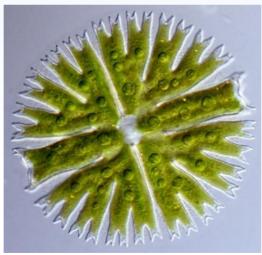
How to live in a microssm

Hidden diversity, speciation, and evolution of protists





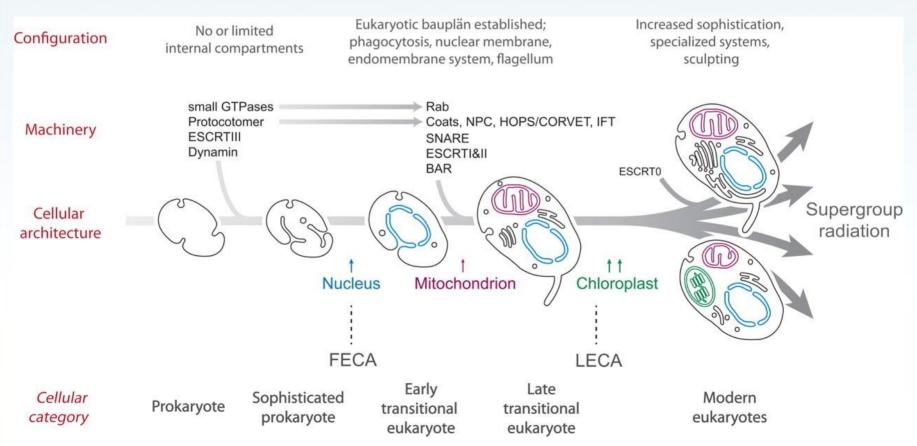


Pavel Škaloud Charles University, Prague

 Eukaryotic microorganisms with simple thalli (unicellular, colonial or filamentous).

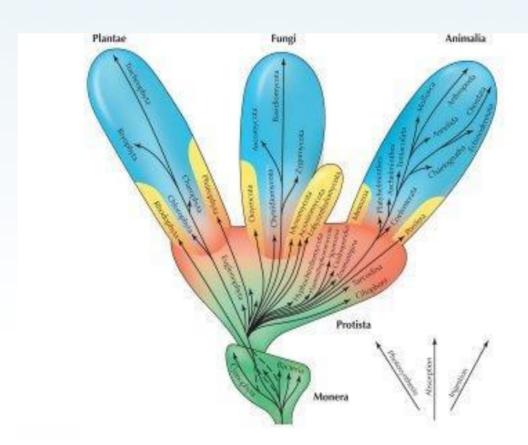


- Age of protists 1.2 3.5 billion years
- Last common eukaryotic ancestor (LECA) ca 1 billion years

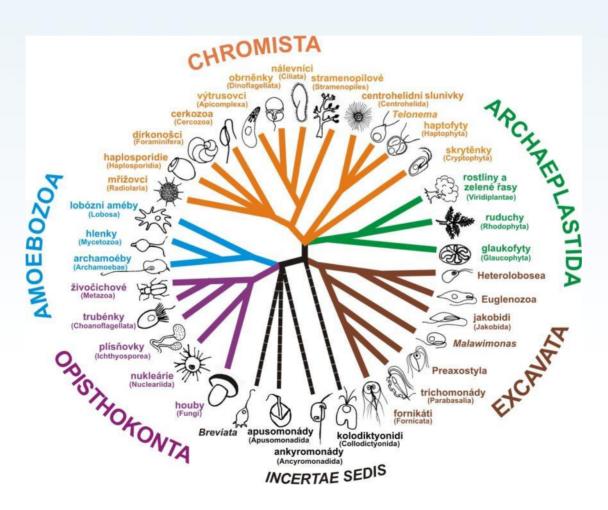


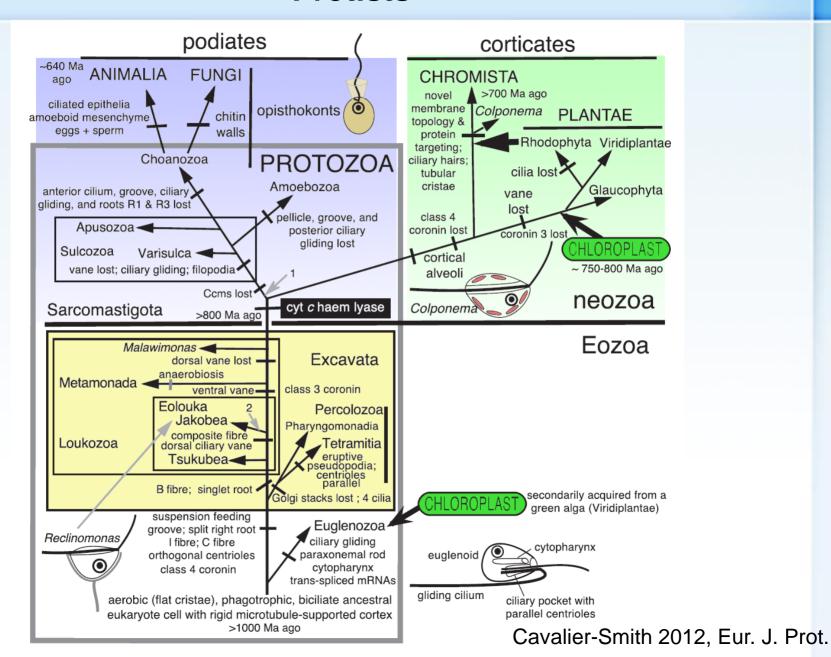
Field et al. 2011, JCB

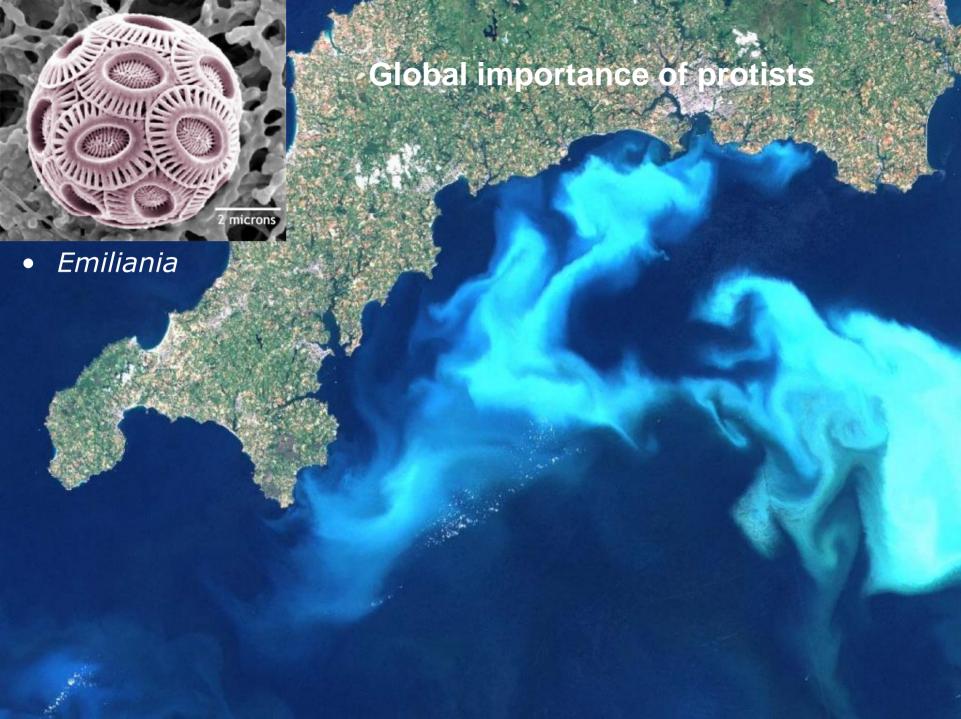
• Traditionally classified to the supergroup Protista (concept of 5 kingdoms)



