Diversity and desiccation tolerance of *Zygnema* and *Zygnemopsis* from the Arctic and Antarctica

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Introduction

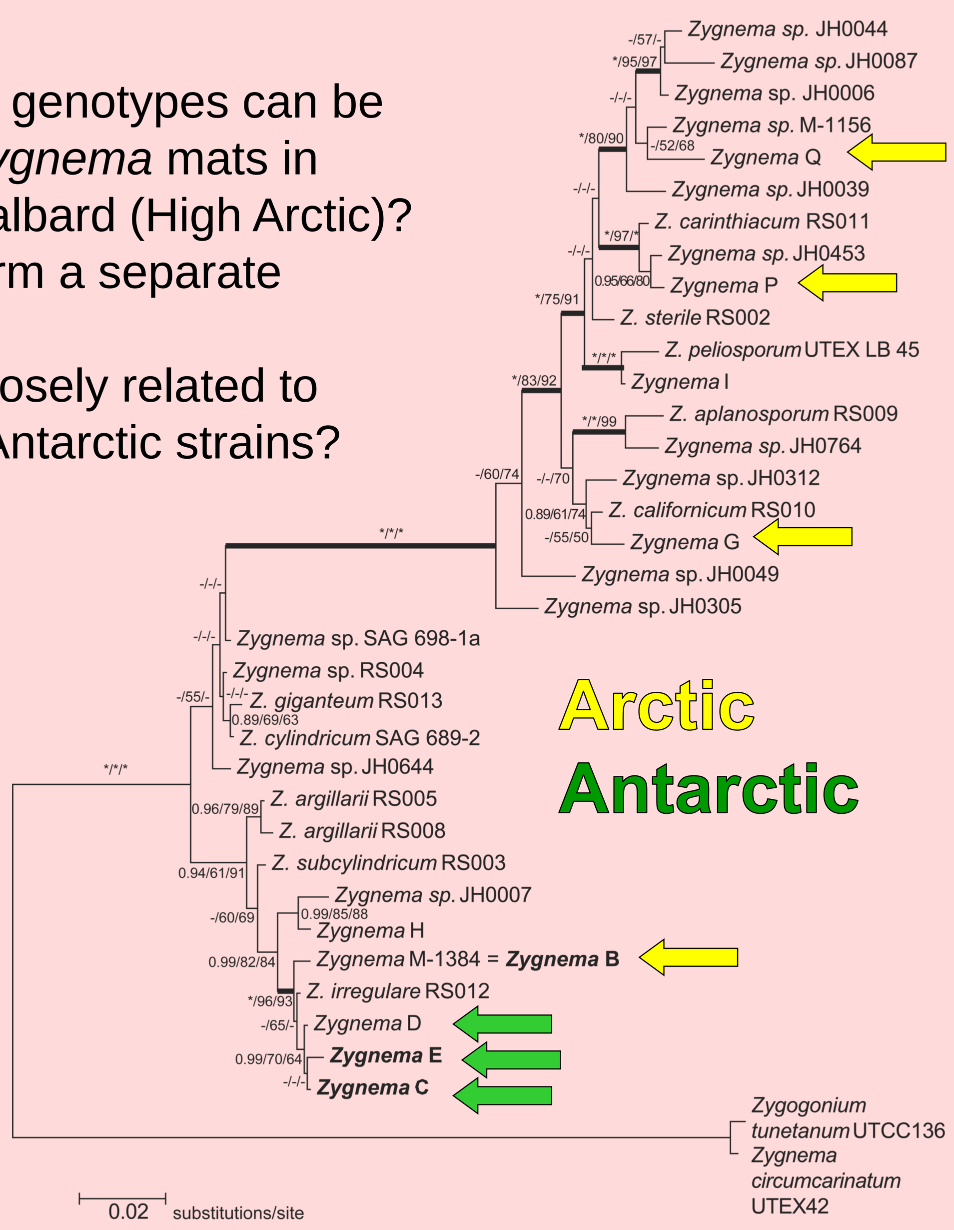
Arctic *Zygnema* mats in various stages of natural desiccation

