2. Speciation mechanisms in microalgae
3. Testing the significance of different speciation models in a microalgal model





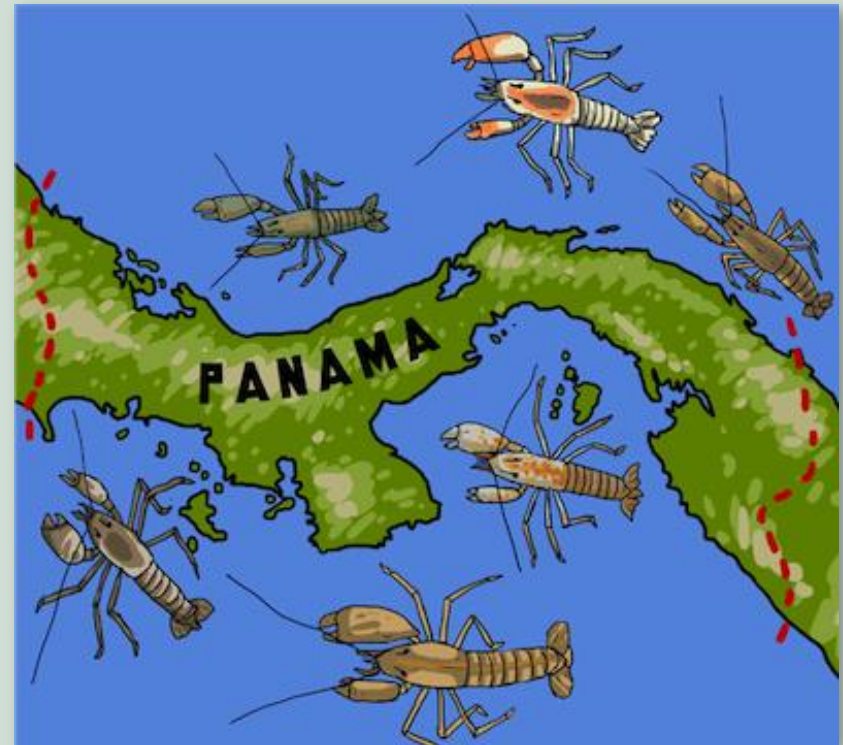
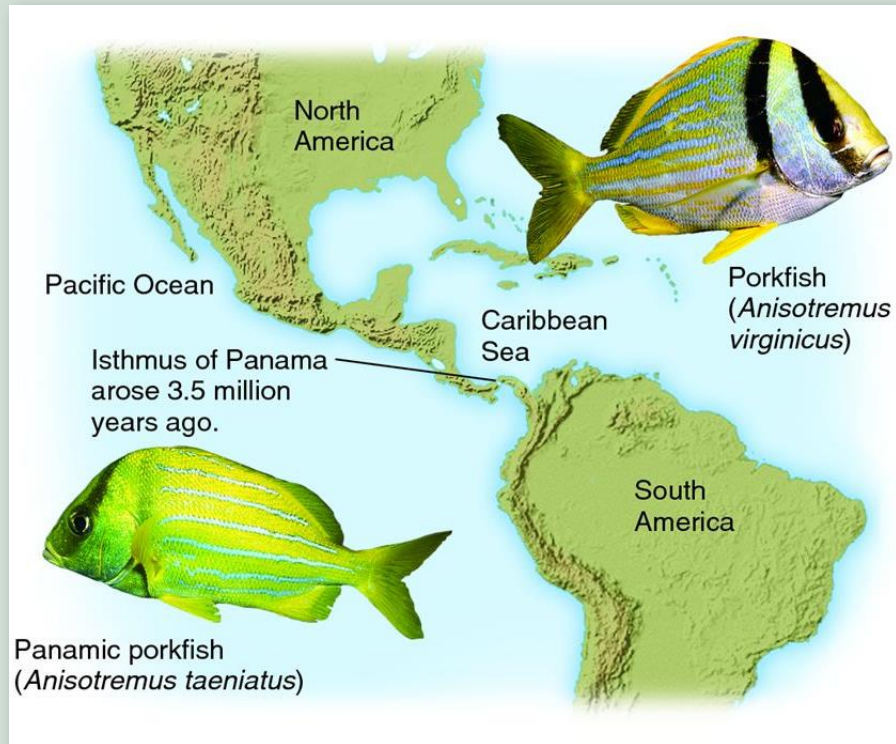
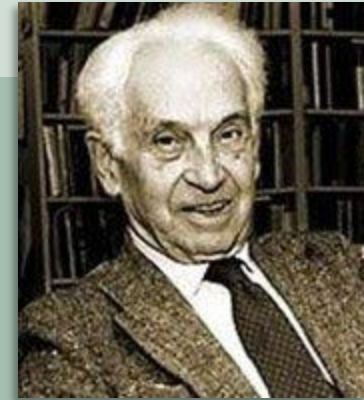
# Models of speciation

- Species = populations with distinguishing characteristics
- Barriers to gene flow as a key factor in speciation



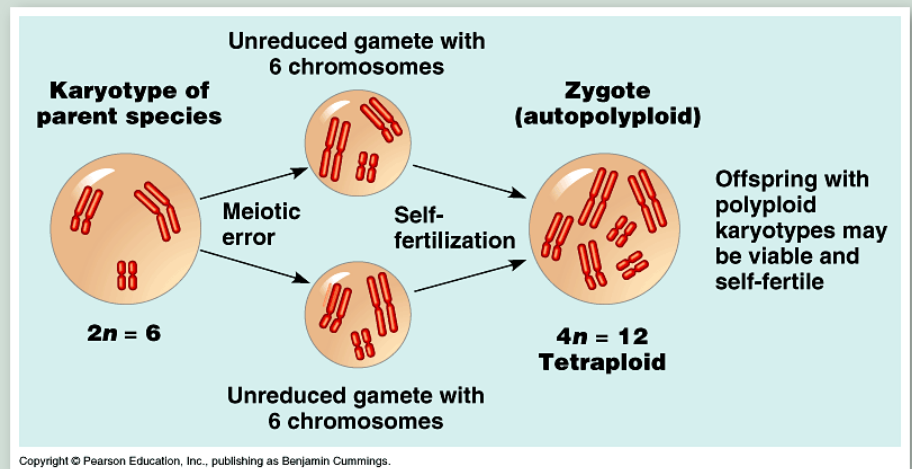
# Models of speciation

- **Allopatric speciation** = geographical barrier to gene flow
- Ernst Mayr (1946): “*differentiation of populations must be preceded by geographical or other means of physical isolations*”



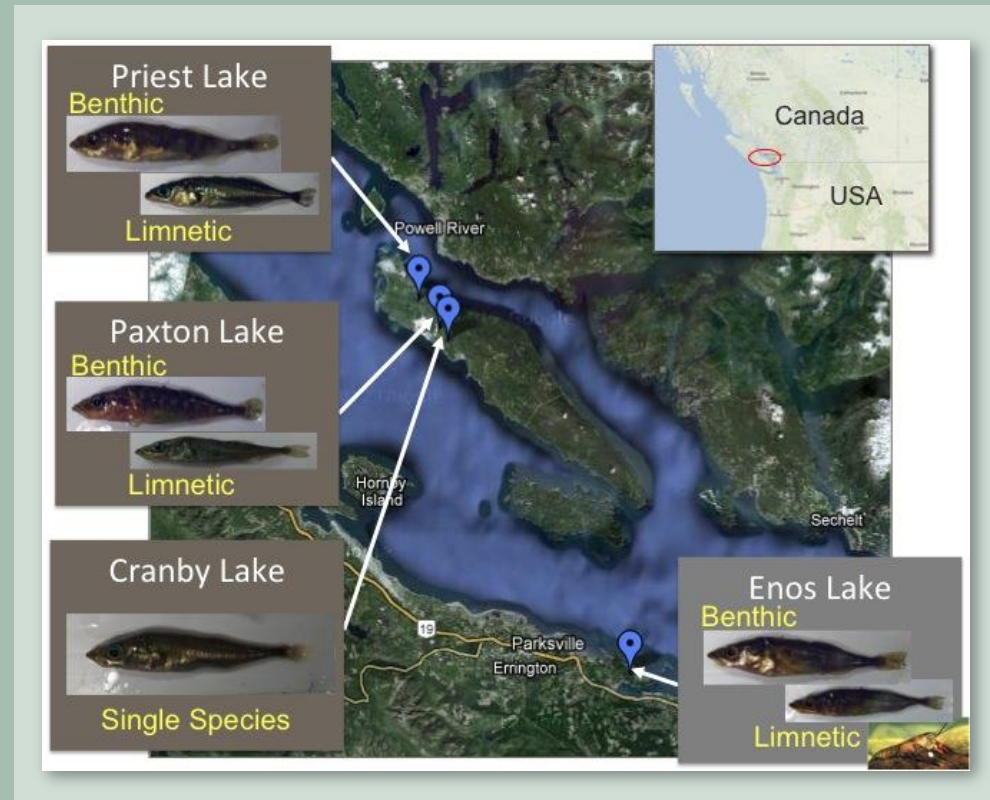
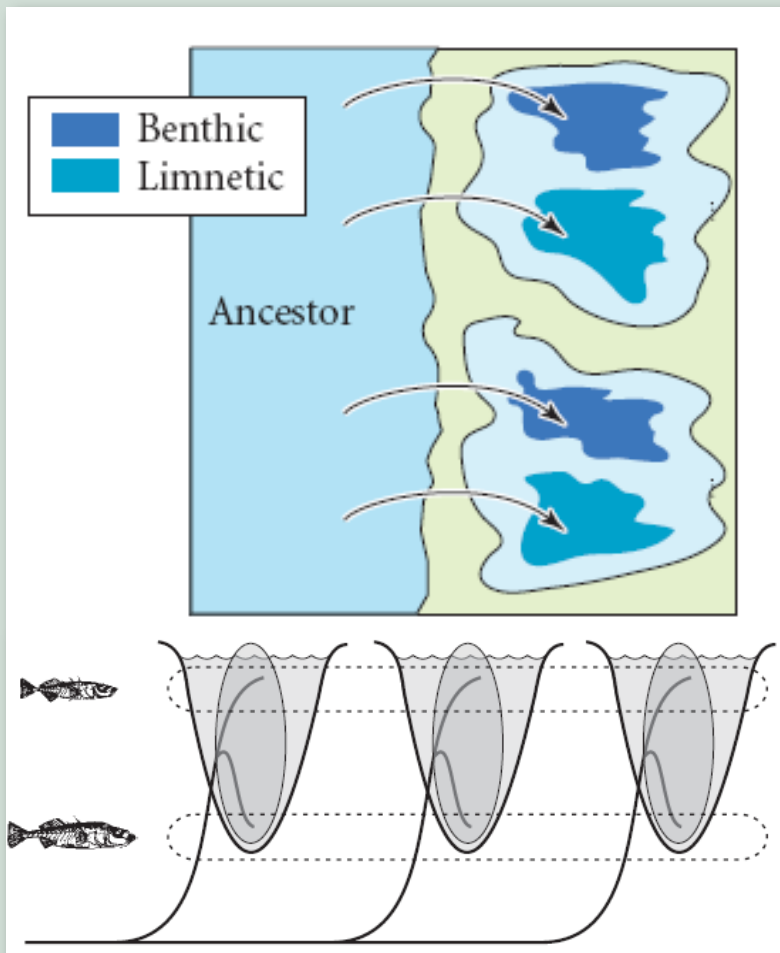
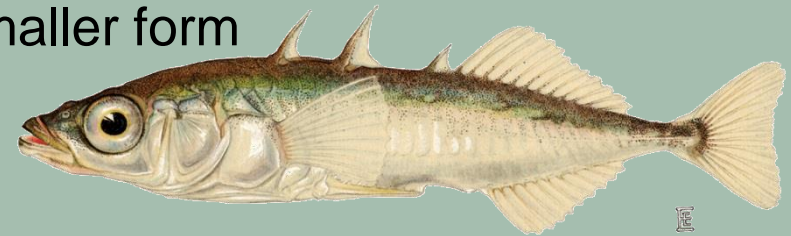
# Models of speciation

- **Sympatric ecological speciation** = barrier to gene flow as a result of ecologically-based divergent selection
- Ecological factors of speciation:
  - Habitat isolation (salinity levels, substrate types, host species ...)
  - Temporal isolation (flowering times)
  - Sexual isolation (selection for body size)
  - Gametic isolation (gamete recognition mechanisms)
  - Postzygotic isolation (hybrid low fitness / lethality)
  - Cytological isolation (polyploidisation)



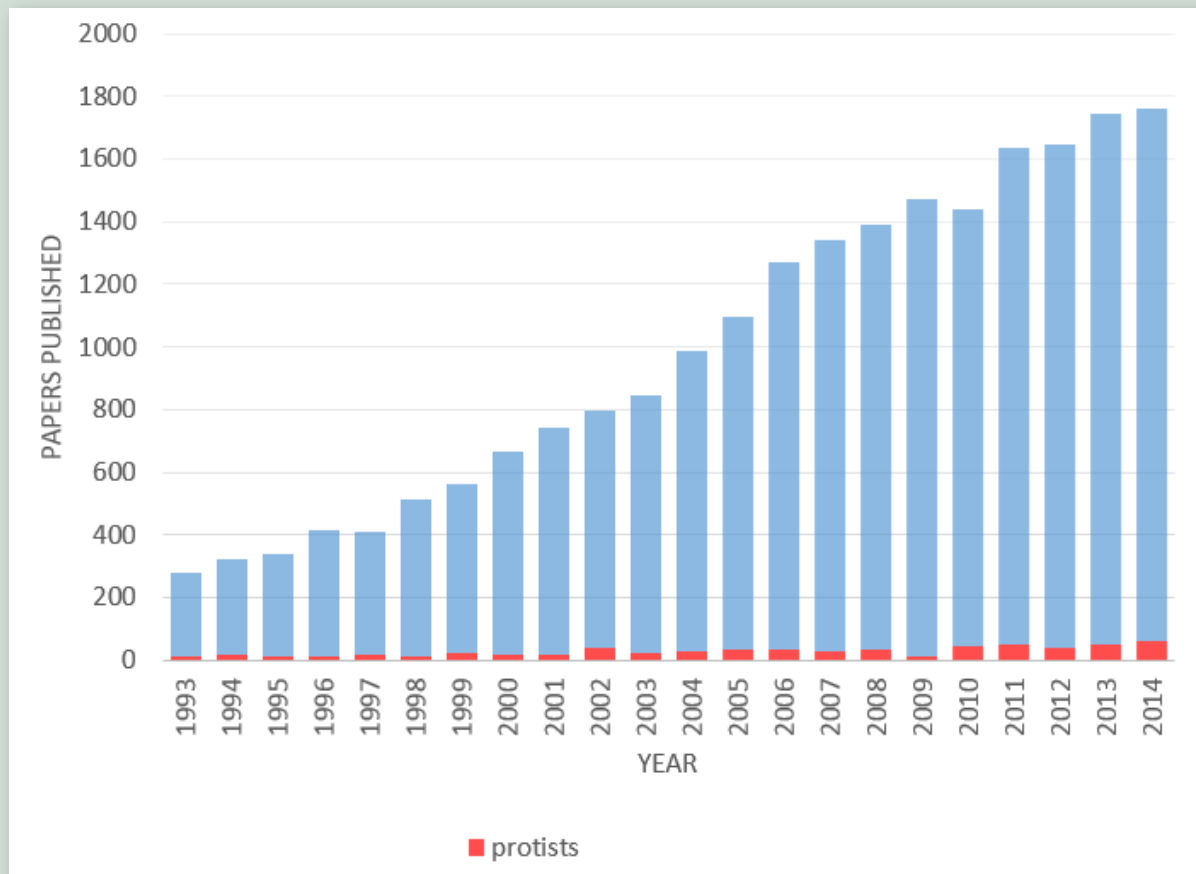
# Models of speciation

- Parallel speciation of three-spined stickleback in Canadian lakes
  - Limnetic species = open-water, larger form
  - Benthic species = bottom/feeding, smaller form



# Speciation in microalgae

- Only a very small fraction of papers focusing on speciation is dealing with eukaryotic microorganisms.
- Thought microalgae are extremely numerous and essential in global ecosystem functioning, our knowledge of their speciation is vastly limited.



*Web of Science literature search for papers of the topic “speciation” for the years 1993–2014, refined to subject areas “evolutionary biology,” “ecology,” or “genetics and heredity.” Papers refined to topic “algae”, “protist” or “protozoa” are given in orange.*

# Specificity of protist organisms

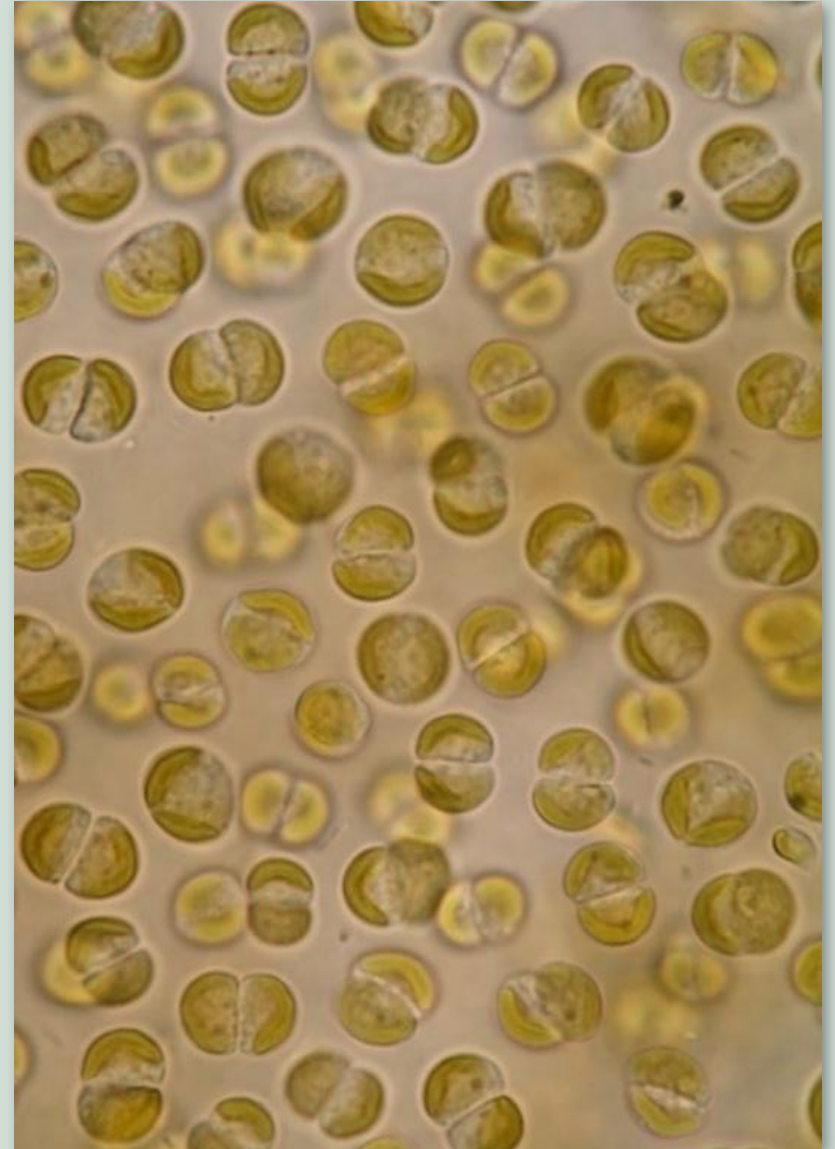
- Short generation time
- Enormous population sizes
- Unlimited dispersal & gene flow



## Unlike the macroorganisms:

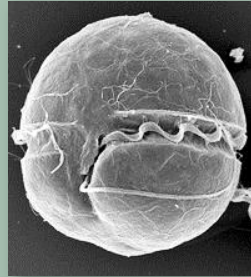
- Ubiquitous distribution
- Absence of population differentiation
- Barriers to gene flow extremely rare
- Very low speciation rate

## How do the microalgae speciate?

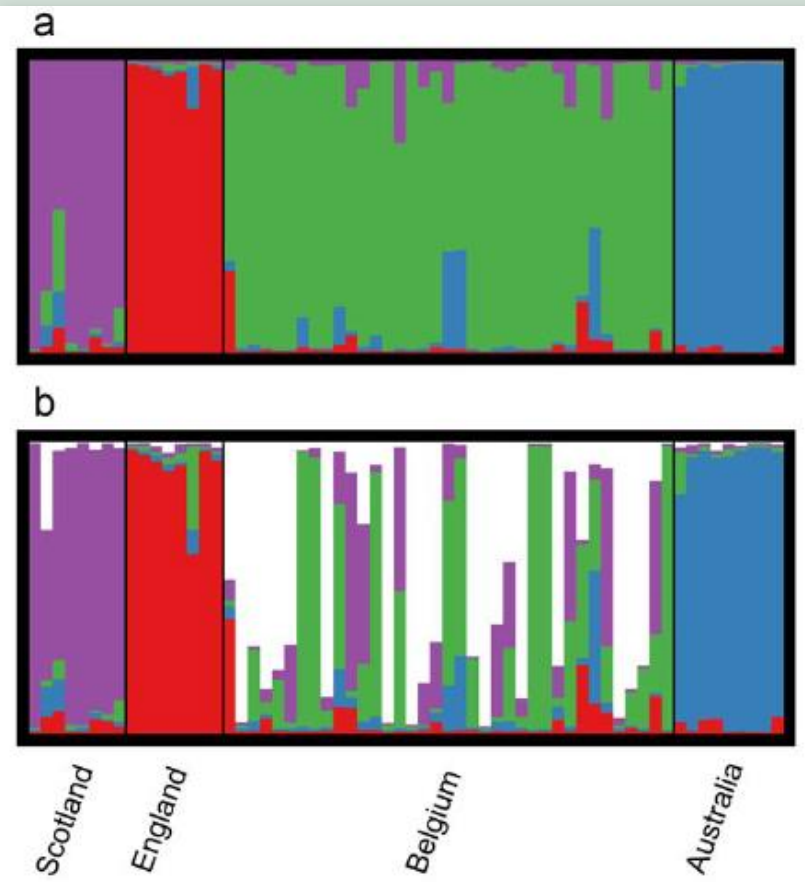
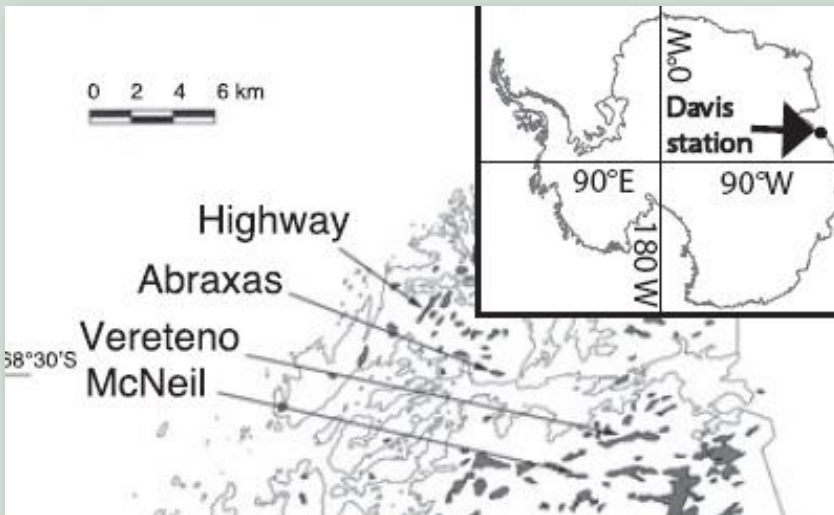
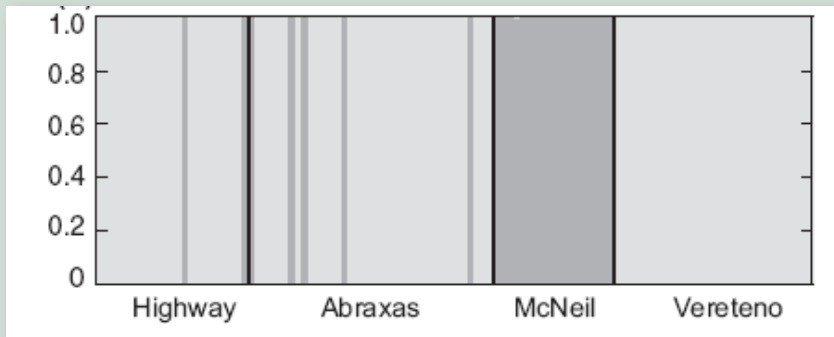
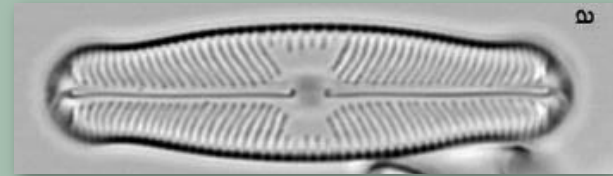


# Genetic structure of microalgal populations

- *Scrippsiella hangoei*



- *Sellaphora capitata*



# Mechanisms of microalgal speciation

Genetic differentiation of microalgal species into distinct populations



Mechanisms of gene flow restriction?