- Current systematics of 5 kingdoms
- A major portion of eukaryotic diversity is comprised by protist organisms



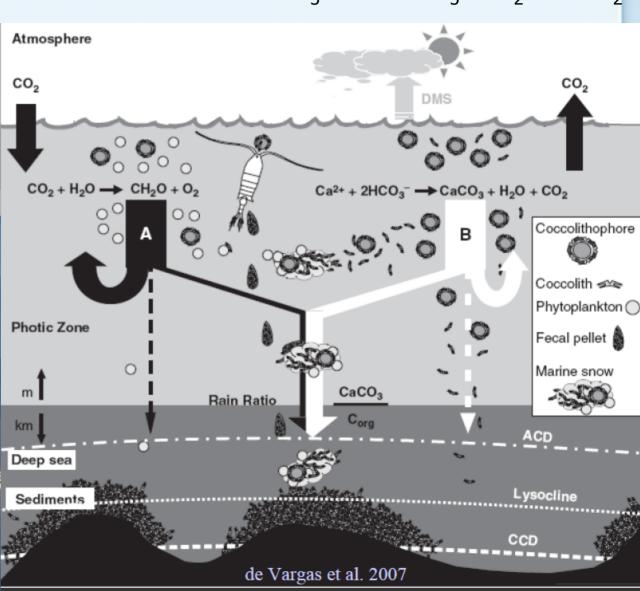




Global importance of protists

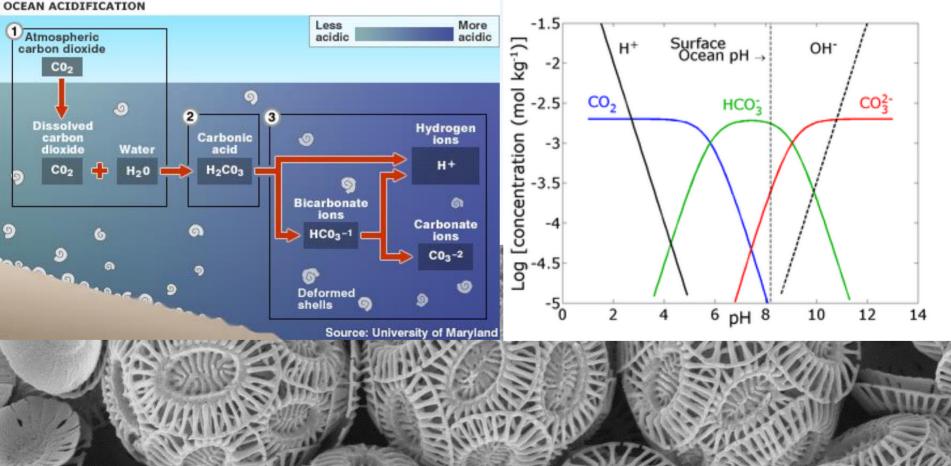
- Emiliania huxleyi coccolithes Ca + 2HCO₃ ---> CaCO₃ + H₂O + CO₂
- Strong influence of global Earth climate
- Limestones (Dover cliffs)





Global importance of protists

- Emiliania huxleyi
 - Sea acidification: carbon dioxide decreases pH of sea water, changing the balanced state of its three soluble forms in favour of CO₂ (not utilized by *Emiliania*)









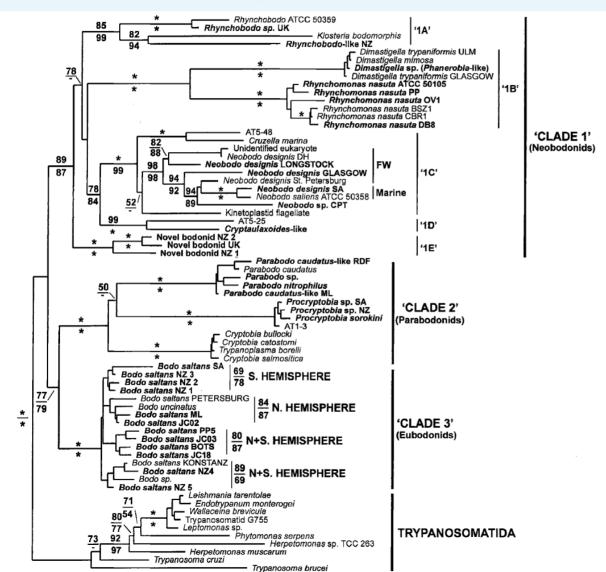
OBAMA'S WAR FOR ALGAE

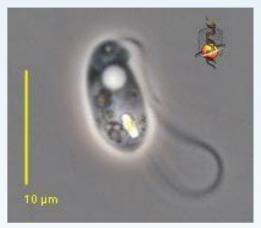
ThePeoplesCube.com

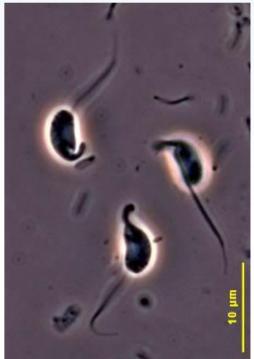


Hidden diversity of protists

Genetic diversity of bodonids (Kinetoplastida)



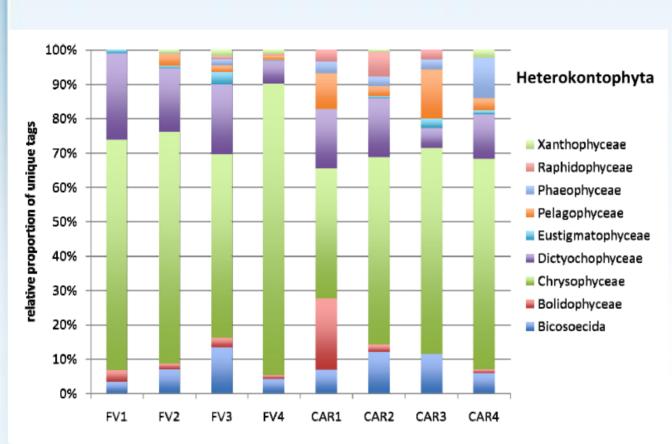




von der Heyden et al. 2004, J. Euk. Microb.

