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Freezing tolerance of freshwater diatoms as a key to their success in polar regions

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Introduction

Diatoms are microscopic algae characterised by a golden-brown colour and ornamented silica shells. They thrive in various environments worldwide ranging from aquatic marine and freshwater to terrestrial. Together with cyanobacteria and green algae, they are important primary producers in both polar regions (Arctic and Antarctica). The polar environment is characterised by extreme natural conditions, which microalgae have to overcome, such as low and freezing temperatures, desiccation, long periods of dark and irregular nutrient and liquid water availability. In general, microorganisms survive unfavourable period using dormancy and produce stress resistant stages. Yet, freshwater pennate diatoms are not known to form morphologically distinct forms. Despite this fact, they thrive in many polar habitats very well.

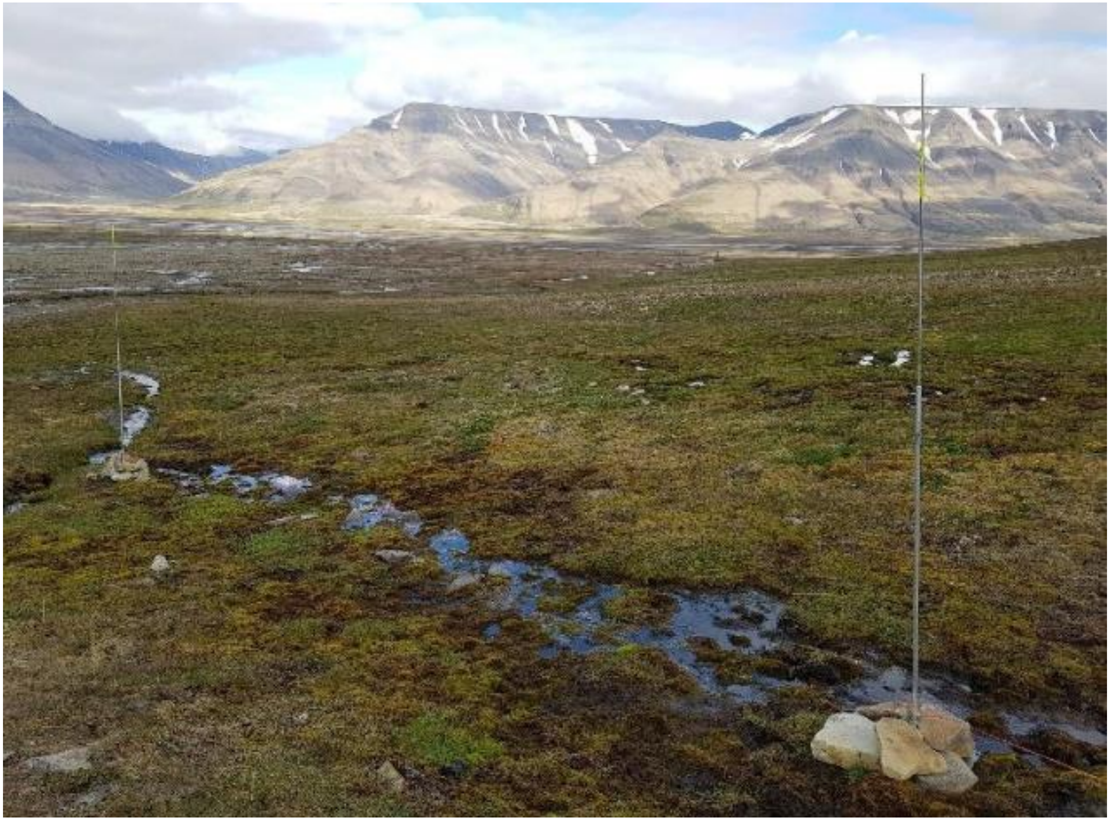

Figure 1. One of the original localities included in the field study.

Research objectives

The aim was to achieve a deeper insight into the survival strategy of freshwater pennate diatoms in the harsh conditions of the polar environments. One part is dedicated to testing of diatom tolerance limits to freezing under laboratory conditions and focused on evaluation of the survival of polar and temperate strains using different freezing regimes, induction of stress resistant dormant stages by simulation of changes in their natural environment by temperature, light and nutrient availability manipulation, comparison of tolerance between laboratory induced dormant stages and vegetative cells and introduction of a multiparameter fluorescent staining protocol for diatom viability evaluation, which enables a more precise assessment of physiological activity on a single cell level. The other part dedicated to diatom communities in their natural environment in Svalbard (High Arctic) studied the seasonal development of natural diatom populations with a focus on their morphology and physiological activity using the multiparameter fluorescent staining protocol and tested the potential effect of repeated freeze- thaw cycles, depth of ice and snow cover on freezing survival.

