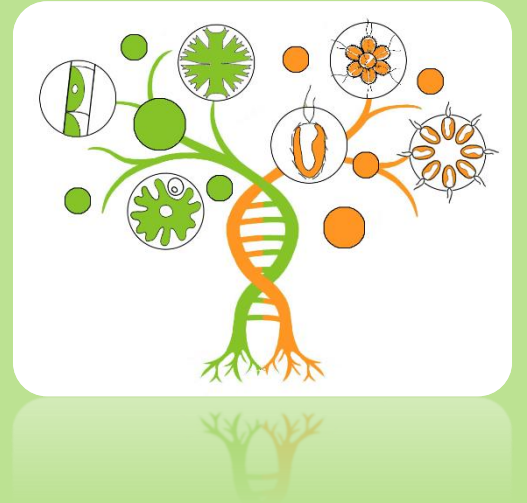


Speciation in protists

Spatial and ecological divergence processes cause rapid species diversification in a freshwater chrysophyte



Škaloud P.¹, Škaloudová M.¹, Doskočilová P.¹, Kim J.I.², Shin W.², Dvořák, P.³

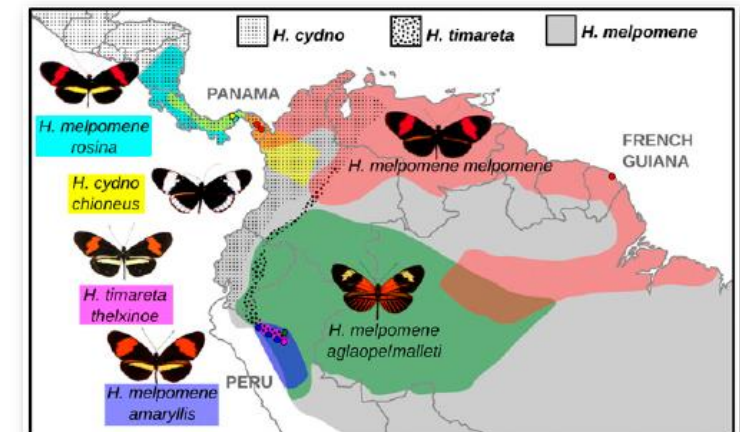
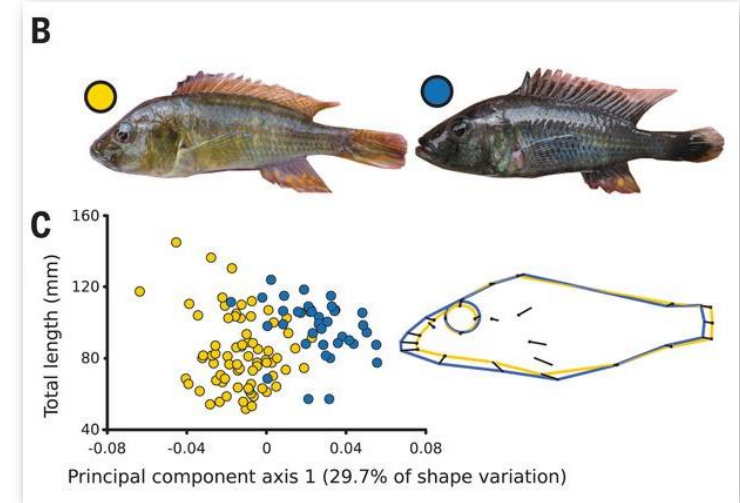
¹Charles University, Czech Republic

²Chungnam National University, Korea

³Palacký University, Czech Republic

Speciation mechanisms

- Despite the growing number of published papers focusing to speciation, only a very small fraction is dealing with protists.

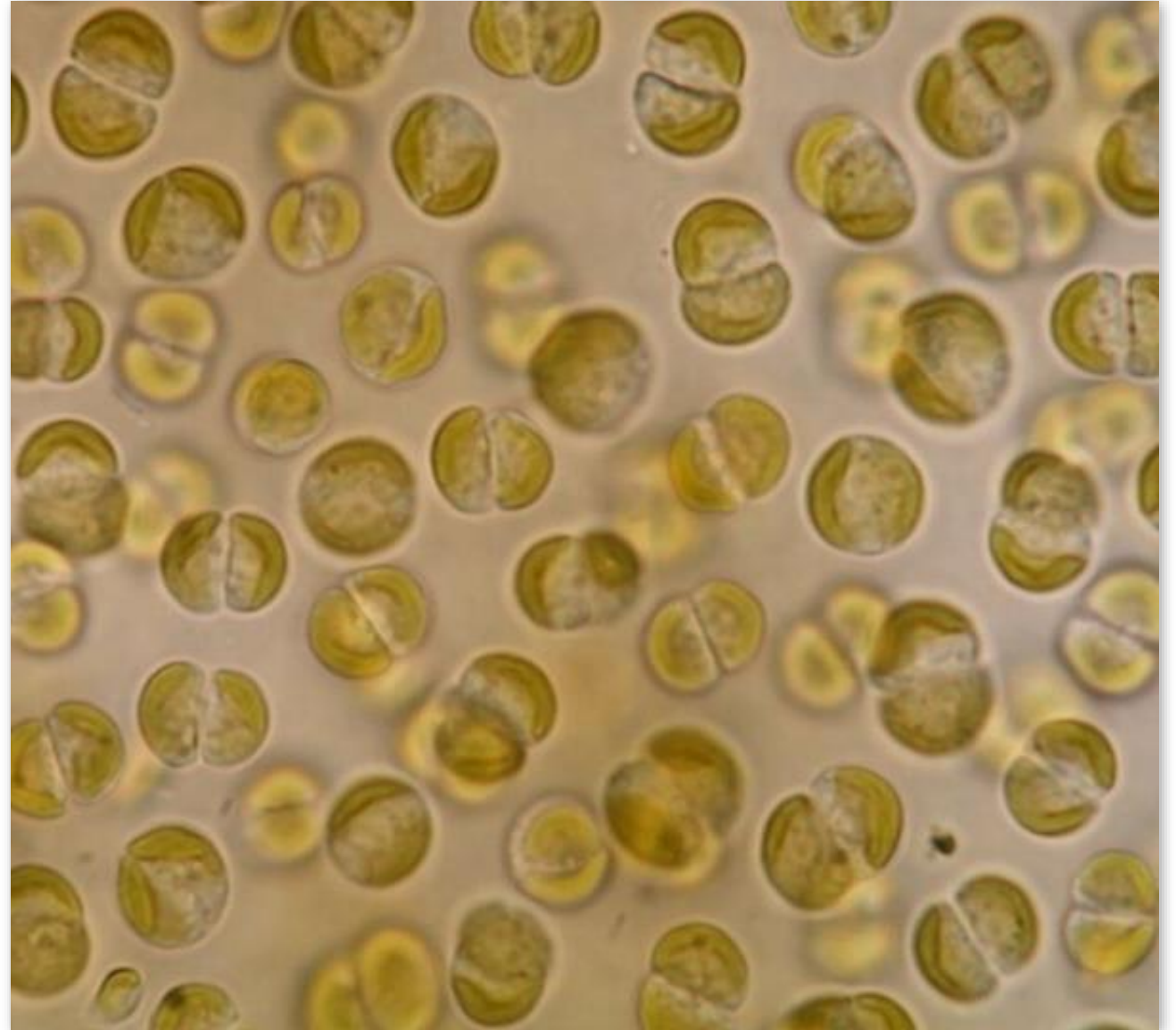
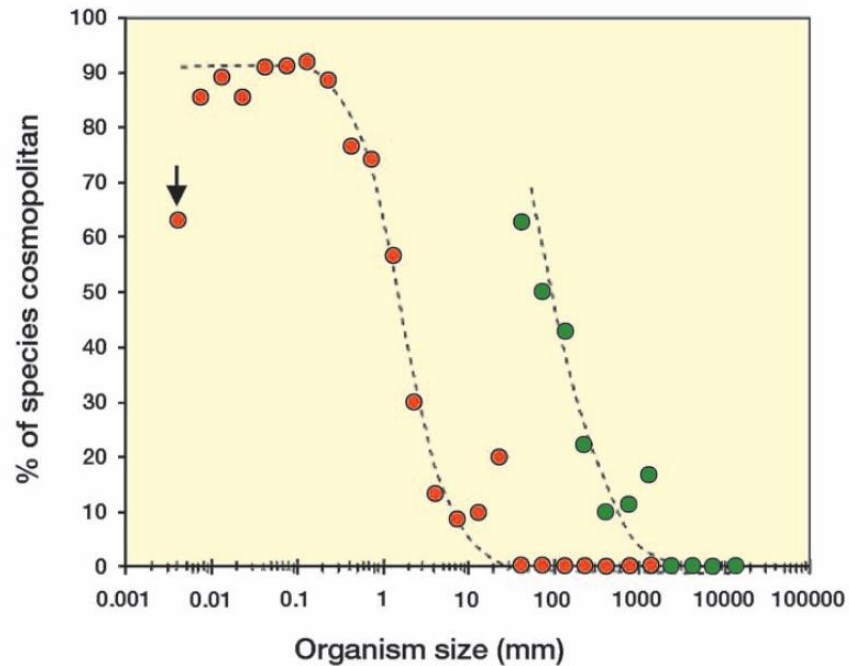


