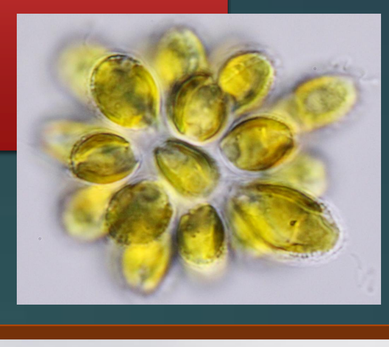
The intricacy of population structure estimates – a comparison of organellar haplotype nets of freshwater alga *Synura petersenii* (Chrysophyceae, Stramenopiles)



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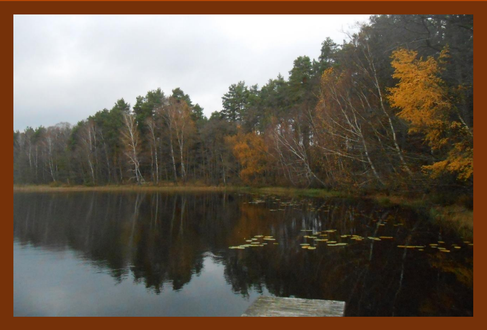
Background:

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 phototrofic protist – cosmopolitan planktonic alga *Synura petersenii*? Are the organellar markers eligible for this kind of questions?

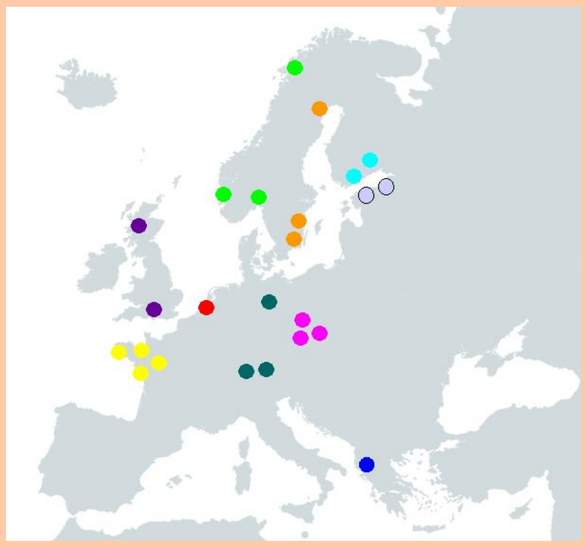


Typical sampling localities – a pond during the autumn (left), a spilling downstream river (right)

Aims:

- to reveal the existence of population structure in 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

Sampling map:



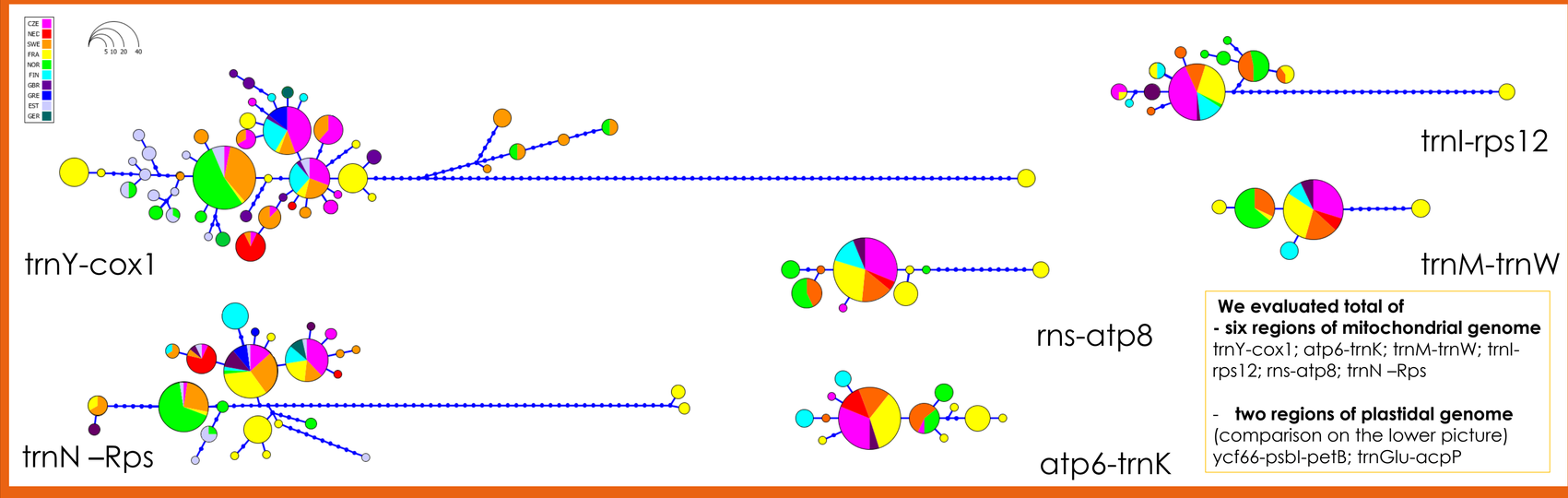
Methods:

- isolation of algal colony from natural samples by ‚micropippete method' to the unialgal clonal culture
- *in vitro* cultivation -> yielding enough of algae biomass
- DNA isolation
- amplification of chosen organellar DNA region by 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

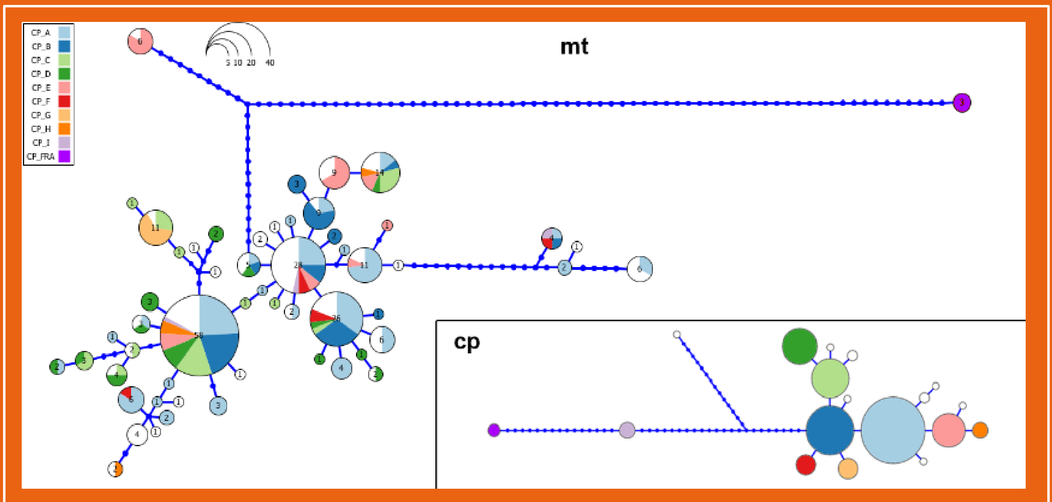
Haplotype nets based on mitochondrial molecular data :



We evaluated total of
- six regions of mitochondrial genome
trnY-cox1; atp6-trnK; trnM-trnW; trnI-rps12; rns-atp8; trnN –Rps

- **two regions of plastidal genome**
(comparison on the lower picture)
ycf66-psbI-petB; trnGlu-acpP

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 generally characterized three individuals (clonal cultures) per each locality. About 200 cultures (50 localities) were evaluated for two haplotype nets on the right, for the rest of the nets just half of them.

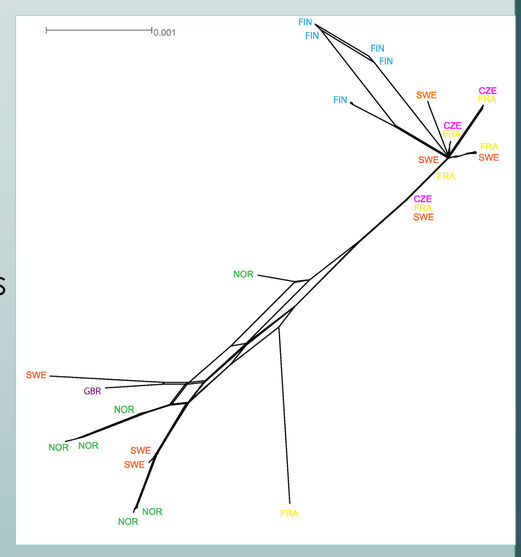


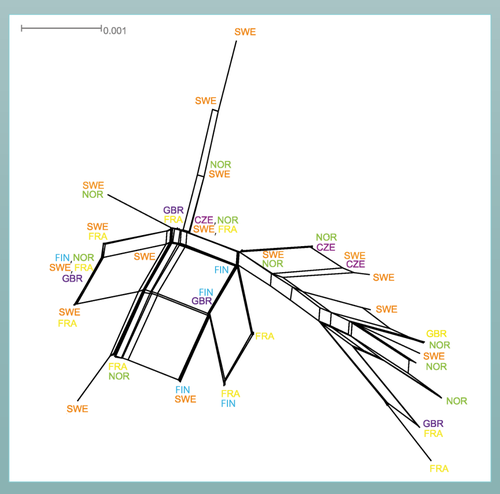
The balancing mitochondrial haplotype net (upper part) is coloured according its related plastid haplotype (lower part).

Neighbor-joining net:

- another way how to compare genetic diversity
- networks are constructed according to genetical distances

- NJ based on mitochondrial SNPs (four molecular markers)
- for chosen localities shows quite clear topology of the net -> the importance of geographical origin





- NJ net based on plastidial SNPs (two genomic markers)

(similar haplotypes of same country are grouped together)

Conclusions:

- a considerable intraspecific haplotype diversity in both organellar genomes was detected
- the haplotype networks based on mitochondrial and plastidial loci were highly incongruent suggesting distinct evolution history of oganelles
- although the individual markers within one organelle exhibit great variability, they are incongruent with each other – possible influence of neutral evolution and incomplete lineage sorting
- the identity of chosen molecular marker is crucial for any result and therefore more markers are necessary for analysis to distinguish the real population structure

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