Summary

The thesis provided new insights into freshwater pennate diatoms freezing tolerance and their survival strategy in relation to polar environments. The data of diatom freezing survival were acquired by experimental testing conducted under laboratory conditions and by a field study performed in their natural habitats in Svalbard (Fig. 1). Furthermore, the stress factors related to polar habitats, microalgal adaptation mechanisms, their life strategies and other issues were summarized in a book chapter.

Temperate diatoms previously appeared to be sensitive to several stresses such as desiccation, heat and freezing. Regarding freezing stress, the only surviving strains were those belonging to terrestrial genera. Our study comparing −20 °C freezing survival between temperate and polar diatom strains found a slightly higher tolerance in the polar diatoms (Fig. 2). Tolerance was revealed not only in strains originating from terrestrial habitats, but even among freshwater species. All the diatoms tested (Fig. 4) appeared to be capable of surviving mild −4 °C freezing without any harm. In contrast, survival to extreme freezing (−40 °C and liquid nitrogen) was shown only in strains belonging to the *Pinnularia borealis* species complex, which is generally regarded as terrestrial, despite the fact that some of them originated from freshwater habitats and both temperate and polar environments. Moreover, the results of laboratory studies found that freezing conditions in natural habitats are not necessarily that severe, which is supported by temperature data taken during the field study.

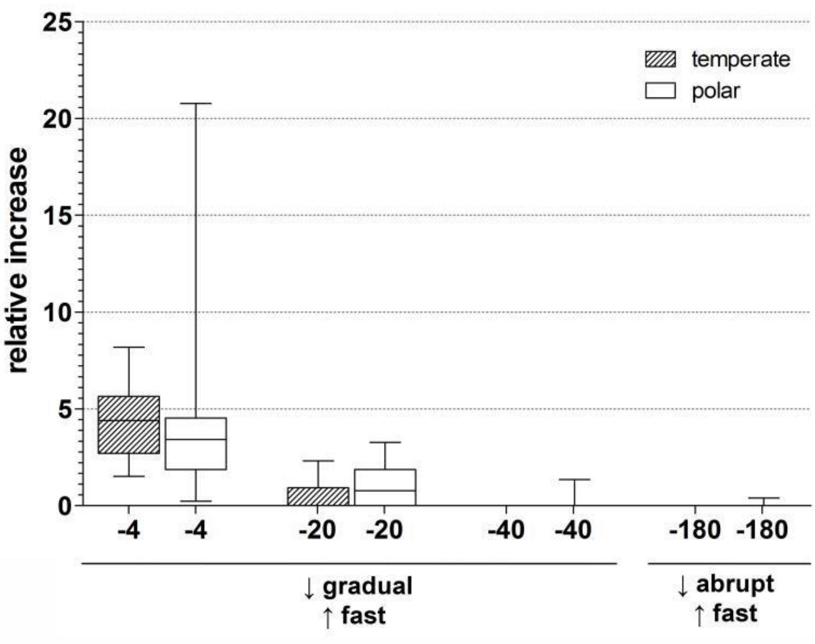

Figure 2. Box plots of relative increase of chlorophyll fluorescence six days after freezing treatments for freshwater strains from temperate (shaded bars) and polar (clear bars) habitats (line: median, box: first and third quartiles, whiskers: Min to Max). Freezing (↓), thawing (↑).

Development of vegetative-looking resting cells in pennate diatoms was shown under laboratory conditions when incubated at cool temperatures, under light and nutrient limitations. Such resting cells appeared to positively influence the survival of polar diatoms after exposure to freezing in comparison to resting cells induced by just dark and cool, or with a concurrent phosphorus limitation. Furthermore, the importance for survival was proven for polar and temperate diatoms under mild below-zero temperatures. No morphologically distinct diatom resting stages were observed. However, even vegetative cells of diatoms tolerated various freezing stresses relatively well. Vegetative cells of the polar strains were remarkably viable in contrast to formerly tested temperate diatoms.

In addition, it was shown that experimental conditions, namely rates of thawing and freezing, significantly influenced the final survival, while the thawing rate appeared to be of approximately the same or even greater importance as freezing.