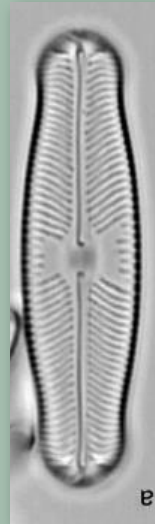




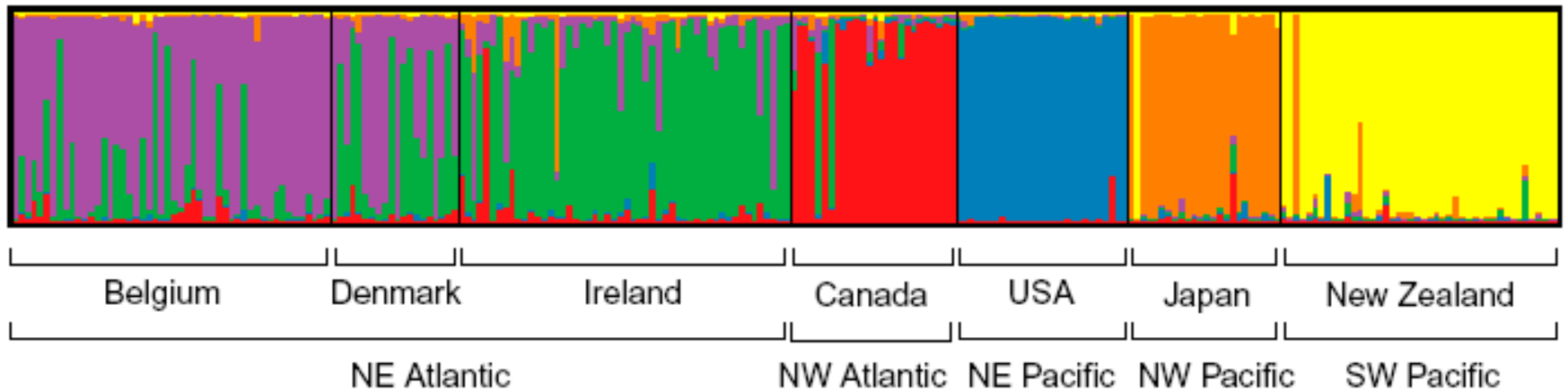
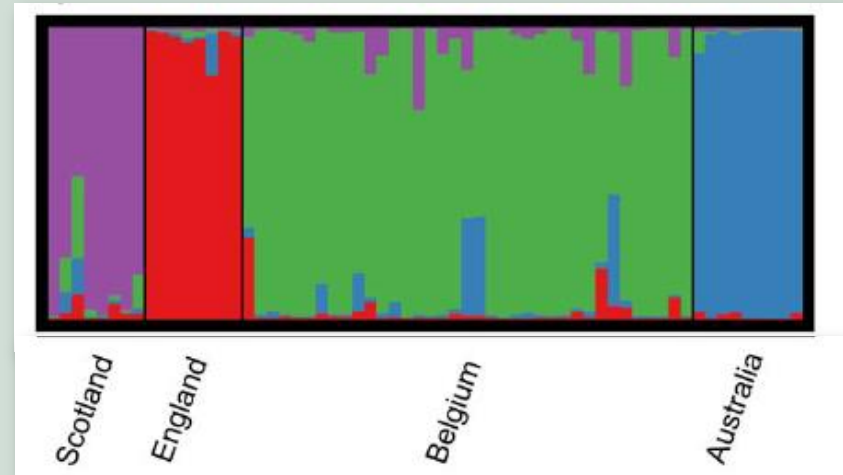
# Mechanisms of microalgal speciation

## 1. Geographic isolation

Evans et al. (2009). *Protist* **160**: 386–396



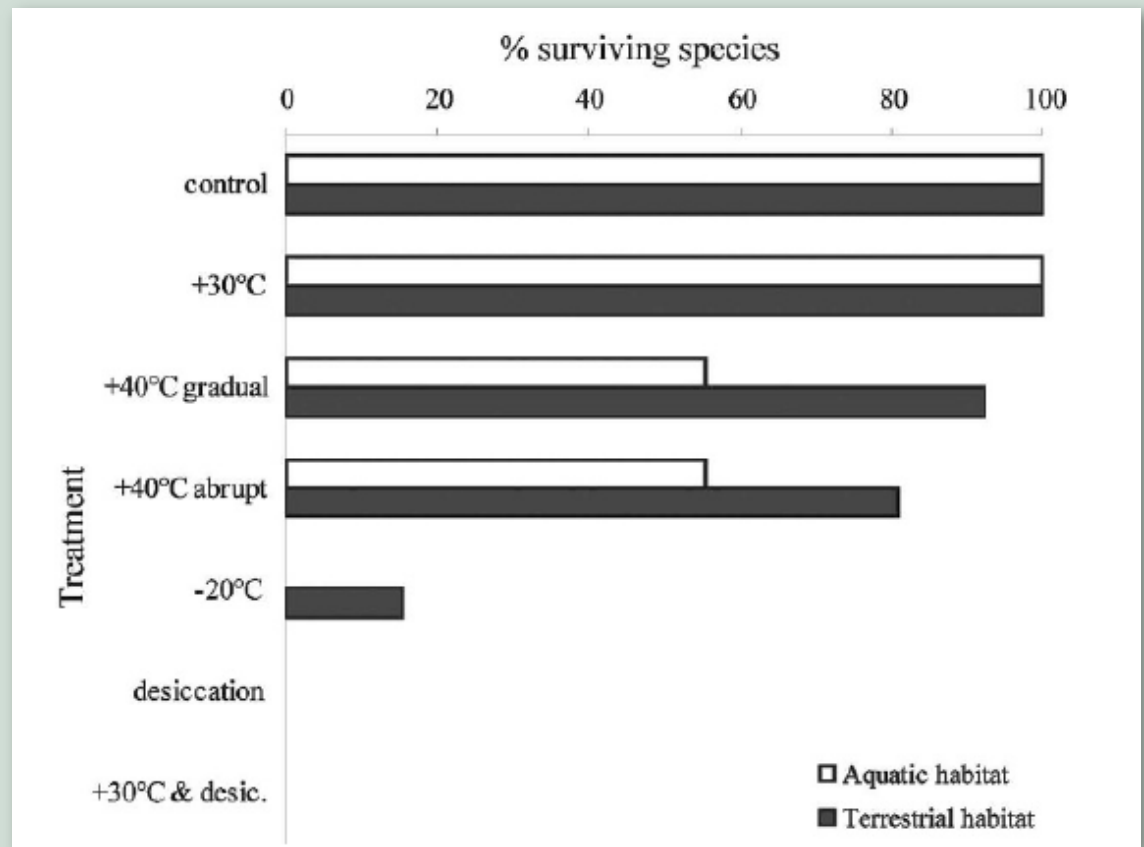
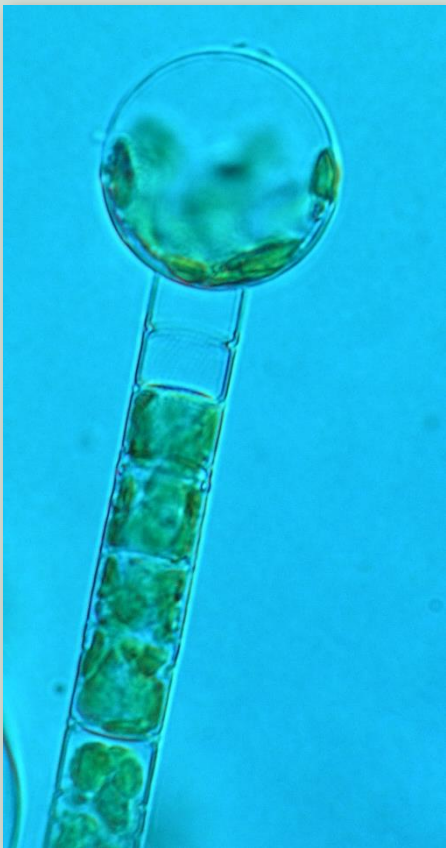
Casteleyn et al. (2010). *PNAS* **107**: 12952-7



# Mechanisms of microalgal speciation

## 1. Geographic isolation

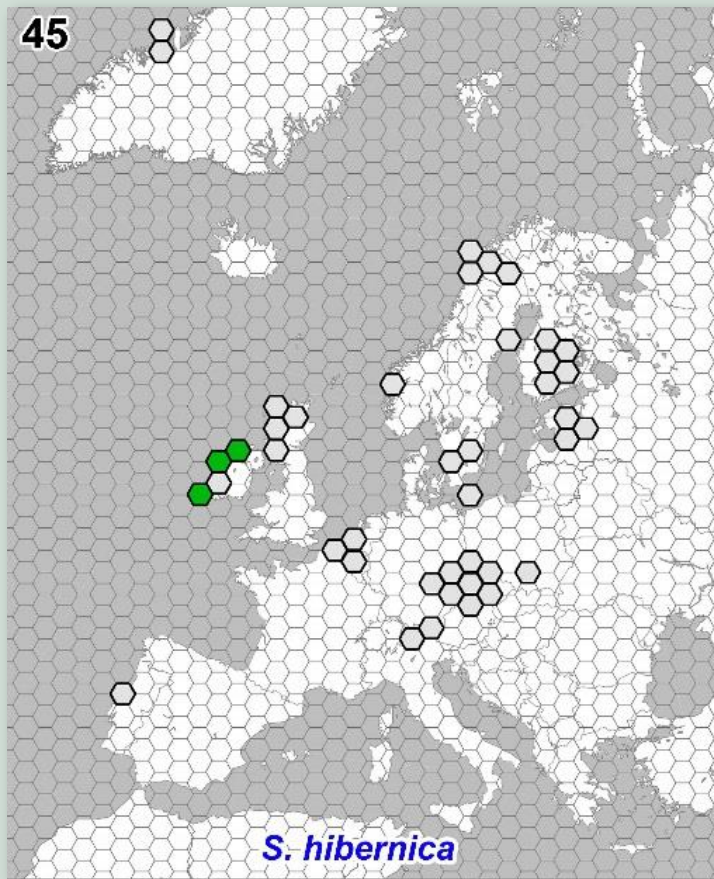
- Freshwater and terrestrial diatoms = the absence of desiccation-resistant cysts can cause the limited dispersal.



# Mechanisms of microalgal speciation

## 1. Geographic isolation

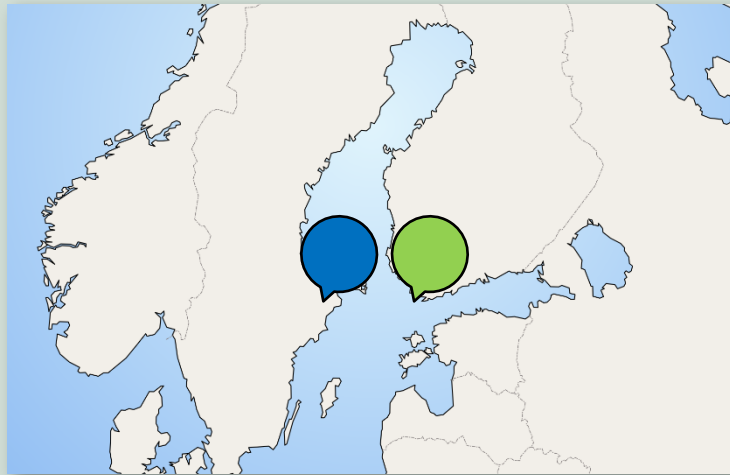
- *Synura hibernica* – restricted to western Ireland



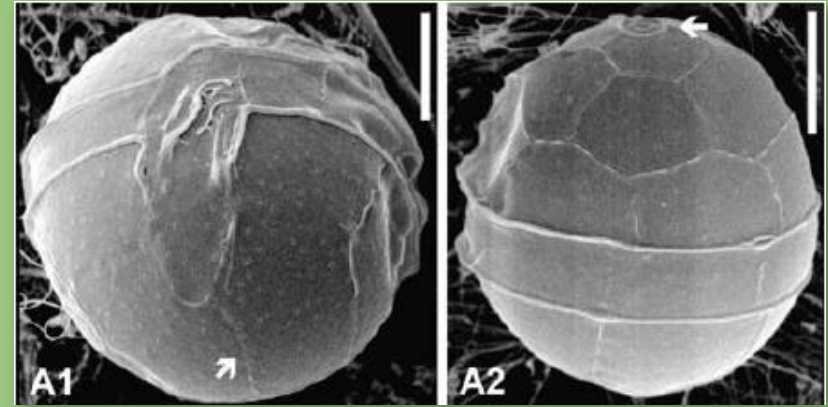
# Mechanisms of microalgal speciation

## 2. Ecological isolation

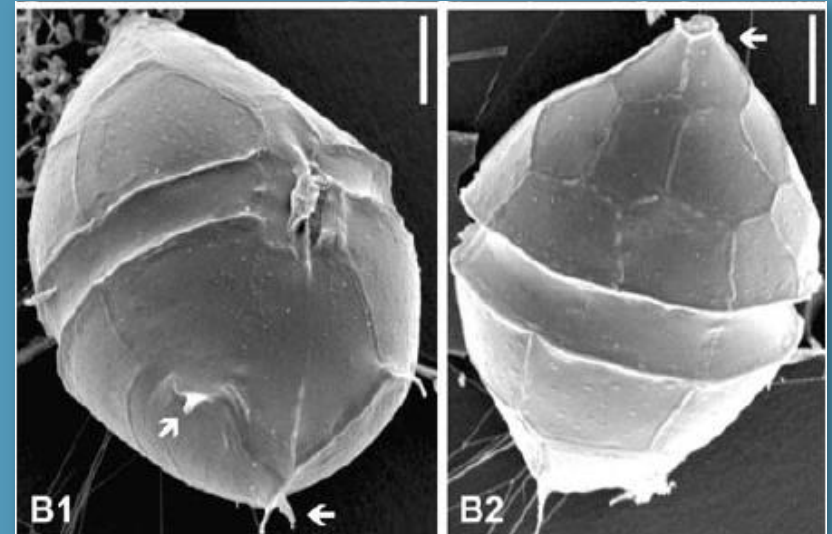
- Recent marine-freshwater transition of a dinoflagellate accompanied with a significant morphological differentiation
- Identical ITS and SSU rDNA
- Probably a rapid postglacial disruptive ecological selection (~ 10,000 years BP)



*Scrippsiella hangoei* - brackish



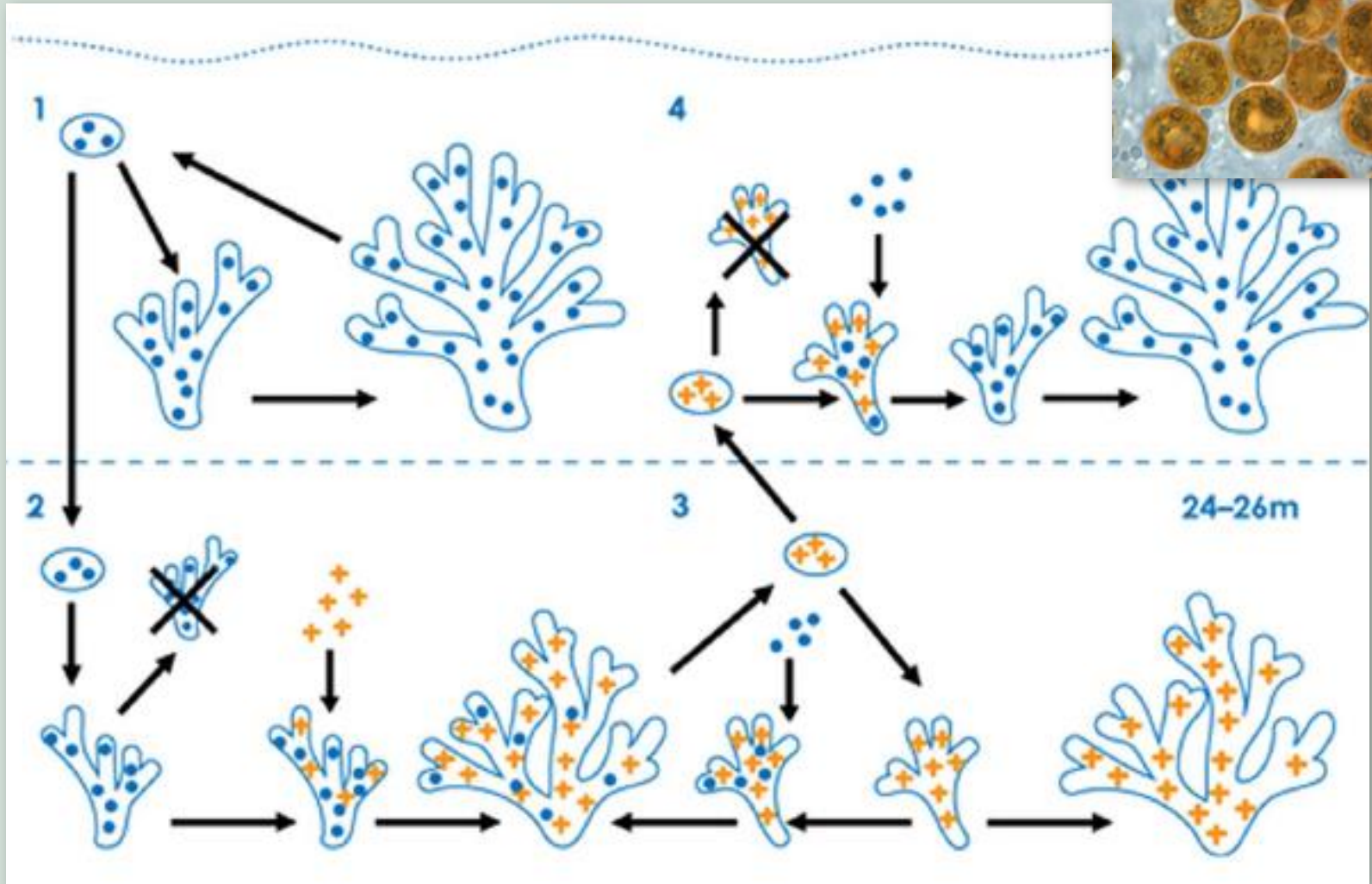
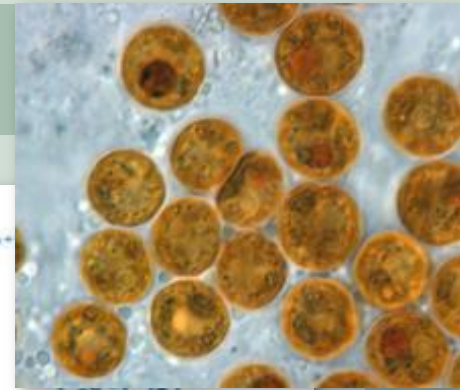
*Peridinium aciculiferum* - freshwater



