



University of South Bohemia
in České Budějovice
Faculty of Science



Molecular phylogeny and taxonomic revision of chaetophoralean algae (Chlorophyta)

Ph.D. Thesis
Mgr. Lenka Caisová

Supervisor

RNDr. Jiří Neustupa, Ph.D.
Department of Botany, Faculty of Sciences, Charles University in Prague

Formal supervisor

Prof. RNDr. Jiří Komárek, DrSc.
University of South Bohemia, Faculty of Science, Institute of Botany, Academy of
Sciences, Třeboň

Consultants

Prof. Dr. Michael Melkonian
Biozentrum Köln, Botanisches Institut, Universität zu Köln, Germany
Mgr. Pavel Škaloud, Ph.D.
Department of Botany, Faculty of Sciences, Charles University in Prague

České Budějovice, 2011

Caisová, L. 2011: Molecular phylogeny and taxonomic revision of chaetophoralean algae (Chlorophyta). PhD. Thesis, composite in English. University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic, 110 pp, shortened version 30 pp.

Annotation

Since the human inclination to estimate and trace natural diversity, usable species definitions as well as taxonomical systems are required. As a consequence, the first proposed classification schemes assigned the filamentous and parenchymatous taxa to the green algal order Chaetophorales sensu Wille. The introduction of ultrastructural and molecular methods provided novel insight into algal evolution and generated taxonomic revisions based on phylogenetic inference. However, until now, the number of molecular phylogenetic studies focusing on the Chaetophorales s.s. is surprisingly low. To enhance knowledge about phylogenetic relationships among taxa within the order, the nuclear-encoded SSU rDNA sequences from 30 strains covering all three chaetophoralean families have been investigated. All revealed monophyletic groupings were further screened for molecular non-homoplasious synapomorphies within the Viridiplantae. To address the question of the correspondence between morphological characters traditionally used for taxonomical delimitation of the Chaetophorales and the tree topology favored by molecular data, the list of morphological/ultrastructural/ecological characters was elaborated and further analyzed. In addition, to obtain a close-up view into the evolution of Compensatory Base Changes (CBCs) of the second internal transcribed spacer (ITS2) which is currently often used to delimit putative biological species, 86 newly obtained/published sequences of ITS2 for five families of the order Ulvales were analyzed. Furthermore, a detailed comparative study of all ITS2 substitutions has been done. Subsequently all revealed CBCs and hemi-CBCs have been mapped upon the ITS2 phylogenetic tree topology. Finally, CBCs/hCBCs taxonomic inference in the Ulvales has been discussed.

Key words

Aphanochaete, *Caespitella*, CBC, CBC-clade, CBC-grade, *Chaetophora*, Chaetophorales, Chaetophoralean algae, Chlorophyceae, *Fritschiella*, evolution, hemi-CBC, ITS2, molecular, NHS, nuclear-encoded SSU rDNA, phenetic, phylogeny, polyphyly, *Schizomeris*, secondary structure, species concept, *Stigeoclonium*, Ulvales, Ulvophyceae, *Uronema*

Financial support

This work was supported by the University of Cologne, by the grant CR no. 206/09/0697, and by JU 038/2008P. Further support came from NERC Oceans 2025 (NF3 *CCAP) and NERC Molecular sequencing grant NERC MGF 154. Many strains were kindly providing by the culture collections CCAP, CCALA, and SAG.

Declaration

I declare that this dissertation was fully worked out by myself and the named coauthors using the cited literature only. I declare that in accordance with the Czech legal code § 47b law No. 111/1998 in valid version.

I consent to the publication of my dissertation in an edition made by removing marked parts

archived by Faculty of Science in an electronic way in the public access section of the STAG

database run by the University of South Bohemia in České Budějovice on its web pages.

Translation into Czech language:

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. V platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejich internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 30th March 2011

Caisová Lenka

Authors' contribution to the article:

Polyphyly of *Chaetophora* and *Stigeoclonium* within the Chaetophorales (Chlorophyceae), revealed by sequence comparisons of nuclear-encoded SSU rRNA genes

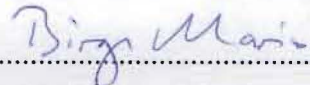
Lenka Caisová, Birger Marin, Nicole Sausen, Thomas Pröschold and Michael Melkonian

Lenka Caisová, Birger Marin and Nicole Sausen evaluated the sequence data, prepared the alignment, performed the analyses and the synapomorphy search, draw the presented figures, and wrote the manuscript. Lenka Caisová performed DNA extraction, amplification of the majority of investigated strains. Thomas Pröschold prepared sequence reactions and performed initial phylogenetic analyses. Michael Melkonian conceived the study, contributed to the design, and critically revised the manuscript.

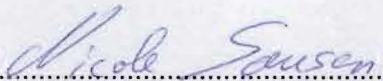
All of the authors read the final manuscript.



Mgr. Lenka Caisová



Dr. Birger Marin



Dipl.-Biol. Nicole Sausen



Dr. Thomas Pröschold



Prof. Dr. Michael Melkonian (MM)

Authors' contribution to the article:

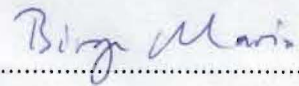
**A close-up view on ITS2 evolution and speciation – a case study in the
Ulvophyceae (Chlorophyta, Viridiplantae)**

Lenka Caisová, Birger Marin and Michael Melkonian

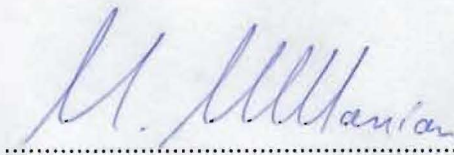
Lenka Caisová (LC) prepared most new sequences, and prepared the ITS2 alignment, consensus secondary structures, and various ITS2 analyses. Birger Marin (BM) contributed two new sequences, and prepared the 18S rDNA alignment and analysis. BM and LC wrote the manuscript and together developed ideas and methods to analyze ITS2 data concerning CBC mapping, quantification, and comparison with taxonomic concepts. Michael Melkonian proposed the ITS2 numbering system, provided many ideas concerning evolutionary approaches of CBC/hCBC analyses, and critically read the manuscript.



Mgr. Lenka Caisová



Dr. Birger Marin



Prof. Dr. Michael Melkonian

Acknowledgements

I would like to express many thanks to my supervisor and consultant – Jiří Neustupa and Pavel Škaloud, respectively – for their support and helpfulness during my study.

My deep thanks go to Prof. Jiří Komárek who provoked my fascination in Phycology already during the first lecture at the University. I am very grateful for his never-ending enthusiasm, hours of interesting discussions and his help and support for the whole time. It was a great pleasure for me!

I do wish to express my deepest gratitude to Prof. Michael Melkonian who opened my mind to another exciting view of science, and induced the ‘Scientific Revolution’ in my life. With his enthusiasm, patience, ideas, advices, suggestions, countless questions all the time, and broad knowledge of the Protist world, he has helped me to understand how great is to be a member of a group and how exciting it can be to create scientific stories. Thank you very much!

I wish to express my special gratefulness to Birger for his enthusiasm, patience, ideas and the introduction into many topics, great help and hours of excellent discussions not always scientific. My gratitude goes also to Nicole-Silke for her energy, enthusiasm, great help and discussions by a cup of tea. Special thanks go to Kerstin Brachhold and Dorothee for very nice English lectures, and especially for their patience. I would like to say thanks to my friends/colleagues, in particular Nicole S., Birger, Karin, Björn, Stuart, Kerstin H.-F., Ram, Sebastian, Kerstin B., Zehra, Nicole F., Stefani, Helga, Lisa, Sabina, Ruben, Weiqun, Annu, Denis, Burkhard, Mrs. Melkonian and Mrs. Esser and Linne von Berg for the exciting time in Cologne. It was great pleasure to be a member of the Melkonian lab!

I owe special thanks to Stuart Sym as well as Dorothee Langenbach for the language corrections of my thesis.

I am also very grateful to Kristýna Hrčková and Dana Švehlová for help with the administration, and with the office work. I would like to thank Terka Stachová, Pavel Rychtecký, Kristýna Hrčková, Alena Lukešová, Romča Potclanová, Dana Švehlová, Jana Šnokhousová, Eliška Zapomělová, Klára Řeháková and Zdeněk Paris for their enthusiasm, support, cups of tea and a nice time whenever I am in the Czech Republic.

Last but not least I would like to thank to my family for overall support during my studies.

Thesis content

| | |
|------------------------------------------|-----------|
| 1. Introduction..... | 1 |
| 2. Summary | 11 |
| 3. Objectives of the thesis | 12 |
| 4. Outline of the thesis..... | 13 |
| 5. Paper 1 | 14 |
| 6. Paper 2 | 17 |
| 7. Conclusions of the thesis..... | 20 |
| References of the thesis..... | 21 |
| Curriculum vitae | 28 |

1. Introduction

Many new taxa of green algae from various biotopes were first described in the late 18th, and during the 19th and 20th centuries (e.g. Schrank 1783, Kützing 1843, Kützing 1845, Rabenhorst 1864, Pascher 1931, Printz 1921, Iyengar 1932, Vischer 1933, Geitler 1942, Bourrelly 1972). These descriptions were based on morphological features such as the type of thallus, the branching pattern, the manner of attachment to the substratum, the type of chloroplast, the presence or absence of the pyrenoid/s, etc. Therefore it is not surprising that one of the first proposed classification schemes of green algae was largely based on the level of organization of the vegetative organism, assuming an underlying phylogenetic trend from unicellular → colonial → coenobial → to filamentous, siphonaceous or thallose taxa (e.g. Blackman 1900, Wille 1901, Pascher 1914; summarized by Round 1984); (Fig. 1).

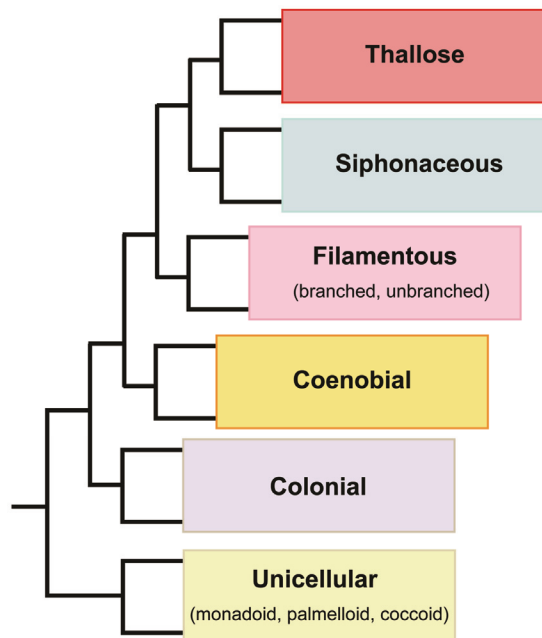


Fig. 1.: Traditional view of the green algal evolution based on the level of organization, displaying the phylogenetic trend from the simplest organisms towards the most complex one.