Specificity of protist organisms

- Enormous population sizes
- Unlimited dispersal & gene flow



- Lack of population differentiation
- Very low speciation rate

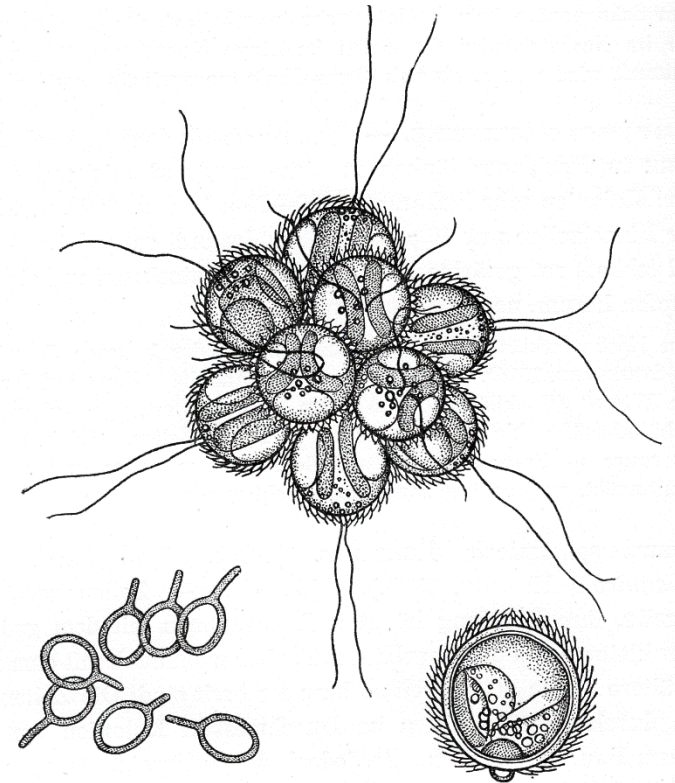
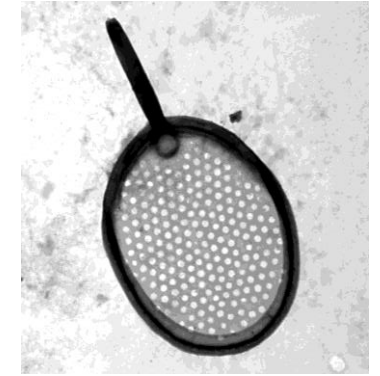
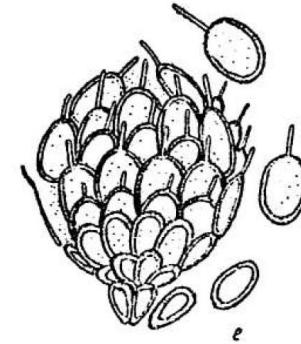
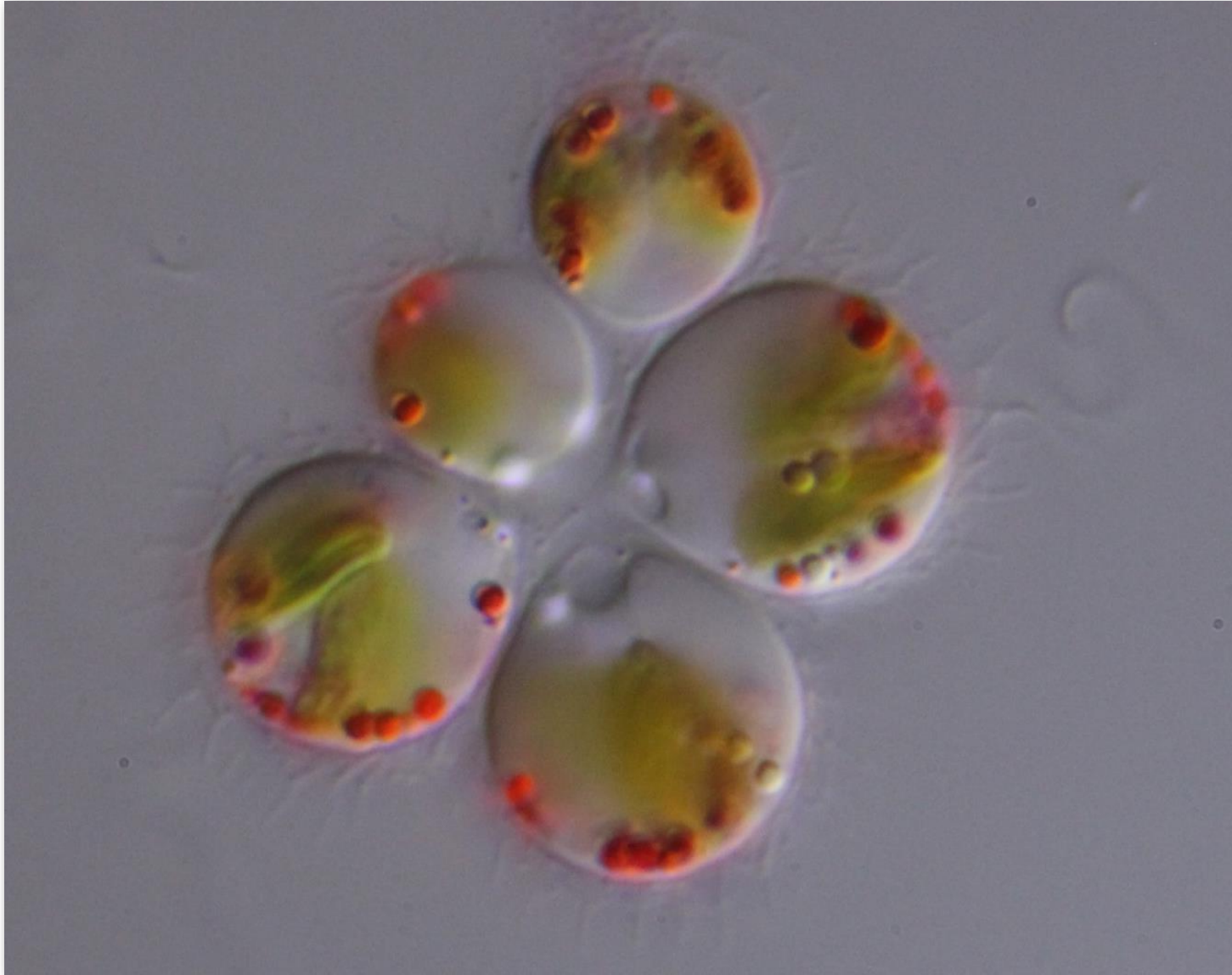


Main goals

- Which are the main factors structuring the extant diversity in a protist species?
- Are the speciation mechanisms in protists different from those proposed for macroorganisms?

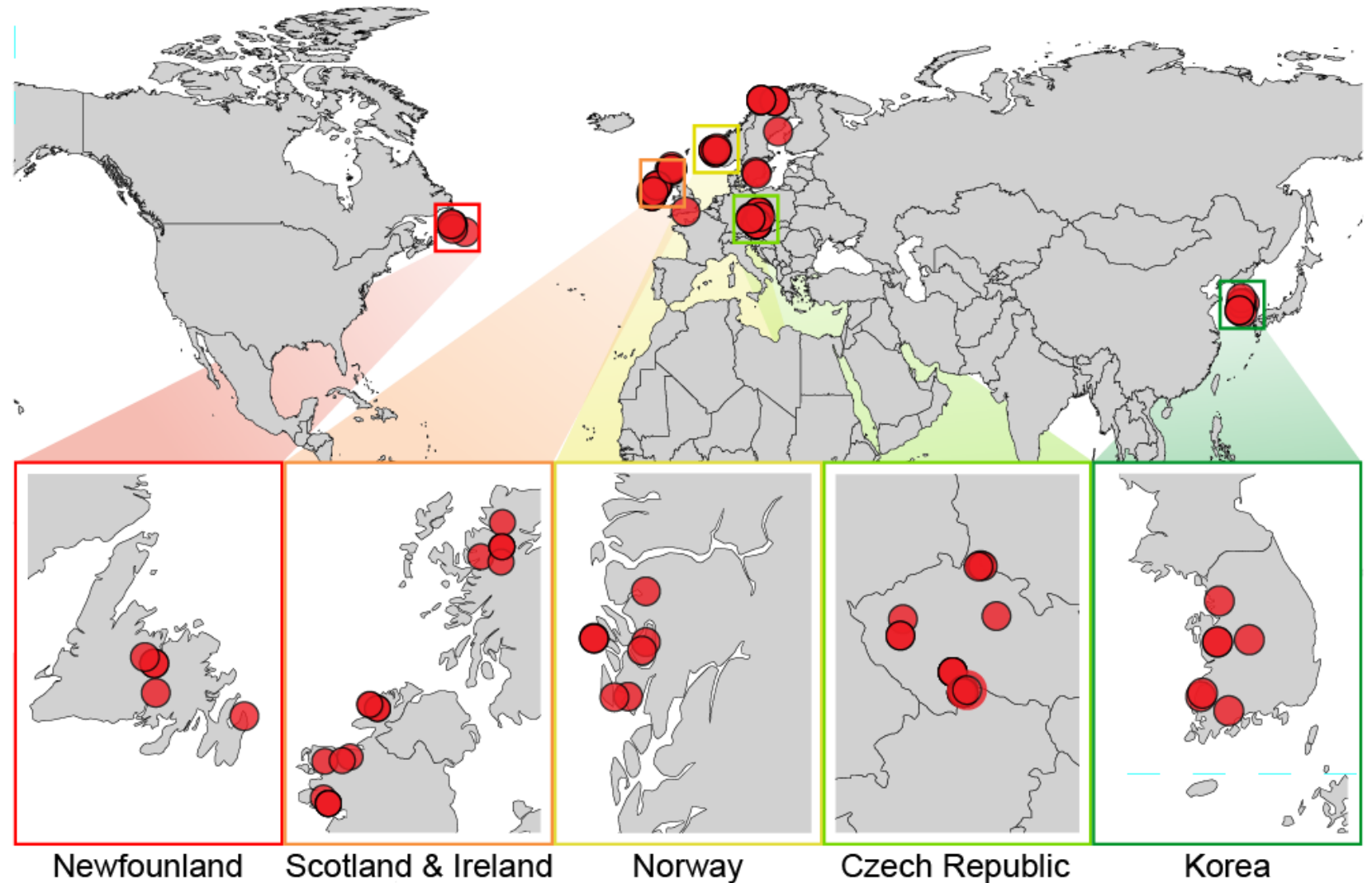


Synura sphagnicola (Chrysophyceae)