Hidden diversity of protists

Diversity of protists in marine benthos (454 sequencing)

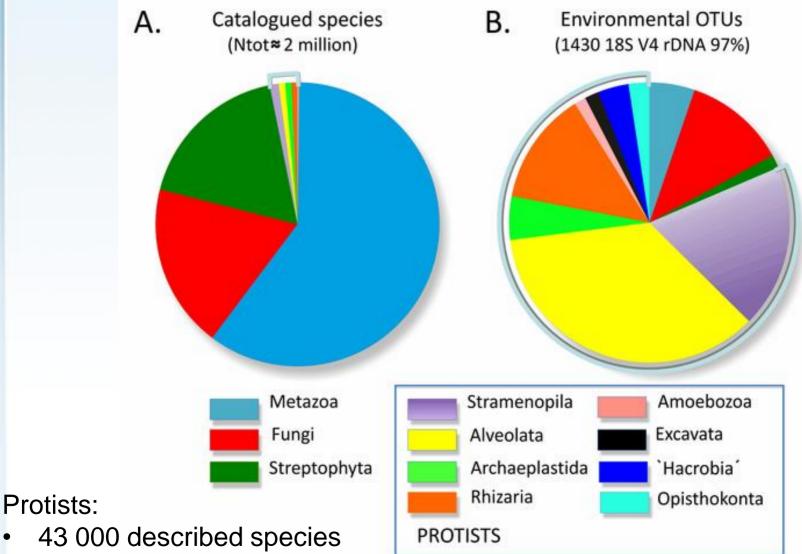




Stoeck et al. 2009, BMC Biology

Hidden diversity of protists

Great proportion of undescribed protist species (genotypes)



more than 1 mil of undescribed species

Pawlowski et al. 2012, PLOS Biology

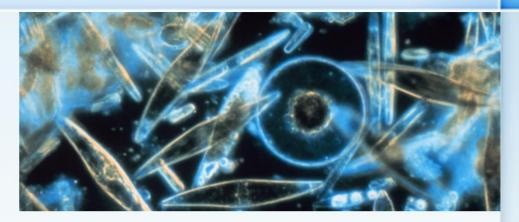
Finlay & Fenchel

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

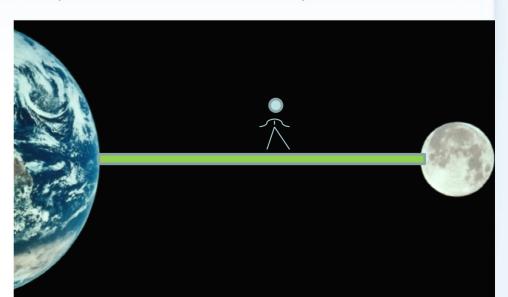


Unlike the macroorganisms:

- Ubiquitous distribution
- Very low speciation rate
- Absence of population differentiation

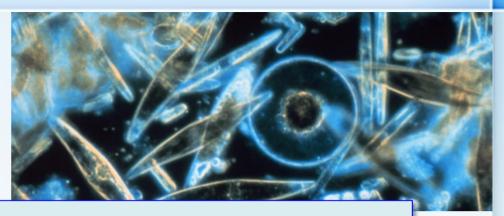


- Marine phytoplankton 10²⁵ of cells
- Average cell size 2 μm
- A pavement 30 cm wide and 8 cm thick (150 000 x 40 000 cells)



Finlay & Fenchel

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

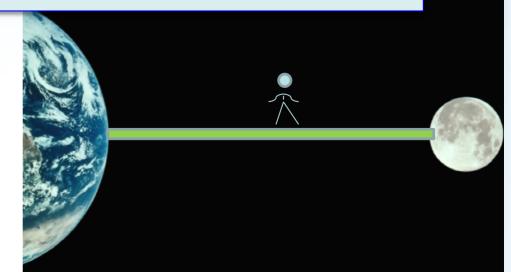


thick

Are the protists really so specific?

Unlike

- Ubiquitous distribution
- Very low speciation rate
- Absence of population differentiation



- Are the "hidden" protist species real?
- Are the protists ubiquitous?
- Could they form different populations?
- How do the protists speciate?

Comparable global and local diversity

Globally low number of species 100,000 Nivå Bay Choanoflagellates Heterotrophic flagellates Rotifers Amoebae • Percentage of global species pool Prasinophytes 10,000 Dinoflagellates Turbellarians Bivalves Number of species - Polychaetes 1000 Malacostraca Nematodes Polychaetes 100 Crustaceans Molluscs 10 0.01 0.01 0.1 0.001 100 1000 Mean size (mm) 1 km² 1000,000 km² 100 1 ha Heliozoa Priest Pot Amoebae Testate amoebae Percentage of global species pool Log area (ha) flagellates Ciliates Daphnians Triclads Diatoms 0.1 Fish 0.01 0.001 0.01 0.1 10 100 1000

Mean size (mm)

Fenchel & Finlay 2004, BioScience

 The most important is the functional (incl. phenotypic) species differentiation in nature, we are confused by molecular data

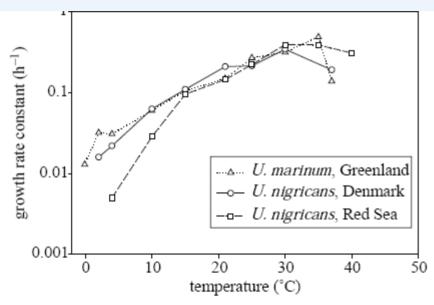
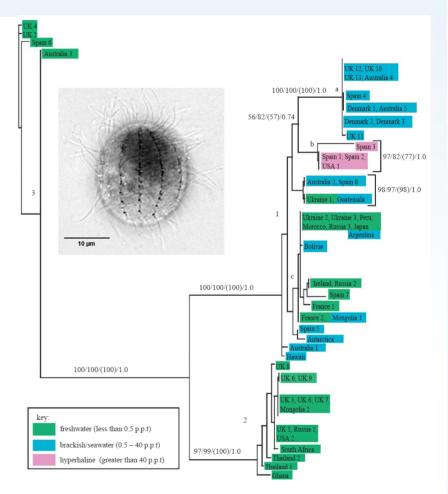
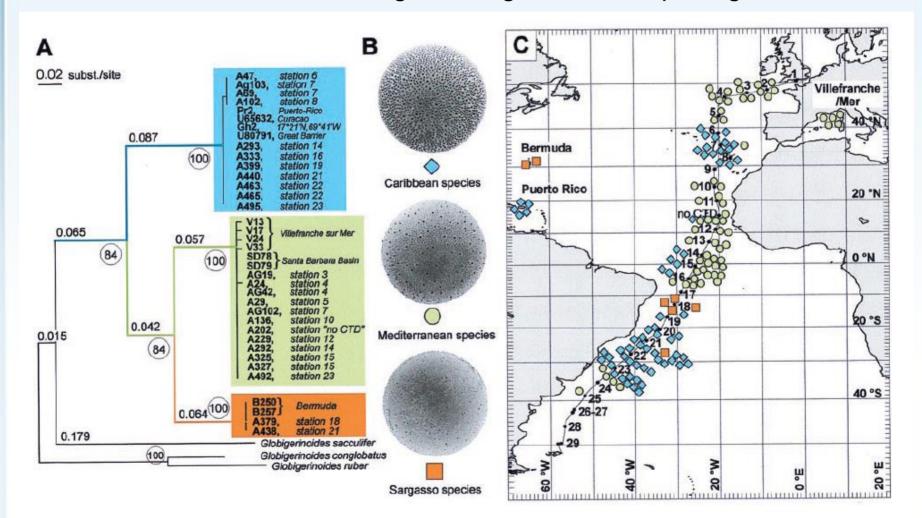


Figure 1. Maximum growth rate constants of the ciliates *Uronema marinum* isolated from the east coast of Greenland and *Uronema nigricans* isolated from the Red Sea (Eilat) and from Denmark, as a function of temperature. The tropical isolate grows significantly slower at temperatures below 10°C, but it is more striking that all three strains show balanced growth within a temperature range that far exceeds that of the habitats from which they were isolated (T. Fenchel, unpublished data).