# Mechanisms of microalgal speciation

## 2. Ecological isolation

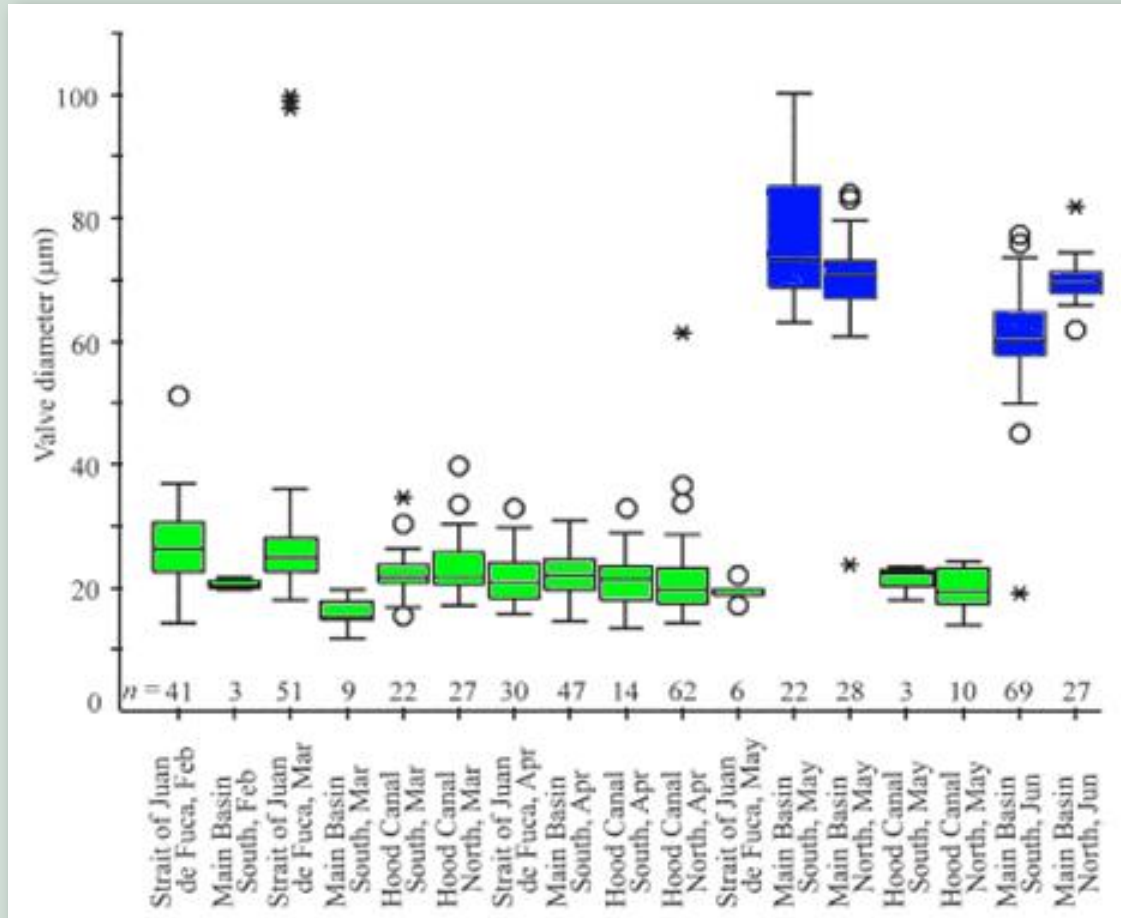
- *Symbiodinium*



# Mechanisms of microalgal speciation

## 3. Temporal isolation

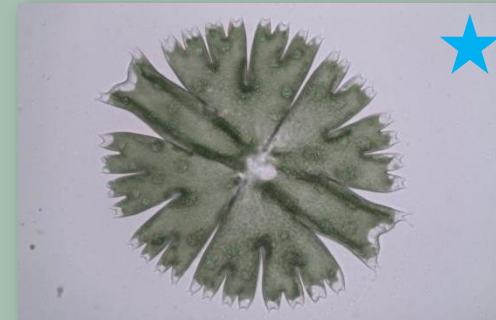
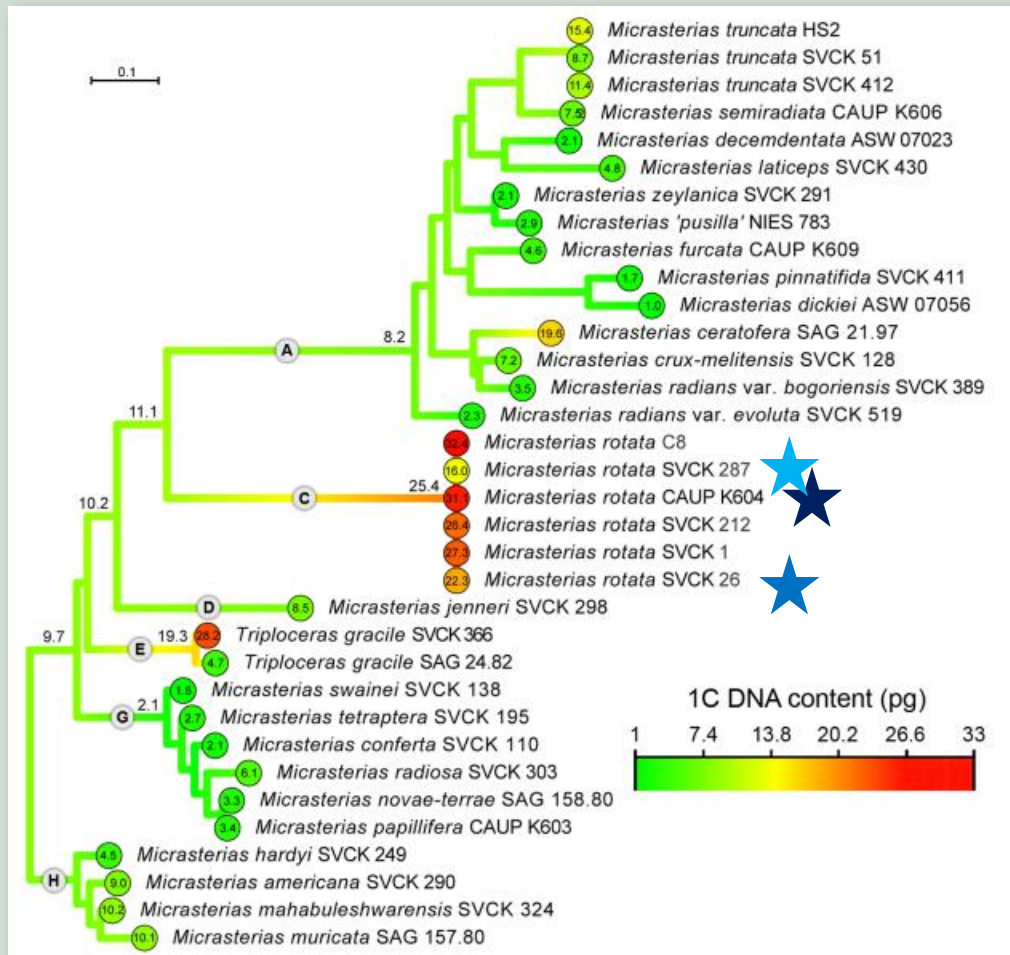
- Population of *Ditylum brighwellii*



# Mechanisms of microalgal speciation

## 4. Polyploidisation

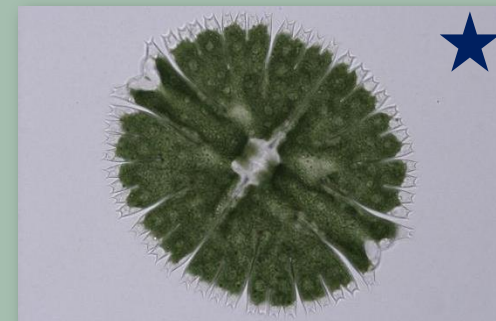
- Micrasterias* = DNA content 2.1-39.2 pg  $\approx$  17-250 chromosomes



SVCK 287  
 $\approx$  159 chromosomes



SVCK 26  
 $\approx$  226 chromosomes



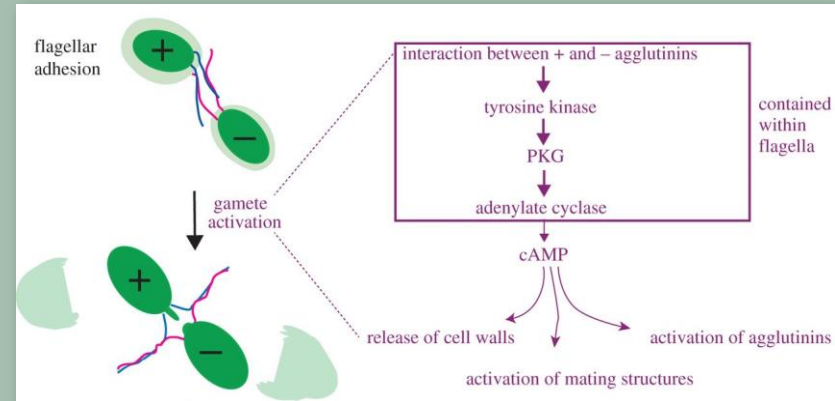
CAUP K604  
 $\approx$  250 chromosomes

# Mechanisms of microalgal speciation

## 5. Gametic incompatibility

- *Chlamydomonas*, *Pandorina*

Coleman (2001). *J. Phycol.* **37**: 836-851



## 6. Host specificity

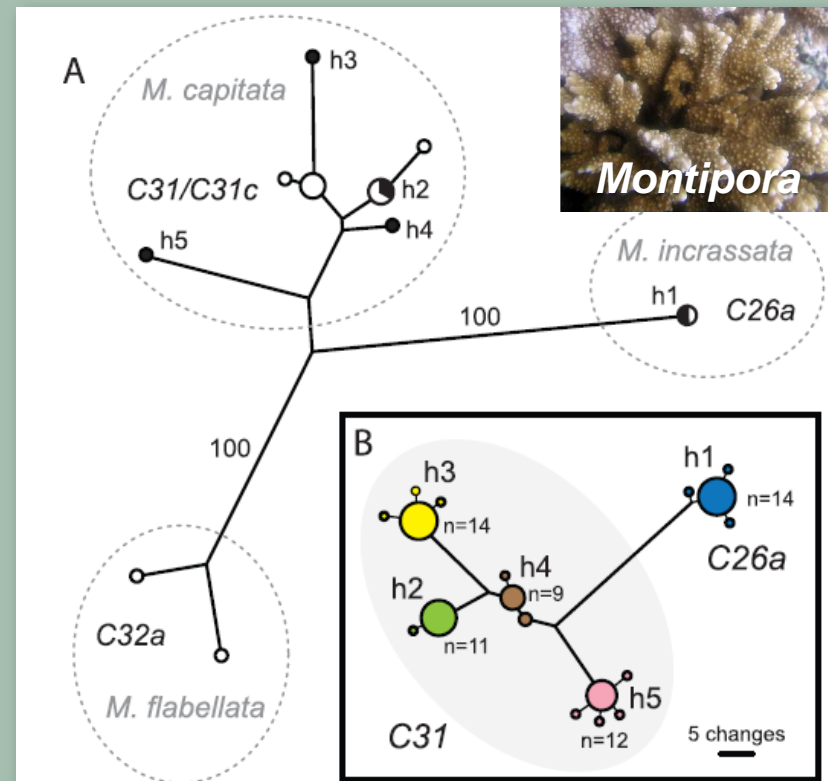
- *Symbiodinium*

LaJeunesse & Thornhill (2011). *Plos ONE* **6**: e29013

## 7. Monopolization

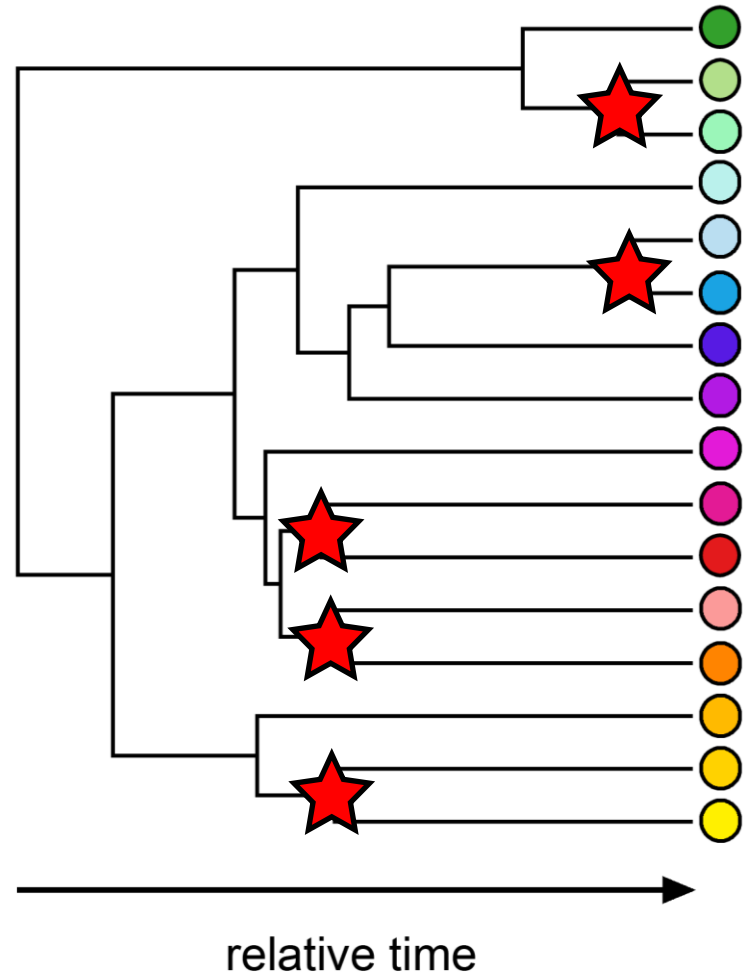
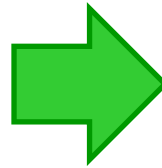
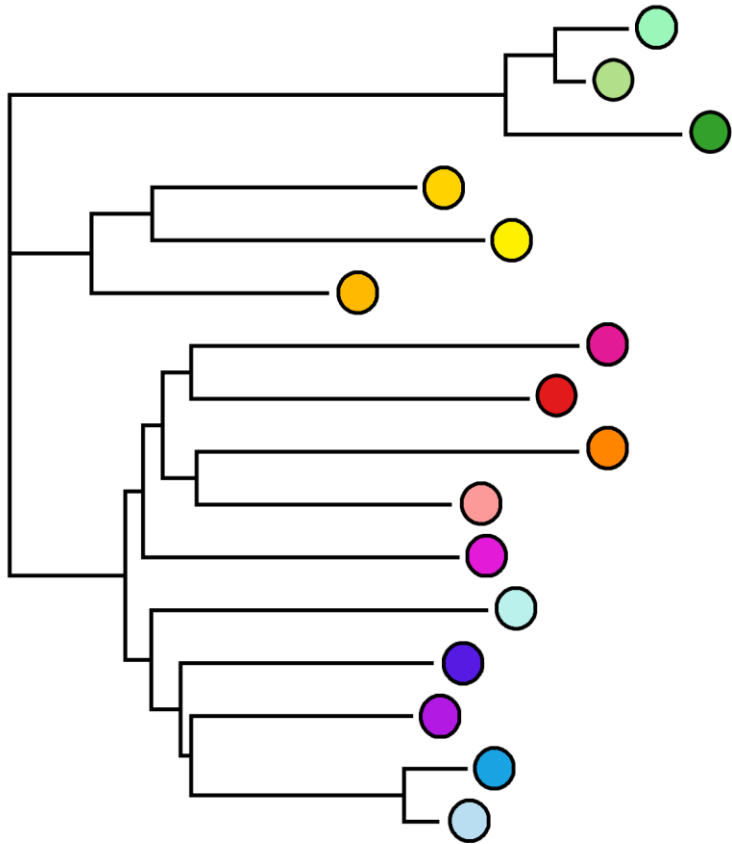
- *Chlamydomonas*, *Ochromonas*, *Oxytricha*

Weisse et al. (2011). *Ecosphere* **2**: 134



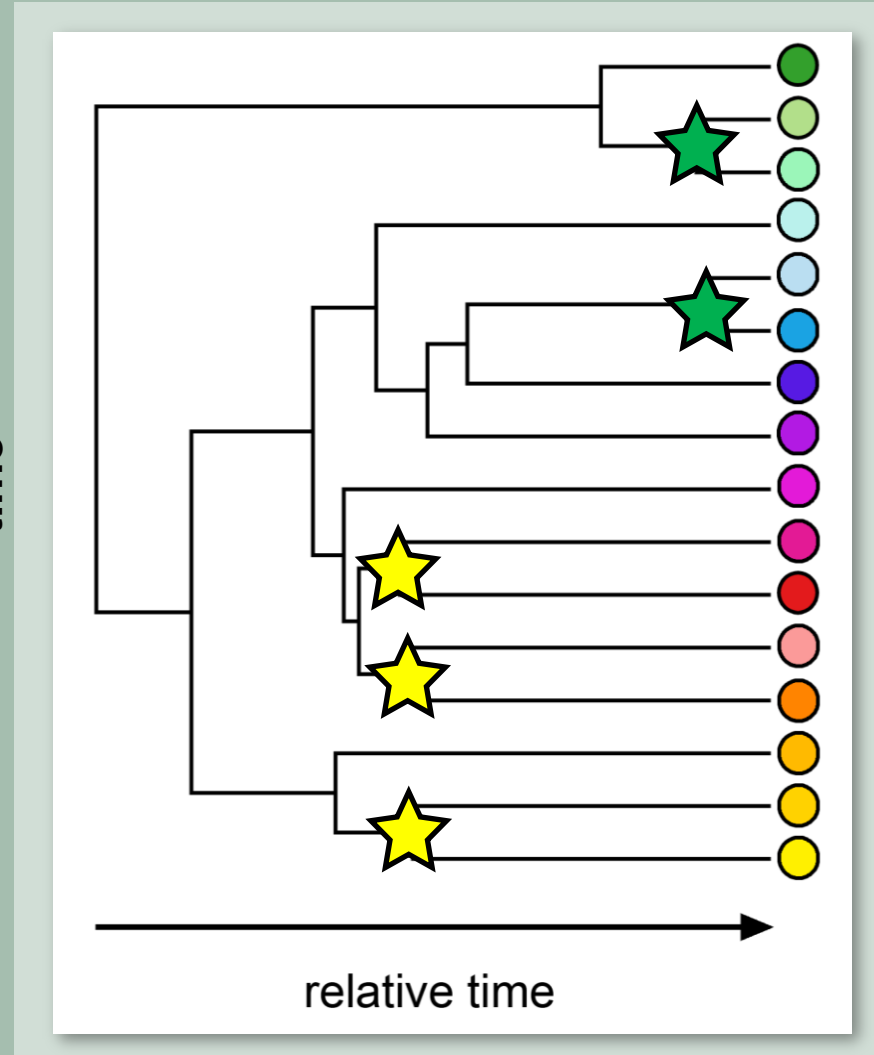
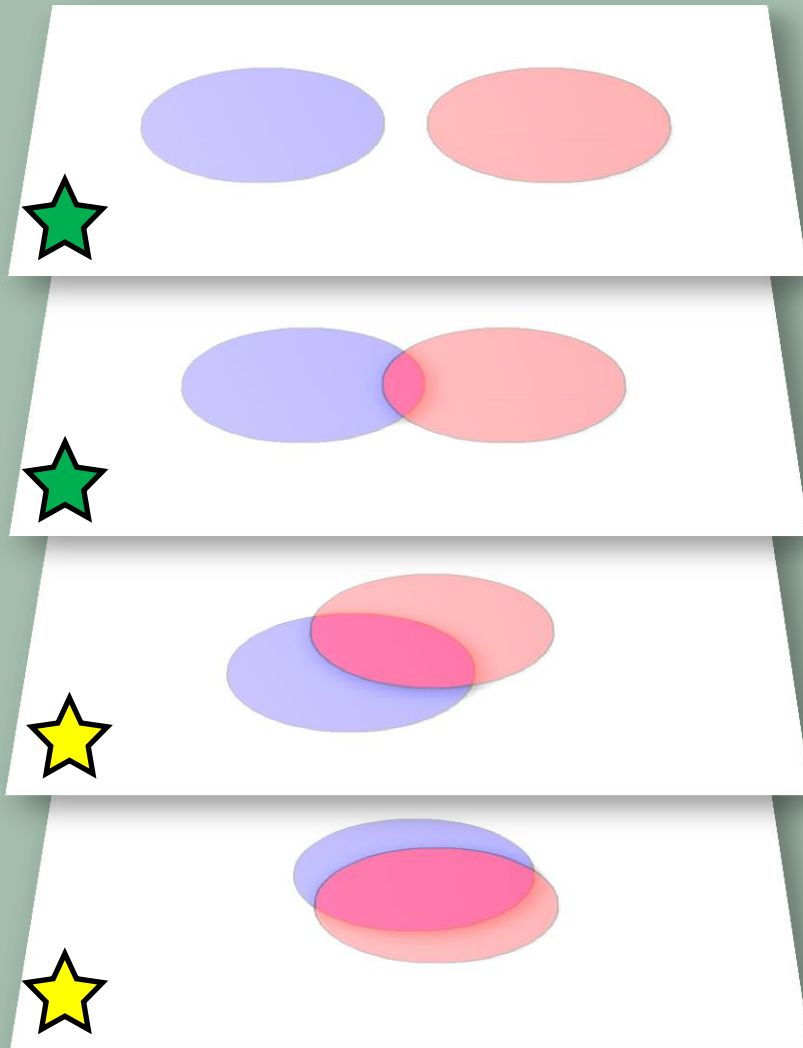