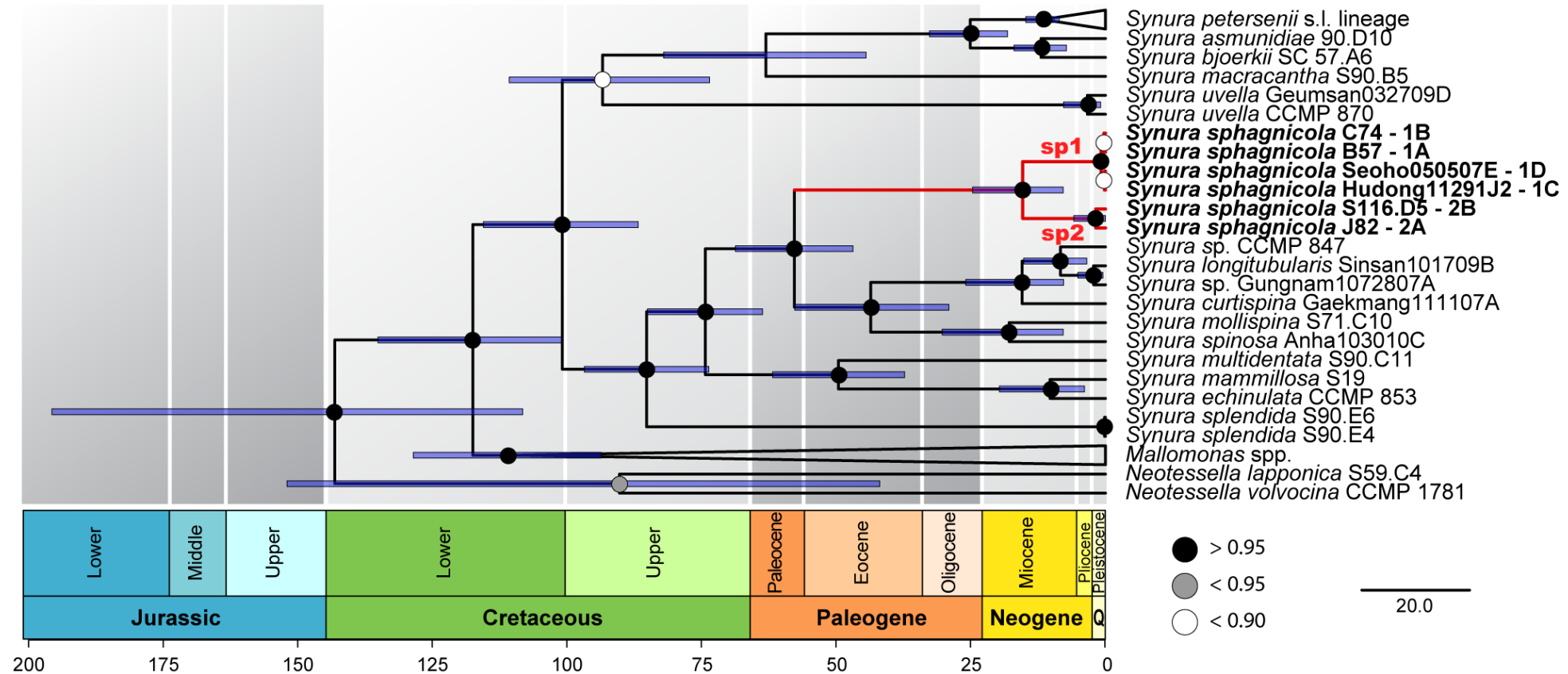
Sampling

- A total of 71 newly established strains
 - ITS, cox, psaA ,SSU, rbcL sequences
 - Geographic coordinates
 - Environmental data (19 bioclimatic variables, 7 habitat variables)
 - Morphological analyses (morphometry of silica scales)



Phylogenetic relationships and timing of divergence

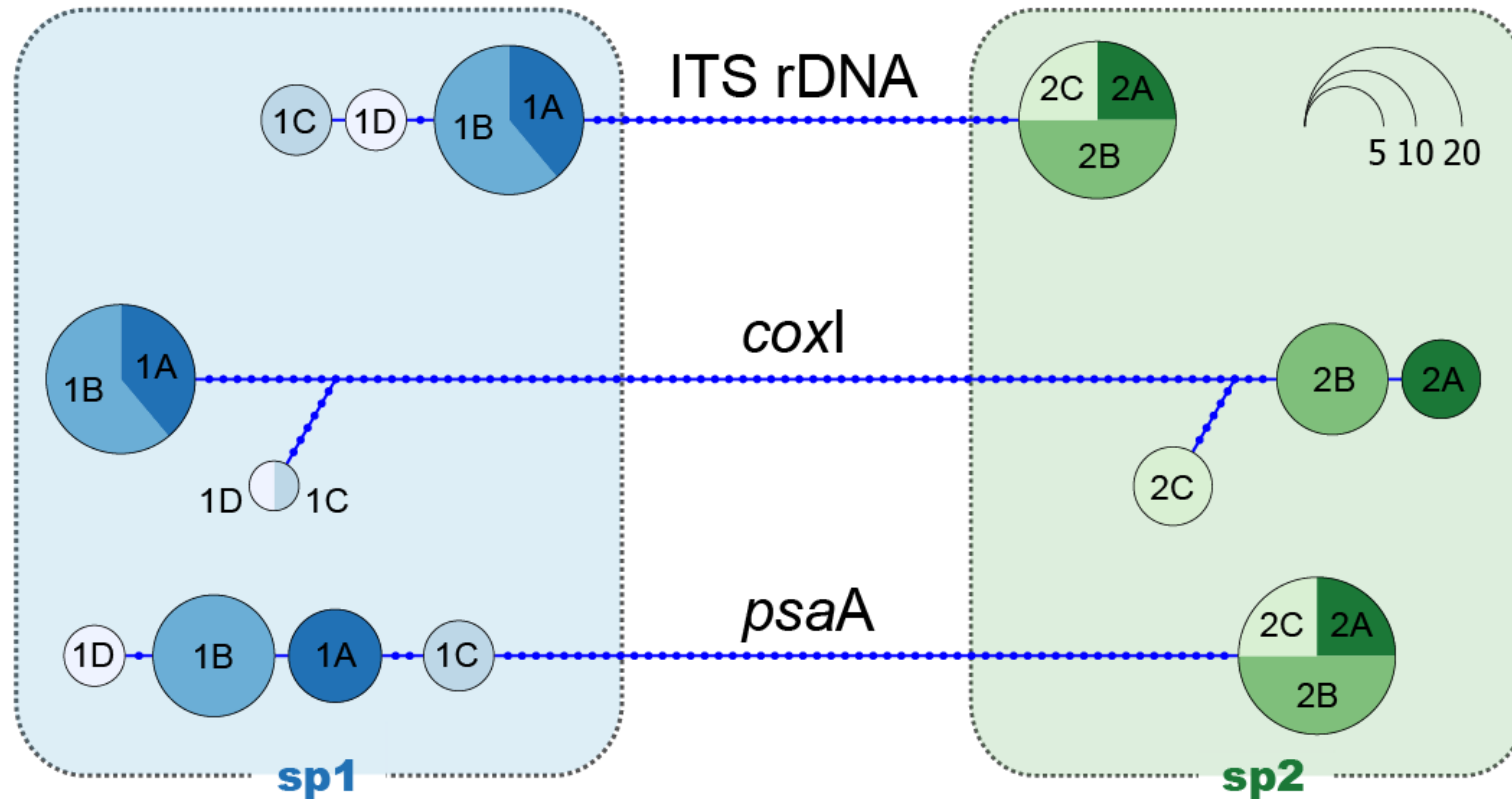
- All strains form a single, monophyletic lineage
- The lineage was split into two distinct clades at about 15 - 18 Mya



Time-calibrated phylogeny for the Synurales based on concatenated *SSU rDNA* and *rbcl* sequences