Fenchel & Finlay 2006, Phil. Trans. R. Soc. B

- Functional differentiation of cryptic species
 - Orbulina universa slight ecological and morphological differences



- Functional differentiation of cryptic species
 - Sellaphora pupula morphological differentiation of hidden species

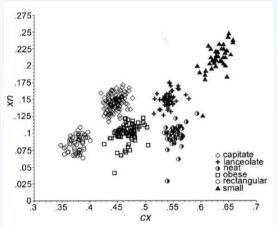
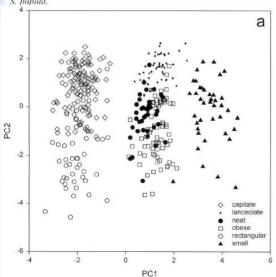
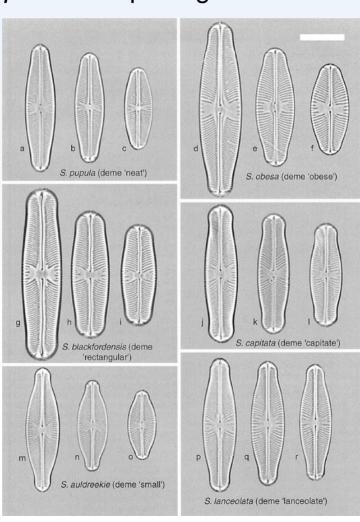
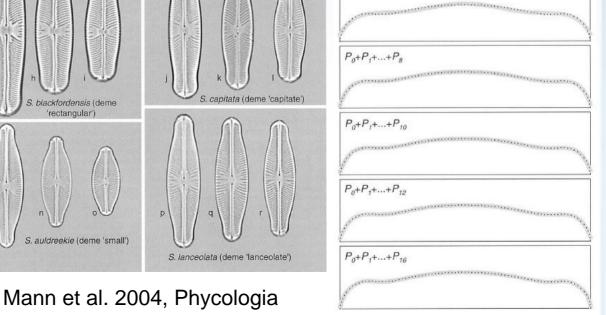


Fig. 16. Contour segment analysis: maximum curvature of all contour points (*cx*) plotted against the maximum of all segment minimum curvatures (*xn*), giving six clusters corresponding to the six demes of *S. pupula*.

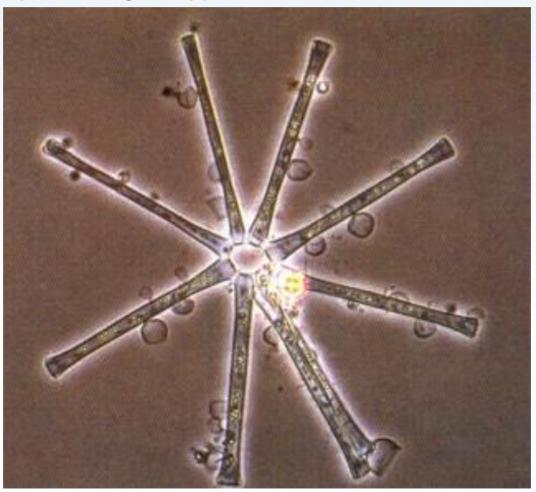






 $P_0 + P_1 + ... + P_4$

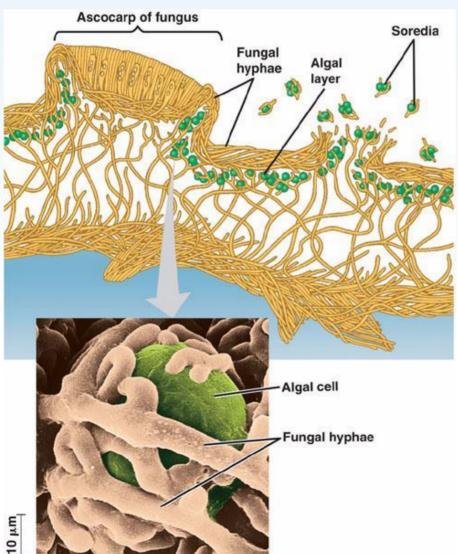
- Functional differentiation of cryptic species fungal parasites
 - Asterionella formosa genetically different strains are infected by specific parasite genotypes



De Bruin et al. 2004, J. Phycol.

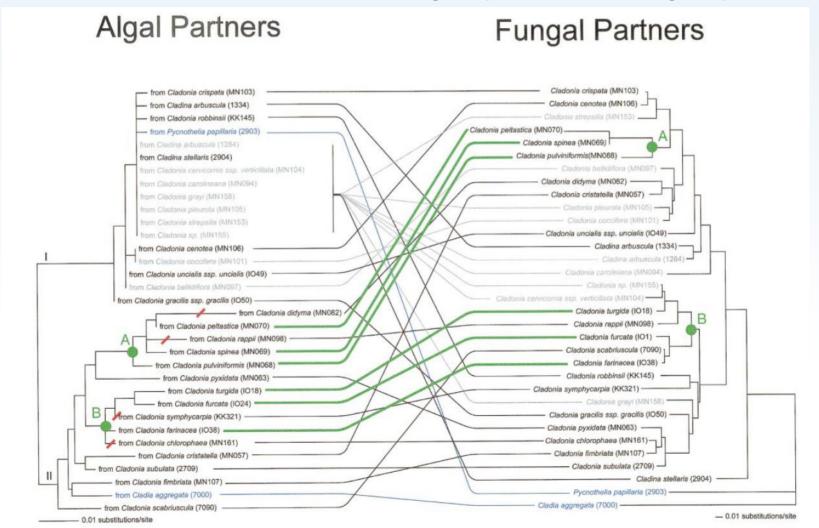
Lichen photobionts

• Lichen association: 15 000 fungal species + 100 algal species





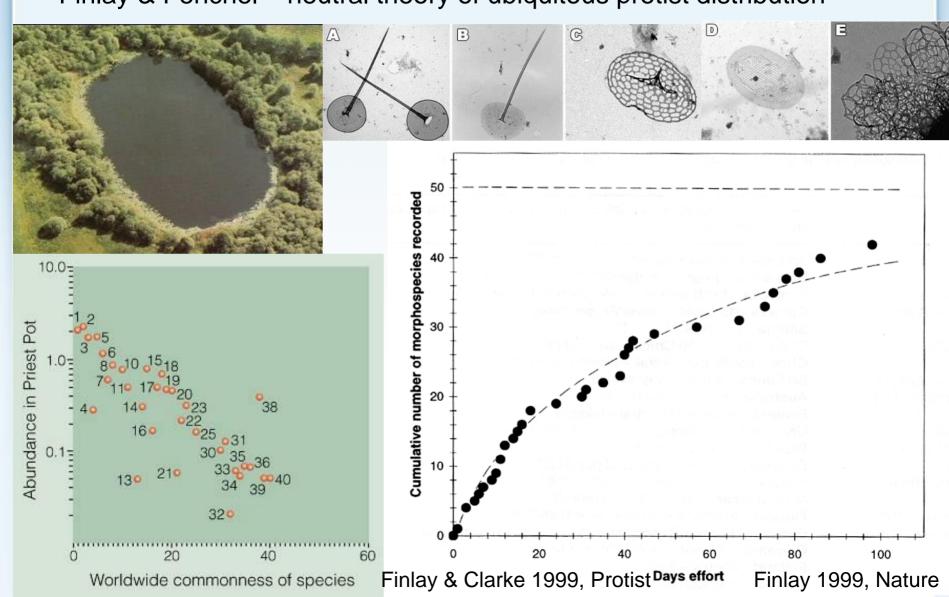
- Lichen photobionts
 - Lichen association: 15 000 fungal species + 100 algal species