# Testing speciation models



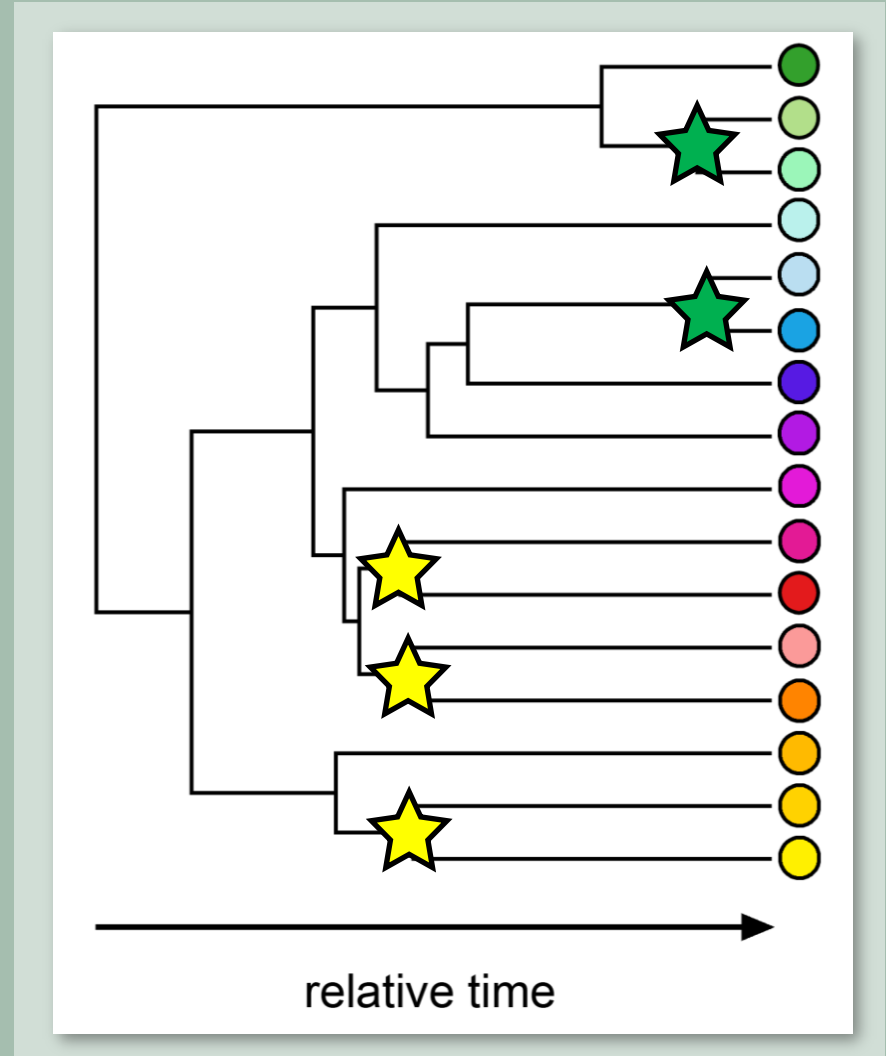
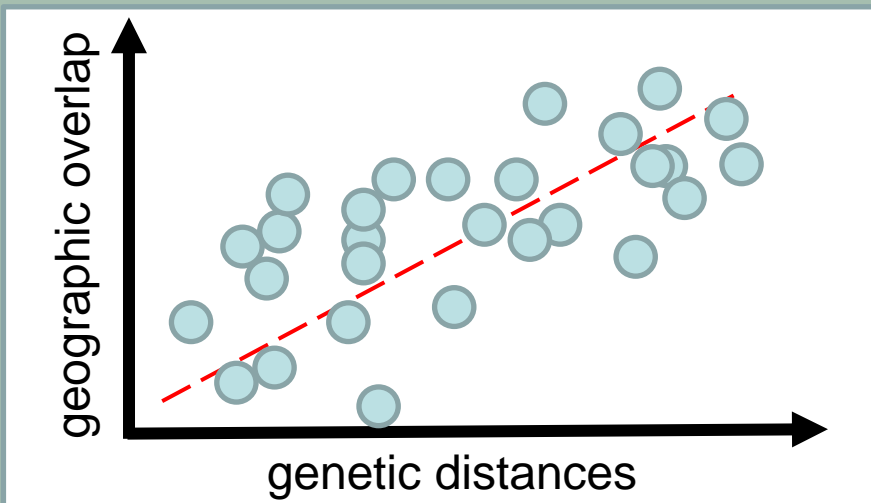
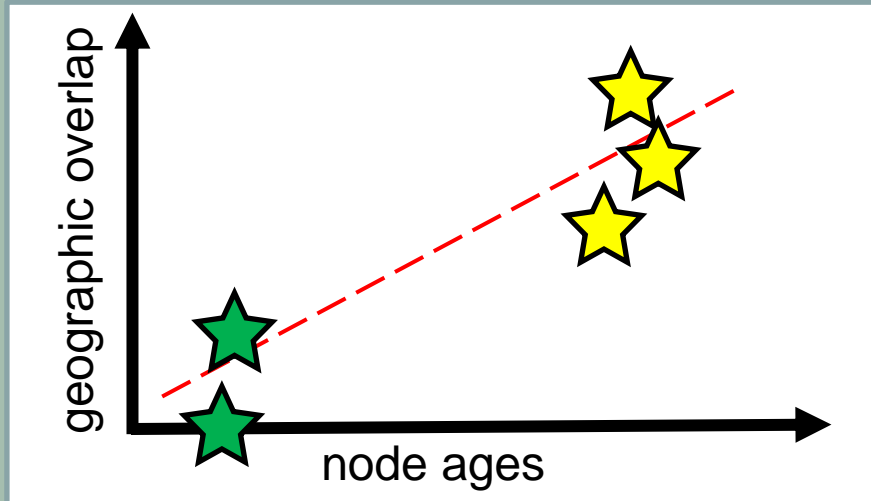
# Testing speciation models

- **Speciation by geographic isolation** – over time, the amount of overlap between the geographic ranges of species can only increase from zero.



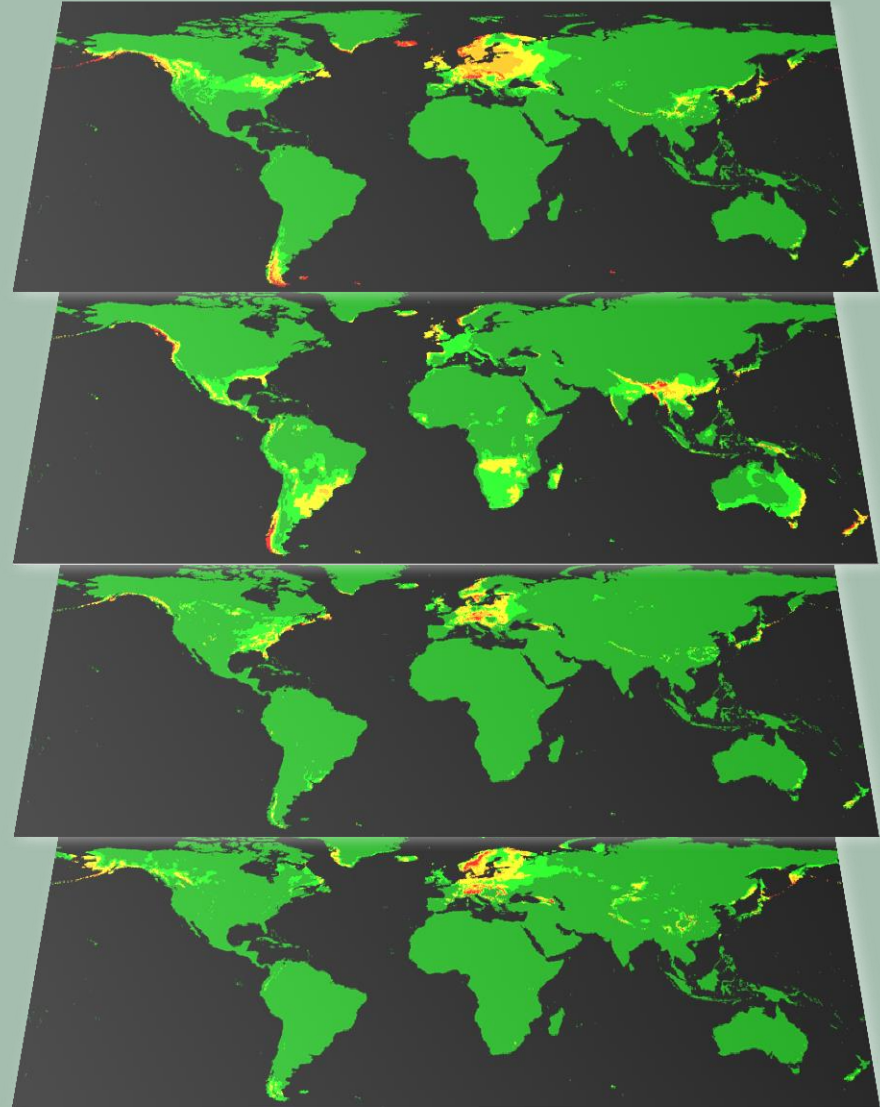
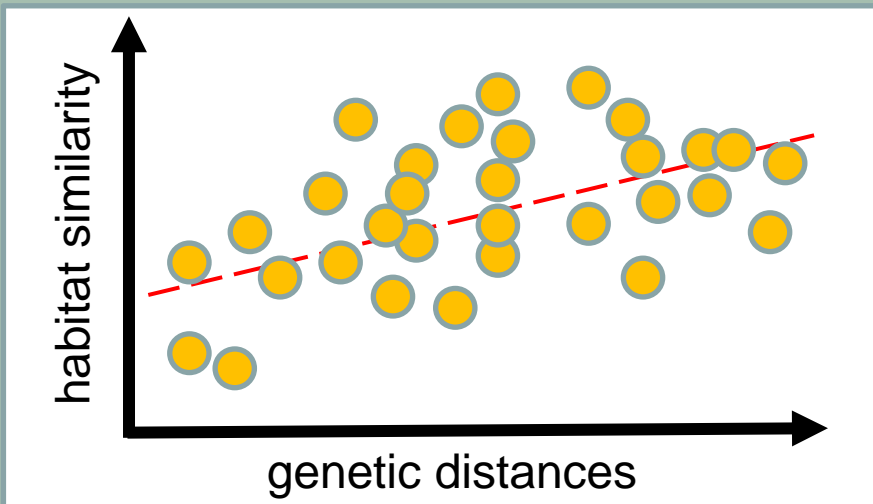
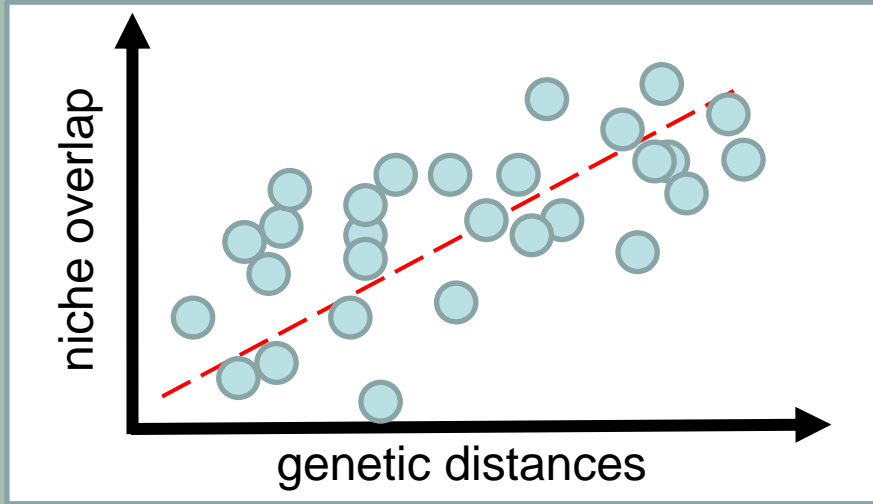
# Testing speciation models

- **Speciation by geographic isolation** – over time, the amount of overlap between the geographic ranges of species can only increase from zero.



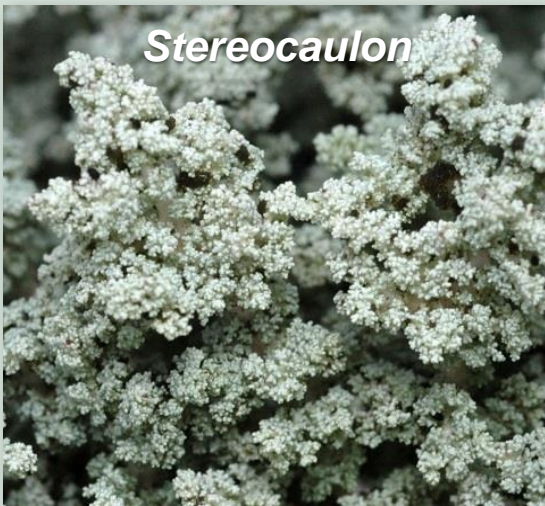
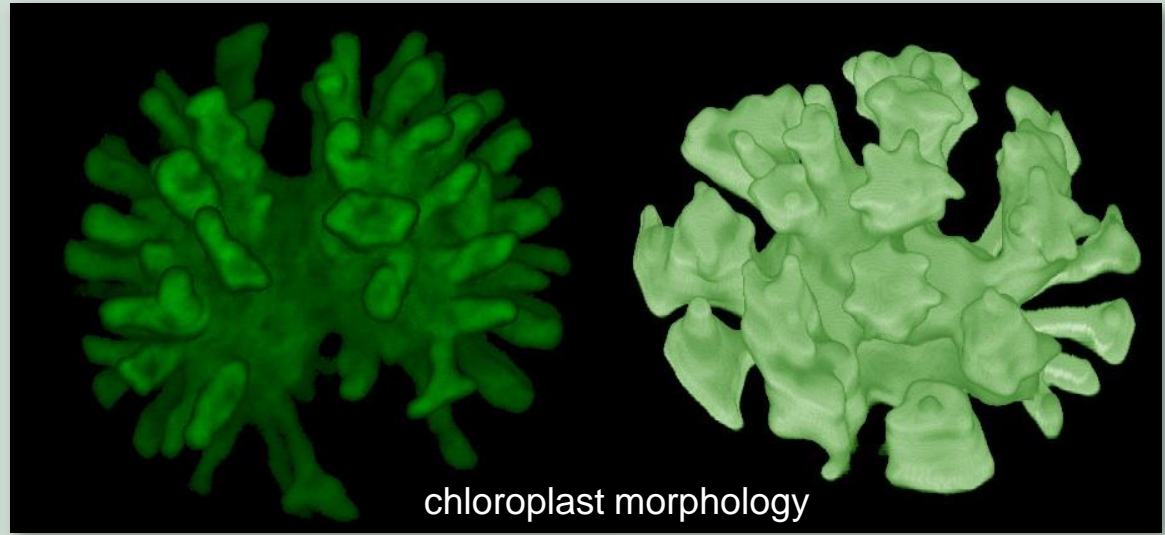
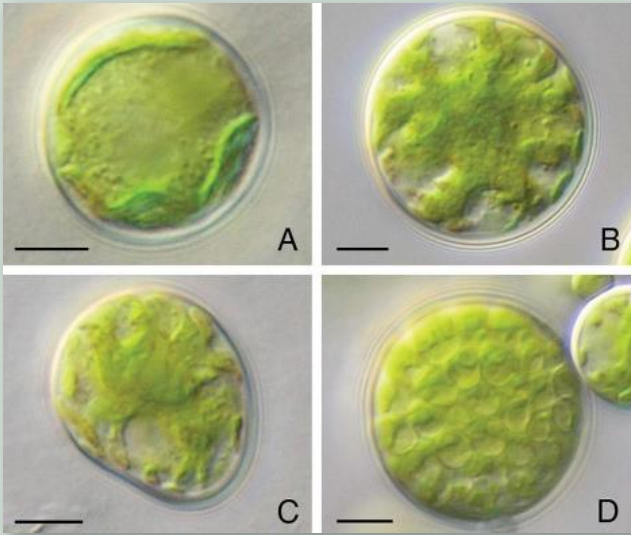
# Testing speciation models

- **Speciation by ecological isolation** - niche differentiation, habitat similarity



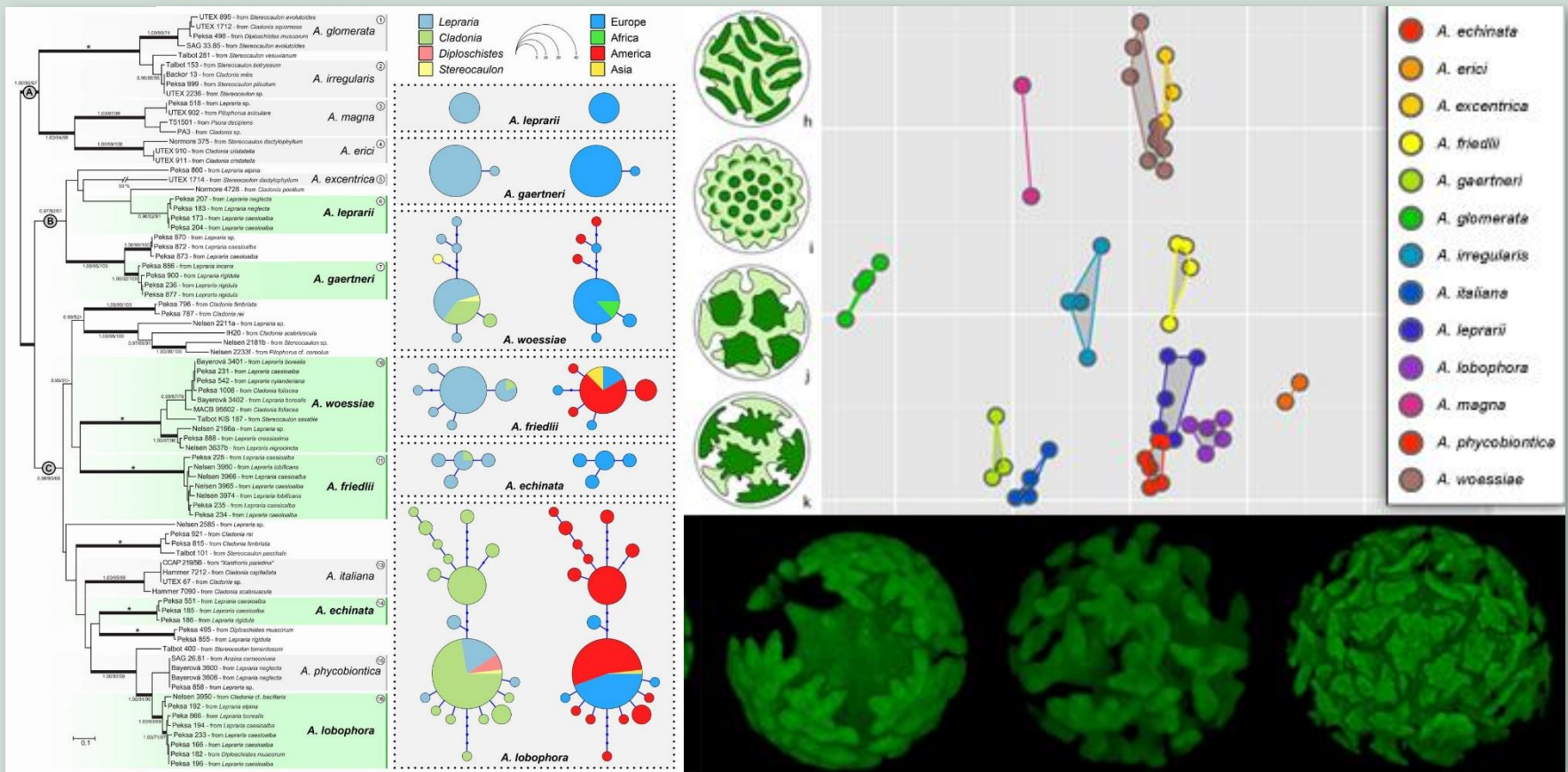
# Testing speciation models

- *Asterochloris* (Trebouxiophyceae, Chlorophyta)



# Testing speciation models

- ***Asterochloris*** (Trebouxiophyceae, Chlorophyta)
  - A robust species concept = species characterized genetically, morphologically, ecologically, and with respect to their mycobiont partners.



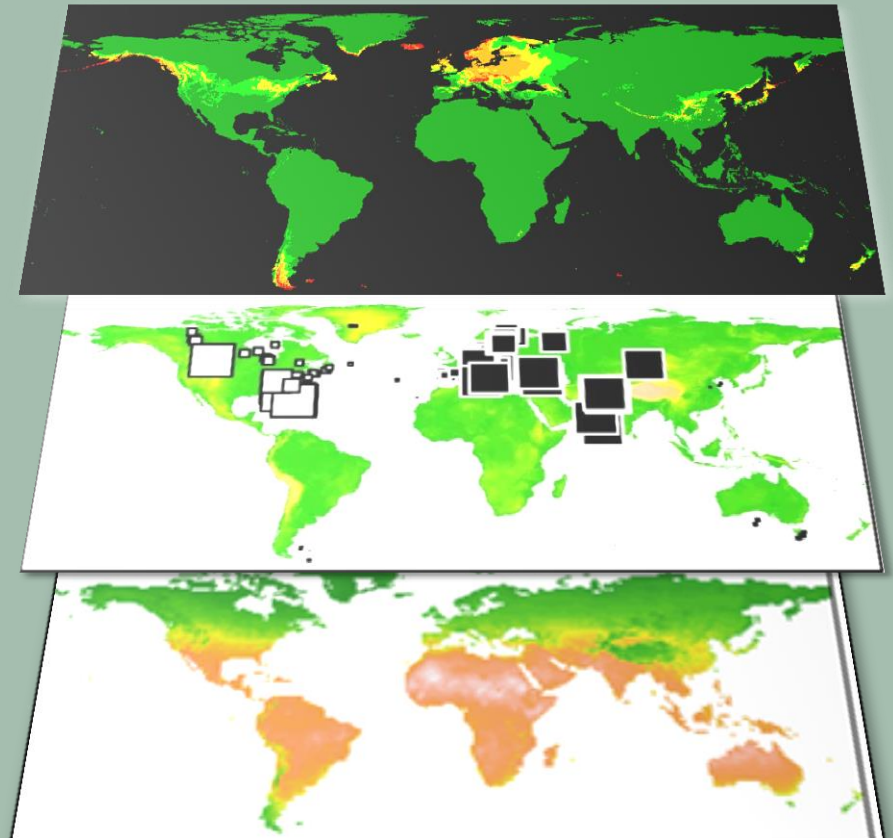
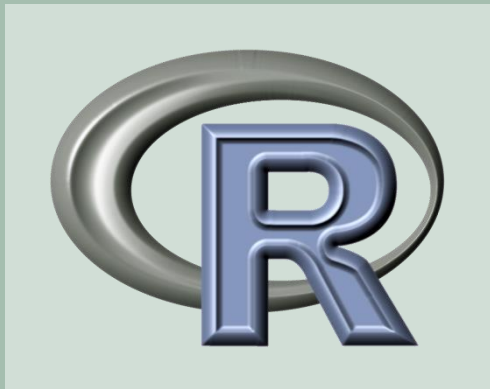
# Testing speciation models

- ***Asterochloris*** (Trebouxiophyceae, Chlorophyta) as a model genus
  - A robust species concept
  - A well known overall diversity
  - Worldwide sampling
  - “Visible” in the nature as a lichen inhabitant
  - Lichens serve us as very effective algal containers