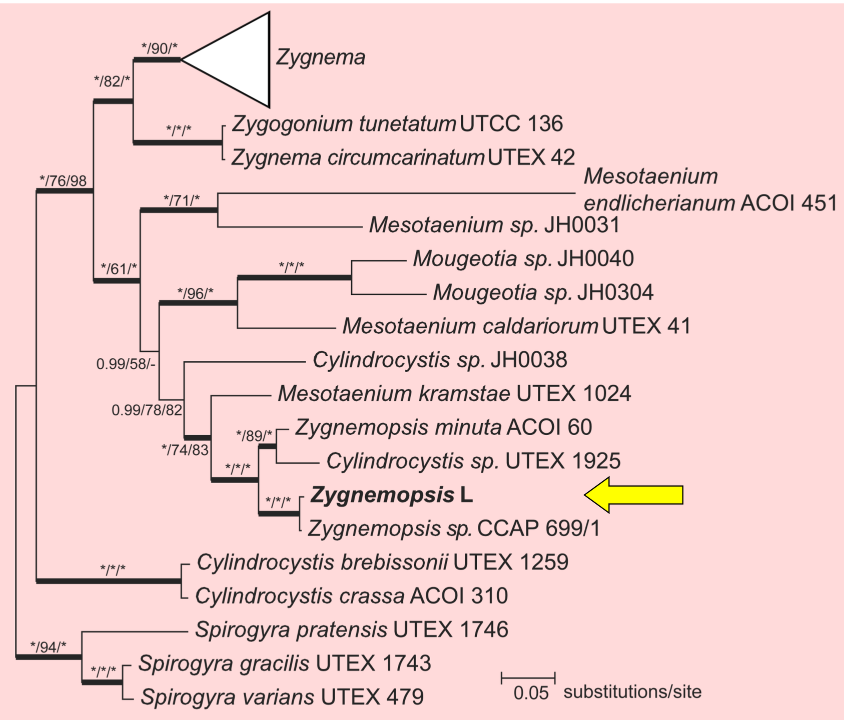
Extensive *Zygnema* mats have often been reported from shallow freshwater localities in both polar regions. In such an extreme environment the algae belong to the main primary producers. Special adaptations are expected that enable them to cope with various environmental stresses, e.g. UV radiation, freeze-thaw cycles and drought. McLean & Pessoney (1971) showed on experimental material from Texas that *Zygnema* survives desiccation by formation of so called akinetes which corresponded to starved, stationary phase cells. However, the akinete formation and desiccation tolerance in *Zygnema* have not been studied in detail with respect to various environmental factors. *Zygnema* also produces another type of specialized, highly resistant cells, the zygospores. Nevertheless, sexual reproduction and zygospore formation have never been observed in the Arctic and Antarctic *Zygnema* strains. This fact complicates also the species determination, because zygospore morphology is one of the basic characters in the traditional taxonomy of the genus (Kadlubowska 1984). First molecular analyses, however, showed that traditional systematics of the genus does not correspond with molecular phylogeny and that the real species diversity is still far from being explored (Stancheva et al. 2012).
Therefore, we aimed at two basic topics: **molecular diversity of *Zygnema* in polar regions** and their **desiccation tolerance**.

Diversity of polar *Zygnema* mats:

Questions:

- How many genotypes can be found in *Zygnema* mats in central Svalbard (High Arctic)?
- Do they form a separate lineage?
- Are they closely related to *Zygnema* Antarctic strains?





Rooted Bayesian trees of a) *Zygnema* strains and b) closely related Zygnematales based on own and GenBank rbcL sequences. Values at the branches indicate MrBayes posterior probabilities, maximum likelihood bootstrap and maximum parsimony bootstrap values. Values of BI PP = 1.00 and ML & MP BS = 100 are marked with asterisk, values of BI PP < 0.8 and ML & MP BS < 50 are marked with dash. Branches with BI PP = 1.00 are thickened; strains used in desiccation experiments are given in bold. Arrows indicate positions of Arctic and Antarctic strains.