Phylogenetic relationships and timing of divergence

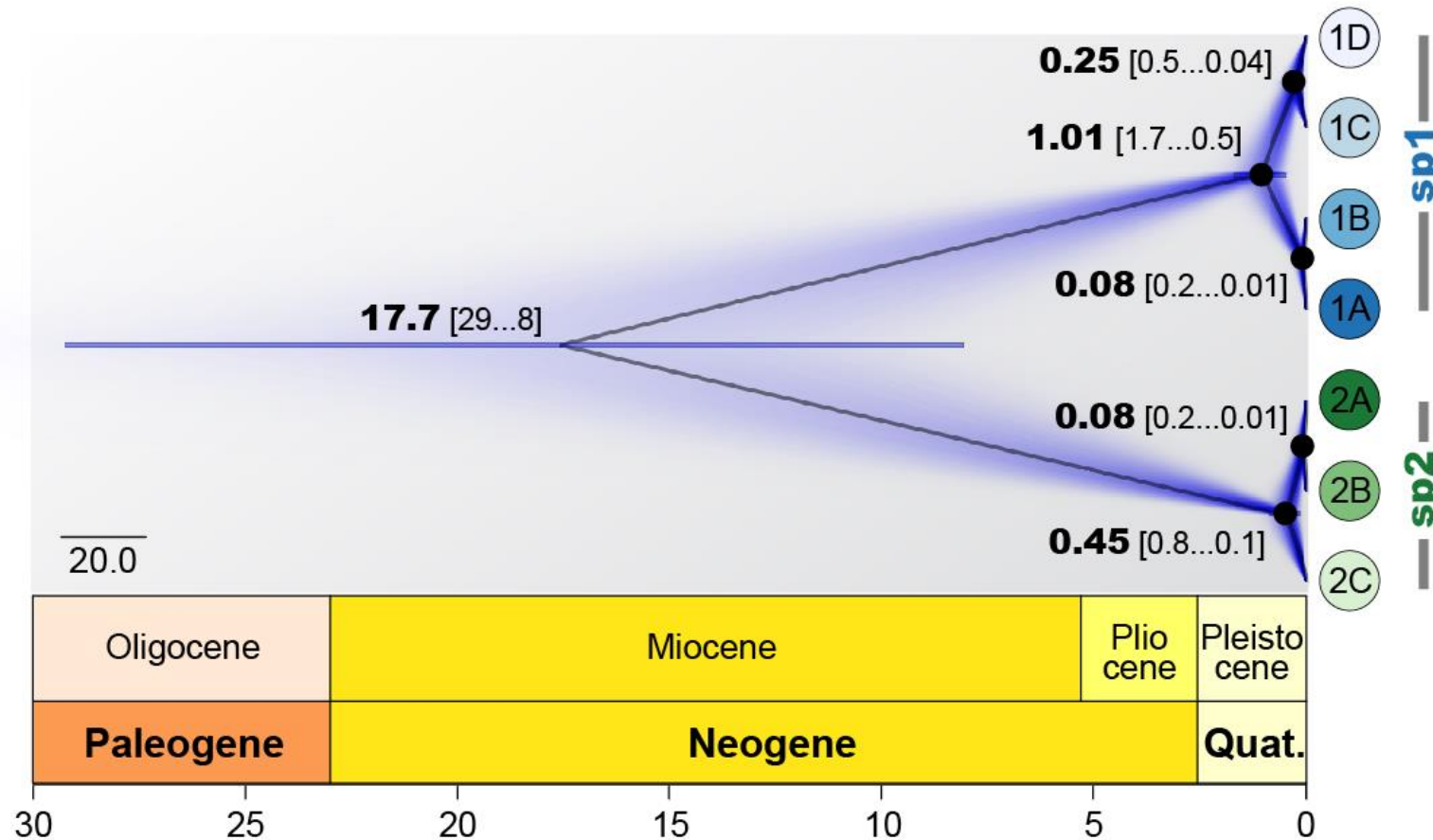
- A fine molecular diversity was revealed within the clades sp1 and sp2
- Seven haplotypes recovered: 1A, 1B, 1C, 1D, 2A, 2B, 2C



Statistical parsimony networks of four sp1 and three sp2 haplotypes based on the analysis of three loci sequences. Circle sizes are proportional to haplotype frequency. Lines between haplotypes are single mutational steps; small dots indicate missing haplotypes.

Phylogenetic relationships and timing of divergence

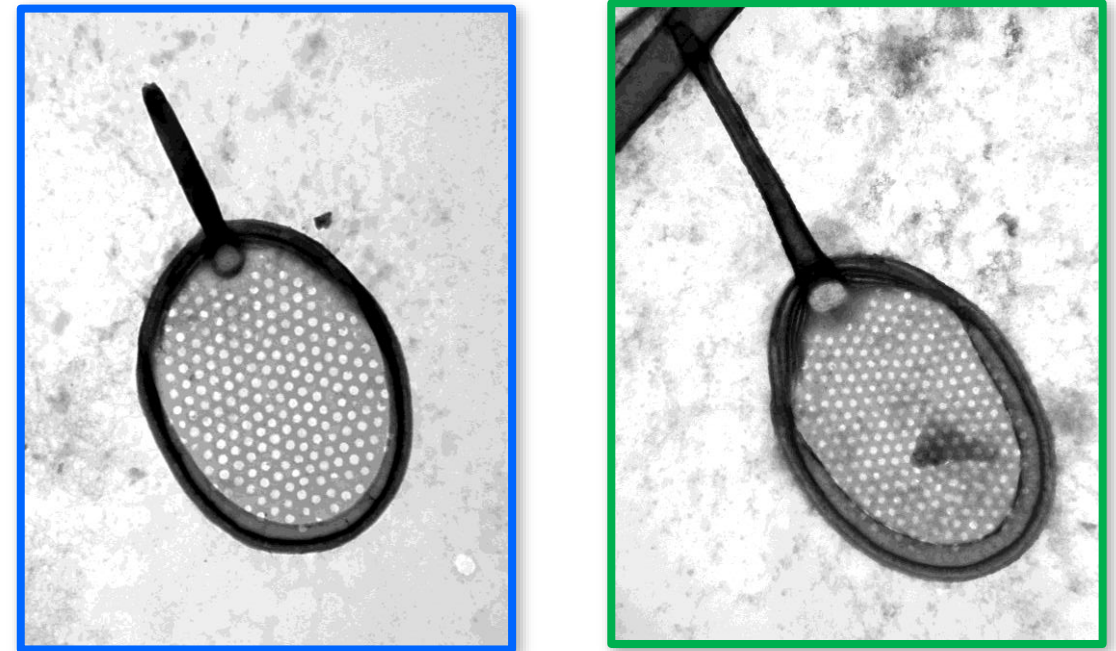
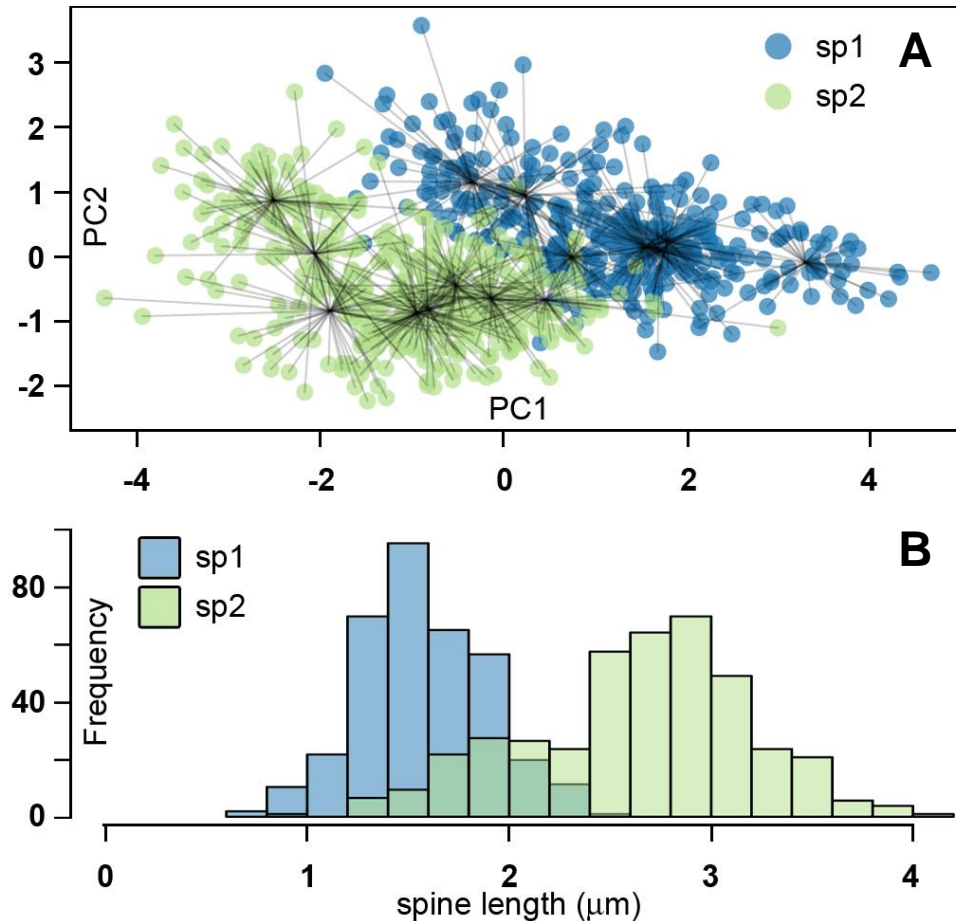
- Recent radiation of haplotypes in the Pleistocene



Time-calibrated coalescence species tree with the visualisation of the most probable topologies.

Morphological analyses

- Sp1 and sp2 clades are morphologically well differentiated, with the spine length selected as the best discriminating character.
- Haplotypes morphologically indistinguishable.



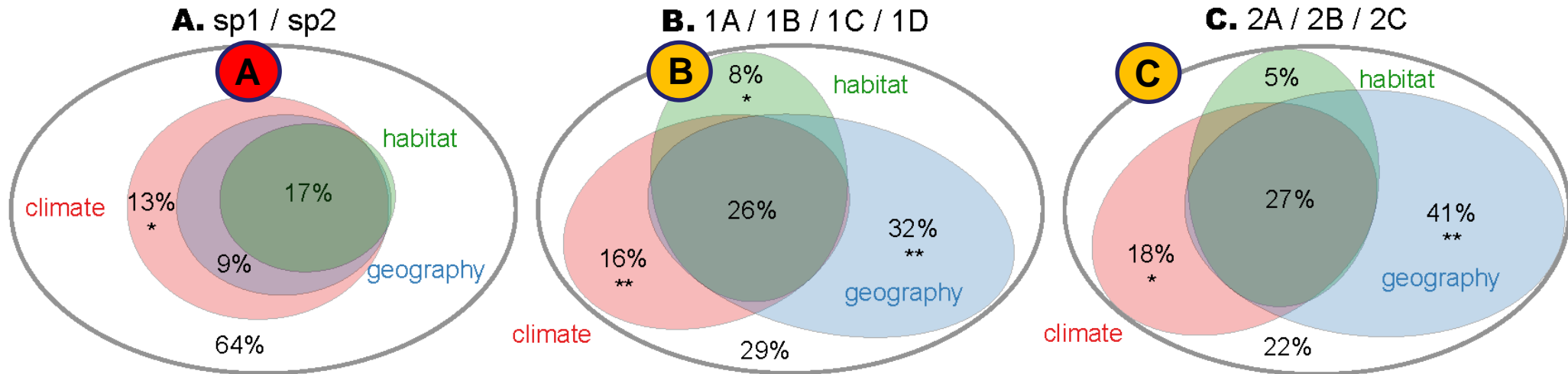
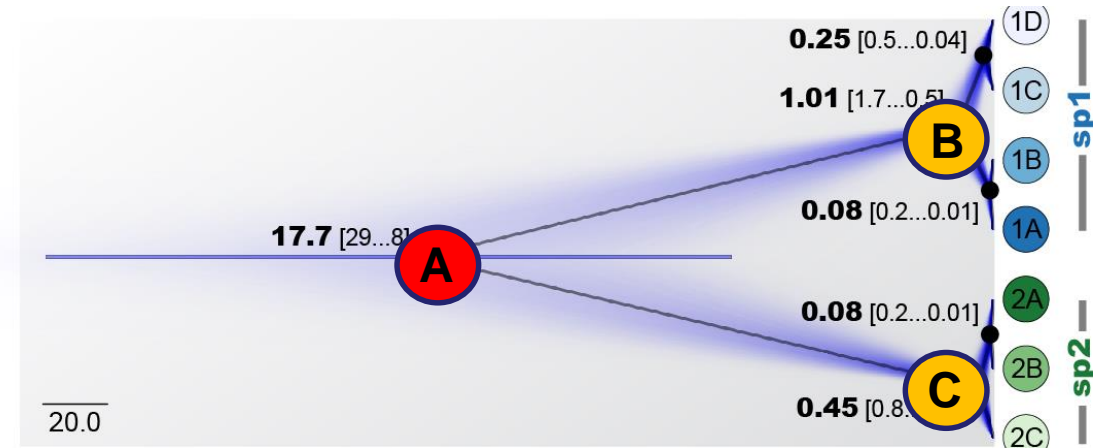
A: PCA ordination of 810 silica scales showing the morphological distinction of the lineages sp1 and sp2. The scales clustered based on their affiliation to 15 investigated strains.