The physiological activity detected using multiparameter fluorescence staining allowed for distinguishing the physiological status of diatoms at the single-cell level. It was revealed that, immediately after thawing, only a minority of the cells of surviving diatoms were metabolically active in both the laboratory experiments and the field study. The cells were mostly found as inactive and intact (presumably dormant cells), injured and inactive, or dead.

The fluorescent staining methodology applied on freshwater communities five times throughout the one-year period following key events for algal survival (summer vegetative season, autumn dry-freezing, winter frozen state, spring thawing, and summer again) provided the first detailed evidence of diatom annual development in the extreme conditions of the High Arctic. Understandably, the physiological activity of natural diatom communities reflects the period of the year (Fig. 3). The highest level of active cells appeared in their vegetative season in summer while winter freezing significantly increased the population mortality. A remarkable portion of inactive dormant cells was examined during periods of autumn dry-freezing and after spring thawing. A surprising number of non-active dormant cells appeared after thawing of winter samples as well. However, the thicker the ice layer was, the more dead cells were present. The assumption of the harmful effect of autumn freeze-thaw cycles on spring survival was not confirmed. To our knowledge, this is the first field study focused on freshwater diatom survival in their natural environment of the polar regions.

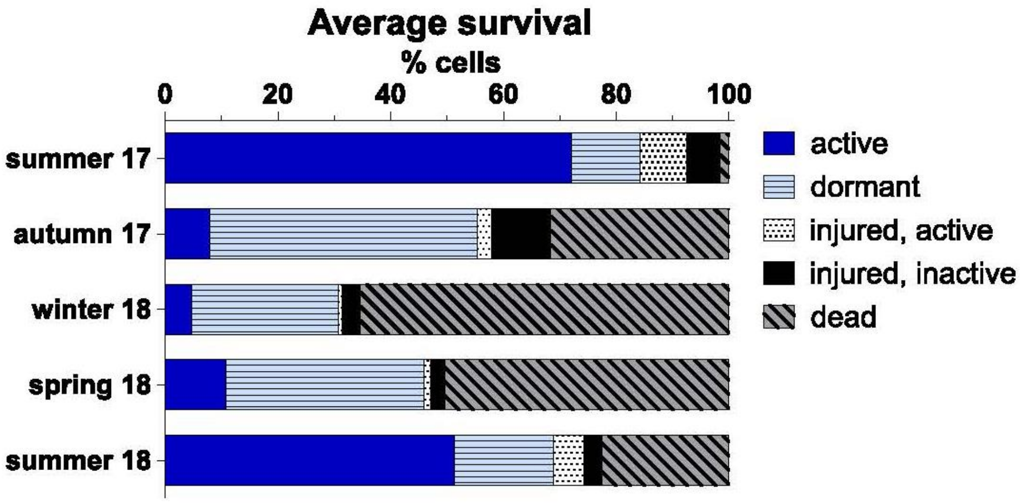

Fig. 3. Seasonal development of diatom viabilities for each site (average percentage per site).

Conclusion

Diatoms originating from freshwater polar environments are sensitive to freezing stress as both vegetative and vegetative-looking resting cells. Their success in polar environments seems to be associated with the ability to withstand unfavourable conditions as small numbers of vegetative cells or adapted vegetative-looking resting cells. These overcome the winter periods and provide an inoculum for establishment of new communities each vegetative season. Future studies focused on diatom survival, their ecophysiology and molecular mechanisms, could help to reveal other possible roles in ecosystems and to better understand the polar environment. Many aspects of life in such extreme environments still await further investigation.

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List of publications:

Hejduková E, Elster J, Nedbalová L (2020). Annual cycle of freshwater diatoms in the High Arctic revealed by multiparameter fluorescent staining. *Microb Ecol* 80:559–572. https://doi.org/10.1007/s00248-020-01521-w