Subsequent ultrastructural characterization (e.g. studies of motile cell ultrastructure and/or studies of mitosis and cytokinesis) resulted in the recognition of two major lineages of green algae, the Chlorophyceae and the Charophyceae (Pickett-Heaps 1975), and showed incongruence with the traditional delimitation of many of the previously-proposed taxonomic entities (e.g. Melkonian 1975, Stewart and Mattox 1975, O'Kelly et al. 1994). In the 1990s, the introduction of molecular methods in green algal taxonomy provided a fresh data set to enable novel ways of thinking and questioned the use of certain traditional morphological and ultrastructural characters and their resulting phylogenies. Molecular phylogenetic analyses, mostly based on small subunit ribosomal DNA (SSU rDNA) (e.g. Kantz et al. 1990, Buchheim and Chapman 1991, Friedl and

Zeltner 1994, Friedl 1996, Nakayama et al. 1996), were largely congruent with ultrastructural knowledge. Additionally, sequence comparisons clearly revealed that (1) identical forms of thallus morphology evolved independently in different lineages, and that (2) members of one clade, i.e. phylogenetically-related organisms, displayed diverse levels of thallus organization. For example, taxa with unbranched filaments traditionally belonged to the order Ulotrichales *sensu* Borzi or Chaetophorales *sensu* Wille, but now they are placed in Trebouxiophyceae (*Geminella*), Ulvophyceae (*Ulothrix zonata*), Chlorophyceae (*Uronema*) as well as in Streptophyta (*Klebsormidium*); (Fig. 2), and taxa with different levels of organization are to be found within a single class; e.g. the Trebouxiophyceae include coccoid (*Trebouxia*), colonial (*Dictyosphaerium*), unbranched (*Geminella*), branched (*Microthamnion*) and foliose (*Prasiola*) forms; (Fig. 3). Moreover, some derived lineages also show evidence of a reduction in complexity (e.g. unbranched thalli in the genus *Uronema* while all other members of the Chaetophoraceae are branched).

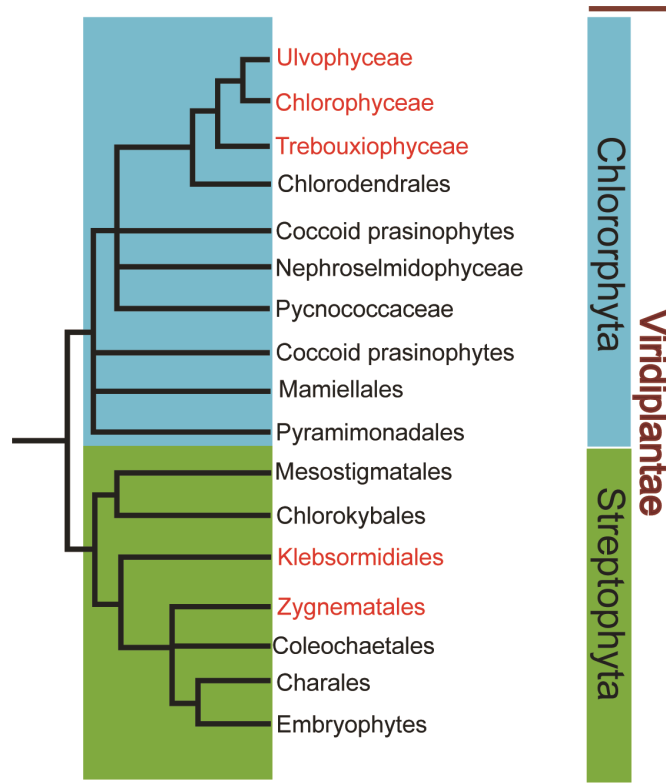


Fig. 2.: Independent evolution of unbranched filamentous type of thallus within different lineages: the Chlorophyta and the Streptophyta. The groups including unbranched taxa are marked in red. (modified after Becker and Marin 2009)

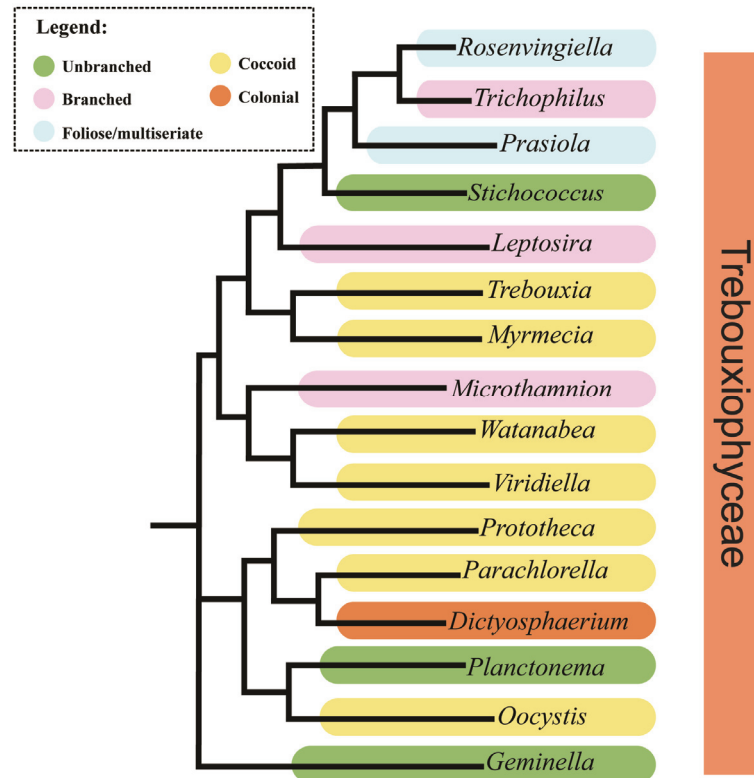


Fig. 3.: The great variability of the thallus organization within the class Trebouxiophyceae. The simplified phylogenetic tree demonstrates coccoid, colonial, unbranched, branched and foliose/multiseriate morphological organization in the main clades of the class. (modified after Friedl 1996, Lewis and McCourt 2004, Pröschold and Leliaert 2007, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>)

In summary, a combination of ultrastructural and molecular evidence has clearly revealed that the level of thallus organization and other traditionally-used morphological features are not useful characters for phylogenetic inference, and the resultant current system is in conflict with many of the original circumscriptions of genera and species (summarized by Melkonian and Surek 1995, Lewis and McCourt 2004, Pröschold and Leliaert 2007).

Especially, the ‘chaetophoralean’ algae provide a good example of a taxon in constant flux over the years. The first record of ‘chaetophoralean’ taxa is to be found in the Species Plantarum where all green algae were recognized in the ‘class’ Cryptogamia (Linnaeus 1753). Later on, with the advent of ‘systematic groupings’, parenchymatous and filamentous forms of green algae were separated as a new ‘group’, the Confervoideae (Agardh 1817), a group which has subsequently been resurrected and even re-named many times (e.g. Kützing 1843, De Bary 1858). Green algae with a parenchymatous, or a branched or unbranched filamentous thallus with parietal chloroplasts were assigned to the order Chaetophorales *sensu* Wille (Wille 1901). However, some authors (e.g. West and Fritsch 1927) have separated filamentous

unbranched taxa into a separate order, the Ulotrichales Borzi, but retained taxa with a branched thallus in the Chaetophorales. In 1982, the concept of the Chaetophorales was further refined by Silva, based on ultrastructural data. His circumscription included taxa (1) with filamentous, or rarely-parenchymatous thalli with uninucleate cells containing parietal chloroplasts with pyrenoid/s; (2) with ultrastructural features of the Chlorophyceae (presence of a phycoplast and a collapsing telophase spindle; cruciate flagellar roots with basal bodies which are more or less clockwise (CW) in displacement)); (3) with asexual (zoospores) and/or sexual (gametes/heterogametes) reproduction.

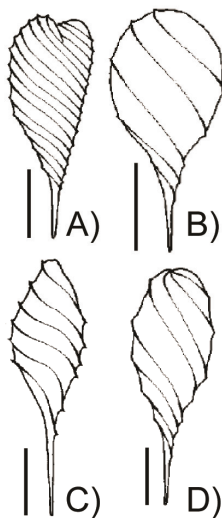
As a result of the ultrastructural studies (summarized in Melkonian 1982, Mattox and Stewart 1984) and later molecular phylogenetic analyses (e.g. Friedl and Zeltner 1994, Friedl 1995, Friedl 1996, Marin and Melkonian 1999, Sanchez-Puerta and Leonardi 2006), many taxa of chaetophorean algae (e.g. *Chaetosphaeridium*, *Coleochaete*, *Klebsormidium*, *Leptosira*, *Microthamnion*), traditionally restricted to the Chaetophorales, were re-classified into different orders or even different classes. Currently, the order Chaetophorales comprises an assemblage of (1) mainly-freshwater, rarely-terrestrial algae with a parenchymatous, or branched or unbranched filamentous thallus and with cells with parietal chloroplasts; (2) algae with a phycoplast associated with cell plate formation, with daughter cells kept interconnected by plasmodesmata, and with centrioles not involved in cell division (John 1984, Melkonian 1990). The order contains three families (Chaetophoraceae, Aphanochaetaceae and Schizomeridaceae), and more than 20 described genera (for details see John 1984). However, until now, the number of molecular phylogenetic studies focusing on this order is surprisingly low. The first molecular study, based on nuclear-encoded SSU rDNA sequences included four taxa (*Fritschiella*, *Chaetophora*, *Stigeoclonium*, *Uronema*) of the Chaetophoraceae, and confirmed both the monophyly of this family and the position of the Chaetophorales within the Chlorophyceae (Booton et al. 1998). The addition of sequences from *Aphanochaete* and *Schizomeris*, to provide a 'complete' order, increasing the sequences to include nuclear-encoded SSU + partial large subunit ribosomal DNA (LSU rDNA), led to the clear elucidation of the relationship between the Chaetophorales and the Chaetopeltidales (Buchheim et al. 2001). Recent phylogenetic analyses based on partial and complete chloroplast genomes of six members of the Chlorophyceae: *Chlamydomonas reinhardtii*, *C. moewusii* (Chlamydomonadales), *Scenedesmus obliquus* (Sphaeropleales), *Oedogonium cardiacum* (Oedogoniales), *Stigeoclonium helveticum* (Chaetophorales), and *Floydiella terrestris* (Chaetopeltidales) resulted in relationships among Chaetophorales, Chaetopeltidales and Oedogoniales (Brouard et al. 2008, Turmel et al. 2008, Turmel et al. 2009).