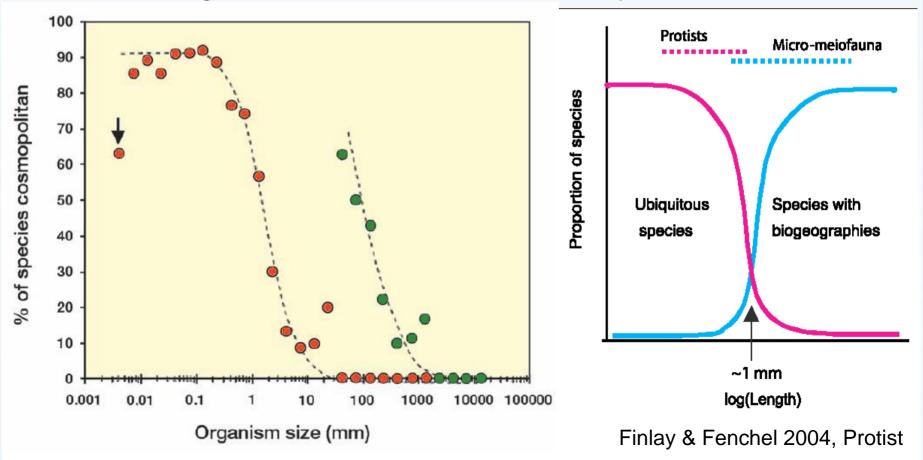
Piercey-Normore & DePriest 2001, Am. J. Bot.

- Are the "hidden" protist species real?
- Are the protists ubiquitous?
- Could they form different populations?
- How do the protists speciate?

Finlay & Fenchel – neutral theory of ubiquitous protist distribution



- Finlay & Fenchel neutral theory of ubiquitous protist distribution
 - Everything is everywhere, but many species are extremely rare (enormous populations with unlimited dispersal)
 - Conservation of protists is not needed, the diversity could be investigated in the closest available locality

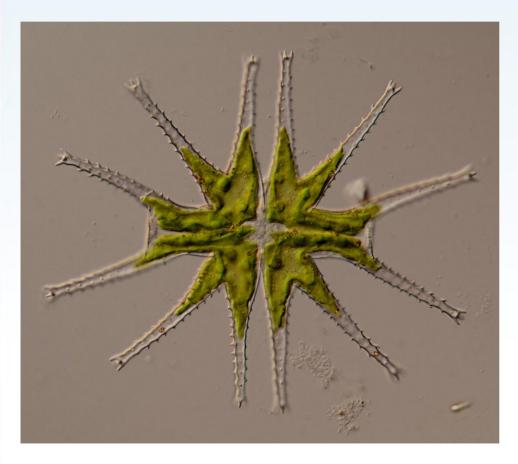


- Foissner moderate endemicity model
 - Even if many species are qbiquitous, one third of known species have limited distribution

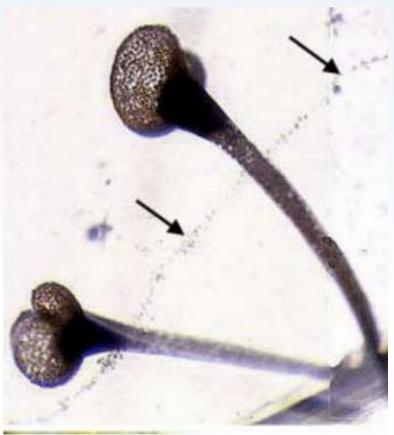
Table 5.3 Percentages of dispersal routes of protists. Based on the calculation of Foissner (2008) that one-third of ciliates possibly have restricted distribution.

Dispersal routes	Amount (%)
Cosmopolitan distribution due to step-by-step dispersal and human introductions	35
Cosmopolitan distribution due to geological processes, euryoecious lifestyle and others	30
Restricted distribution due to morphological and physiological peculiarities of the resting cysts, break-up of Pangaea and insufficient time to disperse in young species	35

- Foissner moderate endemicity model
 - Flagship species

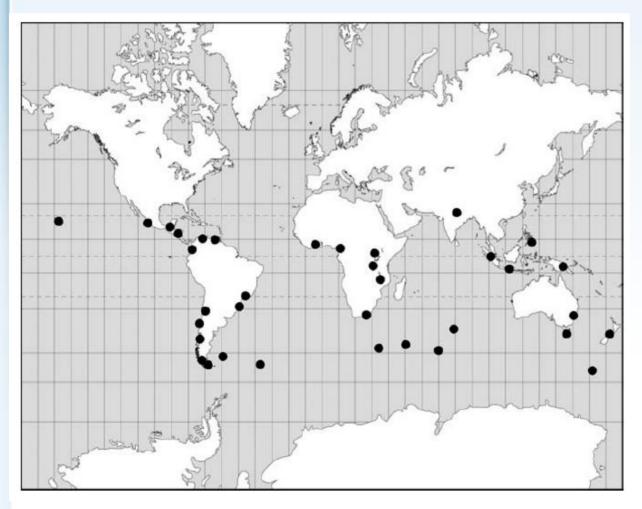


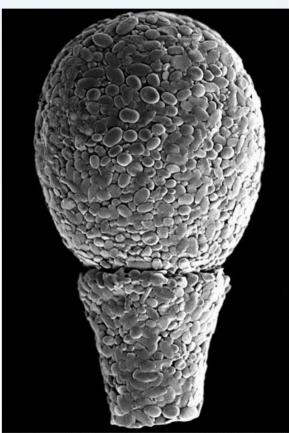
Micrasterias hardyi - Australia



Maristentor dinoferus – S America

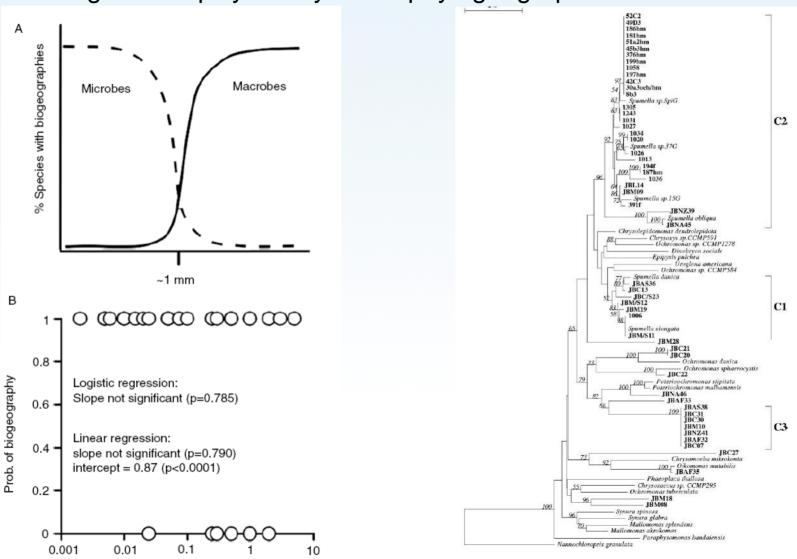
- Foissner moderate endemicity model
 - Apodera vas Gondwanan distribution