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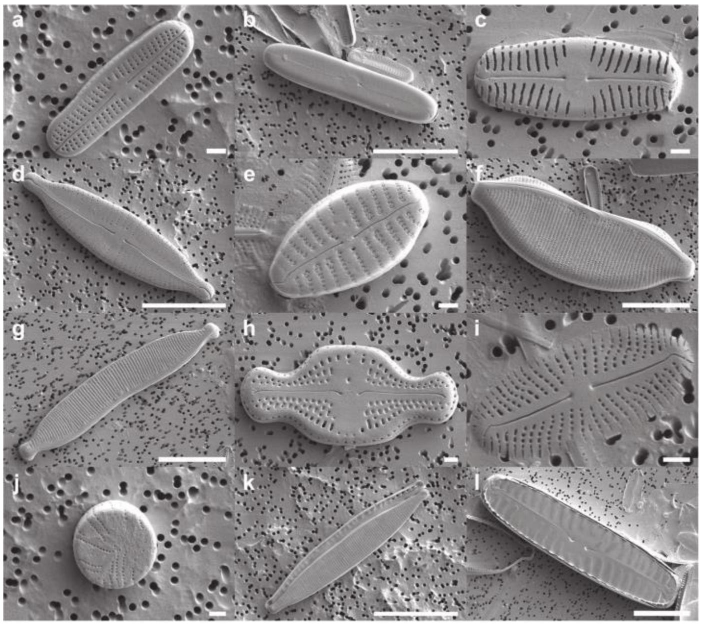

Figure 4. Scanning electron microscopy pictures of the experimental strains: a *Achnanthidium lineare*, b *Caloneis falcifera*, c *Chamaepinnularia krookiformis*, d *Encyonopsis descripta*, e *Gomphonema* sp., f *Hantzschia abundans*, g *Hantzschia amphioxys*, h *Luticola muticopsis*, i *Mayamaea atomus*, j *Meridion circulare*, k *Nitzschia palea*, l *Pinnularia catenaborealis*, scale bar a, c, e, h, i, j = 1 µm, b, d, f, g, k, l = 10 µm

Systematics of coccal green algae of the classes Chlorophyceae and Trebouxiophyceae

Lenka Štenclová

Annotation

The aim of the review part of the thesis is to summarize the current situation of the systematics of green coccal algae. Those were traditionally assembled in only one order: Chlorococcales and their distribution into the lower taxonomical unites (suborders, families, subfamilies, genera) was based on the classic morphological criteria as a shape of cells and a character of colonies. Introduction of molecular methods caused radical changes in our insight to the system of green (not only coccal) algae and green coccal algae were redistributed in two of newly described classes: Chlorophyceae and Trebouxiophyceae. Representatives of individual morphologically delimited families, subfamilies and even genera or species were commonly split in several lineages, often in both of mentioned classes.

For the practical part, two problematic groups of green coccal algae were chosen. Family Oocystaceae and family Scenedesmaceae – specifically its subfamily Crucigenioideae were revised using polyphasic approach. Based on molecular data, relevance of some old traditional morphological traits was revaluated and replaced by newly defined significant characteristics.

Introduction

Systematics of green algae had been for a long time based only on morphological observations. All coccal green algae were assumed to belong together, and their distribution in the lower taxonomic unites was made according to morphological characteristics as colony/coenobia formation and a shape of the cell. Molecular analyses performed in last decades revealed that this arrangement does not reflect the actual phylogeny of green algae. Coccal forms often clustered among filamented algae and coccal algae with the similar cell shape or the common colonial organization proved not to be related to each other.

The main variability of coccal green algae is nowadays concentrated in the chlorophytean classes Chlorophyceae and Trebouxiophyceae. Their further categorization is based on the molecular phylogeny and not uncommonly is restricted. Particular taxa are commonly being labelled as *uncertae sedis* or classified only in clades. Modern systematics must resist multiple problems. Particularly challenging is species delimitation because united species concept does not exist. Also, numerous uncertainties persist in classification on the higher levels, due to unresolved phylogeny as a result of missing molecular data of many described taxa, or insufficient phylogenetic signal in commonly used molecular markers.

The substantial part of the system had already been revised, nevertheless, some partial questions remained unanswered. One of the basic current topics are abovementioned species concept of green coccal algae and associated cryptic variability. Since the monophyletic concept of higher taxonomic units had been mostly accepted, the classification got complicated because of ubiquitous paraphyly and multiple isolated taxa on the basis of phylogenetic trees, moreover, often possessing long branches. The paraphyly, together with a polyphyletic origin of many morphological characteristics previously applied as the systematic criteria resulted in reassessment of many traditional categories. Modern genera, families and orders are rather smaller and more specifically defined. Discovered multiple conversions of filamented and coccal forms in both two directions were commonly discussed. The more detailed microscopy helped to find novel, more useful morphological characteristics, often ultrastructural, which helped to make sense the newly determined phylogeny.

Aims of the thesis

The main aims of the thesis are to resolve chosen parts of the system of green coccal algae. For molecular analyses, the poorly examined family Oocystaceae and family Scenedesmaceae subfamily Crucigenioideae were chosen. Both groups contained colonial green coccal algae and were traditionally defined on the basis of strong morphological characteristics as their unique colonial organization or specific cell shape.