# Testing speciation models - *Asterochloris*

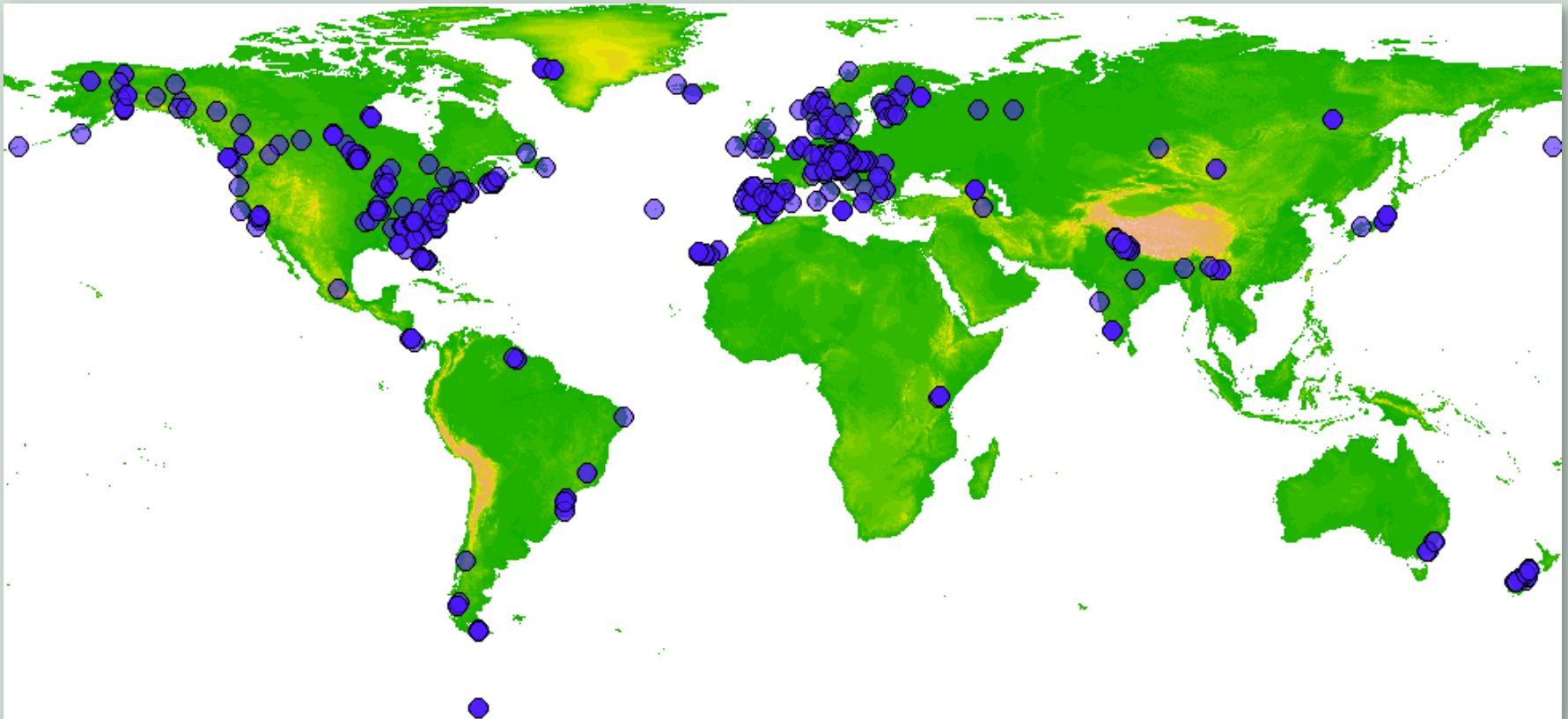
- ***Which are the main factors structuring the extant diversity?***
  - Variation partitioning analysis (relative contribution of geography, climate, substrate, and mycobiont to the algal distribution)
- ***What are the predominant speciation mechanisms?***
  - Phylogenetic comparative methods (overlaps in geographic distributions, niche models, substrate and mycobiont similarity)





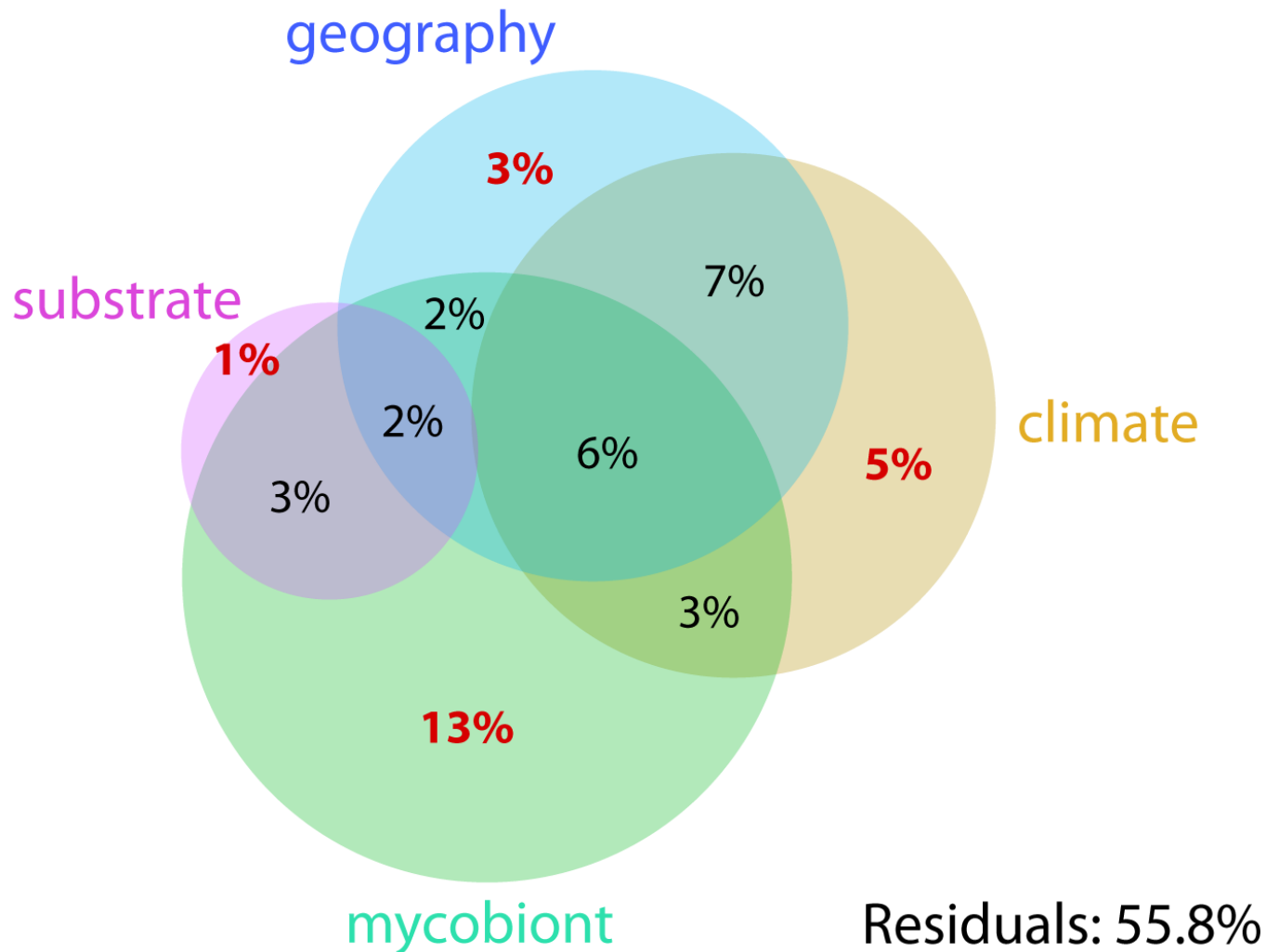
# Testing speciation models

- Total of 740 algal samples
  - algal ITS rDNA + actin sequences
  - precise geographic coordinates
  - climatic data (20 bioclimatic variables)
  - substrate data
  - mycobiont identity (ITS rDNA sequence)



# Testing speciation models - *Asterochloris*

- Which are the main factors structuring the extant diversity?



$P$  geography  
= **0.001**

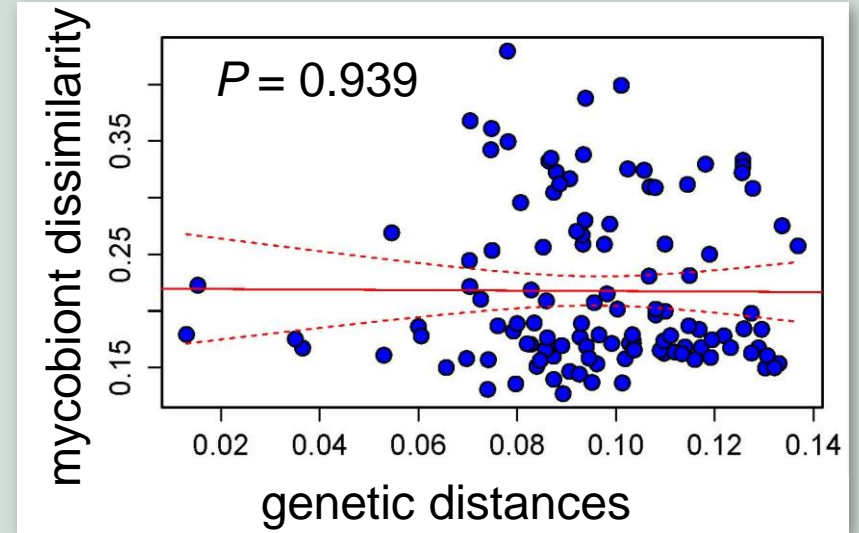
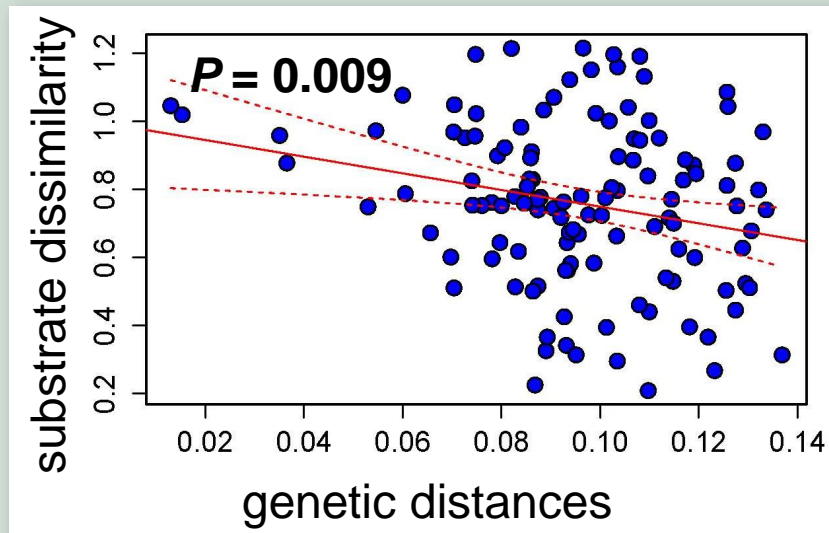
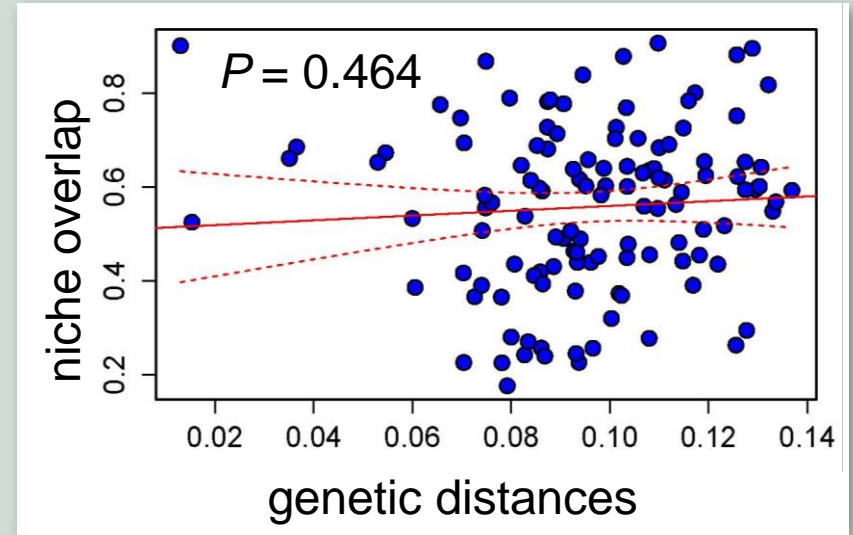
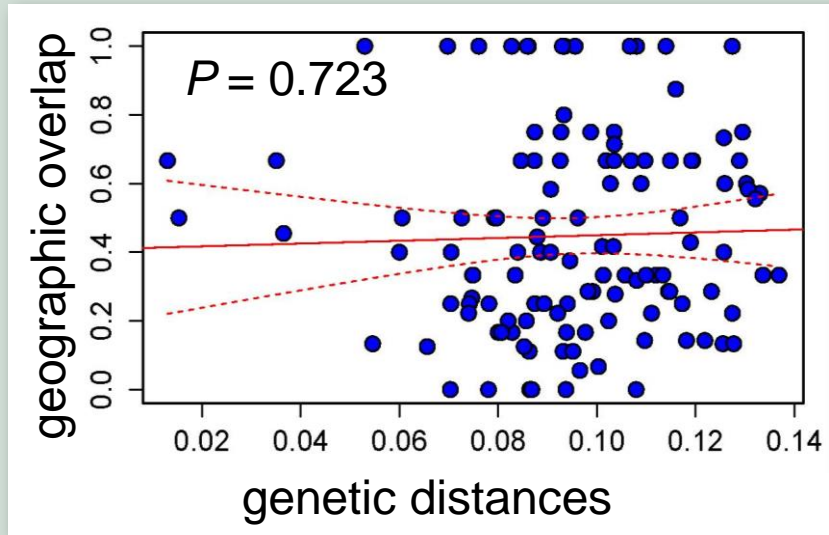
$P$  climate  
= **0.001**

$P$  mycobiont  
= **0.001**

$P$  substrate  
= **0.002**

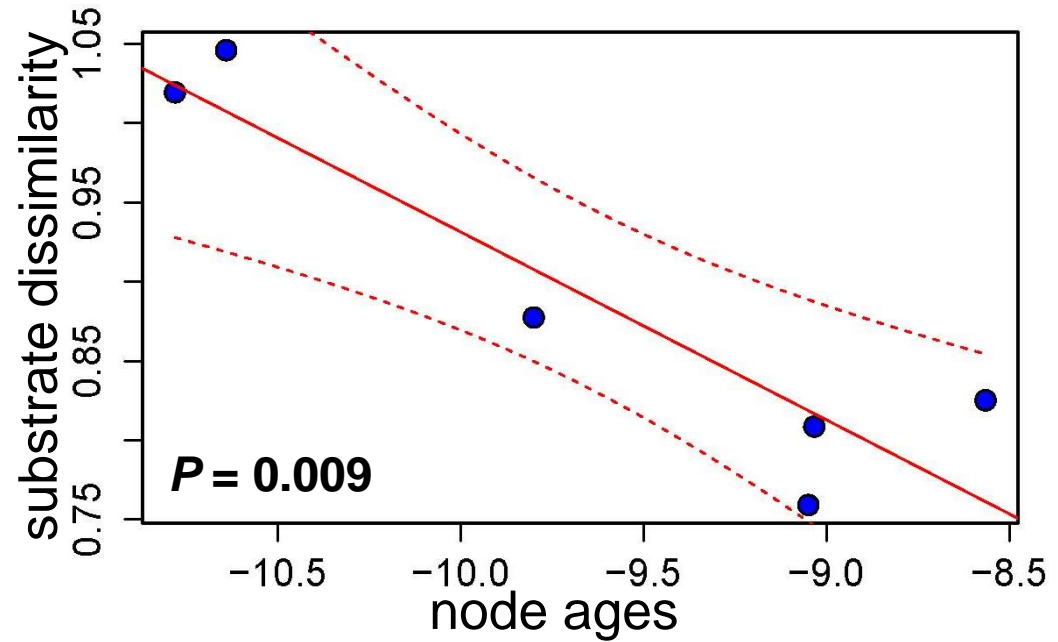
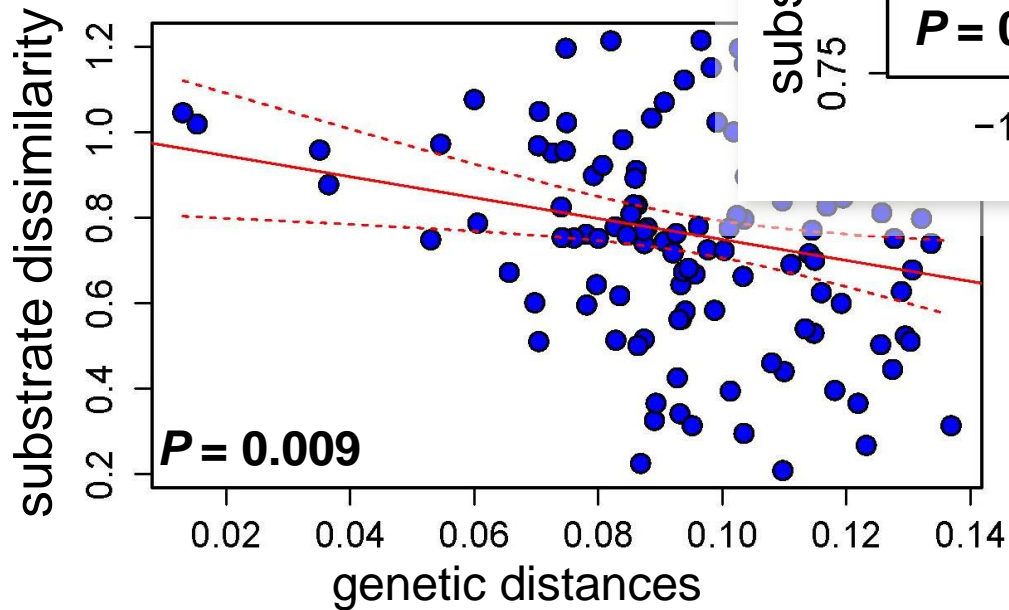
# Testing speciation models - *Asterochloris*

- *What are the predominant speciation mechanisms?*



# Testing speciation models - *Asterochloris*

- *What are the predominant speciation mechanisms?*

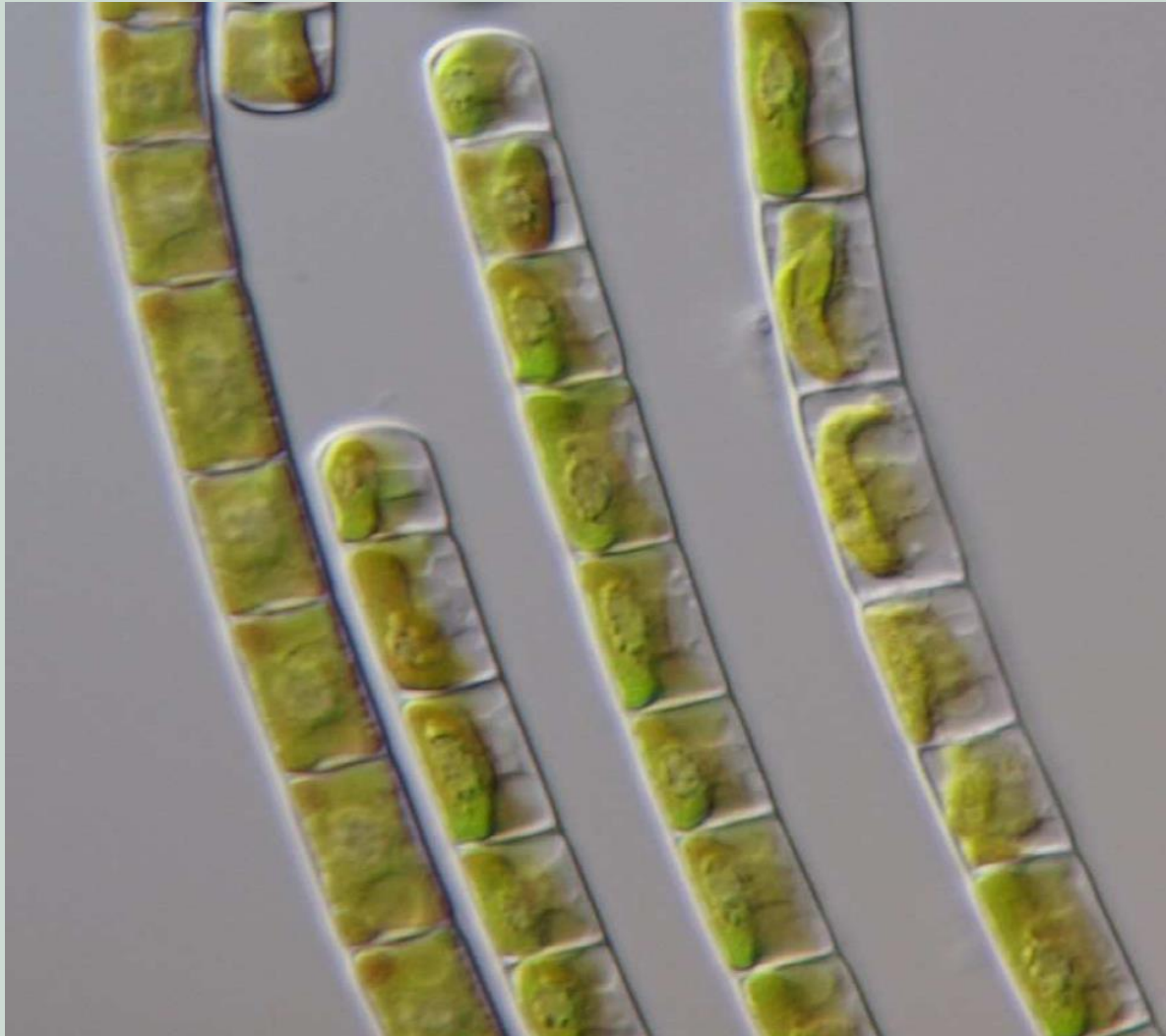


# What are the general causes of speciation?

- Analysis of extant data only, without applying the phylogenetic comparative methods, can be misleading in estimating the speciation mechanisms
- In *Asterochloris*, species are generating by ecological speciation via divergent habitat selection. Their distribution is, however, shaped to a large extent by mycobionts
- Ecological speciation (microallopatry) can have a major role in microalgal species diversification, especially in groups exhibiting unlimited dispersal
- In defining microalgal species, we should primarily focus on ecological differentiation of genetic lineages.