Conclusions:

- Based on rbcL sequences, we revealed 4 *Zygnema* genotypes (3 of them new). Surprisingly one strain of *Zygnemopsis* with *Zygnema* morphology occurred within the mats of *Zygnema*.
- The Arctic strains of *Zygnema* fall into different clades within the genus and thus are not monophyletic.
- All Antarctic strains used in this study form a separate lineage. Genetic variability of these strains most likely represent intraspecific variation

Desiccation tolerance of selected strains:

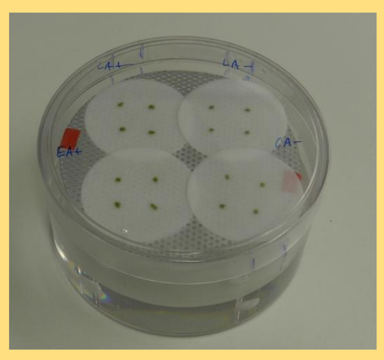
Questions:

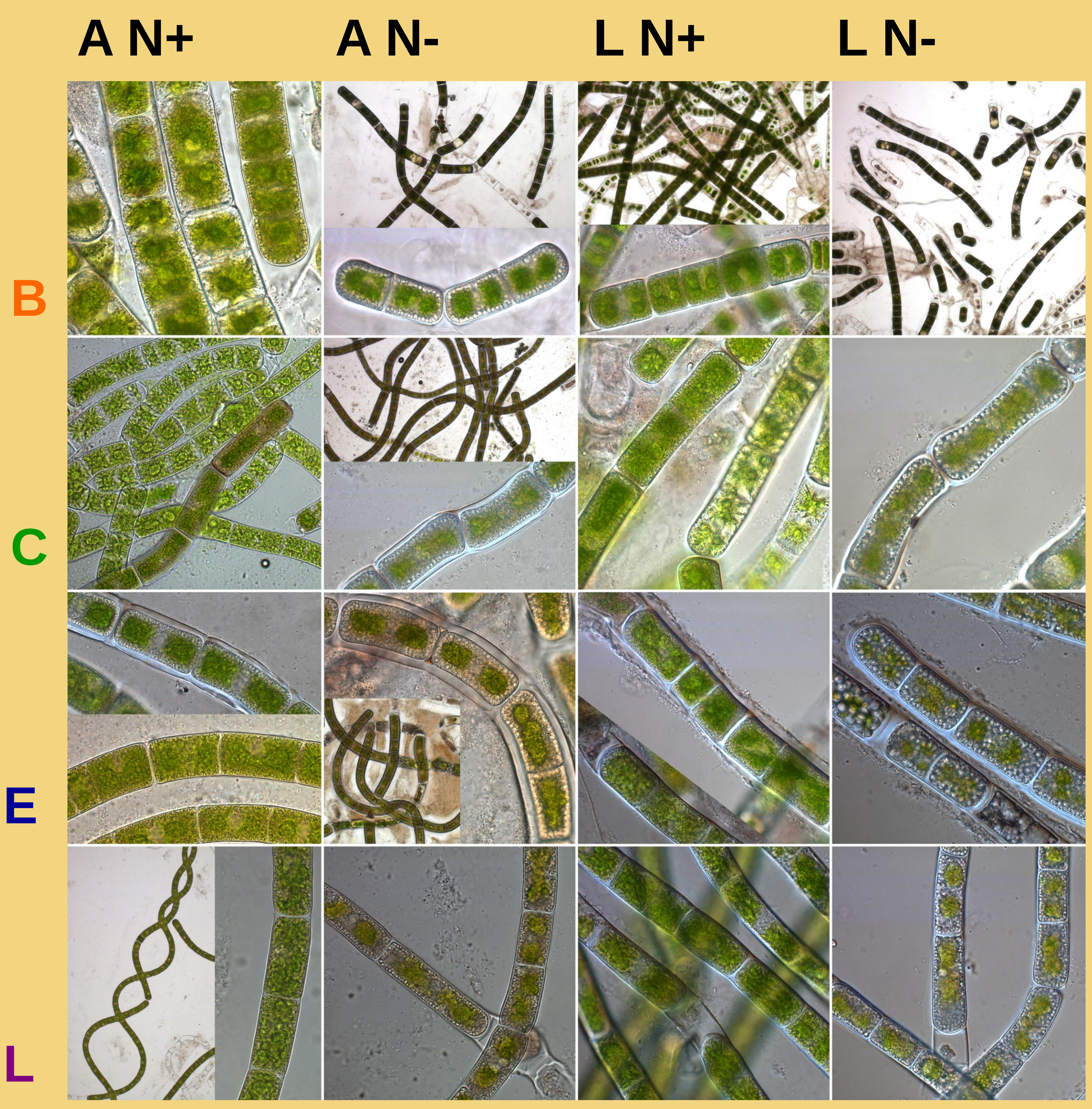
- Is the desiccation tolerance of akinetes induced solely by nitrogen starving or do they need to experience prolonged periods of mild osmotic stress as well?
- Does the speed of desiccation influence survival and recovery rate?
- Can the algae be acclimated by slow desiccation?
- Is there a difference in desiccation tolerance between the selected strains of *Zygnema* and *Zygnemopsis*?