The family Oocystaceae is defined on the basis of the oval to elliptical or lemon like cell shape and propagation by autospores retained in the mother cell wall for longer time, making sometimes composed multigenerational colonies. Typical is its ultrastructure of the multi- layered cell wall with the network of cellulose fibril.

The crucial morphological characteristic of Scenedesmaceae Crucigenioideae is its formation of the typical coenobium, which is described as the four-celled colony of cells of the same generation. Crucigenioid coenobia are flat and cells in tetrads are arranged in cross shape. Composed multigenerational coenobia are often developed as well.

Results

Crucial results of the thesis are adjusted parts of the system of green algae accompanied by submitted and proposed taxonomical changes in each of four included papers. Executed phylogenetic analyses revealed some additional insights to the mentioned recent problems in systematics of green (coccal) algae.

Paper 1: **Molecular and morphological delimitation and generic classification of the family Oocystaceae** (Trebouxiophyceae, Chlorophyta) (Chapter 2).

On the morphological data established family Oocystaceae (Fig. 1) was confirmed by molecular phylogeny. Nevertheless, changes occurred in delimitation and definition of the family. Definition is rather strict in the cell shape but wider in the cell organisation, including pseudo- filamentous forms. Molecular phylogeny combined with newly evaluated morphology supported concept of three subfamilies Eremosphaeroideae, Oocystoideae and Makinelloideae *subf. nova*. However, Eremosphaeroideae is paraphyletic or polyphyletic and long branches together with poor sampling complicates its phylogeny. Distinct subfamily Makinelloideae is represented by coenobial and pseudo-filamentous clade. Oocystoideae consisted of former Oocystoideae and Lagerheimioideae, divided in five morphologically and phylogenetically well-defined clusters accompanied by algae of genera *Oocystis* and *Tetrachlorella* (*Oocystis sensu lato* group) with unresolved relations. Taxonomic changes in several genera including genus *Oocystis* were executed, other proposed or expected to be done in future.

Paper 2: **Revised phylogenetic position of genus *Nephrocytium* Nägeli (Sphaeropleales, Chlorophyceae), with description of Nephrocytiaceae *fam. nov*. and *Nephrocytium vieirae sp. nov.*** (Chapter 3)

Genus *Nephrocytium* Oocystaceae shares with the family Oocystaceae similar colonial morphology, propagation by autospores, nevertheless, differs in the shape of the cell, which is crescent or lunar and especially in the ultrastructure of the cell wall. Recently, traditional position of *Nephrocytium* in Oocystaceae was questioned by molecular phylogeny. Previous studies did not resolve the exact place and *Nephrocytium* was designed as *incertae sedis* inside the order Sphaeropleales. Phylogenetic analyses support position of the genus *Nephrocytium* distinct from other described families which led to the definition of new family Nephrocytiaceae *fam. nov.* Unique combination of morphological and ultrastructural characteristics of *Nephrocytium* supports the novel position. Phylogeny and morphology of studied strains in comparison of the morphology of previously described *Nephrocytium* species resulted in description *Nephrocytium vieirae spec. nova.*