B: The distribution of spine lengths in sp1 and sp2 lineages.

Explaining patterns in molecular diversity

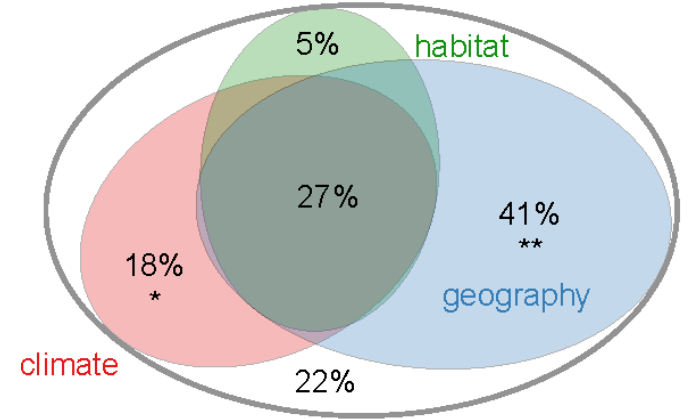
- The haplotypes are ecologically and biogeographically much more differentiated than the old clades, presumably because of their persistent differentiation after the allopatric speciation events.
- The patterns of variation partitioning of sp1 and sp2 lineages are very similar

Venn's diagrams showing the relative effects of climate, habitat and geographical distance on the variance in *Synura* diversity:

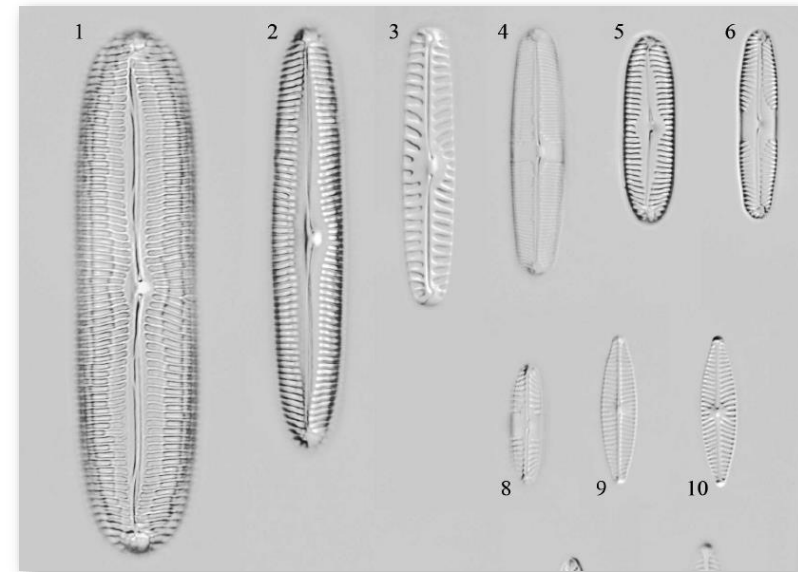
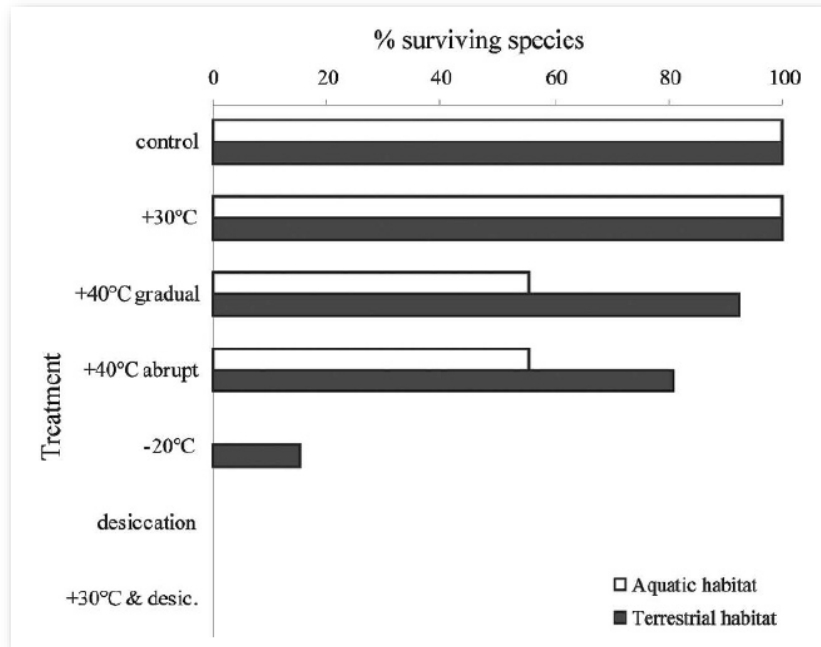


Protist speciation

- Both allopatric and divergent ecological speciation may occur in protists.
- How can populations be resistant to gene flow under the continuous influx of immigrant cells?



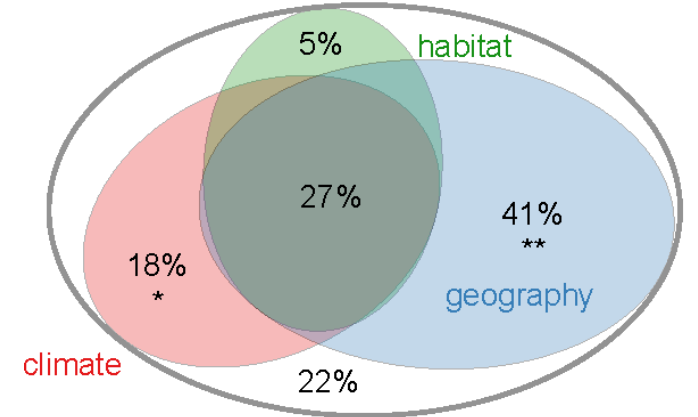
1. Unfavourable conditions during the long-distance transport



Souffreau et al. (2010). *Phycologia* **49**: 309-324

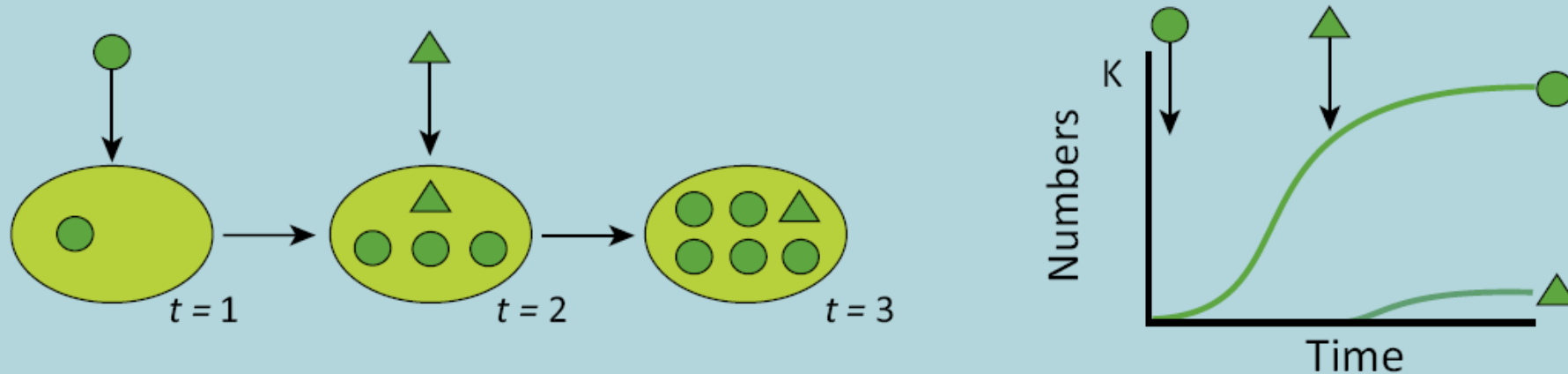
Protist speciation

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2. Priority effect of resident species