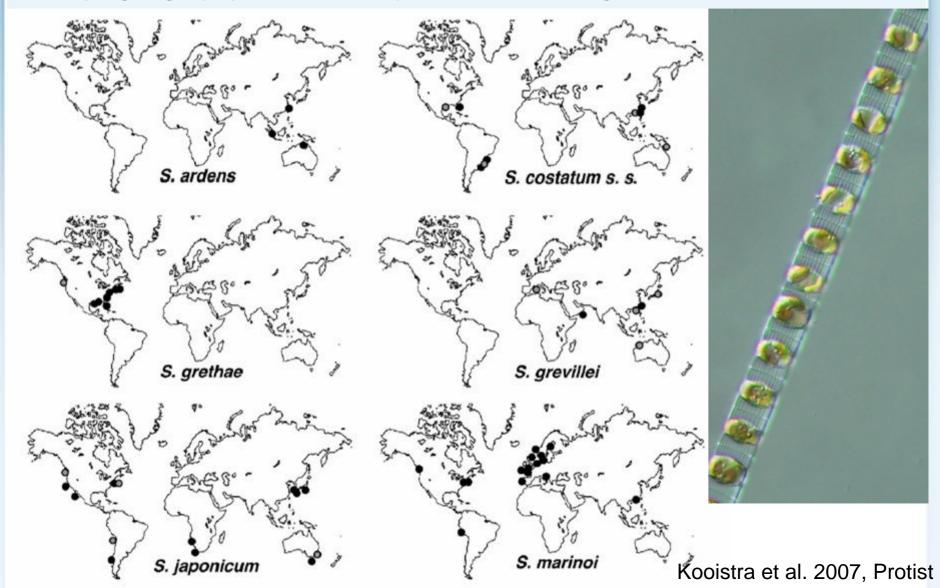
Testing the ubiquity theory on 51 phylogeographic studies

log (propagule size, mm)

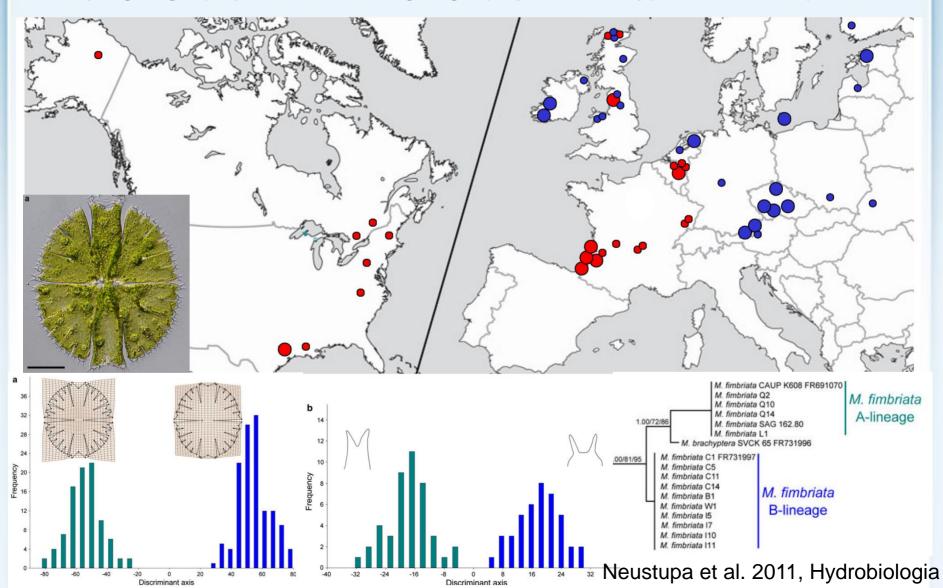


Jenkins et al. 2011, Biogeography of Microscopic Organisms

• Phylogeography – endemic species within the genus Skeletonema



Phylogeography – distinct biogeography of two cryptic desmid species



- Are the "hidden" protist species real?
- Are the protists ubiquitous?
- Could they form different populations?
- How do the protists speciate?

Genetic structure of protist populations

- Very few studies
- Finlay unlimited dispersal and continuous gene flow imply the absence of differentiated populations

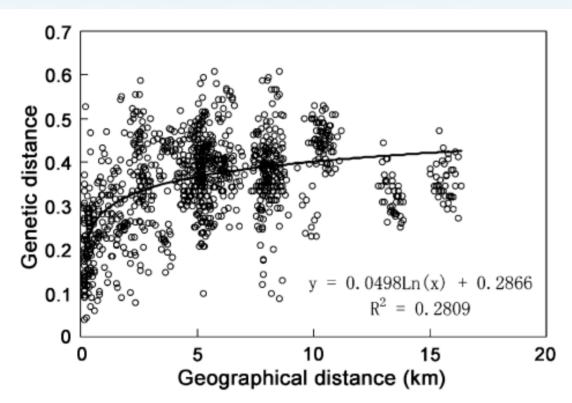
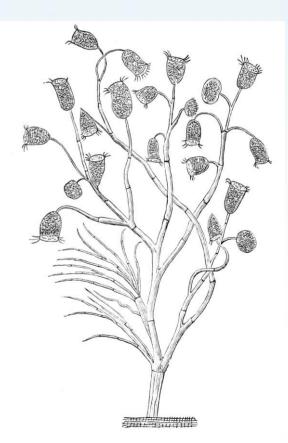


Fig. 5. Logarithmic correlation between genetic distance (Nei and Li 1979) and geographical distance (r = 0.5300, $P < 10^{-6}$).



Carchesium polypinum

Zhang et al. 2006, J. Eukar. Microbiol.

Genetic structure of protist populations

• Spatial population structure – *Pseudo-nitzschia pungens*

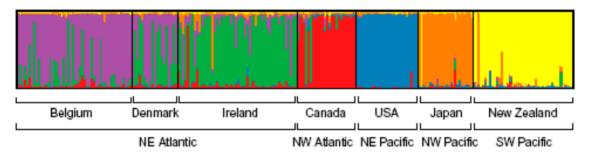


Fig. 3. Structure plot for K = 6. Each individual is depicted by a vertical line that is partitioned into K colored sections, with the length of each section proportional to the estimated membership coefficient (q_{ind}) of the isolate to each cluster.

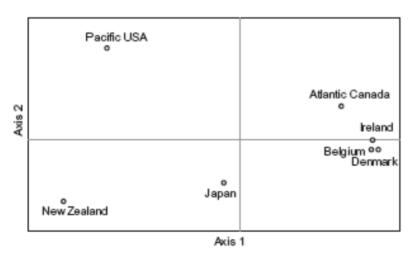


Fig. 4. Principal component analysis of pairwise F_{ST} values among the seven predefined populations of P. pungens clade I. The first and second principal components account for 59.47% and 24.58% of the total variation, respectively.



Genetic structure of protist populations

• Spatial population structure – Scrippsiella hangoei

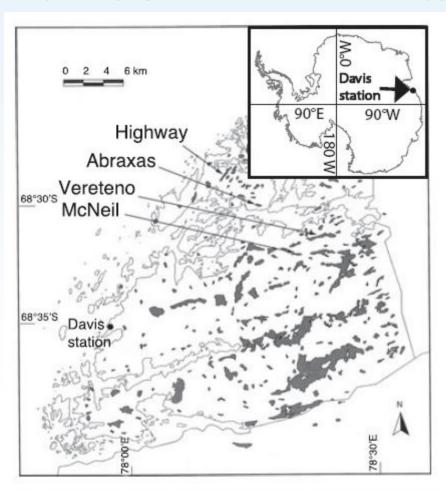


Fig. 1. Map of Vestfold Hills showing location of study lakes and Davis Station.