Experimental design:

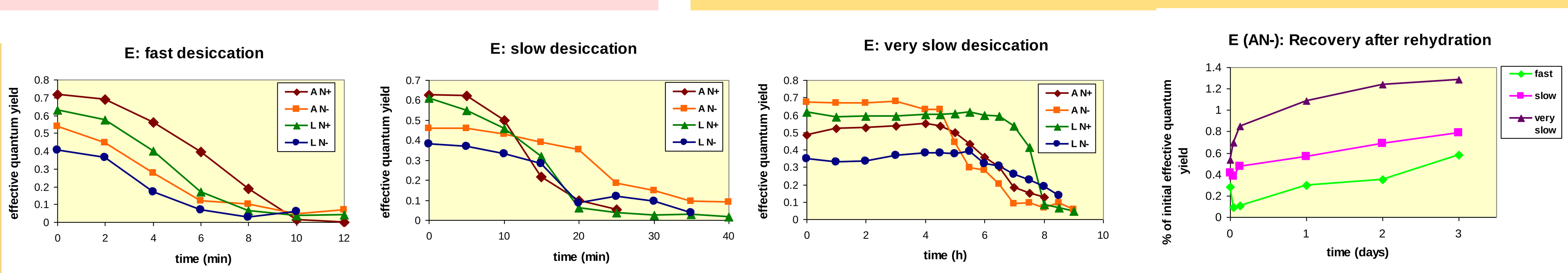
For each strain, we used 4 combinations of culture conditions: **AN+**, **AN-**, **LN+** and **LN-** (**A** = grown on agar, **L** = liquid medium, **N+** = BBM medium with nitrate, **N-** = BBM medium without nitrate) This pre-cultivation lasted 2 months, in order to induce the formation of stationary phase cells.

Small pieces of biomass were desiccated on glass fibre filters in sealed plastic boxes.
We tested 3 desiccation regimes:
FAST: desiccation with silicagel
SLOW: with sat. KCl solution (RH 86%).
VERY SLOW: using KCl solution +10 µl of medium was added to each piece of biomass before drying





All cultures prior to desiccation experiments.



The speed of desiccation depicted as decline of the effective quantum yield of PSII. Differences between various types of cultures and desiccation protocols are shown on the strain E as an example.

A comparison of recovery rate among samples desiccated at different speed.

strain name	culture	desiccation rate fast	slow	very slow	very slow + fast
B	A N+	x	x	✔	✔
	A N-	x	✔	✔	✔
	L N+	x	x	✔	ND
	L N-	x	✔	✔	ND
C	A N+	x	✔	✔	✔
	A N-	✔	✔	✔	✔
	L N+	x	✔	✔	ND
	L N-	x	✔	✔	ND
E	A N+	x	✔	✔	✔
	A N-	✔	✔	✔	✔
	L N+	x	✔	✔	ND
	L N-	x	✔	✔	ND
L	A N+	x	✔	✔	x
	A N-	x	✔	✔	x
	L N+	x	✔	✔	ND
	L N-	x	x	✔	ND

Summary of the results from all desiccation experiments. Treatments with at least one observed surviving cell are marked with a tick.

Conclusions:

- Desiccation tolerant akinetes were formed in all starved cultures. However, even after two months of pre-cultivation a significant proportion of non-tolerant vegetative cells was still present in N+ cultures
- The slower is the desiccation, the higher number of cells survives and the faster is the recovery of physiological activity.
- Only strains C and E pre-cultivated on agar without nitrate were able to survive fast desiccation
- After acclimation during very slow desiccation, also the other cultures of *Zygnema* were able to survive fast desiccation. *Zygnemopsis* was not capable of such acclimation and can be regarded as less stress tolerant than *Zygnema.*

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