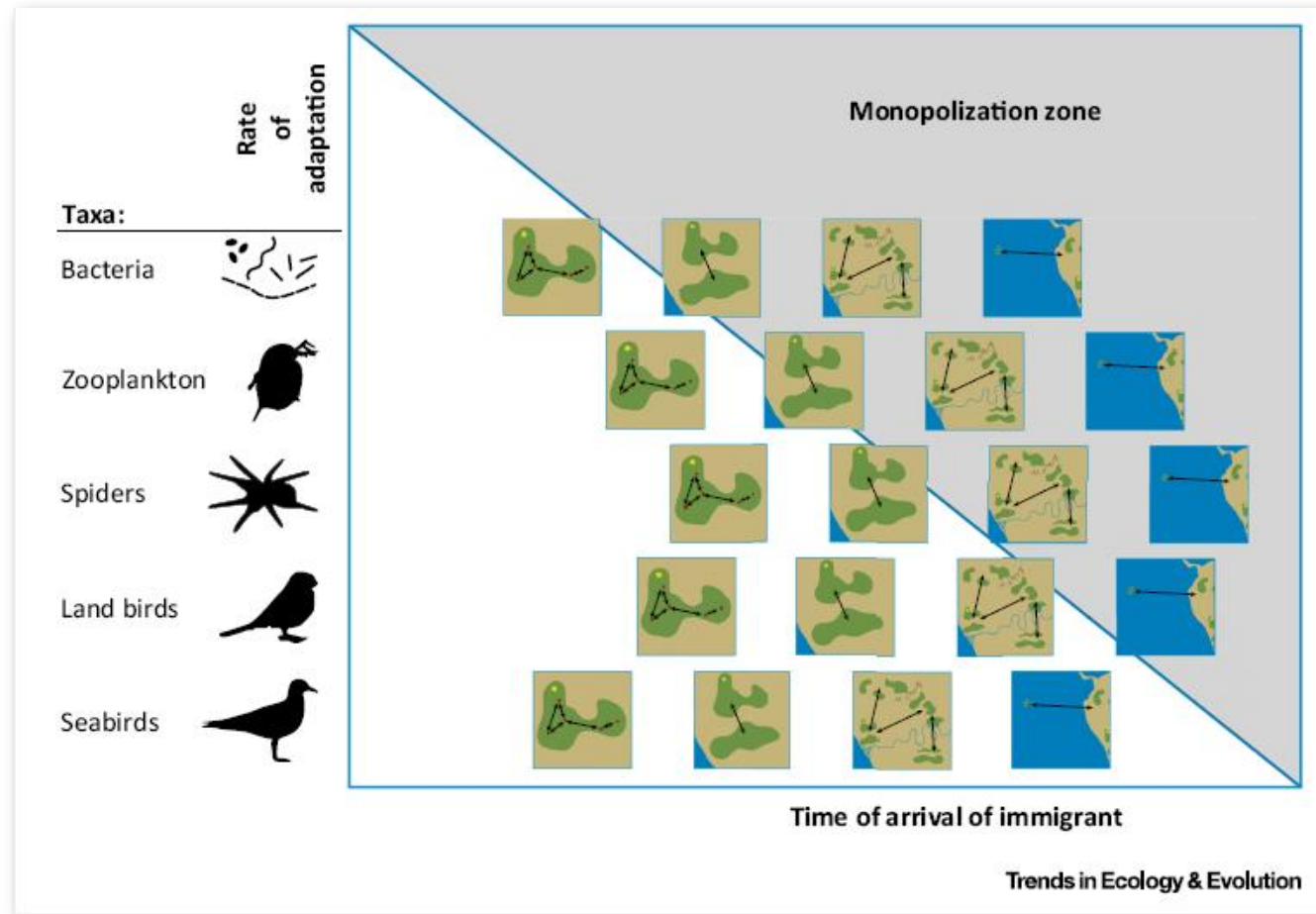
(B) Priority effect – pure numerical effects – fitness equivalence



De Meester et al. (2016). *Trends Ecol Evol* **31**: 136-146

Priority effect

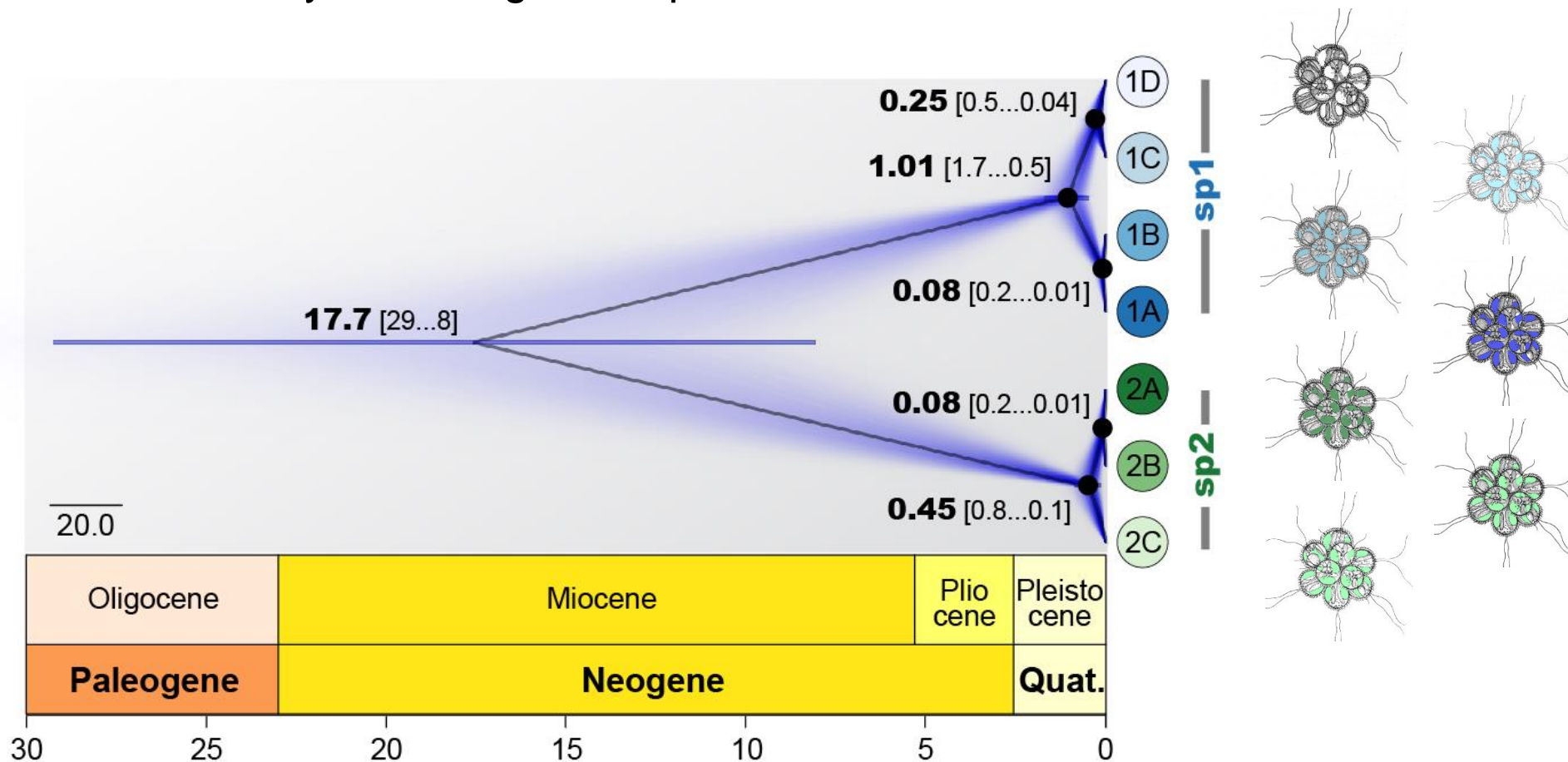
- Priority effect (monopolization of resources) much probable for microorganisms.