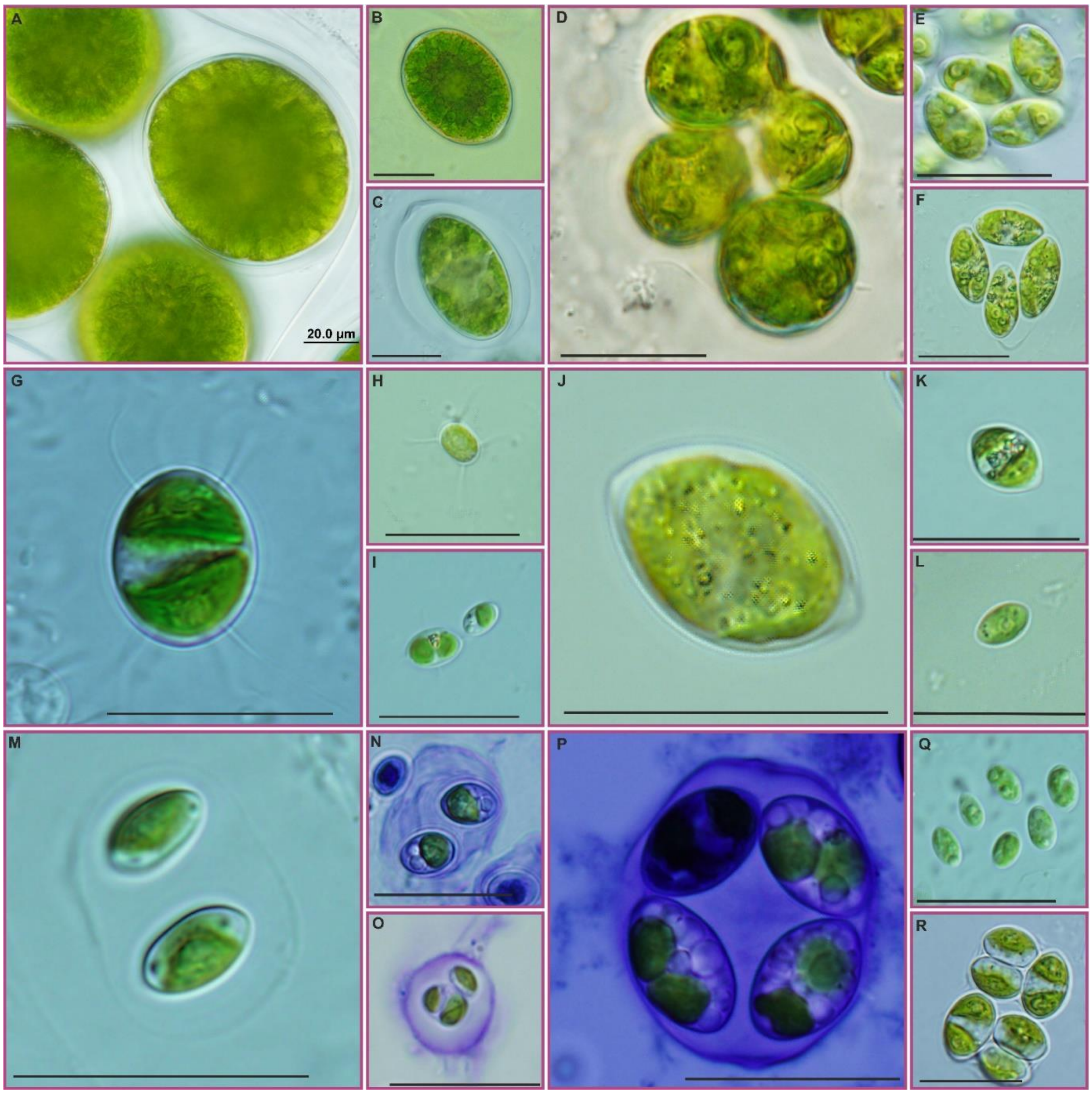

Figure 1: Morphological traits characteristic for each subfamily and morphological clade.
A-C: Eremosphaeroideae – large cells with numerous chloroplasts.
D-F: Makinoelloideae – coenobia.
G-I: Oocystoideae – spines.
J-L: Oocystoideae – granules.
M-O: Oocystoideae – mucilage covers (stained with methylene blue).
P-R: Oocystoideae

Paper 3: **Distribution of the Crucigenioid algae inside the classes Chlorophyceae and Trebouxiophyceae.** (Chapter 4)

Subfamily Crucigenioideae of family Scenedesmaceae is typical by cross-shaped coenobial morphology and its genera were expected to belong together. In recent molecular studies, were crucigenioid taxa redistributed into multiple lineages inside classes Chlorophyceae and Trebouxiophyceae. In present study, two crucigenioid algae *Crucigenia lauterbornii* and *Komárekia rotundata* clustered together inside the family Chlorellaceae (Trebouxiophyceae) and the genus *Komárekia* was restored in its original sense, including three species: *Komarekia appendiculata*, *Komárekia lauterbornii* and *Komárekia rotundata*. Rest of analysed strains represented traditional genera *Crucigenia* (*C. mucronata* and the type species of *Crucigenia*: *C. quadrata*) and *Crucigeniella* (*C. apiculata* and *C. saguei*) and clustered together distinctly in the family Scenedesmaceae (Chlorophyceae). *Crucigeniella apiculata* and *Crucigeniella saguei* were recently combined in the genus *Willea*, nevertheless, our analyses both species clustered distant from other *Willea* taxa, in close relations with the genus *Crucigenia*. Therefore, species were combined to the genus *Crucigenia* as *Crucigenia apiculata* and *Crucigenia saguei comb. nov.*