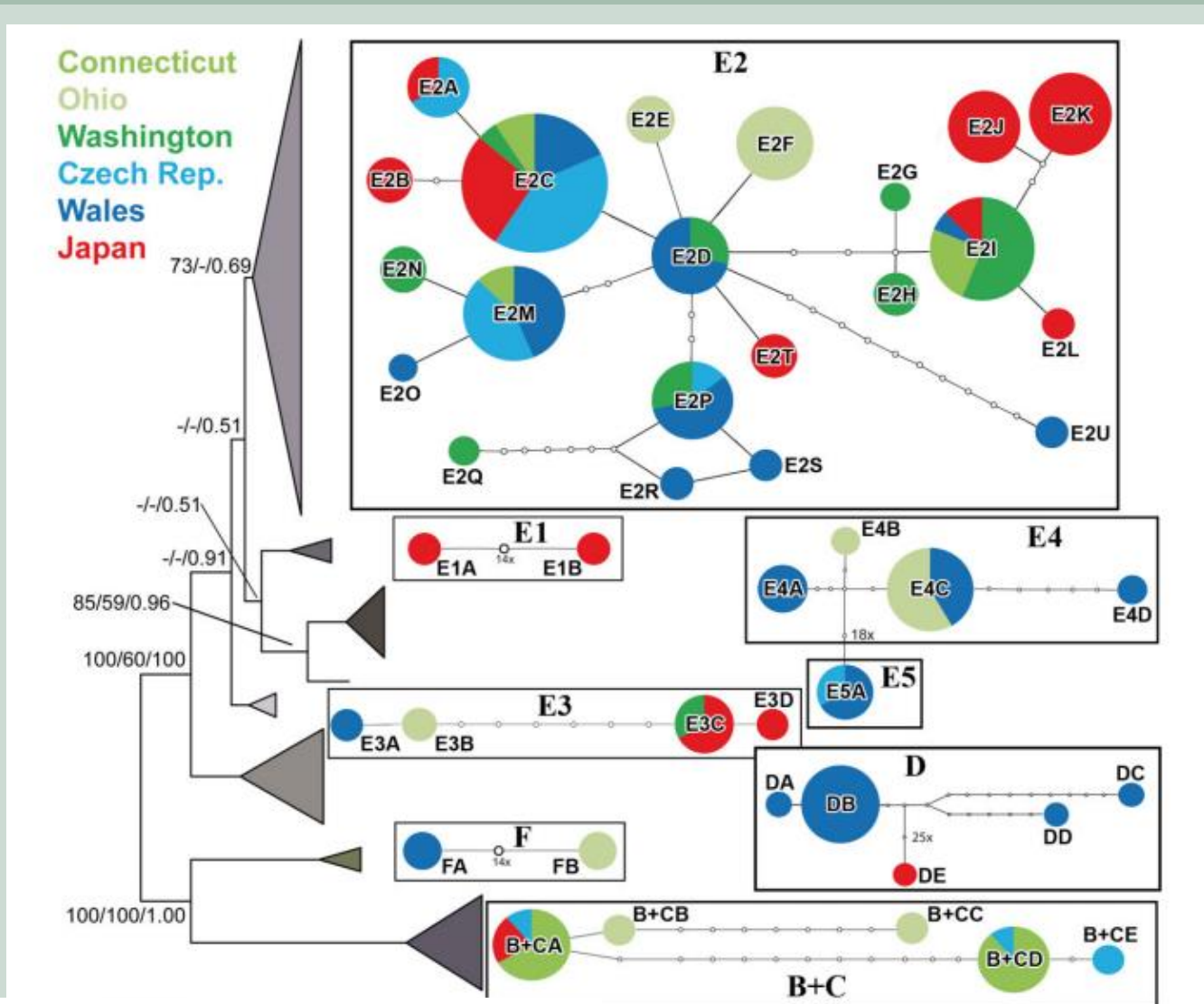
# Ecological speciation

- *Klebsormidium* (Klebsormidiophyceae, Streptophyta)



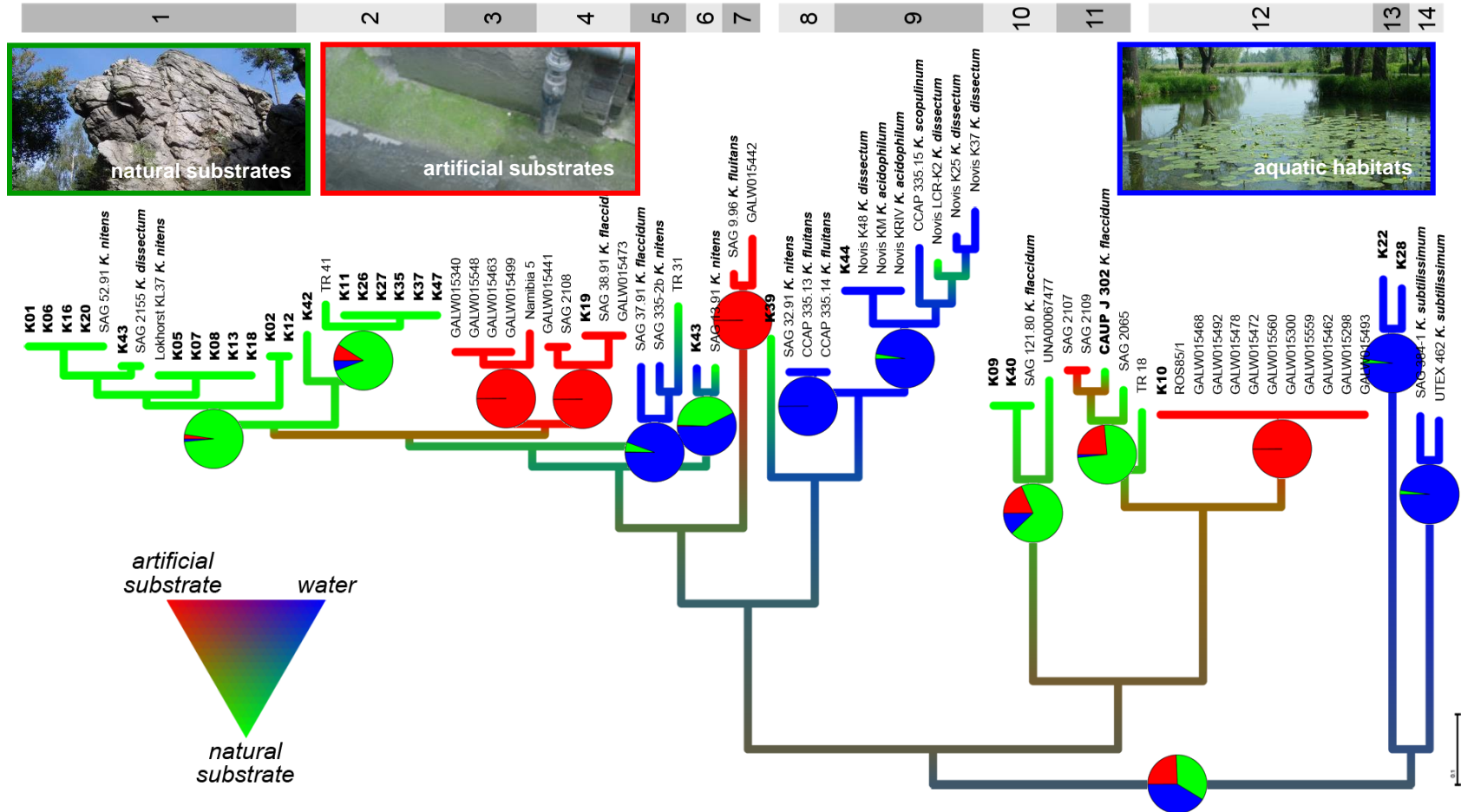
# Ecological speciation

- *Klebsormidium* (Klebsormidiophyceae, Streptophyta)



# Ecological speciation

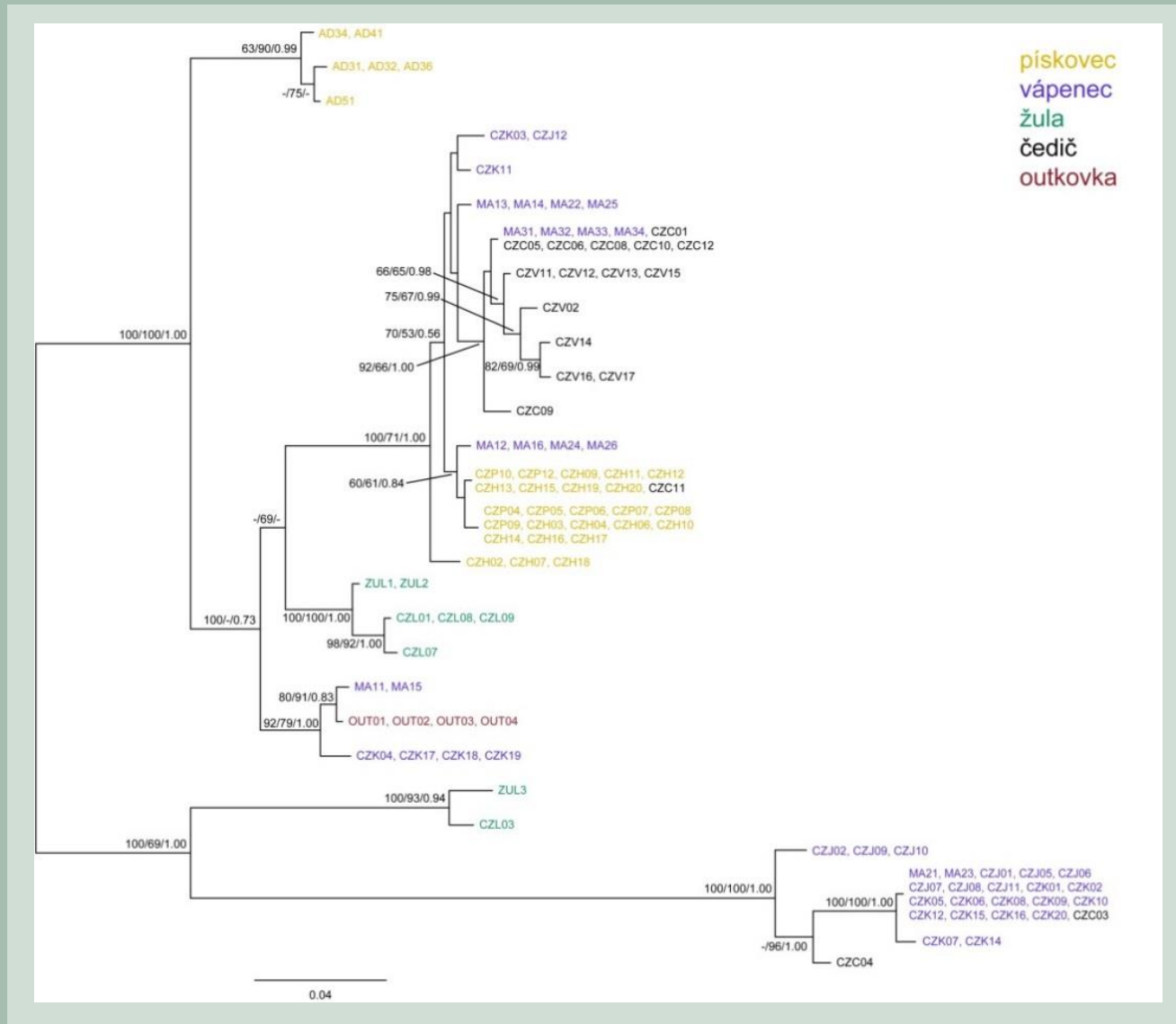
- *Klebsormidium* (Klebsormidiophyceae, Streptophyta)
  - Strong ecological preferences of the lineages to one of three habitat types





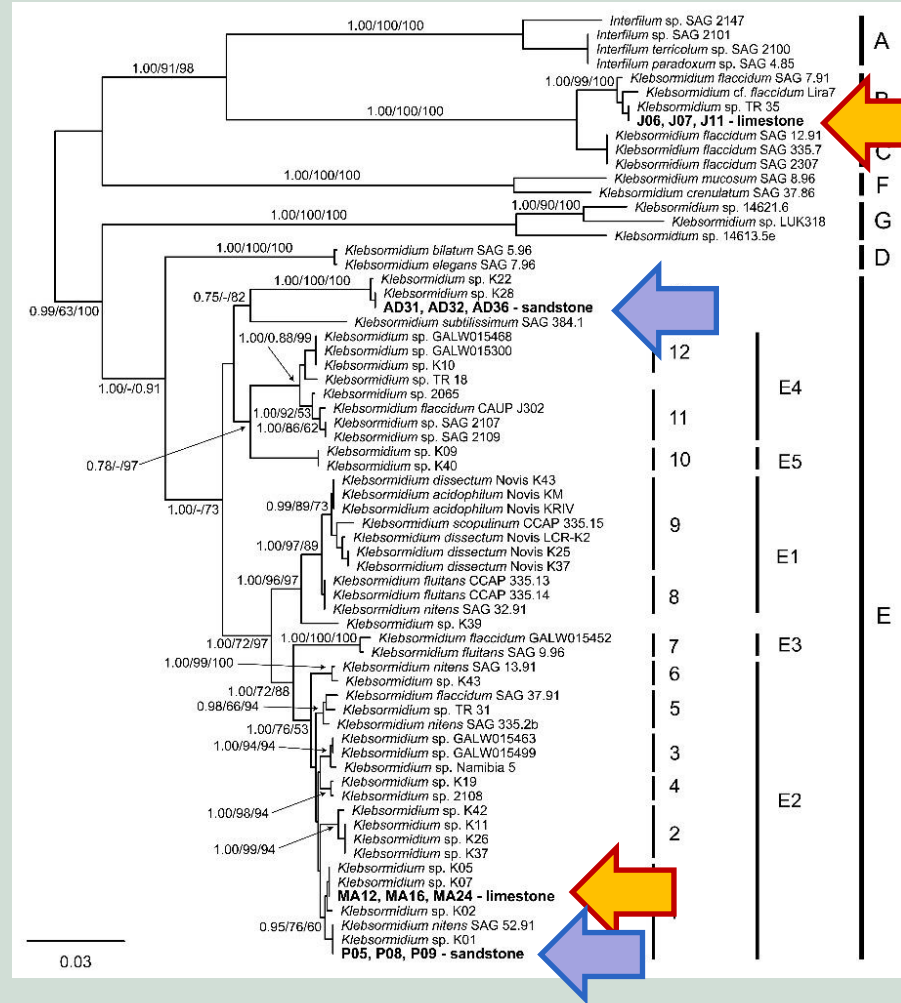
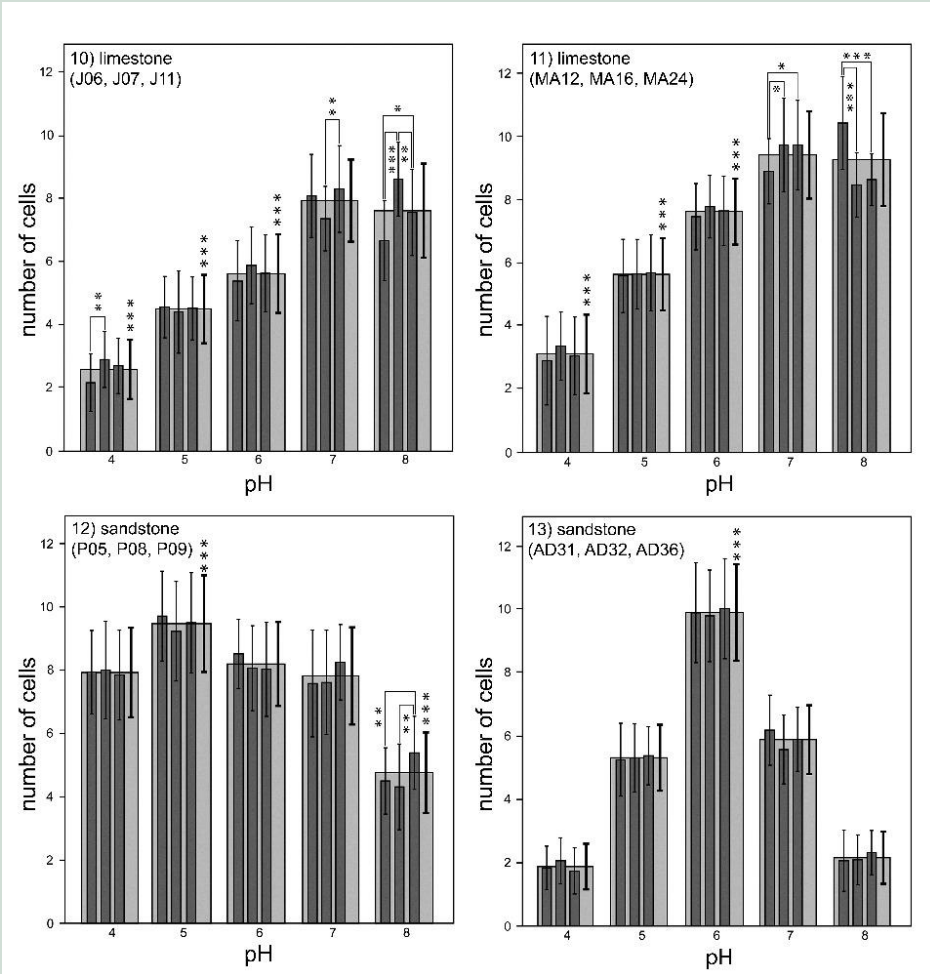
# Ecological speciation

- *Klebsormidium* (Klebsormidiophyceae, Streptophyta)



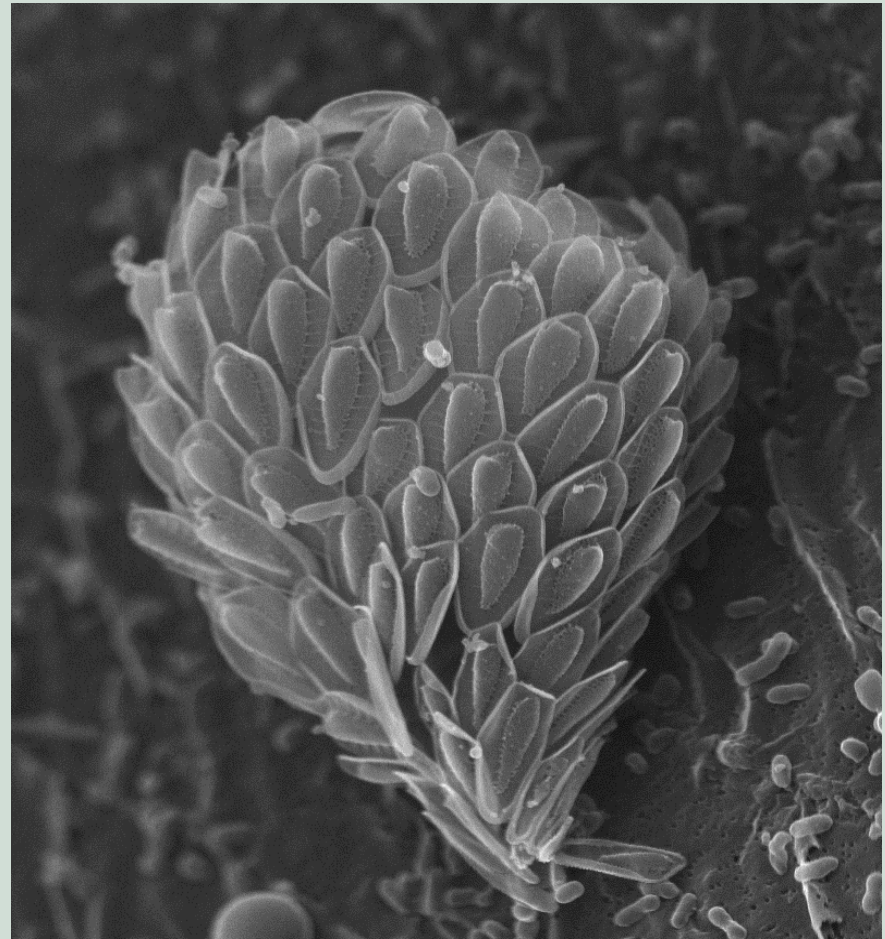
# Ecological speciation

- Klebsormidium*** (Klebsormidiophyceae, Streptophyta)



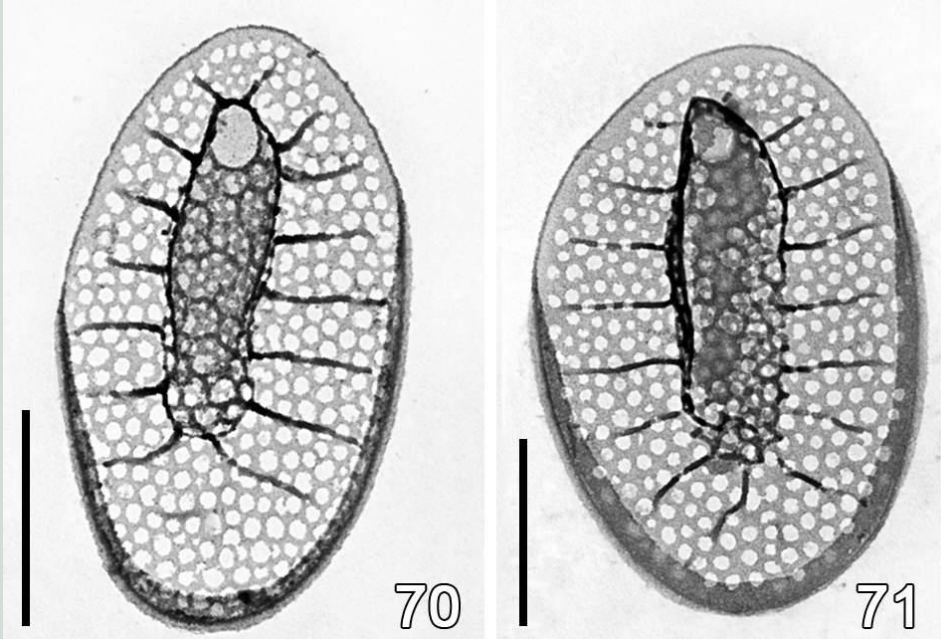
# Ecological speciation

- *Synura* (Chrysophyceae, Stramenopiles)



# Ecological speciation

- *Synura* (Chrysophyceae, Stramenopiles)