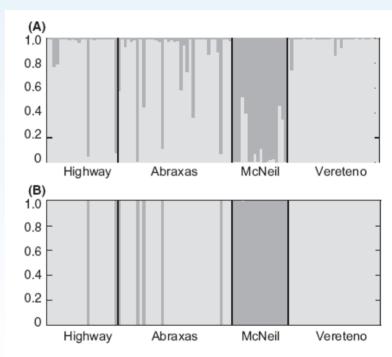


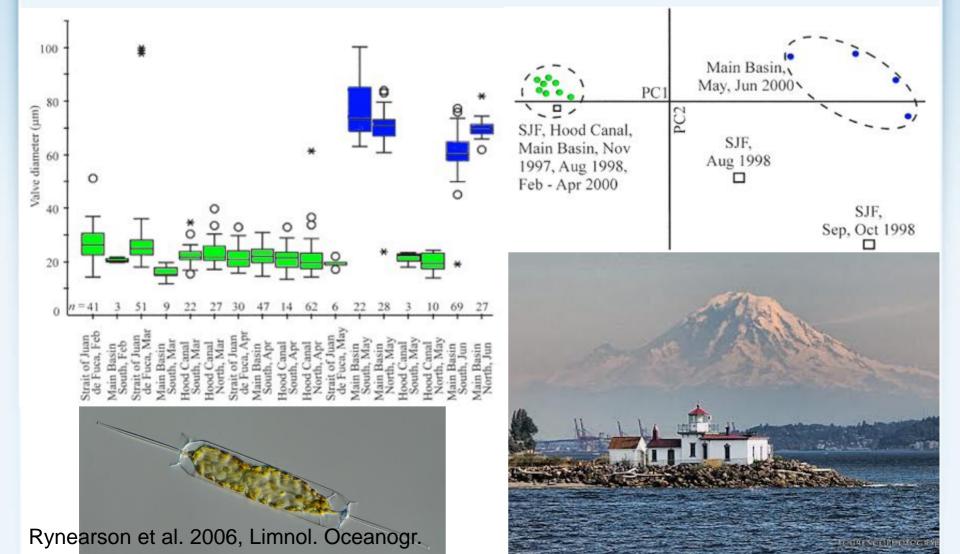
Fig. 3. Population analyses results from STRUCTURE shown as bar plots. Only the models showing the highest likelihood



Rengefors et al. 2012, Mol. Ecol.

Genetic structure of protist populations

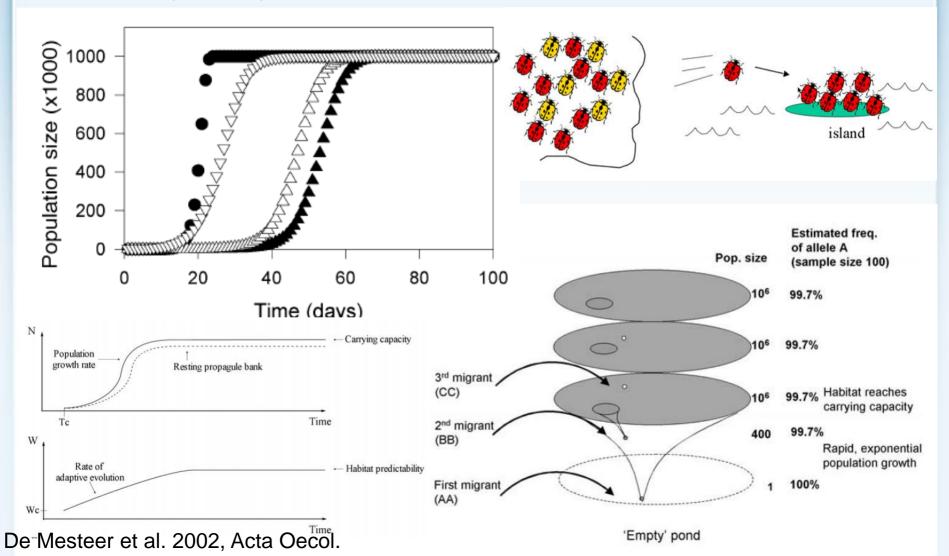
- Temporal population structure *Ditylum brightwellii*
- Annual change of two blooms



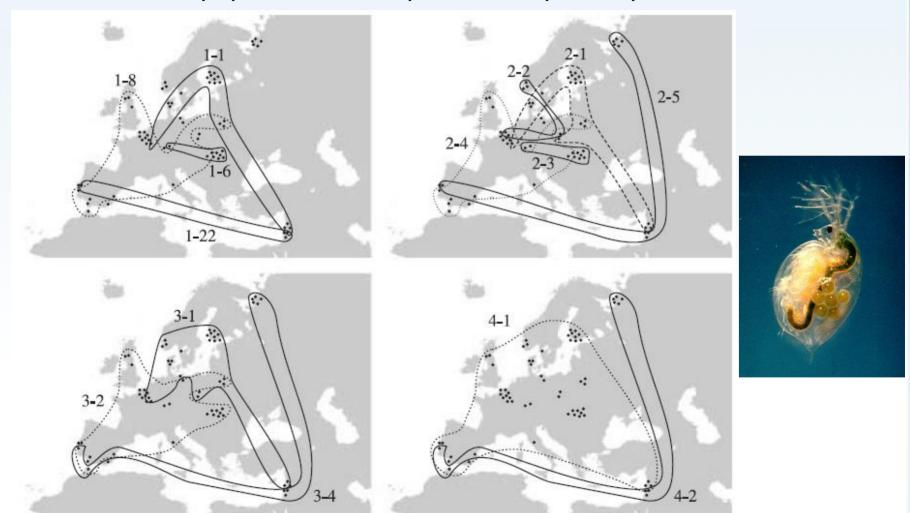
Specificity of protist organisms

- Are the "hidden" protist species real?
- Are the protists ubiquitous?
- Could they form different populations?
- How do the protists speciate?

- Monopolisation hypothesis founder efect (bank of dormant cysts)
 - Rapid adaptation to local environmental conditions



- Monopolisation hypothesis
 - Mosaic populations in Daphnia = allopatric speciation

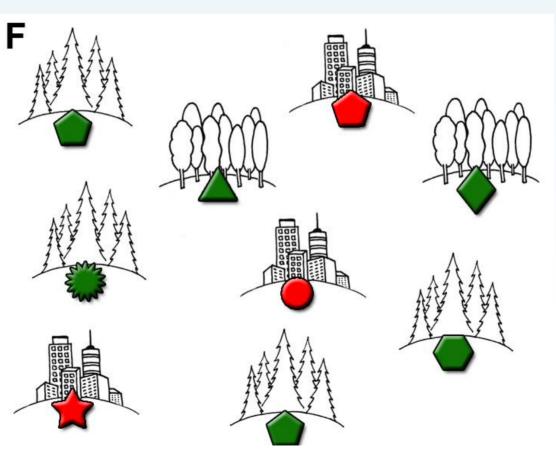


De Gelas & De Meester 2005, Mol. Ecol.