De Meester et al. (2016). *Trends Ecol Evol* **31**: 136-146

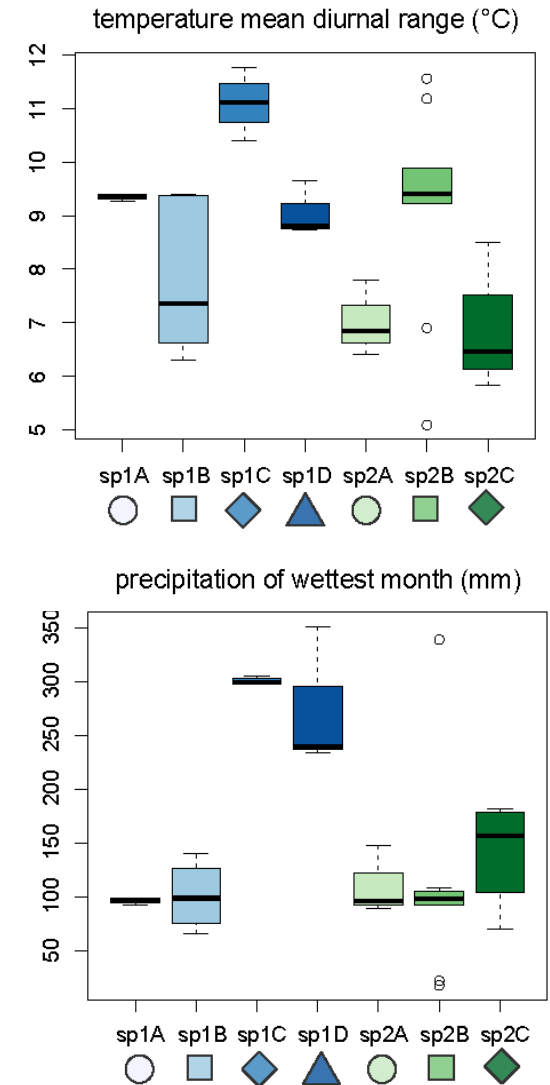
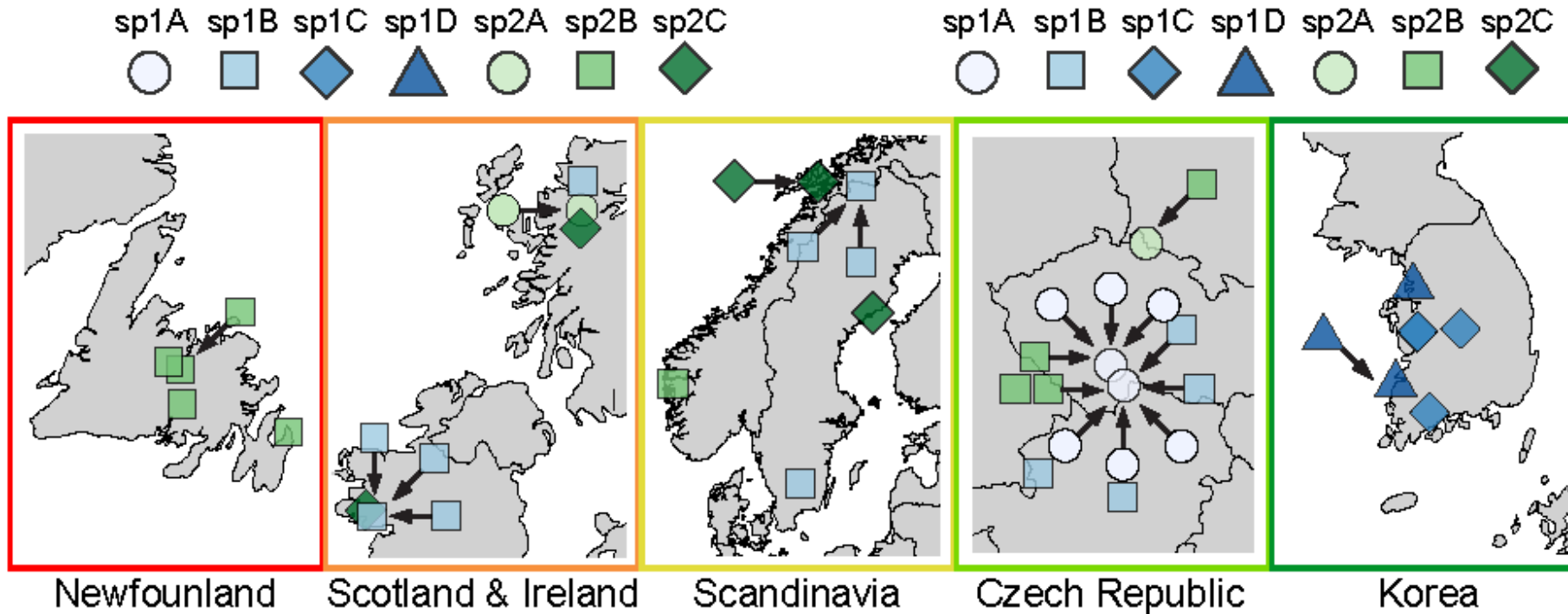
Revisiting species concept of protists

- The haplotypes in fact represent young species diverging in late Pleistocene.
- The scarcity of morphological features in protists lead to ignoring recently diverged lineages and recognizing rather evolutionary old lineages as species units.



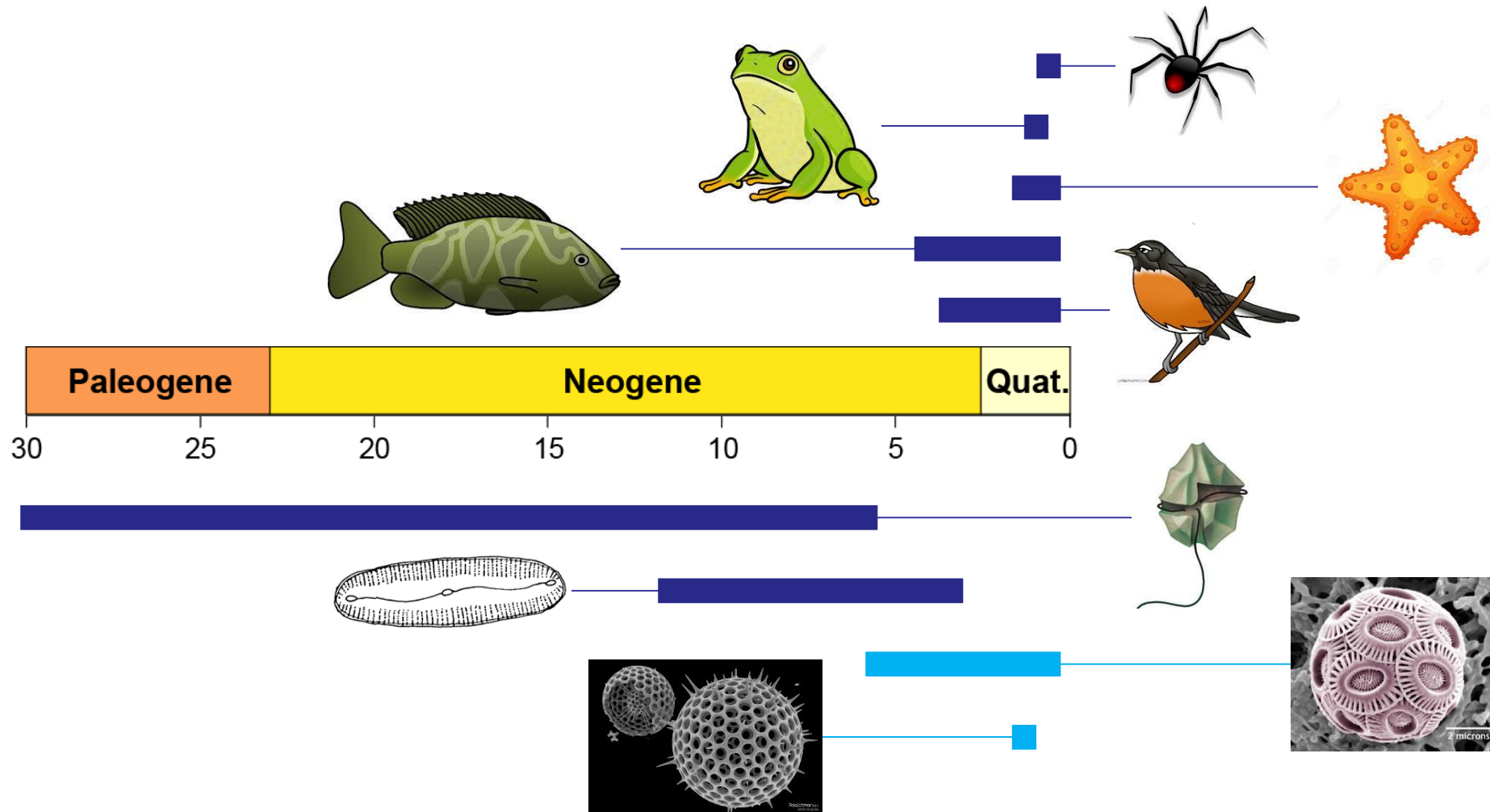
Revisiting species concept of protists

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Revisiting species concept of protists

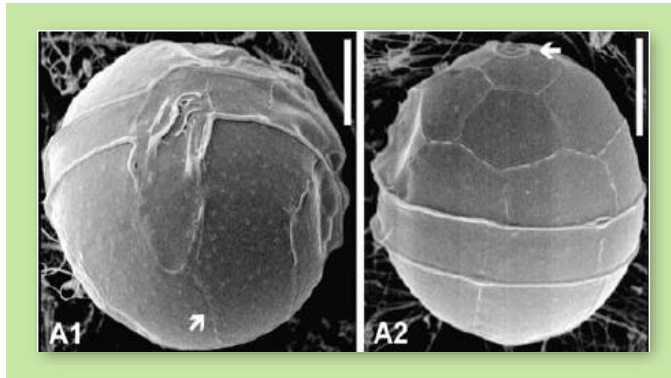
- In contrast to macroorganisms, the estimated divergence times of sister protist species are usually much older.



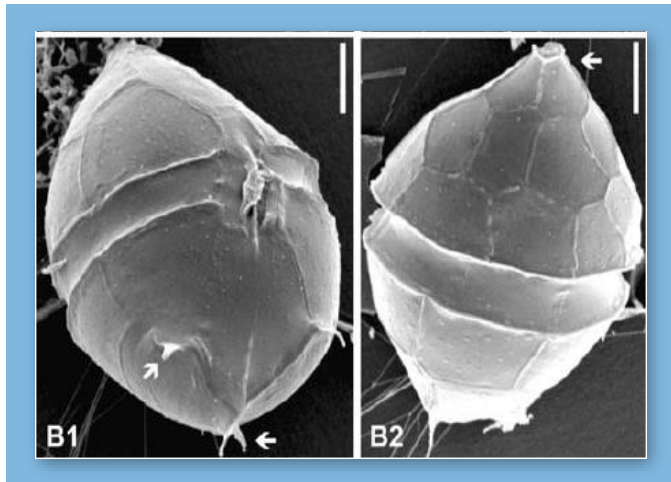
Revisiting species concept of protists

- It is highly probable that numerous protist species originated during the Quaternary