Paper 4: ***Dispora speciosa*, a new addition to the genus *Parallela* and the first coccoid member of the family Microsporaceae.** (Chapter 5)

Colonial coccal green algal species *Dispora speciosa* (Fig. 2) has traditionally been classified into the Radiococcaceae family due its mucilaginous envelopes or to relatives of the Scenedesmaceae family (subfamily Crucigenioideae) due to the formation of four-cell coenobia reminiscent the genus *Willea*. The phylogenetic analysis of four molecular markers determined its position in the Microsporaceae family, so far consisted of only multicellular representatives. A detailed morphological and ultrastructural study showed congruent features with the genus *Parallela,* and the algae was moved to the genus as *Parallela speciosa*. Probable position of other *Dispora* species according to its morphology is discussed.

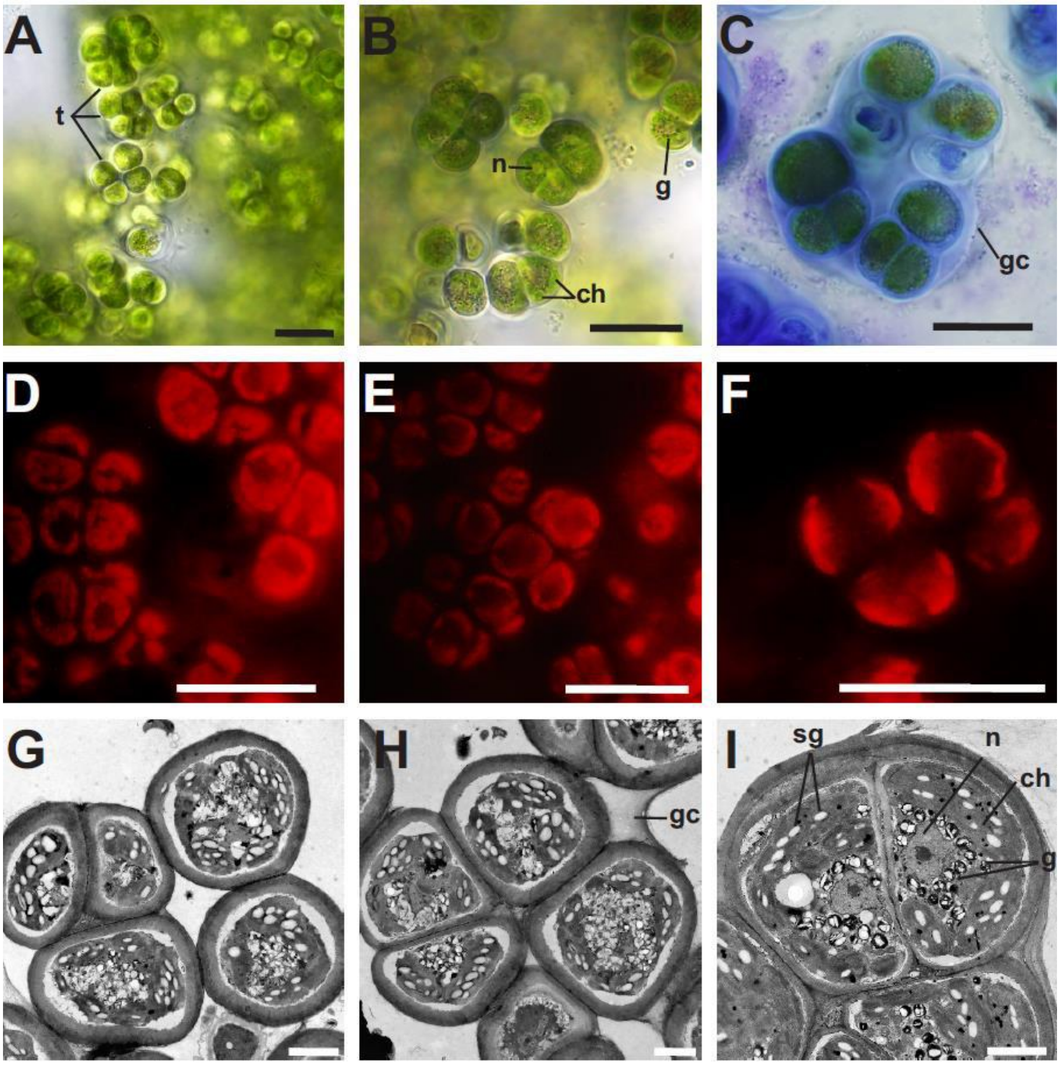

Figure 2. Gross morphology of *Dispora speciosa* strain ACOI 1508. Microscopical observation were carried out using: light microscopy (A–C), fluorescence microscopy: observing autofluorescence of chlorophyll (D–F) and transmission electron microscopy (G–I). A: arrangement of the cells into tetrads in the culture, B: distribution of individual organelles inside cells, C: gelatinous cover around the cell aggregation highlighted by methylene blue, D–F: shape of autofluorescent chloroplasts inside cells, G: dividing cells in a tetrade, H: tetrade conjoined to others by the mucilage cover, I: detailed content of the cell. Description: ch=chloroplast, g=granules, gc=gelatinous cover, n=nucleus, sg=starch grain, t=tetrads of cells. The scale bars indicate 20µm (A–F) or 2 µm (G–I).

Conclusion

Though the big boom of the modern systematics of green algae already passed, there still exist groups with unresolved relations and commonly unsuspected phylogeny. The system is incomplete, concerning the delimitation of classes, orders and families. For many described species and genera is missing relevant material or molecular data. In present study species delimitation (Paper 2) and generic classification (Paper 1, 3) tending to split of traditional wide genera into smaller ones was discussed, new higher taxonomical unites were erected (Paper 1, 2) and old on morphology delimited ones were shown as polyphyletic (Paper 1, 3). Taxonomical relevance of traditional morphological traits was re-evaluated (Papers 1-4), including the colonial characteristics (Papers 1-4) and conversions of filamentous or other multicellular and coccal types (Paper 1, 4). New morphological and ultrastructural criteria were found.