- Monopolisation hypothesis founder efect (bank of dormant cysts)
 - Rapid adaptation to local environmental conditions



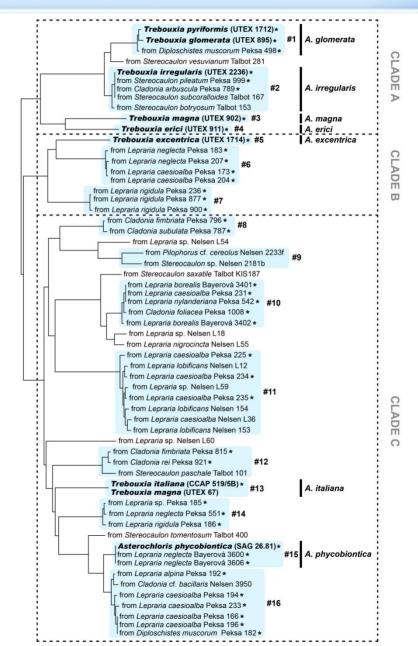
Ecological speciation



- Asterochloris
 - Genetic diversity



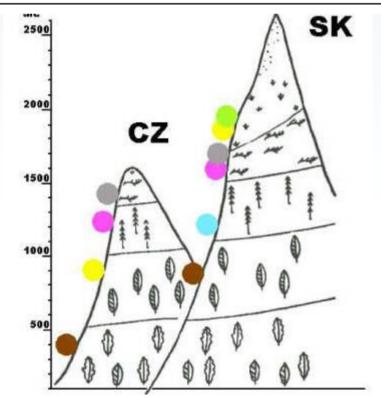
Škaloud & Peksa 2010, Mol. Phyl. Evol.

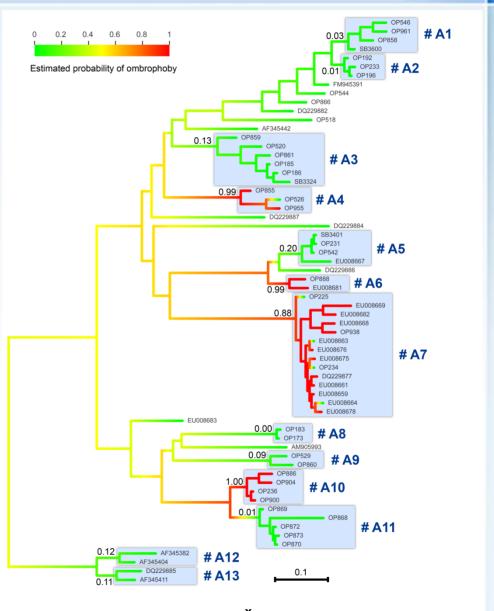


Asterochloris

Ecological speciation

Trait	Pagel's λ		
	λ	Likelihood ratio	P-value
Exposure to rain	0.946	1.53	< 0.0001
Altitude	0.045	1.01	< 0.0001
Substrate type	0.652	1.05	0.0011

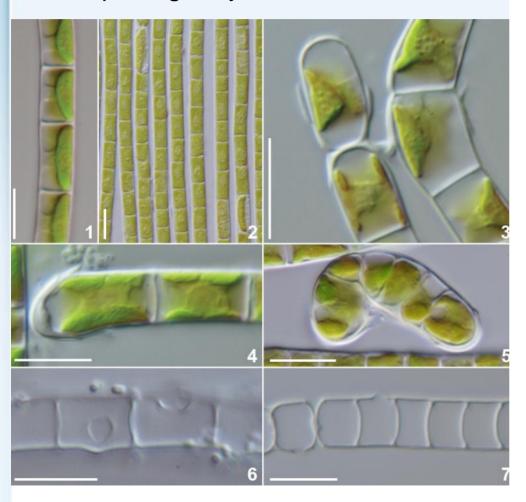


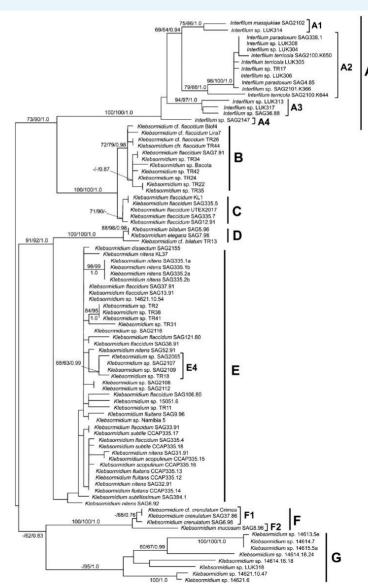


Peksa & Škaloud 2011, Mol. Ecol.

Klebsormidium – the high level of cryptic diversity, particular clades are

morphologically indiscernable

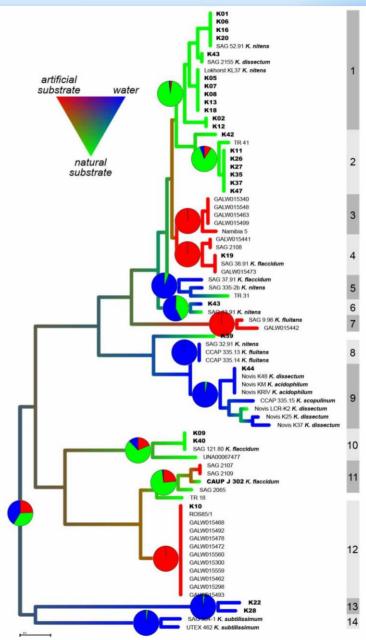




Rindi et al. 2011, Mol. Phyl. Evol.

• Klebsormidium – ecological speciation





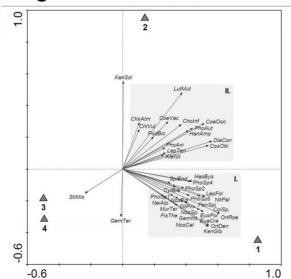
Škaloud & Rindi 2013, J. Euk. Microb., in press

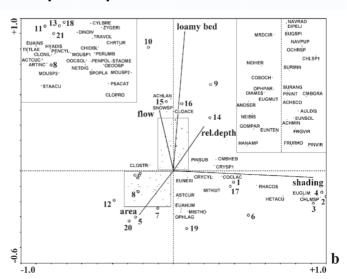
Summary

- Protists could speciate even if they have enormous populations and unlimited dispersal
- We should keep in mind the existence of a high portion of cryptic, so far undescribed species od protists
- Species are often differentiatied by their ecological preferences



Ekological studies based on protist morphospecies?





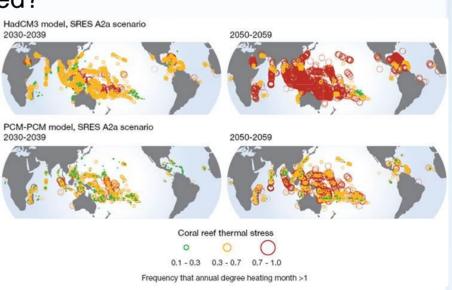
Summary

- Protists could speciate even if they have enormous populations and unlimited dispersal
- We should keep in mind the existence of a high portion of cryptic, so far undescribed species od protists
- Species are often differentiatied by their ecological preferences



Conservation of protists – is it needed?





Acknowledgements

- Phycology lab, Prague, Czech Republic
 - Jiří Neustupa
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