Generally, many of the discrepancies leading to misunderstandings or conflicts in taxonomy, might be a consequence of impoverished original diagnoses which only consider characters like the colour of the alga, the season when the alga was collected, and the statement that the life history was unclear or unknown. Furthermore, many features have been derived from observations on cultured material under non-specified conditions (mostly soil algae, see in Chodat 1894, Deason and Bold 1960, Brown and Bold 1964, Deason 1969; but also some freshwater taxa, for example *Stigeoclonium helveticum* described by Vischer 1933) or even on dried or fixed specimens (see *Helicodictyon* in Whitford 1960) which can severely impact on the recognition of viable diagnostic characters. Another reason might be that characters (e.g. formation of a distinct type of thallus, the presence or absence of pyrenoid/s or spines/bristles on the cell wall) commonly considered diagnostic for taxonomy are plastic under variable conditions (light or dark, type of medium, grazer effect, bacterial contamination), see e.g. Uspenskaja 1930, Trainor et al. 1971, Provasoli and Pintner 1972, Provasoli and Pintner 1980, Johnstone 1978, Nozaki et al. 1998, Luo et al. 2005, Luo et al. 2006. This has resulted in the formulation of a large number of ambiguous (homoplasious) characters which easily can be applied to more than one taxon, and thus can cause confusion in taxonomy.

To clearly distinguish one taxonomic unit from another, it is necessary to search for unique, shared derived trait/s = **Non-Homoplasious Synapomorphies (NHS)**. These signatures, i.e. characters without known parallels in other taxa, are considered to be essential tools in taxonomy; they are used for taxonomic diagnosis of the various taxa. As NHSs can be defined as morphological traits (e.g. the presence of frustule in diatoms; Round and Crawford 1990), ultrastructural characters (e.g. the suite of characters - the limonoid scale morphology, double eyespots etc. - in subgenus *Pyramimonas*; McFadden et al. 1986), pigment compositions (e.g. chlorophyll d as a major pigment in the cyanobacterial genus *Acaryochloris*; Miyashita et al. 2003) as well as molecular features (e.g. the second base pair of Helix 41 in the plastid-encoded 16S rRNA [H1086: bp 1088/1097] is U-A instead of G-C in the green algal order, Monomastigales; Marin and Melkonian 2010). In the ideal scenario, the newly established or emended taxon may have more than one unique synapomorphy which support its monophyly (e.g. the genus *Monomorphina* can be defined within the Euglenophyceae by a combination of two types of NHS: (1) the peculiar type of pellicle built by hyaline keels associated with the pellicle strips which create a 'relief-like' appearance, and (2) the nuclear-encoded SSU rRNA molecule has a U as the third nucleotide in the terminal loop of Helix 27, and has C as the third nucleotide in the spacer helices 45-47 (Marin et al. 2003)); Fig. 4. However, to detect these unique features often requires a detailed knowledge, not only of the taxon in question, but also of its relatives. To identify all types of NHS, adequate taxon sampling is demanded. The uniqueness of the morphological/molecular/ultrastructural characters may end up being tested against a few or hundreds or thousands of homologous features of taxa,

depending on the taxonomic rank at which one wishes to detect the NHS. However, at least one general rule will come into effect: The number of NHSs will probably decrease with a greater sample of taxa within the dataset. This means, if an NHS synapomorphy search is to be performed, for example, for a genus in the Ulvales, Chlorophyta (e.g. *Acrochaete*), the highest number of the unique signatures, morphological or molecular, would most likely be found within the Ulvaceae than at higher taxonomical levels (i.e. within the Ulvales, the Ulvophyceae or the Chlorophyta or even within the Viridiplantae). In other words, the delimitation of the taxon level within which the synapomorphy search is performed is probably the most important factor.

1) NHS Morphological synapomorphy



2) NHS Molecular synapomorphy

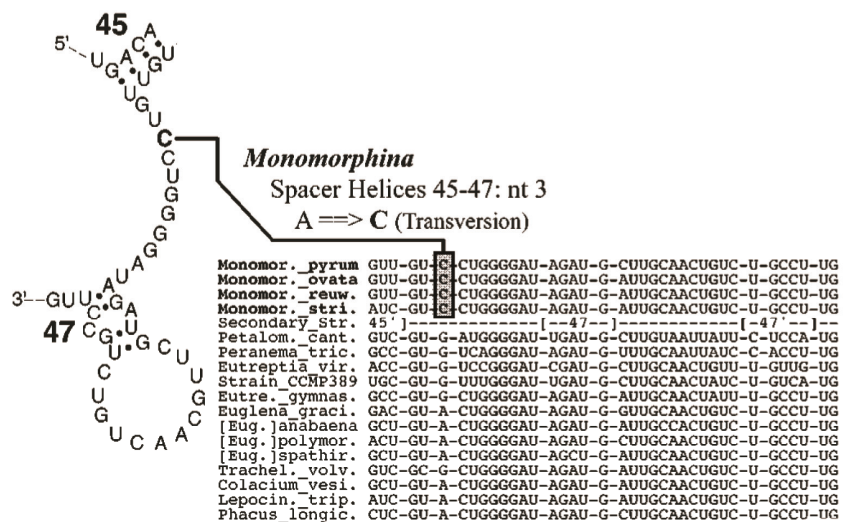


Fig. 4.: Non Homoplasious Synapomorphies (NHS) of the genus *Monomorphina* (Euglenophyceae). 1) NHS morphological synapomorphy – pellicle – demonstrated for different species of *Monomorphina*: A) *Monomorphina striata* (CCAP 1261/9), B) *M. ovata* (SAG 1244-5), C) *M. reeuwykiana* (M 1768), D) *M. pyrum* (UTEX 2354). All scale bars = 10 µm. 2) One of the NHS molecular synapomorphies in the SSU rRNA. *Monomorphina* spp. (in alignment) as well as C (the third nucleotide in the spacer helices 45-47, in alignment and in secondary structure diagram) are in bold. Note that unique synapomorphies were defined within Euglenophyceae, and that the secondary structure is based on *Monomorphina pyrum*. (simplified according to Marin et al. 2003)

More than 20 years have passed since the first molecular approaches had been introduced in algal taxonomy and the conflict between the phenetic system (groupings of organisms based on mutual similarity of phenotypic characters) and the phylogenetic system (groups of organisms based on shared evolutionary heritage) became more and more obvious.

Many phylogenetic markers, such as the SSU and LSU (18S rRNA and 5.8S +28S rRNA), including the internal transcribed spacers (ITS) region, of the nuclear-encoded ribosomal operon; the SSU and LSU (16S rRNA and 23S rRNA) plastid-encoded ribosomal operon; and several chloroplast (*rbcL*, *atpB*), mitochondrial (*coxI*) or nuclear, protein-coding genes (e.g. *actin*), have been employed for studies at various taxonomic levels (e.g. Watanabe et al. 1998, Coleman 2009, Del Campo et al. 2010, Marin and Melkonian 2010, O’Kelly et al. 2010). However, the small subunit nuclear ribosomal operon (SSU rRNA) remains probably to be one of the most frequently-used molecular markers of all (Ouvrard et al. 2000, Meyer et al. 2010) In fact, there are various good reasons for this practice: the existence of (1) a large database, (2) universal primers (3) an appropriate number of nucleotide positions for alignment and over which to perform analyses, and (4), as yet, no evidence for lateral gene transfer within this region in eukaryotes.

Various studies indicate that the nuclear-encoded ribosomal operon (Fig. 5), which shows a mosaic of conserved and divergent regions, has become a popular tool in green algal phylogeny (e.g. Buchheim et al. 2001, Gontcharov et al. 2004, Buchheim et al. 2005).

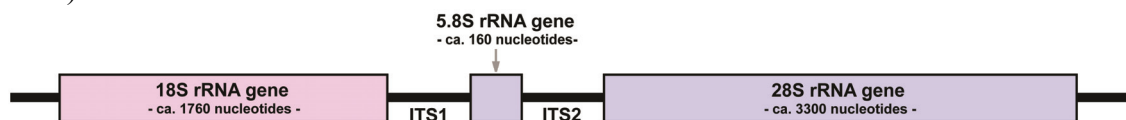


Fig 5. Nuclear-encoded ribosomal operon consisting of three rRNA genes (18S, 5.8S, 28S) and two internal transcribed spacers (ITS1 and ITS2). The small subunit ribosomal DNA (SSU rDNA) and the large subunit ribosomal DNA (LSU rDNA) are indicated by pink and blue color, respectively. (modified after Coleman 2003)

Generally, it is assumed that the slowly-evolving SSU rRNA (18S rRNA) is a marker suitable for evolutionary studies at higher taxonomical levels (e.g. family, order, class). However, the suitability of SSU rRNA to taxonomic classification is highly dependent on the evolutionary rate of sequences investigated.

Many studies also evaluate the utility of the LSU rRNA (28S rRNA) as a potential marker for identification at the various taxonomic levels (e.g. Medina et al. 2001, Sonnenberg et al. 2007). The conservative segments of LSU rRNA are alignable across kingdoms and have probably a comparable potential of resolution as the SSU rRNA gene (Kuzoff et al. 1998). In contrast, the variable segments (e.g. the C domain) evolved more rapidly and thus might be more informative at the lower taxonomic levels, especially at the species level (e.g. Ellegaard et al. 2008, Howard et al. 2009).

In addition, the internal transcribed spacer (ITS), especially ITS2, is a favourite marker in taxonomy. The ITS2 is a fast-evolving part of the nuclear rRNA operon localized between the 5.8S and 28S rRNA genes. Although the primary sequence of ITS2 is highly variable, the typical secondary structure, which comprises four helices (Fig. 6), is

displayed among many eukaryotic organisms (Coleman and Mai 1997, Joseph et al. 1999, Coleman 2007). Of these four helices, Helix 1 and Helix 4 show a degree of variability both in sequence and in length. In contrast Helix 2 and Helix 3 contain motifs that are essential during the excision process of ITS2 (e.g. Thomson and Tollervey 2010) and therefore these two helices are more conserved (Coleman 2007).