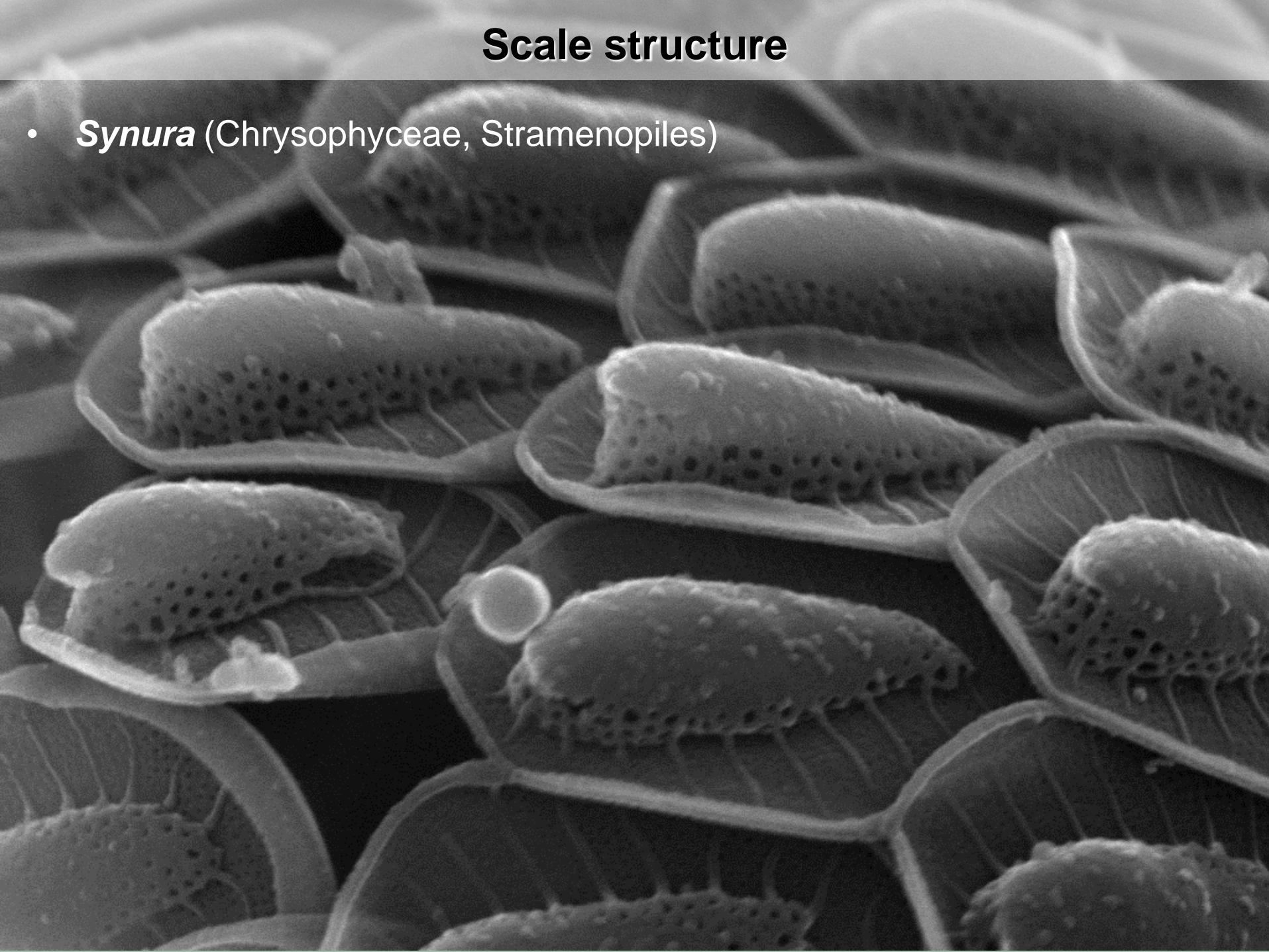


***S. macropora***: adaptation to eutrophic conditions (less availability of silica could cause a shift from heavily to less silicified scales)


***S. hibernica***: adaptation to oligotrophic conditions (less availability of nutrients could cause cell elongation - to achieve a high surface-to-volume ratio)

## Scale structure

- *Synura* (Chrysophyceae, Stramenopiles)



# Scale structure



**READY FOR EUROCODES**


# RFEM 5

**FEM Software for Structural and Dynamic Analysis**

Enjoy Structural Analysis...


**Ready for Windows 8**

Steel, Concrete, Glass, Timber

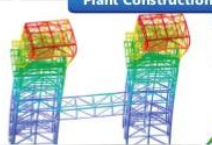


(C) www.lbh-engineering.de

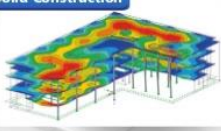
**Stability and Dynamics**




**Plant Construction**



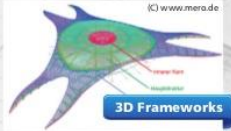
**Solid Construction**



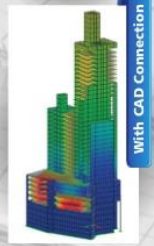
**Steel Construction**




**3D Frameworks**



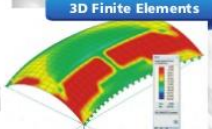
**With CAD Connection**



**Connections**



**3D Finite Elements**




**RFEM 5 - For Calculations in Civil, Mechanical and Plant Engineering.**  
**Intuitive, efficient, universal, powerful.**

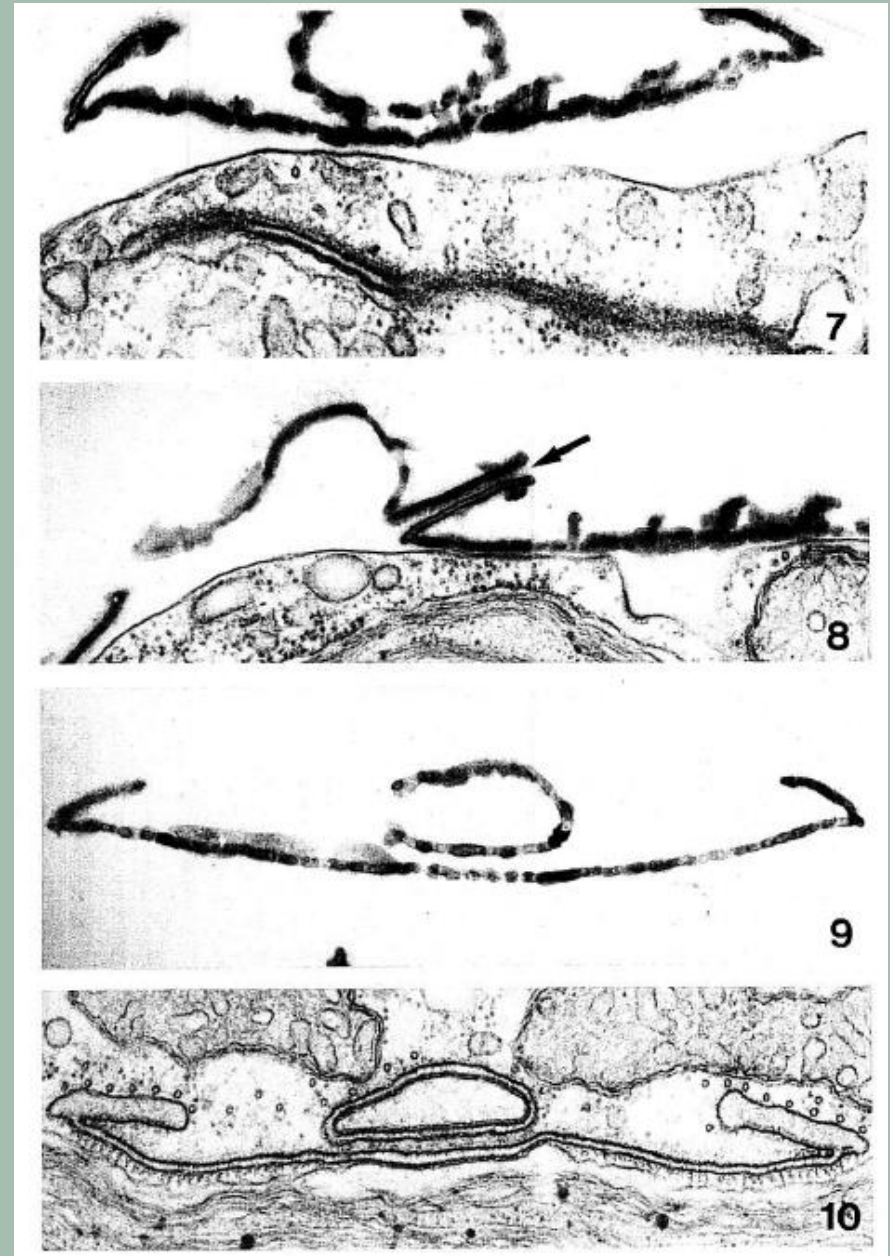
The RFEM program family is based on a modular system. The main program RFEM is used to define structures, materials and loads for planar and spatial structural systems consisting of plates, walls, shells and members. Creating combined systems as well as modeling solid and contact elements is also possible.

RFEM provides deformations, internal and support forces as well as soil contact stresses. For the subsequent design RFEM offers various add-on modules taking into account material- and standard-specific conditions. The modular approach allows you to combine all programs individually according to your needs. Upgrades at a later time are always possible. RFEM offering numerous interfaces represents the perfect tool for a smooth interaction between CAD and structural analysis in Building Information Modeling (BIM).

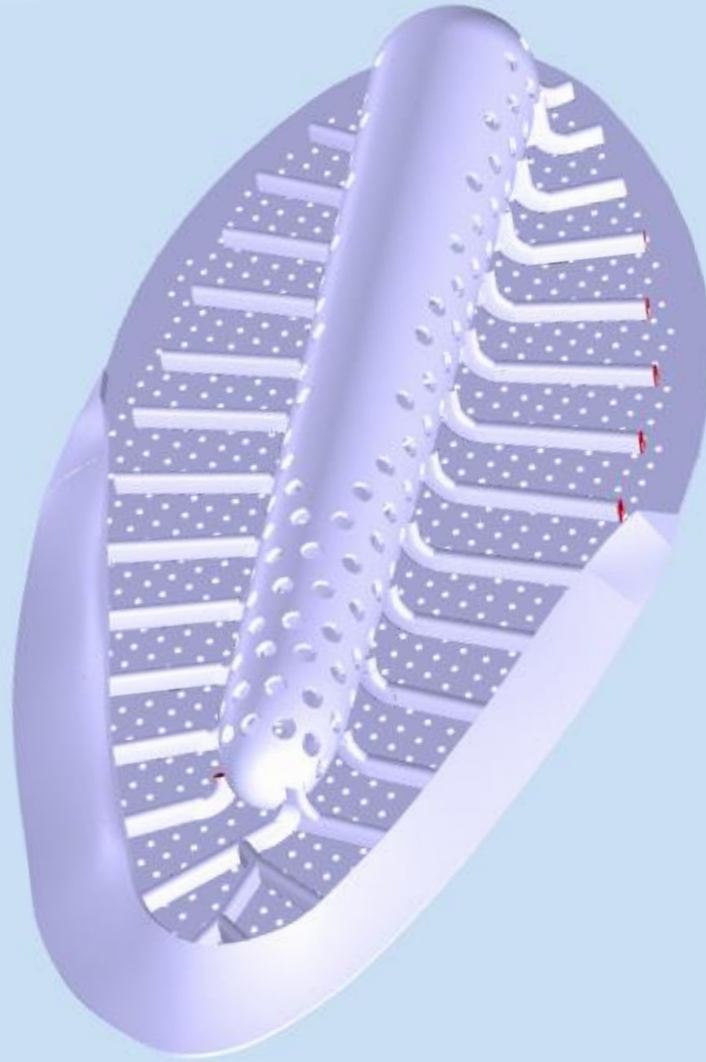
**RFEM - the FEM tool for steel, reinforced concrete, glass and timber structures, plant construction, dynamics, aluminum, design according to Eurocodes**

**Dlubal Engineering Software • Software for Structural and Dynamic Analysis**

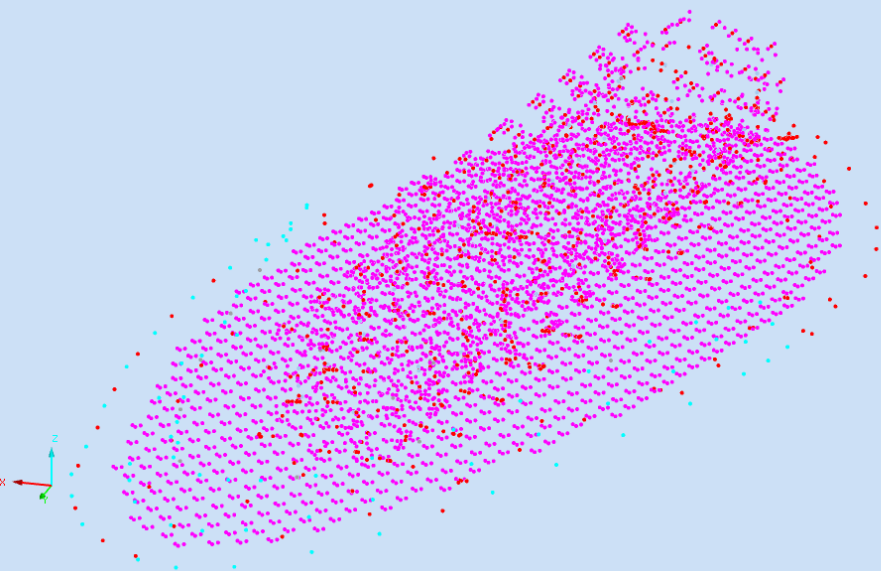




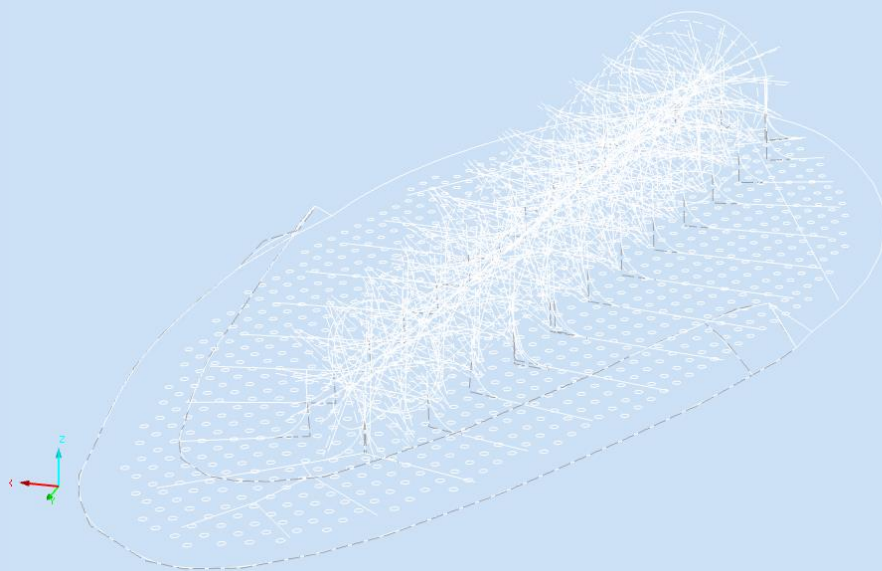
# Scale structure



# Scale structure



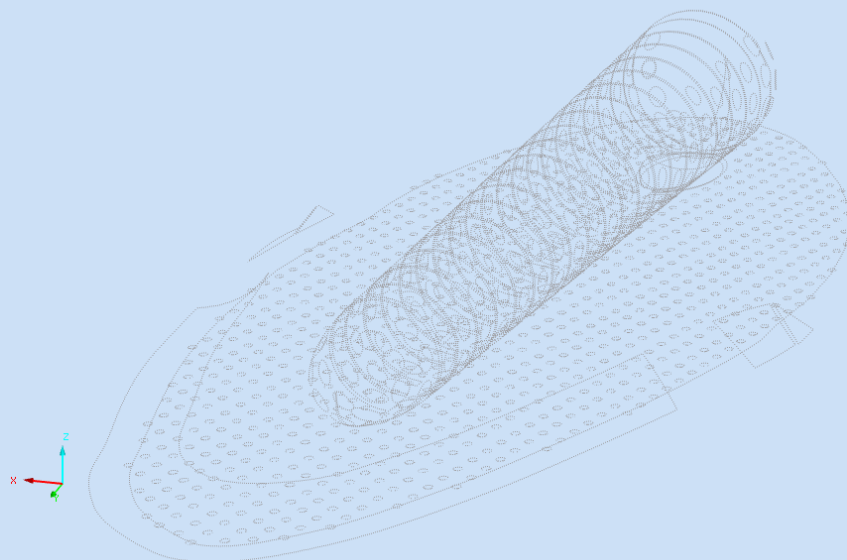
7479 nodes



2527 lines

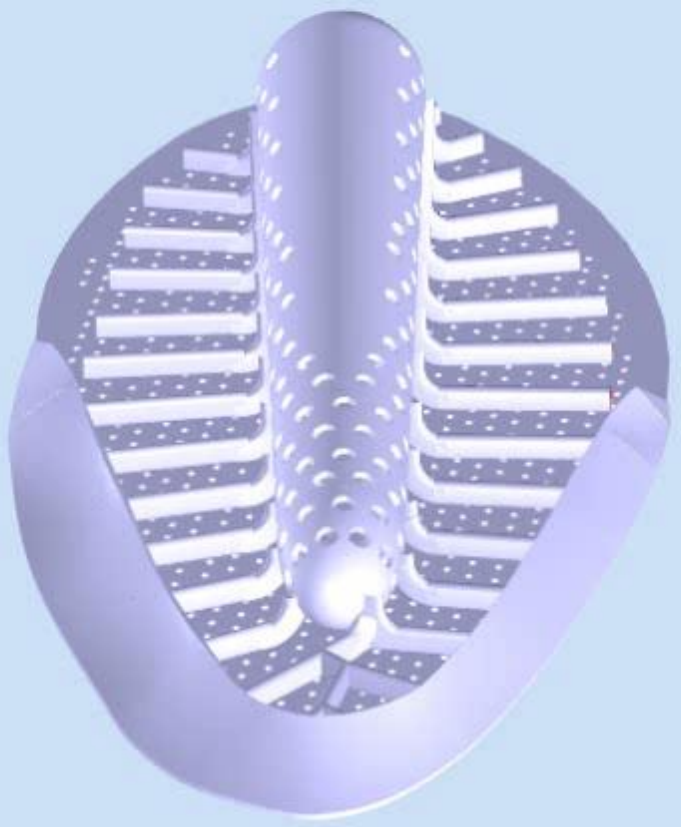


152 rods

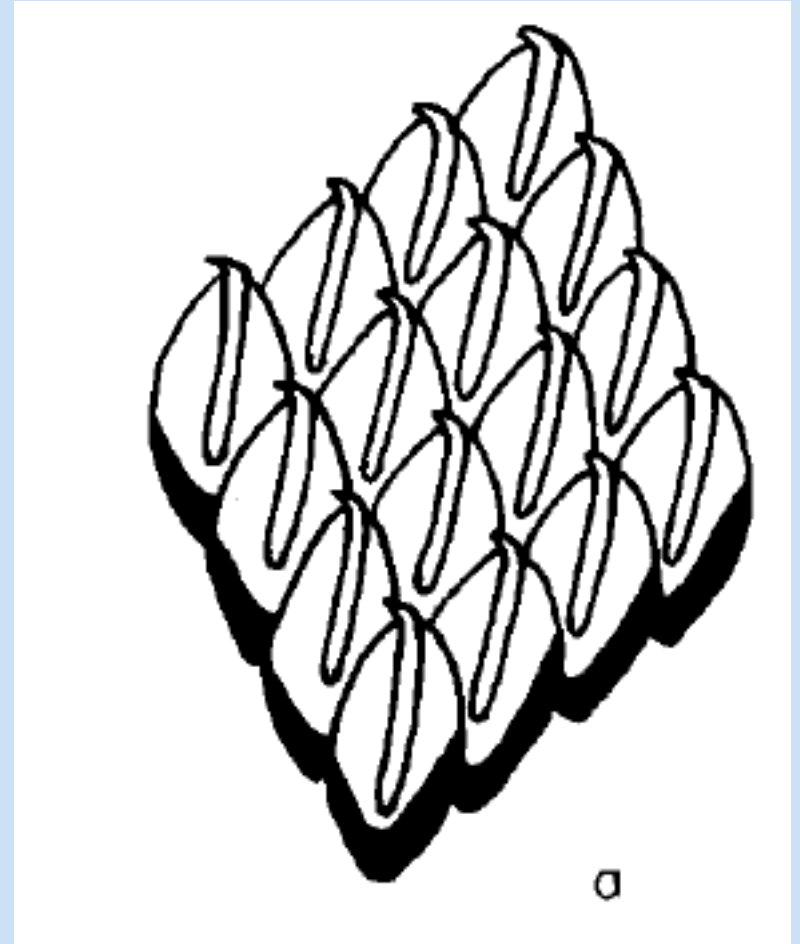
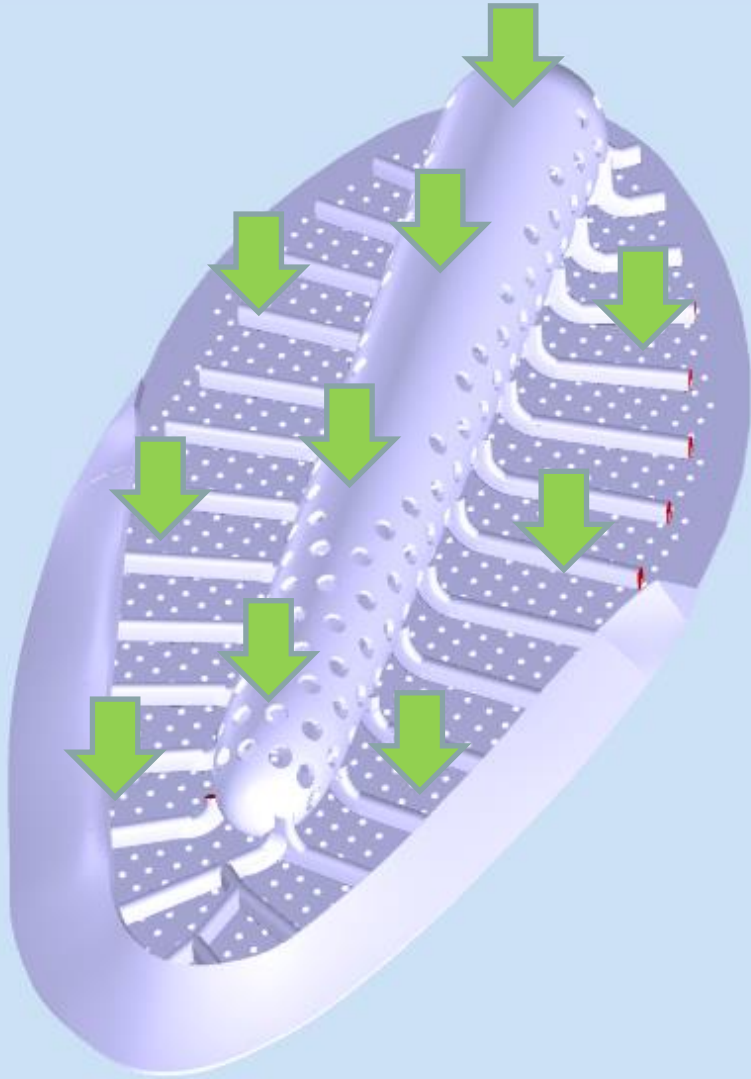