New findings concerning the family Oocystaceae and scenedesmaceaen subfamily Crucigenioideae have been submitted, nevertheless, thesis has also brough some partial results and indicates direction of future prospects. Problematic of delimitation and species concept of the genus *Oocystis*, phylogenetic position of *Juraniella javorkae* and new insides in the phylogeny of the genus *Kirchneriella* and characteristic of the chloroplast and mitochondrial genomes of a microscopic alga *Oonephris obesa* (Chlorophyceae) have been started and are still being examined with promising preliminary results.

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List of publication:

Štenclová, L., Fučíková, K., Kaštovský, J. and Pažoutová, M. (2017) Molecular and morphological delimitation and generic classification of the family Oocystaceae (Trebouxiophyceae, Chlorophyta). Journal of phycology. 53(6):1263-1282. DOI: 10.1111/jpy.12581

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Štenclová L. Distribution of the Crucigenioid algae inside the classes Chlorophyceae and Trebouxiophyceae. Unpublished

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Ohlédnutí za akcemi

Setkání biologů podniků Povodí

Ve dnech 28. – 30. 11. 2022 se uskutečnilo tradiční setkání biologů laboratoří podniků Povodí. Tato setkání jsou pořádána od roku 2010 a pořadatelství se střídá mezi jednotlivými laboratořemi. Letošní setkání bylo jedenácté v pořadí, jelikož v letech 2020 a 2021 je nebylo možné uskutečnit. Tentokrát se pořadatelství ujalo Oddělení hydrobiologie a mikrobiologie Povodí Labe, a zároveň se jednalo o akci odborné skupiny Tekoucí vody České limnologické společnosti. Setkání hostily prostory Ústřední hasičské školy se sídlem na zámku v Bílých Poličanech. Přítomných 32 účastníků je rekordní počet za celou dobu konání setkání.

Na setkání se obvykle řeší celá řada praktických problémů provázejících práci biologa v laboratoři podniku Povodí. Hlavním letošním tématem byla diskuze o exportu biologických dat z monitoringu do databáze IS Arrow a doplnění taxalistů. Toto téma bylo zařazeno na žádost Mgr. Libuše Barešové z ČHMÚ, která se jako host setkání také účastnila. Novinkou byla metoda Stanovení původců cerkáriové dermatitidy ve vodním prostředí, kterou představila Mgr. Zuzana Pokrupová z Povodí Vltavy. Další část setkání byla věnována výměně zkušeností při odběrech a determinaci ve skupinách zabývajících se makrozoobentosem, fytobentosem, fytoplanktonem a makrofyty.

Tradiční krátká exkurze se uskutečnila na přehradě Les království. Příští setkání je plánováno v předjaří roku 2024.

Zámek v Bílých Poličanech

Výměna zkušeností při determinaci (J. Špaček)

Exkurze na přehradu Les království (J. Hotový)

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