Because of the combination of (1) the rapid evolution of the ITS2 region and (2) the presence of conserved regions within it (Helix 2 with at least one pyrimidine-pyrimidine mismatch and Helix 3 with its YGGY motif), it was assumed that the ITS2 might be a highly appropriate marker for taxonomy (Hershkovitz and Lewis 1996, Hershkovitz and Zimmer 1996, Schultz et al. 2005, Coleman 2007, Coleman 2009), and especially useful to differentiate among closely related organisms i.e. to delimit ‘biological species’ (e.g. Coleman 2000).

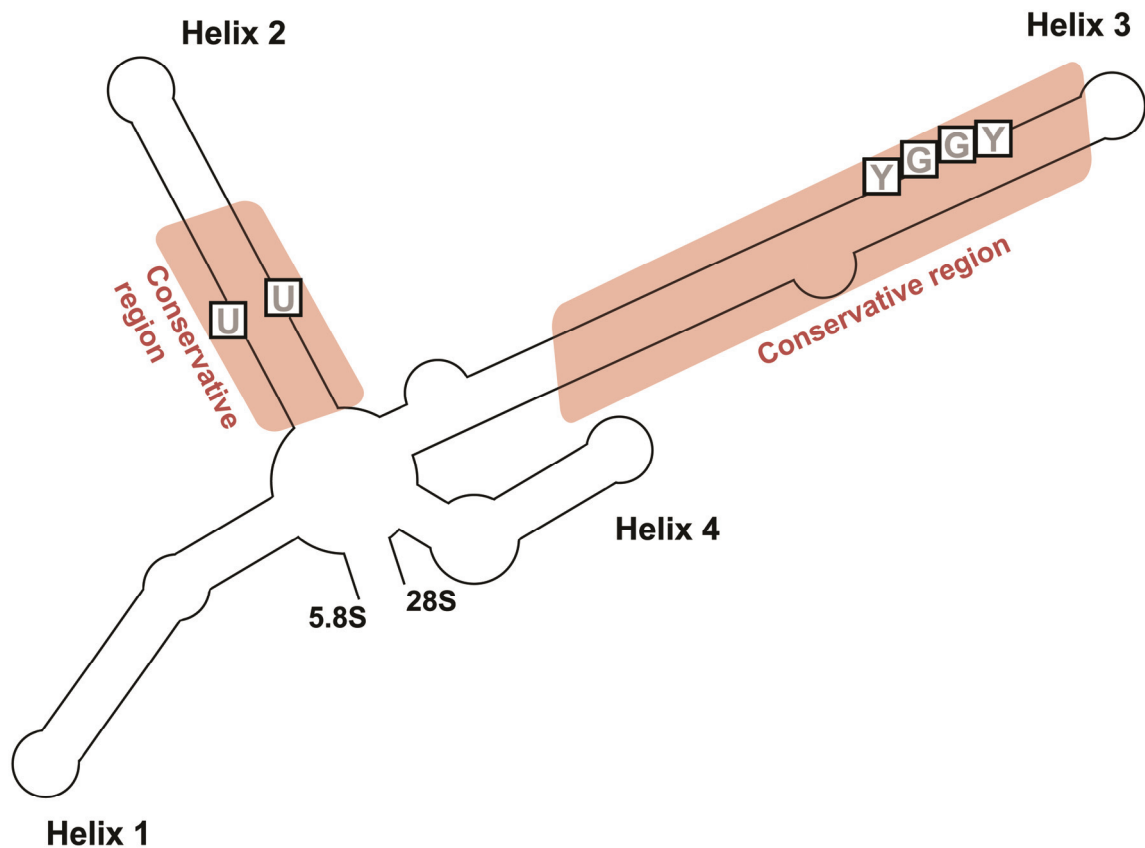


Fig. 6.: Typical secondary structure of ITS2 consisting of four helices (Helix 1 – 4). The important regions for species delimitation (pink colour in Helix 2 and 3) and the conservative motifs (pyrimidin-pyrimidin mismatch in Helix 2 and YGGY in Helix 3) are highlighted. (modified after Mai and Coleman 1997)

As mentioned above, many of the ‘traditionally’ described genera in algae are either paraphyletic or polyphyletic. Since systematics deals with a cladistic approach, and the clade is restricted to a monophyletic group of organisms, all these non-monophyletic genera have to be revised. This begs other questions concerning species delimitation. What is a species and how can different species be defined?

Species, organisms divided into the smallest distinct units, are considered as one of the basic entities of biological classification. Each species is placed within a single genus, and consequently is assumed that the species is more closely-related to other species within its genus than to species of other genera.

To estimate the natural diversity, usable species definitions are required. In the following paragraphs consideration is given to four of the most commonly-known species concepts.

The oldest one is the **morphological species concept**, which characterizes sexual, as well as asexual, species based on differences in structural (morphological) features of the body. Despite the fact that the definition relies on subjective criteria, the morphological species concept is still frequently used in taxonomy (e.g. Johansen and Lowe 2007, Mareš 2010, Siler et al. 2010).

Probably the most widely-accepted species concept among biologists until now is the **biological species concept**, which has been circumscribed by Ernst Mayr in 1942. The biological species is defined as “groups of actually- or potentially- interbreeding natural populations which are reproductively isolated from other such groups” (Mayr 1942).

In summary the biological species concept is highly reproducible but (1) time-consuming mating experiments are required and (2) the biological species is solely-restricted to sexually-reproducing groups of organisms.

The phylogenetic species concept employs different molecular markers to determine a smallest group of related taxa as a separate species. The advantage of this species concept is its applicability to sexual as well as asexual organisms. On the other hand, the phylogenetic species concept is based on groupings of organisms that share the same character/s. This approach may divide organisms into many small groups of species which do not have any biological relevance, i.e. into units which do not correspond with biological species. In practice, some groups of phylogenetic ‘species’ can theoretically still sexually cross with each other and therefore may not be ‘permanent’ species.

The CBC species concept, introduced by Coleman and co-workers, is based on the presence of a Compensatory Base Change (CBC; double sided change which still retains pairing ability, e.g. C-G \leftrightarrow A-U) in the conservative regions of the secondary structure of the second Internal Transcriber Spacer (ITS2). Based on crossing experiments in several organisms, it has been revealed that the presence/absence of even a single CBC in Helix 2 or 3 corresponds to incompatibility/inability to sexually cross and thus determines the limit between biological species (Fabry et al. 1999, Coleman 2000). In contrast, the presence of a hemi-compensatory base change (hCBC; single sided change which retains pairing ability, e.g. C-G \leftrightarrow U-G) in conservative parts of ITS2 theoretically still allows mating, and therefore is not useful in resolving species differences. In other words, the CBC species concept is based on the comparison of the secondary structures of ITS2 between two taxa, and when at least one CBC in the conserved parts of the helices is found, these two taxa are considered to be two different species. The ITS2 region has now become a widely-utilized DNA marker for algae, fungi, plants, and also animals to distinguish among ‘biological species’ (e.g. Coleman 2005, Coleman and van Oppen 2008, Ahvenniemi et al. 2009, Coleman 2009, Agnarsson 2010, Fawley et al. 2011). Furthermore, according to Müller et al. (2007) ‘the correlation between the presence of CBCs and the species concept occurs independently of reproduction and mating affinities’, i.e. it can be used to distinguish species whether they exhibit sexual reproduction or not.

Although, many different theoretical and practical ‘species concepts’ have been established (summarized in Mayden 1997, Wheeler and Meier 2000) until now, no completely-satisfactory, general definition of the species concept exists.

2. Summary

The introduction of ultrastructural and molecular methods clearly revealed that the original circumscription of the order Chaetophorales based on morphological characters is artificial. Many 'chaetophoralean algae' fall into various lineages within the Chlorophyta, often forming paraphyletic or polyphyletic assemblages of the originally-established taxa.

A more refined ordinal concept for the Chaetophorales is defined based on morphological/ultrastructural and molecular characters. Recent studies elucidated the affiliation of the Chaetophorales with the Chlorophyceae, and have also shown its phylogenetic position within this class. Nevertheless, these phylogenetic studies only dealt with one or two representatives of six chaetophoralean genera. Therefore the relationships among the three described families (Schizomeridaceae, Aphanochaetaceae and Chaetophoraceae) remain unknown. The same lack of knowledge holds true for the phylogenetic status of the species-rich genera (*Chaetophora*, *Draparnaldia* and *Stigeoclonium*) of the Chaetophoraceae.

Since most described species (including chaetophoralean algae) have been established solely on the basis of morphological characters, many phycologists are now asking the question: What is the relation between morphospecies, biological species and their phylogenetic position inferred from molecular data? As already recognized, no general rule can be applied.

Nevertheless, phylogenetic studies and crossing experiments revealed that observed CBC-type substitutions in conserved ITS2 regions are linked to an inability to cross and thus may serve as some measure of the limits between biological species. Therefore the CBC ITS2 species concept has become widely applied in algal taxonomy.

However, until now, neither a comparative study of the ITS2 secondary structure nor a relation between CBC ITS2 - clades and the taxonomic level of organisms have been performed in the green algal order Ulvales. Moreover, the mechanistics by which CBCs evolve in pairs in the ITS2 region have never been interrogated, nor has the taxonomic value of non-homoplasious vs. homoplasious CBCs in ITS2 ever been considered. Since there are many ITS2 sequences available for Ulvales algae, data from crossing experiments, and morphologically-diverse taxa (previously assigned to the Chaetophorales) from various habitats, the Ulvales was selected as a suitable taxon to test the questions posed above.

3. Objectives of the thesis

This study has following main aims:

(1) To analyze the relationships among traditionally established families within the order Chaetophorales, and to test the monophyly of species-rich genera. (Paper 1)

(2) To test the relation between CBC ITS2 - clades and the taxonomic level of organisms in the green algal order Ulvales. (Paper 2)

(3) To study the way in which CBCs evolve and to analyze the non-homoplasious and homoplasious history of CBCs and hCBCs-type substitutions. (Paper 2)

4. Outline of the thesis

To address the question of the relationships among the chaetophoralean families, as well as to investigate the monophyly of the species-rich genera, the nuclear-encoded SSU rDNA sequences from 30 strains covering all three described families in the Chaetophorales have been obtained. Subsequently-performed phylogenetic analyses indicate (1) a basal phylogenetic position for the Schizomeridaceae and the weakly-supported Aphanochaetaceae, and (2) that the species-rich genera *Chaetophora* and *Stigeoclonium* are polyphyletic. Moreover, the revealed polyphyly of *Chaetophora* and *Stigeoclonium* has been clearly supported also by NHS synapomorphies in the SSU rRNA secondary structure.