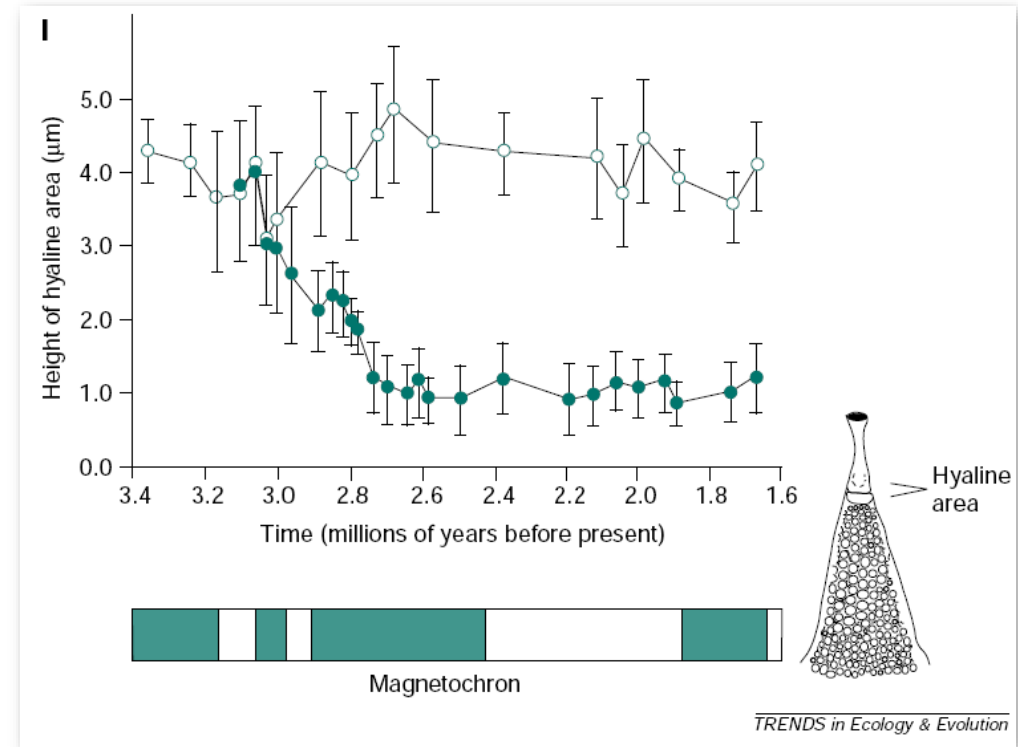
Scrippsiella hangoei - brackish



Peridinium aciculiferum - freshwater



Rhizosolenia praebergonii

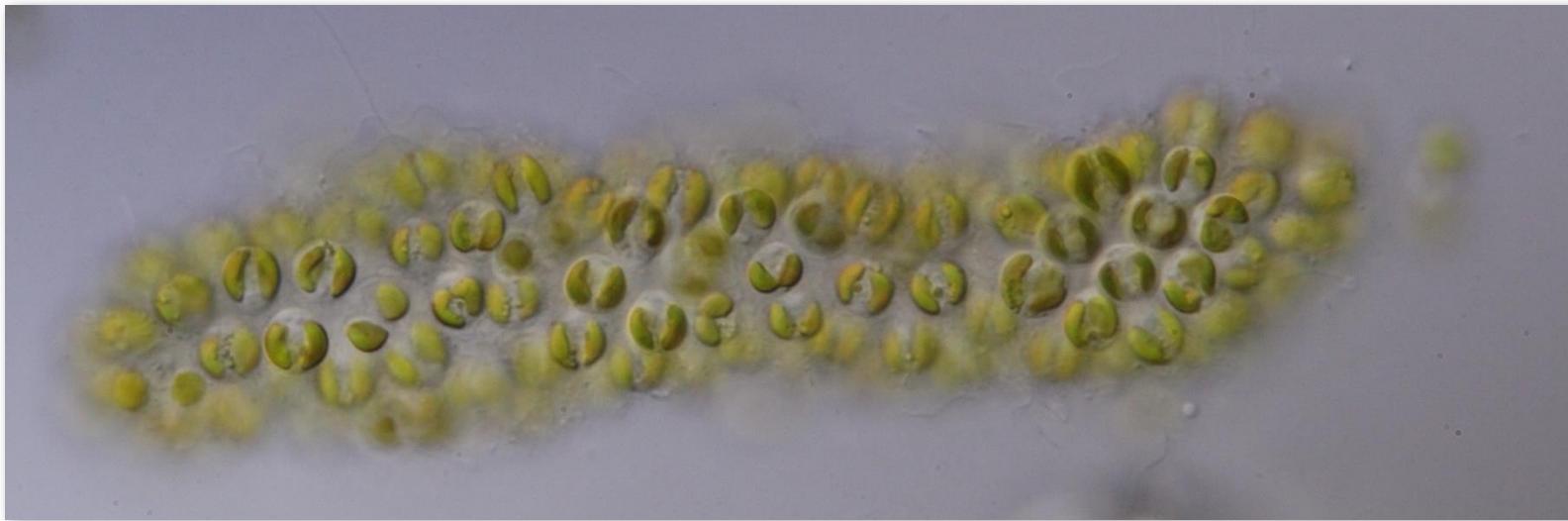


Benton & Pearson (2001). *Trends Ecol. Evol.* **16**: 405-411

Logares et al. (2007). *Microb. Ecol.* **53**: 549-561

Conclusions

- We uncovered rapid species diversification in a freshwater microbial eukaryote that occurred during the late Pleistocene.
- Ecological and allopatric speciation seem to occur much more readily in microbial eukaryotes than was previously thought.
- Speciation times of microorganisms may be in some lineages equivalent to the estimated divergence times of plants and animals.



Future steps

- To recover the population structure and assess the importance of geographical and ecological factors in shaping the populations

The intricacy of population structure estimates – a comparison of organellar haplotype nets of freshwater alga *Synura petersenii* (Chrysophyceae, Stramenopiles)

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¹ Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 01, Prague, Czech Republic
 Email: jadrna@seznam.cz

Background:
 Protists were supposed to be ubiquitous, found everywhere are able to survive. Their presence should be ensured by their small size and large number of individuals who, in addition, produce cysts or spores capable of distribution over long distances.
 Maybe, some protists could be cosmopolitan but show some geographical pattern.
 Do we have enough information to pronounce that there is any population structure in a phototrophic protist – cosmopolitan planktonic alga *Synura petersenii*? Are the organellar markers eligible for this kind of questions?

Aims:
 - to reveal the existence of population structure of an autotrophic protist – freshwater algal species *Synura petersenii* (Chrysophyceae, Stramenopiles)
 - to evaluate the suitability of various organellar markers for estimation of haplotype distribution
 - to compare the plastidial and mitochondrial genetic diversity

Methods:
 Isolation of algal colony from natural samples by 'micro-pipette method' to the unialgal clonal culture
 - *in vitro* cultivation → yielding enough of algae biomass
 - DNA isolation
 - amplification of chosen organellar DNA region with PCR
 - Sanger sequencing

Why to use organellar genetic markers:
 - widely used for biogeography of vascular plants and animals → potential for protists
 - supposed to be non-recombining → easy to track a haplotype over the generations
 - when found relevant and optimal markers → the possibility to do more samples

Haplotype nets based on mitochondrial molecular data:
 Haplotype nets (top picture) show the geographical distribution of each haplotype within Europe. The nets allow to present phylogenetic structure simply – the size of a pie chart mirrors number of samples while the color characterize country of origin.
 We used for each locality ideally three individuals (clonal culture). About 200 cultures (80 localities) were evaluated for two haplotype nets on the right; for the rest of the nets just half of them.

Neighbor-joining net:
 - another way how to compare genetic diversity of samples
 - networks are constructed according to genealogical distances
 - based on mitochondrial SNPs (four molecular markers) for chosen localities show quite clear topology of the net → the importance of geographical

Conclusions:
 - a considerable intraspecific haplotype diversity in both organellar genome was detected
 - the haplotype networks based on mitochondrial and plastidial loci were highly incongruent suggesting distinct evolution history
 - some geographic pattern is visible but more data is needed
 - the identity of chosen marker is crucial for any result and therefore only one marker is not enough to distinguish any population structure

Acknowledgments: The study was supported by the Charles University in Prague, project GA UK [No. 243-251294].

organelar loci
 15PO.1 Iva Jadrná



RAD-Seq
 15PO.3 Petr Dvořák



Population differentiation of the protist *Synura* (Chrysophyceae, Stramenopiles)

Petr Dvořák¹, Zuzana Vaiglová¹, Iva Jadrná², Magda Škaloudová², Martin Puztaří¹, Dora Čertnerová², Helena Bestová², Pavel Škaloud¹
¹Palacky University Olomouc, Faculty of Sciences, Department of Botany, Czech Republic
²Charles University in Prague, Department of Botany, Czech Republic

Introduction
 • Factors driving speciation are still enigmatic, especially among protists.
 • In macrobes, geographical barrier was considered as the most important factor.
 • On the contrary, microbes are often thought to have unlimited dispersal, therefore speciation would take place in sympatry.

Goals
 • To infer population structure of *Synura petersenii* using RADseq
 • To evaluate importance of geographical and ecological factors shaping the population structure using environmental data and gradient experiments with cultured strains

Sample map

Phylogeny IQTREE, 85626 positions

 • Populations cluster by their geographical origin rather than conductivity.
 • Strains from the same regions can prefer different values of conductivity.
 • Conductivity was measured at the time of sampling.

Bioclimatic variables

 • Ancestral state reconstruction of maximal growth rate of cultures in a gradient of temperature.
 • Two groups – first adapted to all temperatures and second adapted to moderate and high.

Structure – population structure

 • Similar pattern revealed for both assumed numbers of populations (K).
 • Strains were mostly separated into two populations.
 • Few strains had mixed origin, which suggests a possible gene flow among them.

Variation partitioning - sampling site data
 • How much of a variability is explained by each factor or together?

 Residual = 0.49 (for Geography and Habitat)
 Residual = 0.45 (for Climate and Conductivity)
 Residual = 0.45 (for Climate and Habitat)
 Residual = 0.45 (for Climate and pH)

Conclusions
 • There are two major groups within *S. petersenii*
 • Group 1 – Czechia and Sweden and group 2 – France, Canada and Norway
 • Both groups contain samples with high and low conductivity. Variation partitioning showed that climate and geography are the most important.
 • Gradient experiments with cultures revealed that group 1 is adapted to all temperatures, while group 2 is adapted and moderate to high temperatures.

Methods
 • Organism – *Synura petersenii* (Chrysophyceae, Stramenopiles)
 • Sampling, environmental condition measurement – conductivity, pH, temperature
 • Culturing in gradient of conductivity and temperature – growth rate.
 • RADseq
 • Phylogeny, Structure and ancestral state reconstruction

Thank you for your attention!



This research was supported by the Czech Science Foundation No. 17-13254S