809 planes

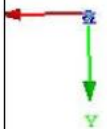
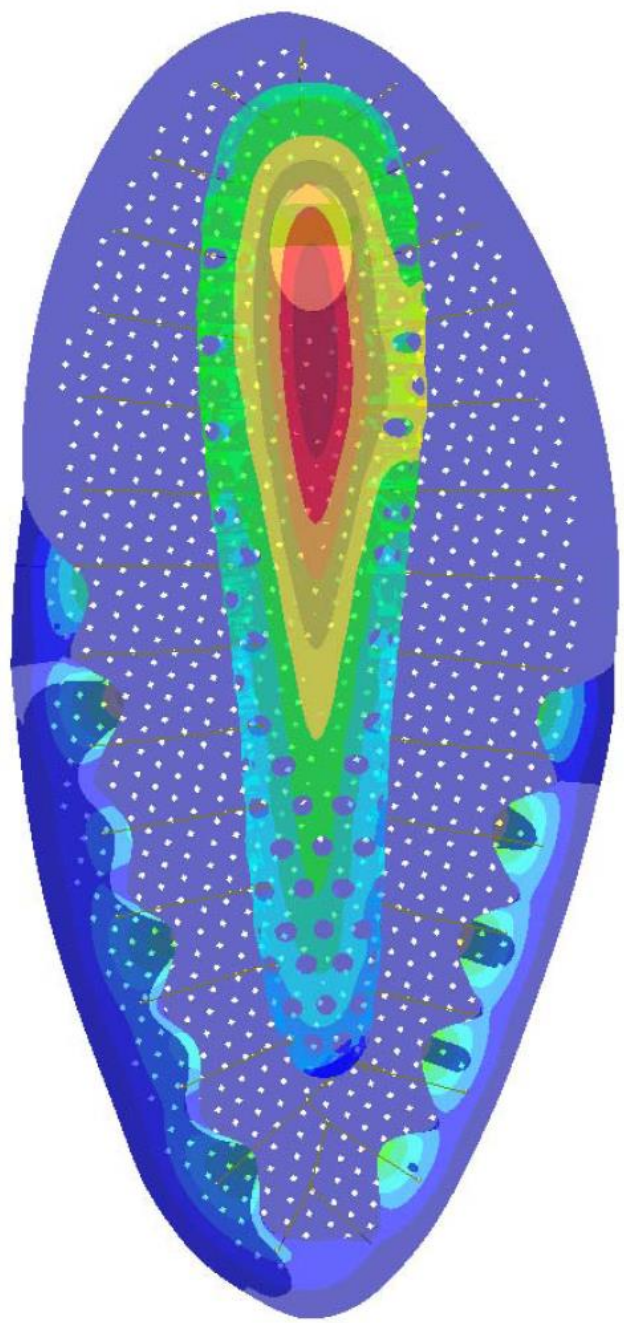
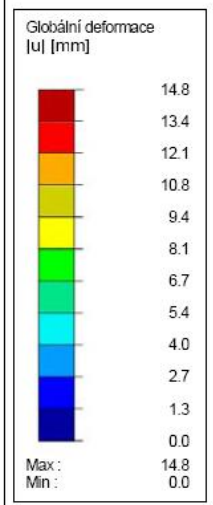




# Scale structure



ZS1  
u



deformace

]

14.8

13.4

12.1

10.8

9.4

8.1

6.7

5.4

4.0

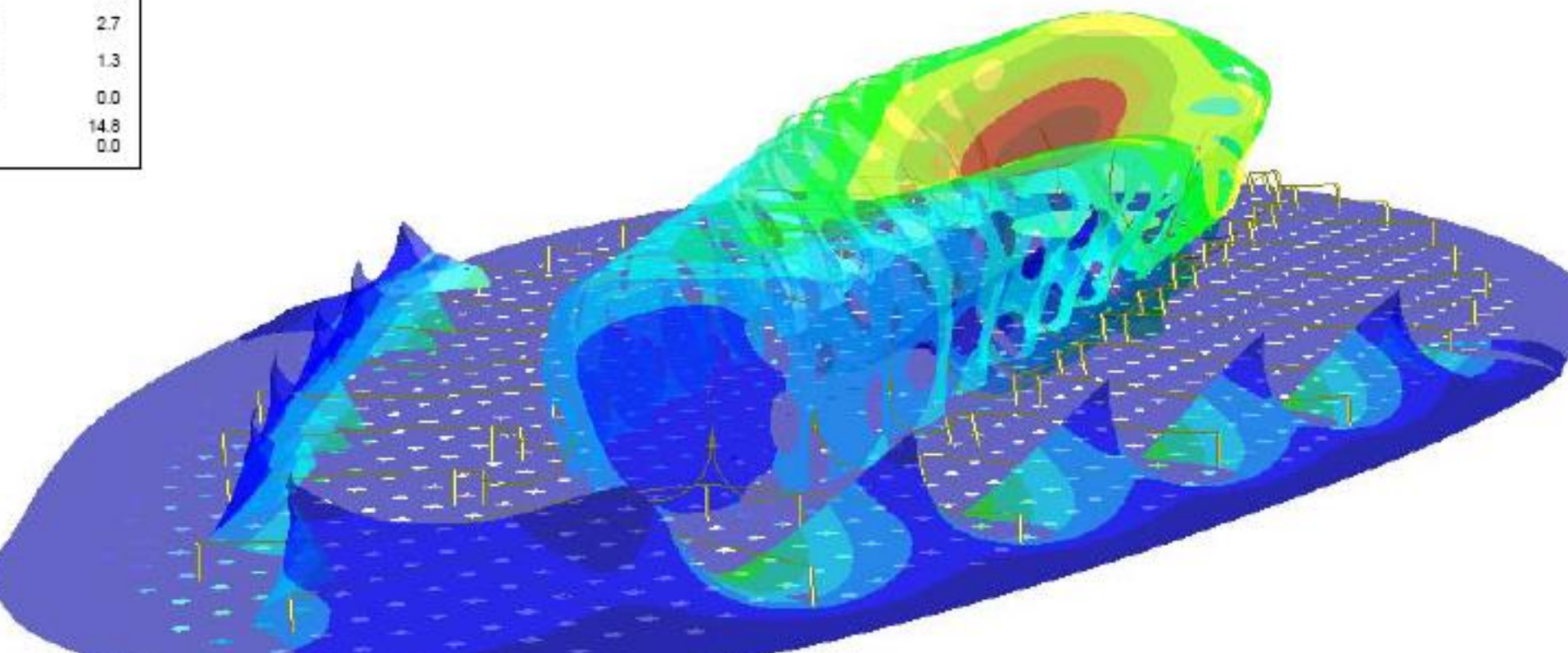
2.7

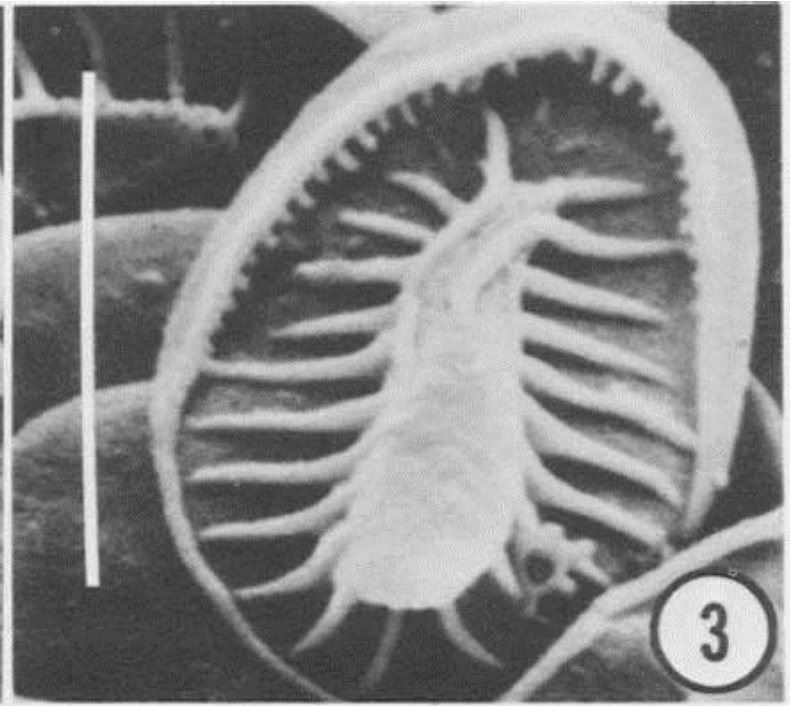
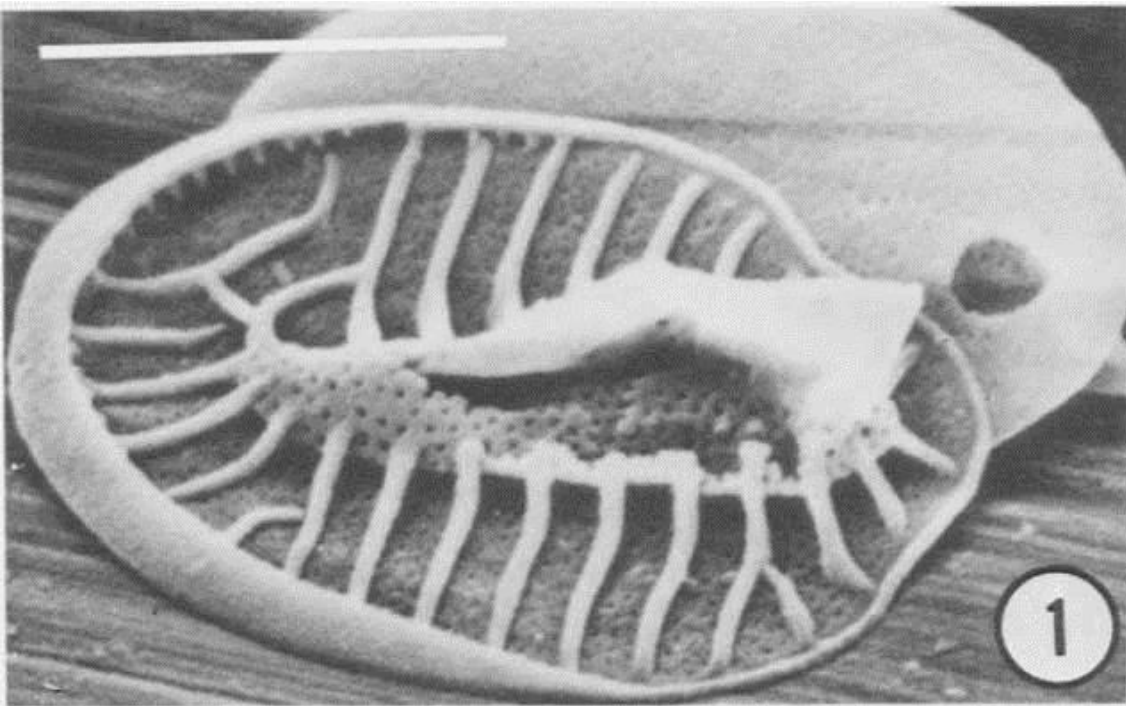
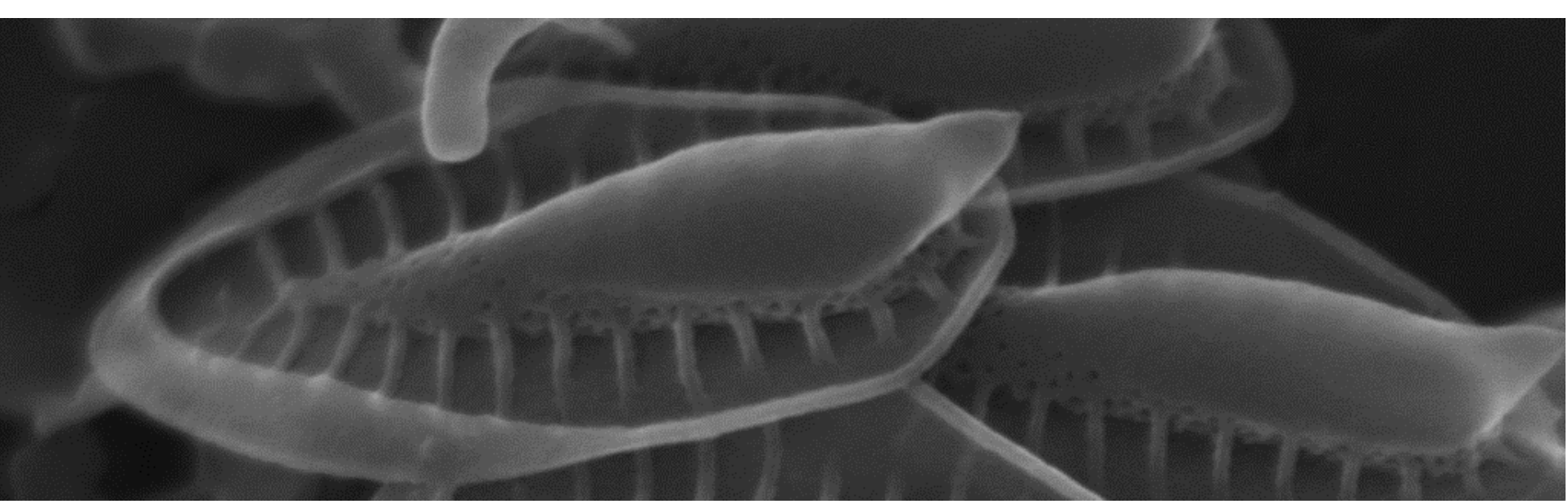
1.3

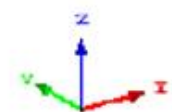
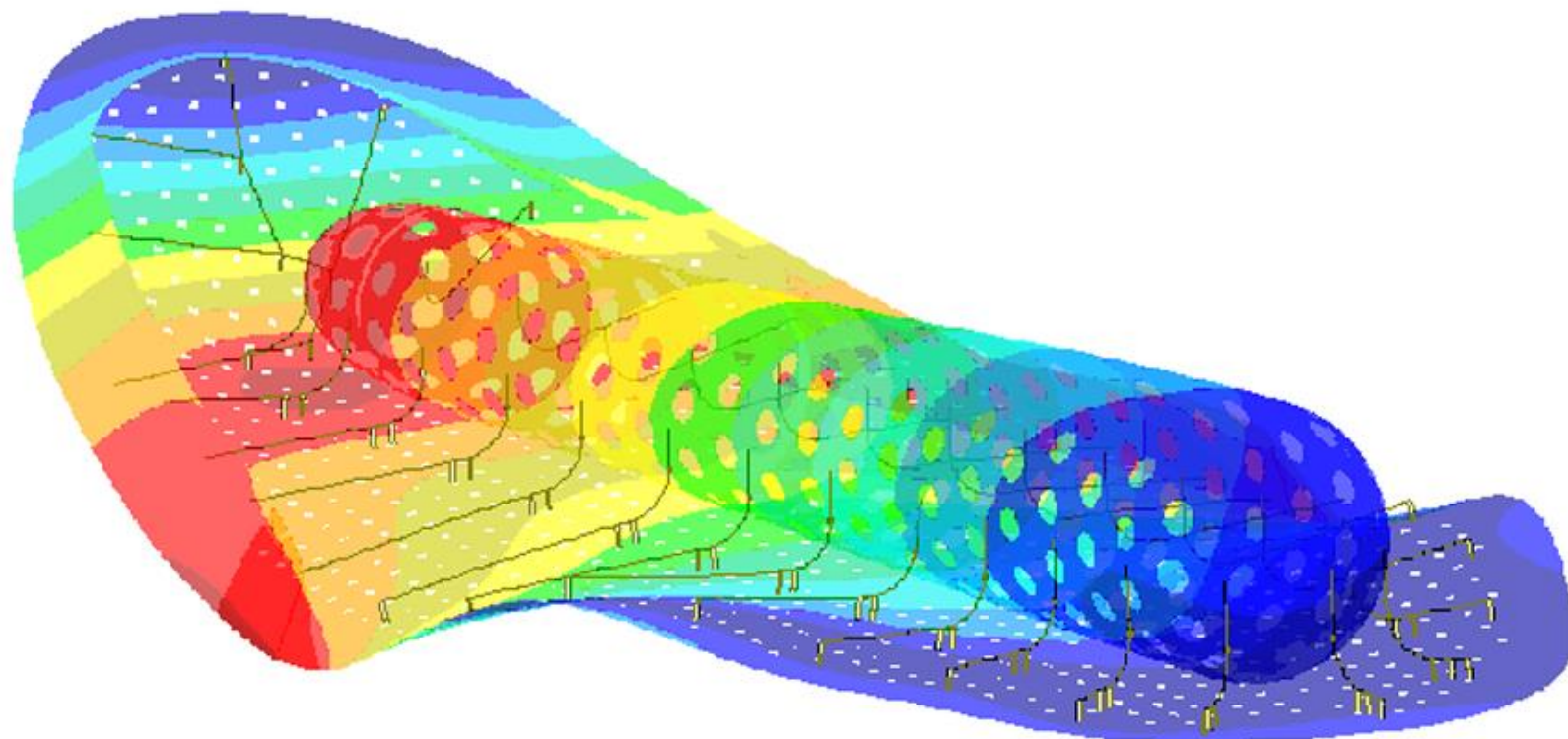
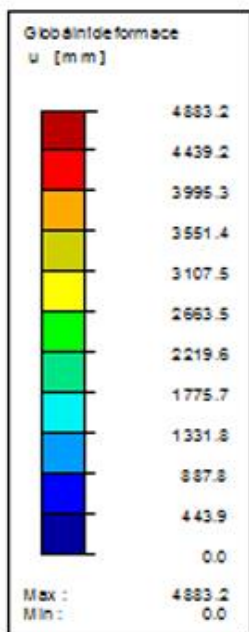
0.0

14.8

0.0



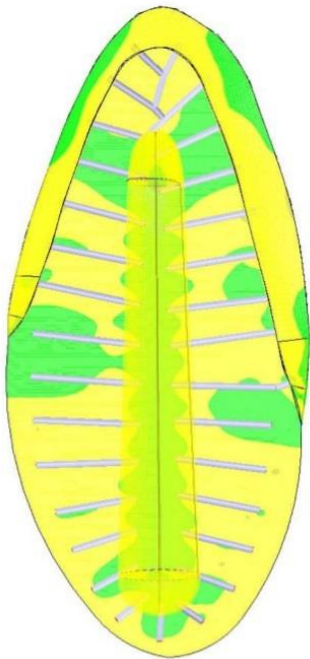
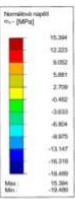




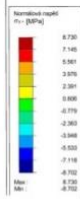
Max u: 4883.2, Min u: 0.0 [mm]  
Součinitel pro deformace: 0.12

# tension (stress)

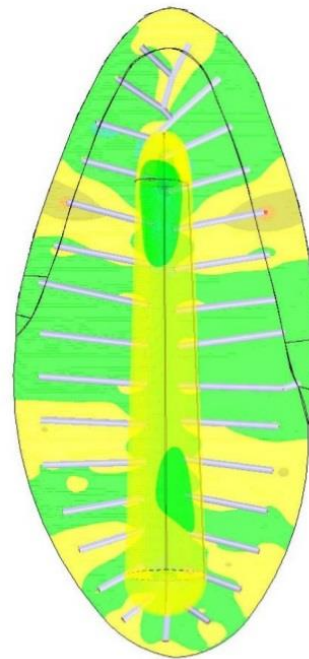
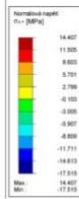
**real scale**



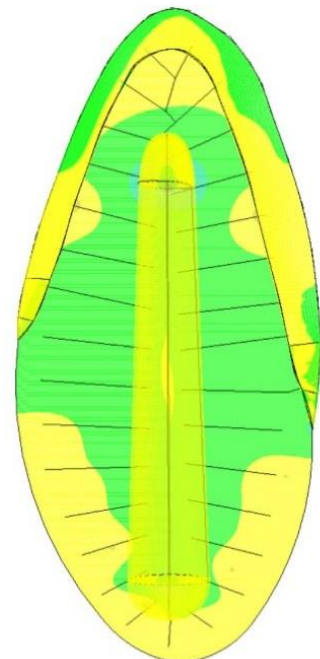
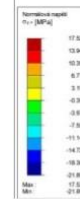
**no keel**



**no rim**



**no struts**



# Acknowledgements



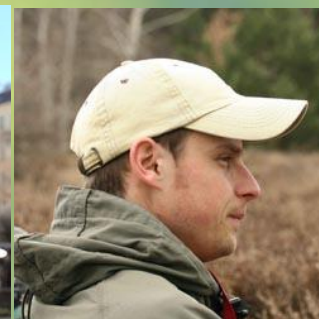
Jana  
Steinová



Tereza  
Řídká



Lucie  
Vančurová



Ondřej  
Peksa



Jiří  
Malíček



Helena  
Bestová

- Lucia Muggia
- Silke Werth
- Jan Vondrák
- Michele Piercey-Normore
- Soili Stenroos
- Mark Allen Wetter
- Ana Rosa Burgaz
- Yvonne Němcová
- Magda Škaloudová
- Tomáš Herben
- Martin Weisser



David  
Ryšánek



Fabio  
Rindi  
(Ancona,  
Italy)



Karel  
Mikeš  
(ČVUT)