The results clearly indicate that traditional morphological criteria for defining genera and species of the order Chaetophorales are either homoplasious or plesiomorphic and need to be re-evaluated (**Paper 1 – Polyphyly of *Chaetophora* and *Stigeoclonium* within the Chaetophorales (Chlorophyceae), revealed by sequence comparisons of nuclear-encoded SSU rRNA genes**).

To test the relation between CBC – clades of ITS2 and the taxonomic level of organisms in the green algal order Ulvales, the ITS2 sequences of 86 taxa covering five families were investigated. Most sequences are conservative in length and facilitated the generation of a consensus model for the ITS2 secondary structure and allowed the establishment of a new numbering system of ITS2 to unambiguously describe and locate base pairs, CBCs and hCBCs. By mapping CBC and hCBC – type substitutions on the tree topology the following results were obtained: (1) In the Ulvales, the presence of CBCs is not restricted to a particular taxonomic level. (2) Most CBC ‘clades’ *sensu* Coleman are paraphyletic and should thus be named CBC ‘grades’. (3) The phenetic approach of species delimitation can be misleading, therefore all CBCs and hCBCs should be mapped on the phylogenetic tree. (4) Hemi-CBCs do not represent an intermediate step in the evolution of CBCs, both CBCs and hCBCs evolved independently (**Paper 2 – Close up view on ITS2 evolution and speciation – a case study in the Ulvophyceae (Chlorophyta, Viridiplantae)**).

5. Paper 1

**POLYPHYLY OF CHAETOPHORA AND STIGEOCLONIUM WITHIN THE
CHAETOPHORALES (CHLOROPHYCEAE), REVEALED BY SEQUENCE
COMPARISONS OF NUCLEAR-ENCODED SSU rRNA GENES**

Lenka Caisová, Birger Marin, Nicole Sausen, Thomas Pröschold & Michael Melkonian

2011

Journal of Phycology. 47: 164–177

DOI: 10.1111/j.1529-8817.2010.00949.x

Abstract

Previously published molecular phylogenetic analyses of the Chaetophorales (Chlorophyceae) suffered from limited taxon sampling (six genera with only a single species per genus). To test the monophyly of species-rich genera, and to analyze the phylogenetic relationships among families and genera in the Chaetophorales, we determined nuclear-encoded SSU rDNA sequences from 30 strains of Chaetophorales, performed phylogenetic analyses using various methods, and screened clades for support by unique molecular synapomorphies in the SSU rRNA secondary structure. The Schizomeridaceae and the weakly supported Aphanochaetaceae were recovered as basal lineages. The derived family Chaetophoraceae diverged into two clades: the ‘*Uronema*-clade’ containing unbranched filaments, and a sister clade designated as ‘branched Chaetophoraceae’ comprising *Chaetophora*, *Stigeoclonium*, *Draparnaldia*, *Caespitella*, and *Fritschiella*. Although some terminal clades corresponded to genera described, e.g. *Caespitella* and *Draparnaldia*, other clades were in conflict with traditional taxonomic designations. Especially, the genera *Stigeoclonium* and *Chaetophora* were shown to be polyphyletic. The globose species *Chaetophora elegans* was unrelated to lobate *Chaetophora* spp., e.g. *C. lobata*. Since the original description of *Chaetophora* referred to a lobate thallus organization, the latter clade represented *Chaetophora* sensu stricto. In consequence, *C. lobata* was designated as lectotype of *Chaetophora*. Two *Stigeoclonium* species, *S. farctum* and *S.* ‘Longipilus’, diverged independently from the type species of *Stigeoclonium*, *S. tenue*. These results indicated that some commonly used taxonomic characters are either homoplasious or plesiomorphic, and call for a re-evaluation of the systematics of the Chaetophorales using novel morphological and molecular approaches.

Translation into Czech language:

Doposud publikované molekulárně-fylogenetické analýzy řádu Chaetophorales (Chlorophyceae) obsahovaly limitovaný počet taxonů (pouze po jednom zástupci ze šesti rodů). Abychom otestovali monofylii druhově bohatých rodů a fylogenetické vztahy mezi popsányými čeleděmi a rody řádu Chaetophorales, sekvenovali jsme 18S rDNA gen ze 30 kmenů řádu Chaetophorales. Získané sekvence byly analyzovány pomocí různých fylogenetických metod. Monofyletické skupiny organismů byly dále testovány, zda jsou charakteristické, tzn. odlišné od ostatních taxonů, přítomností unikátních molekulárních znaků v 18S rRNA sekundární struktuře. Čeleď Schizomeridaceae a slabě podpořená čeleď Aphanochaetaceae byly situovány na bázi fylogenetického stromu. Odvozená čeleď Chaetophoraceae byla rozdělena do dvou monofyletických skupin: “*Uronema* clade” (zahrnující nětvetvené stélky) a “branched Chaetophoraceae” (zahrnující rody *Chaetophora*, *Stigeoclonium*, *Draparnaldia*, *Caespitella*, a *Fritschiella*). Ačkoli některé terminální monofyletické skupiny odpovídaly popsáným rodům (např. *Caespitella* a *Draparnaldia*), jiné monofyletické skupiny ukázaly jasný rozpor s tradičně vymezenými rody. Rody *Stigeoclonium* a *Chaetophora* se ukázaly být polyfyletické. Druh s kulovitou morfologií makroskopických kolonií – *Chaetophora elegans* – nebyl fylogeneticky příbuzný s *Chaetophora* spp. s lalokovitým tvarem kolonií, např. *Chaetophora lobata*. Protože

lalokovitý tvar kolonií je součástí originálního popisu rodu *Chaetophora*, clade obsahující taxony s lalokovitými koloniemi byl označen jako *Chaetophora* sensu stricto. Následně *C. lobata* byla designována jako lektotyp rodu *Chaetophora*. Dva druhy rodu *Stigeoclonium*, *Stigeoclonium farctum* Berthold a *Stigeoclonium 'Longipilus'*, jsou vzdáleně příbuzní typovému druhu rodu *Stigeoclonium*, *Stigeoclonium tenue* (C. Agardh) Kütz. Získané výsledky jasně ukazují, že některé běžně užívané morfologické znaky v taxonomii těchto řas jsou homoplasizické nebo plesiomorfické, a že k přehodnocení taxonomické situace v řádu Chaetophorales je nutná studie širšího spektra morfologických znaků za jasně stanovených podmínek stejně tak sekvenace více genů.

6. Paper 2

A close-up view on ITS2 evolution and speciation – a case study in the Ulvophyceae (Chlorophyta, Viridiplantae)

Lenka Caisová, Birger Marin & Michael Melkonian

2011

BMC Evolutionary Biology – before submission

Abstract

Background

The second Internal Transcriber Spacer (ITS2) is a fast evolving part of the nuclear-encoded rRNA operon located between the 5.8S and 28S rRNA genes. Based on crossing experiments it has been proposed that even a single Compensatory Base Change (CBC) in Helices 2 and 3 of ITS2 indicates sexual incompatibility and thus the limit between biological species. Taxa without any CBC in these ITS2 regions were designated ‘CBC-clade’. However, in depth comparative analyses of ITS2 secondary structures, ITS2 evolution, the origin of CBCs, and their relationship to biological species have rarely been performed. To gain ‘close-up’ insights into ITS2 evolution, (1) 86 sequences of ITS2 including secondary structures have been investigated in the green algal order Ulvales (Chlorophyta, Viridiplantae), (2) after recording all existing substitutions, CBCs and hemi-CBCs (hCBCs) have been mapped upon the ITS2 phylogeny, rather than merely comparing ITS2 characters among pairs of taxa, and (3) the relation between CBCs, hCBCs, CBC-clades, and the taxonomic level of organisms was investigated in detail.

Results

High sequence and length conservation allowed the generation of an ITS2 consensus secondary structure, and introduction of a novel numbering system of ITS2 nucleotides and base pairs. Alignments and analyses were based on this structural information, leading to the following results: (1) in the Ulvales, the origin of a CBC is not linked to any taxonomic level, (2) most CBC ‘clades’ *sensu* Coleman are paraphyletic, and must correctly be named CBC grades. (3) the phenetic approach of pairwise comparison of sequences can be misleading, and thus, CBCs/hCBCs should be investigated in their evolutionary context, including homoplasy events (4) CBCs and hCBCs in ITS2 helices evolved independently, and we found no example for a CBC that originated via two-fold hCBC substitution.

Conclusions

Our case study revealed several discrepancies between ITS2 evolution in the Ulvales and generally accepted assumptions underlying ITS2 evolution as e.g. the CBC-clade concept. Therefore, we developed a suite of methods providing a critical ‘close-up’ view into ITS2 evolution by directly tracing the evolutionary history of individual positions, and we caution against a non-critical use of the ITS2 CBC-clade concept for species delimitation.

Translation into Czech language:

Úvod: ITS2 je rychle se vyvíjející část jaderného rRNA operonu, nacházející se mezi 5.8S a 28S rRNA geny. Na základě výsledků získaných křížením studovaných organismů se předpokládá, že dokonce už jedna změna dvou nukleotidů, které stále zachovávají párování, tzv. Compensatory Base Change (CBC), v ITS2 helixech 2 a 3, vede k blokaci sexuálního rozmnožování, a tudíž určuje hranici mezi biologickými druhy. Organismy bez žádné CBC v ITS2 helixech 2 a 3 jsou označovány jako ‘CBC clade’. Nicméně, detailní srovnávací analýza ITS2 sekundárních struktur, vznik CBCs a jejich vztah/shoda s hranicí vymezení biologického druhu byly jen velmi zřídka studovány. K tomu, abychom získali detailní náhled na ITS2 evoluci jsme: (1) studovali 86 ITS2 sekvencí z řádu Ulvales (Chlorophyta, Viridiplantae) včetně jejich sekundárních struktur, (2) zaznamenali jsme všechny existující substituce na jednotlivých pozicích v ITS2. Poté, místo jednoduchého porovnávání CBC substitucí v sekundárních strukturách mezi dvojicemi studovaných organismů, byly všechny CBCs/hCBCs mapovány na ITS2 fylogenetický strom, a (3) jsme detailně studovali vztahy mezi CBCs, hCBCs, CBC-clades s taxonomicky vymezenými kategoriemi.

Výsledky: Vysoká shodnost sekvencí v motivech a délce umožnila vytvoření consensu sekundární struktury ITS2 a zároveň uvedení jednotného číslovacího systému nukleotidů a bází ITS2. Alignment a analýzy korigované a založené na informaci sekundární struktury ITS2 vedly k následujícím zjištěním: (1) v řádu Ulvales se vznik CBC nevztahuje k žádné taxonomicky vymezené kategorii, (2) většina CBC ‘clades’ sensu Coleman je parafyletických a musí být pojmenovány jako CBC grades, (3) fenetický přístup k porovnávání párů sekvencí může vést k nesprávným závěrům, a proto by vždy měla být studována evoluce CBC/hCBC, zahrnující také homoplázie, (4) CBC a hCBC v ITS2 vznikly nezávisle na sobě. Ve studovaném řádu Ulvales nebyl nalezen jediný příklad, kdy by CBC vznikla pomocí dvou po sobě následujících hCBC substitucí.

Závěry: Naše pilotní studie demonstruje několik zásadních nesrovnalostí mezi ITS2 evolucí v řádu Ulvales a mezi obecně uznávanými předpoklady/pravidly vztahující se k evoluci ITS2 obecně, jako např. CBC-clade koncept. Na základě těchto zjištění jsme vyvinuly skupinu vzájemně propojených metod, které umožňují detailní pohled na ITS2 evoluci díky sledování evolučních změn na jednotlivých pozicích. Naše výsledky jasně varují proti ‘obecnému’ používání ITS2 CBC clade koncept jako markeru pro vymezení druhů bez detailnějšího pohledu na ITS2 evoluci studované skupiny organismů.

7. Conclusions of the thesis

In the present contribution, the first detailed molecular phylogenetic investigation of the order Chaetophorales s.s. based on the nuclear-encoded SSU rRNA gene has been provided. Moreover, it has been clearly demonstrated that the traditional delimitation of the common chaetophorean genera (*Chaetophora* and *Stigeoclonium*) based exclusively on structural characters is inconsistent with the results obtained by the 18S rDNA phylogeny. The revealed polyphyly of the genera *Chaetophora* and *Stigeoclonium* has been further supported by NHS molecular synapomorphies in the SSU rRNA secondary structure. Although the nuclear-encoded SSU rRNA gene has proven to be insufficient to unravel all phylogenetic relationships, the results gained obviously indicate either a homoplasious or plesiomorphic trait of some commonly used taxonomic features, and call for an overhaul of the systematics of the Chaetophorales using new morphological and molecular approaches. In addition, a first detailed comparative investigation of the ITS2 secondary structure as well as tracing the evolutionary history of all ITS2 substitutions in the green algal order Ulvales led to a completely new view into ITS2 evolution demonstrating the weakness of the generally accepted rules delineating the CBC ITS2-clade species concept. It came as surprise that most CBC-clades *sensu* Coleman are not monophyletic, and that at least in the Ulvales, the presence of CBCs is not restricted to a single taxonomic level. Beside that it has been clearly shown that the simple comparison of ITS2 characters among pairs of taxa without consideration of their evolutionary history (i.e. phenetic approach) can be misleading. Therefore a generally applicable suite of methods providing a detailed view into ITS2 by directly tracing the evolution of individual characters has been proposed.

References of the thesis

Agardh, C. A. 1817. *Synopsis algarum Scandinaviae, adjecta dispositione universali algarum*. ex officina Berlingiana, Lundae [Lund], 135 pp.

Agnarsson, I. 2010. The utility of ITS2 in spider phylogenetics: notes on prior work and an example from *Anelosimus*. *Journal of Arachnology*. 38: 377–382.

Ahvenniemi, P., Wolf, M., Lehtonen, M. J., Wilson, P., German-Kinnari, M. & Valkonen, J. P. T. 2009. Evolutionary diversification indicated by compensatory base changes in ITS2 secondary structures in a complex fungal species, *Rhizoctonia solani*. *Journal of Molecular Evolution*. 69: 150–163.

Becker, B. & Marin, B. 2009. Streptophyte algae and the origin of embryophytes. *Annals of Botany*. 103: 999–1004.

Blackman, F. F. 1900. The primitive algae and the flagellata. An account of modern work bearing on the evolution of the algae. *Annals of Botany*. 14: 647–688.

Booton, G. C., Floyd, G. L. & Fuerst, P. A. 1998. Origins and affinities of the filamentous green algal orders Chaetophorales and Oedogoniales based on 18S rRNA gene sequences. *Journal of Phycology*. 34: 312–318.

Bourrelly, P. 1972. *Les Algues d' Eau Douce. I. Les Algues Vertes*. N. Boubée and Cie, Paris, 570 pp.

Brouard, J.-S., Otis, C., Lemieux, C. & Turmel, M. 2008. Chloroplast DNA sequence of the green alga *Oedogonium cardiacum* (Chlorophyceae): unique genome architecture, derived characters shared with the Chaetophorales and novel genes acquired through horizontal transfer. *BMC Genomics*. 9: 290.

Brown, M. R. & Bold, H. C. 1964. *Phycological Studies V. Comparative studies of the algal genera Tetracystis and Chlorococcum*, Vol. 6417. University of Texas, Austin, Texas, 213 pp.

Buchheim, M., Buchheim, J., Carlson, T., Braband, A., Hepperle, D., Krienitz, L., Wolf, M. & Hegewald, E. 2005. Phylogeny of the Hydrodictyaceae (Chlorophyceae): Inferences from rDNA data. *Journal of Phycology*. 41: 1039–1054.

Buchheim, M. A. & Chapman, R. L. 1991. Phylogeny of the colonial green flagellates: a study of 18S and 26S rRNA sequence data. *Biosystems*. 25: 85–100.

Buchheim, M. A., Michalopoulos, E. A. & Buchheim, J. A. 2001. Phylogeny of the Chlorophyceae with special reference to the Sphaeropleales: a study of 18S and 26S rRNA data. *Journal of Phycology*. 37: 819–835.

Coleman, A. W. 2003. ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics*. 19: 370–375.

Coleman, A. W. 2007. Pan-eukaryote ITS2 homologies revealed by RNA secondary structure. *Nucleic Acids Research*. 35: 3322–3329.

Coleman, A. W. 2009. Is there a molecular key to the level of "biological species" in eukaryotes? A DNA guide. *Molecular Phylogenetics and Evolution*. 50: 197–203.

Coleman, A. W. 2005. *Paramecium aurelia* revisited. *Journal of Eukaryotic Microbiology*. 52: 68–77.

- Coleman, A. W. 2000. The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. *Protist*. 151: 1–9.
- Coleman, A. W. & Mai, J. C. 1997. Ribosomal DNA ITS-1 and ITS-2 sequence comparisons as a tool for predicting genetic relatedness. *Journal of Molecular Evolution*. 45: 168–177.
- Coleman, A. W. & van Oppen, M. J. H. 2008. Secondary structure of the rRNA ITS2 region reveals key evolutionary patterns in acroporid corals. *Journal of Molecular Evolution*. 67: 389–396.
- De Bary, A. 1858. *Untersuchungen über die Familie der Conjugaten (Zygnemeen und Desmidiaceen) Ein Beitrag zur physiologischen und beschreibenden Botanik*. A. Förstnersche Buchhandlung (Arthur Felix), Leipzig, 91 pp.
- Deason, T. R. 1969. Filamentous and colonial soil algae from Dauphin Island, Alabama. *Transactions of the American Microscopical Society*. 88: 240–246.
- Deason, T. R. & Bold, H. D. 1960. *Phycological Studies I. An exploratory study of Texas soil algae*. University of Texas, Austin, Texas, 70 pp.
- Del Campo, E. M., Casano, L. M., Gasulla, F. & Barreno, E. 2010. Suitability of chloroplast LSU rDNA and its diverse group I introns for species recognition and phylogenetic analyses of lichen-forming *Trebouxia* algae. *Molecular Phylogenetics and Evolution*. 54: 437–444.
- Ellegaard, M., Godhe, A., Härnström, K. & McQuoid, M. 2008. The species concept in a marine diatom: LSU rDNA-based phylogenetic differentiation in *Skeletonema marinoi/dohrnii* (Bacillariophyceae) is not reflected in morphology. *Phycologia*. 47: 156–167.
- Fabry, S., Köhler, A. & Coleman, A. W. 1999. Intraspecies analysis: comparison of ITS sequence data and gene intron sequence data with breeding data for a worldwide collection of *Gonium pectorale*. *Journal of Molecular Evolution*. 48: 94–101.
- Fawley, M. W., Fawley, K. P. & Hegewald, E. 2011. Taxonomy of *Desmodesmus serratus* (Chlorophyceae, Chlorophyta) and related taxa on the basis of morphological and DNA sequence data. *Phycologia*. 50: 23–56.
- Friedl, T. 1996. Evolution of the polyphyletic genus *Pleurastrum* (Chlorophyta): inferences from nuclear-encoded ribosomal DNA sequences and motile cell ultrastructure. *Phycologia*. 35: 456–469.
- Friedl, T. 1995. Inferring taxonomic positions and testing genus level assignments in coccoid green lichen algae: A phylogenetic analysis of 18S ribosomal RNA sequences from *Dictyochloropsis reticulata* and from members of the genus *Myrmecia* (Chlorophyta, Trebouxiophyceae cl. nov.). *Journal of Phycology*. 31: 632–639.
- Friedl, T. & Zeltner, C. 1994. Assessing the relationships of some coccoid green lichen algae and the Microthamniales (Chlorophyta) with 18S ribosomal-RNA gene sequence comparisons. *Journal of Phycology*. 30: 500–506.
- Geitler, L. 1942. Zur Kenntnis der Bewohner des Oberflächenhäutchens einheimischer Gewässer. In Porsch, O. & Weber, H. [Eds.] *Biologia Generalis, Archiv für die allgemeinen Fragen der Lebensforschung*. Springer-Verlag, Wien, pp. 450–475.
- Gontcharov, A. A., Marin, B. & Melkonian, M. 2004. Are combined analyses better than single gene phylogenies? A case study using SSU rDNA and rbcL sequence

- comparisons in the Zygnematophyceae (Streptophyta). *Molecular Biology and Evolution*. 21: 612–624.
- Hershkovitz, M. A. & Lewis, L. A. 1996. Deep-level diagnostic value of the rDNA-ITS region. *Molecular Biology and Evolution*. 13: 1276–1295.
- Hershkovitz, M. A. & Zimmer, E. A. 1996. Conservation patterns in angiosperm rDNA ITS2 sequences. *Nucleic Acids Research*. 24: 2857–2867.
- Howard, M. D. A., Smith, G. J. & Kudela, R. M. 2009. Phylogenetic relationships of yessotoxin-producing dinoflagellates, based on the large subunit and internal transcribed spacer ribosomal DNA domains. *Applied and Environmental Microbiology*. 75: 54–63.
- Chodat, R. 1894. Matériaux pour servir a l' histoire des Protococcoidées. *Bulletin de l' Herbar Bossier*. 2: 585–616.
- Iyengar, M. O. P. 1932. *Fritschella*, a new terrestrial member of the Chaetophoraceae. *New Phytologist*. 31: 329–335.
- Johansen, J. R. & Lowe, R. L. 2007. *Draparnaldia appalachiana* sp. nov. (Chaetophoraceae, Chlorophyceae) from the Great Smoky Mountains National Park. *Algological Studies*. 123: 35–45.
- John, D. M. 1984. On the systematic of the Chaetophorales. In Irvine, D. E. G. & John, D. M. [Eds.] *Systematics of the Green Algae*. Academic Press, London, pp. 207–232.
- Johnstone, I. M. 1978. Phenotypic plasticity in *Draparnaldia* (Chlorophyta: Chaetophoraceae). I. Effects of the chemical environment. *Journal of Phycology*. 14: 302–308.
- Joseph, N., Krauskopf, E., Vera, M. I. & Michot, B. 1999. Ribosomal internal transcribed spacer 2 (ITS2) exhibits a common core of secondary structure in vertebrates and yeast. *Nucleic Acids Research*. 27: 4533–4540.
- Kantz, T. S., Theriot, E. C., Zimmer, E. A. & Chapman, R. L. 1990. The Pleurostrophyceae and Micromonadophyceae: a cladistic analysis of nuclear rRNA sequence data. *Journal of Phycology*. 26: 711–721.
- Kützing, F. T. 1843. *Phycologia Generalis Oder Anatomie, Physiologie und Systemkunde der Tange*. F. A. Brockhaus, Leipzig, Germany, 458 pp.
- Kützing, F. T. 1845. *Phycologia Germanica, d.i. Deutschlands Algen in Bündigen Beschreibungen. Nebst Einer Anleitung zum Untersuchen und Bestimmen Dieser Gewächse für Anfänger*. Wilh. Köhne, Nordhausen, 340 pp.
- Kuzoff, R. K., Sweere, J. A., Soltis, D. E., Soltis, P. S. & Zimmer, E. A. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. *Molecular Biology and Evolution*. 15: 251–263.
- Lewis, L. A. & McCourt, R. M. 2004. Green algae and the origin of land plants. *American Journal of Botany*. 91: 1535–1556.
- Linnaeus, C. v. 1753. *Species plantarum, exhibentes plantas rite cognitatas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas*, Vol. 1,2. Impensis Laurentii Salvii, Holmiae [Stockholm], 1200 pp.

- Luo, W., Krienitz, L., Pflugmacher, S. & Walz, N. 2005. Genus and species concept in *Chlorella* and *Micractinium* (Chlorophyta, Chlorellaceae): genotype versus phenotypical variability under ecosystem conditions. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie*. 29: 170–173.
- Luo, W., Pflugmacher, S., Pröschold, T., Walz, N. & Krienitz, L. 2006. Genotype versus phenotype variability in *Chlorella* and *Micractinium* (Chlorophyta, Trebouxiophyceae). *Protist*. 157: 315–333.
- Mai, J. C. & Coleman, A. W. 1997. The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *Journal of Molecular Evolution*. 44: 258–271.
- Mareš, J. 2010. *Anabaena fuscovaginata* (Nostocales), a new cyanobacterial species from periphyton of the freshwater alkaline marsh of Everglades, South Florida, USA. *Fottea*. 10: 235–243.
- Marin, B. & Melkonian, M. 2010. Molecular phylogeny and classification of the Mamiellophyceae class. nov (Chlorophyta) based on sequence comparisons of the nuclear- and plastid-encoded rRNA operons. *Protist*. 161: 304–336.
- Marin, B. & Melkonian, M. 1999. Mesostigmatophyceae, a new class of streptophyte green algae revealed by SSU rRNA sequence comparisons. *Protist*. 150: 399–417.
- Marin, B., Palm, A., Klingberg, M. & Melkonian, M. 2003. Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist*. 154: 99–145.
- Mattox, K. R. & Stewart, K. D. 1984. Classification of the green algae: a concept based on comparative cytology. In Irvine, D. E. G. & John, D. M. [Eds.] *Systematics of the Green Algae*. Academic Press, London, pp. 29–72.
- Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In Claridge, M. F., Dawah, H. A. & Wilson, M. R. [Eds.] *Species: The units of diversity*. Chapman and Hall, London, pp. 381–423.
- Mayr, E. 1942. *Systematics and the Origin of Species from the Viewpoint of a Zoologist*. Harvard University Press, Cambridge, Massachusetts. Reprint (1999), 372 pp.
- McFadden, G. I., Hill, D. R. A. & Wetherbee, R. 1986. A study of the genus *Pyramimonas* (Prasinophyceae) from southeastern Australia. *Nordic Journal of Botany*. 6: 209–234.
- Medina, M., Collins, A. G., Silberman, J. D. & Sogin, M. L. 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *Proceedings of the National Academy of Sciences of the United States of America*. 98: 9707–9712.
- Melkonian, M. 1975. The fine structure of the zoospores of *Fritschiella tuberosa* Iyeng. (Chaetophorineae, Chlorophyceae) with special reference to the flagellar apparatus. *Protoplasma*. 86: 391–404.
- Melkonian, M. 1990. Phylum Chlorophyta: Class Chlorophyceae. In Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. [Eds.] *Handbook of Protoctista*. Jones & Bartlett Publishers, Boston, pp. 608–616.

- Melkonian, M. 1982. Structural and evolutionary aspects of the flagellar apparatus in green algae and land plants. *Taxon*. 31: 255–265.
- Melkonian, M. & Surek, B. 1995. Phylogeny of the Chlorophyta: congruence between ultrastructural and molecular evidence. *Bulletin De La Societe Zoologique De France-Evolution Et Zoologie*. 120: 191–208.
- Meyer, A., Todt, C., Mikkelsen, N. T. & Lieb, B. 2010. Fast evolving 18S rRNA sequences from *Solenogastres* (Mollusca) resist standard PCR amplification and give new insights into mollusk substitution rate heterogeneity. *BMC Evolutionary Biology*. 10: 70.
- Miyashita, H., Ikemoto, H., Kurano, N., S., M. & Chihara, M. 2003. *Acaryochloris marina* gen. et sp. nov. (Cyanobacteria), an oxygenic photosynthetic prokaryote containing chl d as a major pigment. *Journal of Phycology*. 39: 1247–1253.
- Müller, T., Philippi, N., Dandekar, T., Schultz, J. & Wolf, M. 2007. Distinguishing species. *RNA*. 13: 1469–1472.
- Nakada, T. & Nozaki, H. 2009. Taxonomic study of two new genera of fusiform green flagellates, *Tabris* gen. nov. and *Hamakko* gen. nov. (Volvocales, Chlorophyceae). *Journal of Phycology*. 45: 482–492.
- Nakayama, T., Watanabe, S. & Inouye, I. 1996. Phylogeny of wall-less green flagellates inferred from 18SrDNA sequence data. *Phycological Research*. 44: 151–161.
- Nozaki, H., Ohta, N., Morita, E. & Watanabe, M. M. 1998. Toward a natural system of species in *Chlorogonium* (Volvocales, Chlorophyta): a combined analysis of morphological and rbcL gene sequence data. *Journal of Phycology*. 34: 1024–1037.
- O’Kelly, C. J., Watanabe, S. & Floyd, G. L. 1994. Ultrastructure and phylogenetic relationships of Chaetopeltidales ord. nov. (Chlorophyta, Chlorophyceae). *Journal of Phycology*. 30: 118–128.
- O’Kelly, C. J., Kurihara, A., Shipley, T. C. & Sherwood, A. R. 2010. Molecular assessment of *Ulva* spp. (Ulvophyceae, Chlorophyta) in the Hawaiian Islands. *Journal of Phycology*. 46: 728–735.
- Ouvrard, D., Campbell, B. C., Bourgoïn, T. & Chan, K. L. 2000. 18S rRNA secondary structure and phylogenetic position of Peloridiidae (Insecta, Hemiptera). *Molecular Phylogenetics and Evolution*. 16: 403–417.
- Pascher, A. 1914. *Die Süßwasserflora Deutschlands, Österreichs und der Schweiz. Heft 6: Chlorophyceae III. Ulothrichales, Microsporales, Oedogoniales*. Gustav Fischer, Jena, Germany, 250 pp.
- Pascher, A. 1931. Systematische Übersicht über die mit Flagellaten in Zusammenhang stehenden Algenreihen und Versuch einer Einreihung dieser Algenstämme in die Stämme des Pflanzenreiches. *Beihefte zum Botanischen Centralblatt*. 48: 317–332.
- Pickett-Heaps, J. D. 1975. *Green algae: Structure, Reproduction and Evolution in Selected Genera*. 1st. Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A., 606 pp.
- Printz, H. 1921. *Subaerial algae from South Africa*. Det Kongelige Norske Videnskabers Selskabs Skrifter, Vol. 1. 41 pp.
- Pröschold, T. & Leliaert, F. 2007. Systematics of the green algae: conflict of classic and modern approaches. In Brodie, J. & Lewis, J. [Eds.] *Unraveling the Algae: The Past,*

- Present, and Future of Algal Systematics*. Taylor & Francis, Boca Raton, Florida, pp. 123–153.
- Provasoli, L. & Pintner, I. J. 1972. Effects of bacteria on seaweed morphology. *Journal of Phycology*. 8: 10.
- Provasoli, L. & Pintner, I. J. 1980. Bacteria induced polymorphism in an axenic laboratory strain of *Ulva lactuca* (Chlorophyceae). *Journal of Phycology*. 16: 196–201.
- Rabenhorst, L. 1864. *Flora europaea algarum aquae dulcis et submarinae*, Vol. 1, Sect. I. Eduardum Kummerum, Lipsiae [Leipzig], 359 pp.
- Round, F. E. 1984. The systematics of the Chlorophyta: an historical review leading to some modern concepts (The taxonomy of the Chlorophyta III). In Irvine, D. E. G. & John, D. M. [Eds.] *The Systematics of Green Algae*. Academic Press, London, pp. 1–28.
- Round, F. E. & Crawford, R. M. 1990. Phylum Bacillariophyta. In Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. [Eds.] *Handbook of Protozoology*. Jones & Bartlett Publishers, Boston, pp. 574–598.
- Sanchez-Puerta, M. V. & Leonardi, P. I. 2006. *Pseudulvella americana* belongs to the order Chaetopeltidales (class Chlorophyceae), evidence from ultrastructure and SSU rDNA sequence data. *Journal of Phycology*. 42: 943–950.
- Schrank, F. v. P. 1783. Botanische Rhapsodien. *Der Naturforscher (Halle)*. 19: 116–126.
- Schultz, J., Maisel, S., Gerlach, D., Müller, T. & Wolf, M. 2005. A common core of secondary structure of the internal transcribed spacer 2 (ITS2) throughout the Eukaryota. *RNA*. 11: 361–364.
- Siler, C. D., Diemos, A. S., Linkem, C. W., Diemos, M. L. & Brown, R. M. 2010. A new species of limestone-forest frog, genus *Platymantis* (Amphibia: Anura: Ceratobatrachidae) from central Luzon Island, Philippines. *Zootaxa*. 2482: 49–63.
- Silva, P. C. 1982. Chlorophycota. In Parker, S. [Ed.] *Synopsis and Classification of Living Organisms*, Vol. 1. McGraw-Hill, New York, pp. 133–161.
- Sonnenberg, R., Nolte, A. W. & Tautz, D. 2007. An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. *Frontiers in Zoology*. 4: 6.
- Stewart, K. D. & Mattox, K. R. 1975. Some aspects of mitosis in primitive green algae: phylogeny and function. *Biosystems*. 7: 310–315.
- Thomson, E. & Tollervey, D. 2010. The final step in 5.8S rRNA processing is cytoplasmic in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*. 30: 976–984.
- Trainor, F. R., Rowland, R. C., Lylis, J. C., Winter, P. A. & Bonanomi, P. L. 1971. Some examples of polymorphism in algae. *Phycologia*. 10: 113–119.
- Turmel, M., Brouard, J.-S., Gagnon, C., Otis, C. & Lemieux, C. 2008. Deep division in the Chlorophyceae (Chlorophyta) revealed by chloroplast phylogenomic analyses. *Journal of Phycology*. 44: 739–750.
- Turmel, M., Gagnon, M. C., O’Kelly, C. J., Otis, C. & Lemieux, C. 2009. The chloroplast genomes of the green algae *Pyramimonas*, *Monomastix*, and *Pycnococcus* shed new light on the evolutionary history of prasinophytes and the origin of the secondary chloroplasts of euglenids. *Molecular Biology and Evolution*. 26: 631–648.

- Uspenskaja, W. J. 1930. Über die Physiologie der Ernährung und die Formen von *Draparnaldia glomerata* Agardh. *Zeitschrift Botany*. 22: 337–393.
- Vischer, W. 1933. Über einige kritische Gattungen und die Systematik der Chaetophorales. *Beihefte zum Botanischen Centralblatt*. 51: 1–100.
- Watanabe, K. I., Ehara, M., Inagaki, Y. & Ohama, T. 1998. Distinctive origins of group I introns found in the COXI genes of three green algae. *Gene*. 213: 1–7.
- West, G. S. & Fritsch, F. E. 1927. *A Treatise on the British Freshwater Algae*. Cambridge Univ. Press, Cambridge, UK, 534 pp.
- Wheeler, Q. D. & Meier, R. D. 2000. *Species concepts and phylogenetic theory: a debate*. Columbia Univ. Press, New York, 230 pp.
- Whitford, L. A. 1960. *Heterodictyon planctonicum* L. Whitford and *Chlorosaccus fluidus* Luther: Further notes and corrections. *Transactions of the American Microscopical Society*. 79: 227–229.
- Wille, N. 1901. Algologische Notizen VII, VIII. *Nyt Magazin for Naturvidenskapens*. 39: 1–24.

Curriculum vitae

Name: Lenka Caisová
University of South Bohemia, Faculty of Sciences
Branišovská 31
České Budějovice
CZ – 370 05
Czech Republic
e-mail: lcaisova@gmail.com

Education

- 2002 – 2005 University of South Bohemia, Faculty of Biological Sciences: Bc. studies, Biology, bachelor thesis, Přehled větvených vláknitých řas vybraných lokalit jižních Čech a porovnání jejich variability [A review of branched filamentous green algae in selected locations in South Bohemia and a comparison of their variability]; supervisor Prof. RNDr. Jiří Komárek, DrSc.
- 2005 – 2007 University of South Bohemia, Faculty of Biological Sciences: MSc. studies, Biology, diploma thesis, Taxonomie rodu *Stigeoclonium* v České Republice [A taxonomy of the genus *Stigeoclonium* in the Czech Republic]; supervisor Prof. RNDr. Jiří Komárek, DrSc.

Professional experience

- 2005 – present Researcher; Institute of Botany v.v.i., Czech Academy of Sciences of the Czech Republic, Třeboň, CZ – 379 82, Czech Republic (part-time employment).

Membership in scientific organizations

Czech Algological Society (CAS)
Phycological Society of America (PSA)

International cooperation

- 2006, 2007 biological survey in Altai, Kazakhstan, Irbis project (3 months)
- 2008 archeological survey in Belo Horizonte, Brasil, Lagoa Santa project (4 weeks)
- 2008 Experimental Phycology and Culture Collection of Algae (SAG), Georg-August-Universität Göttingen (Prof Dr. Thomas Friedl), Germany, Sokrates/Erasmus program (3 months)
- 2009 The Culture Collection of Algae at the Botanical Institute of the University at Innsbruck (Dr. Georg Gärtner), Austria (1 week)

- 2009 Culture Collection of Algae and Protozoa (CCAP), (Dr. Thomas Pröschold), Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Dunbeg by Oban, Scotland (4 weeks)
- 2009 – 2011 Botanisches Institut – Universität zu Köln, Cologne (Prof. Dr. Michael Melkonian), Germany (19 months)

Publications:

- Caisová, L.** 2006. *Pleurocapsa cuprea*, originally described as blue – green alga, is a eukaryotic alga similar to the species *Hildenbrandia rivularis* (Rhodophyta) *Czech Phycology*. 6: 69–76.
- Caisová, L.** 2007. Diversity of Cyanophyta and Algae of the Katon-Karagay National Park Territory (East Kazakhstan). In: *Modern Approaches to Biodiversity Protection in the Context of Steady Development Achievement of Republic Kazakhstan, International Science Conference Papers*. East-Kazakhstan State University, Uskamenogorsk, pp. 46–52.
- Caisová, L.** 2008. Sinice a řasy na kůře stromů [Cyanobacteria and algae growing on the bark tree]. *Veronica*. 22: 9 pp.
- Caisová, L.**, Husák, Š. & Komárek, J. 2008. *Nitella mucronata* (Br.) Miquel (Charophyta) in the Czech Republic. *Fottea*. 8: 105–107.
- Caisová, L.** & Kopecký, J. 2008. Relation of "*Pleurocapsa cuprea*" to the genus *Hildenbrandia* (Rhodophyta). *Phycologia*. 47: 404–415.
- Zapomělová, E., Hrouzek, P., Řeháková, K., Šabacká, M., Stibal, M., **Caisová, L.**, Komárková, J. & Lukešová, A. 2008. Morphological variability in selected heterocystous cyanobacterial strains as a response to varied temperature, light intensity and medium composition. *Folia Microbiologica*. 53: 333–341.
- Caisová, L.**, Bešta, T., Chlachula, J., Komárek, J. & Husák, Š. 2009. Taxonomic investigations of cyanobacterial and algal flora from the Southern Altai, East Kazakhstan. *Biodiversity Research and Conservation*. 15: 13–22.
- Caisová, L.** & Gąbka, M. 2009. Charophytes (Characeae, Charophyta) in the Czech Republic: taxonomy, autecology and distribution. *Fottea*. 9: 1–43.
- Caisová, L.**, Marin, B., Sausen, N., Pröschold, T. & Melkonian, M. 2011. Polyphyly of *Chaetophora* and *Stigeoclonium* within the Chaetophorales (Chlorophyceae), revealed by sequence comparisons of nuclear-encoded SSU rRNA genes. *Journal of Phycology*. 47: 164–177.

Projects

- 2009 The phylogeny of ecologically important polymorphic filamentous green algae, 038/2008P GAJU - Grant Agency of the University of South Bohemia (GA JU), awarded to – L. Caisová

2009 – 2012 The phylogeny of polymorphic filamentous green algae, Grant Agency CR no 206/09/0697, awarded to Prof. RNDr. Jiří Komárek, DrSc.

International conferences and meetings

- 2009 9th International Phycological congress in Tokyo (Japan), **Caisová, L.**: Taxonomy of the genus *Rhexinema* (Ulvophyceae) based on phylogeny of the 18S rRNA and morphology, poster presentation.
- 2009 13th Evolutionary Biology Meeting at Marseilles (France): **Caisová, L. & Kopecký, J.**: Relation of *Pleurocapsa cuprea* Hansgirg to the genus *Hildenbrandia* (Rhodophyta), oral presentation.
- 2010 13th Wissenschaftliche Tagung der Sektion Phykologie, Insel Reichenau im Bodensee (Constance), **Caisová, L., Marin, B. Sausen, N., Pröschold, T. & Melkonian, M.**: Do traditional morphological features correspond to phylogenetic relationships of distinct genera of the order Chaetophorales?, oral presentation.
- 2011 59th Annual meeting of the British Phycological Society at Cardiff (Wales), **Caisová, L., Marin, B. & Melkonian, M.**: Is ITS2 evolution linked to speciation? A comparative analysis of the Ulvales as a case study, oral presentation.

Other activities

- Summer term 2008 Phycology, practice (teaching, University of South Bohemia)
- June 2008 Determination course for limnologists and hydrobiologists (Czech Algological Society) – oral presentation: Morphotypes of chaetophoralean genera *Chaetophora*, *Draparnaldia* and *Stigeoclonium* and their determination, Chlum u Třeboně, Czech Republic.
- February 2009 Determination course for limnologists and hydrobiologists (Czech Algological Society) – oral presentation: Green algae in the Czech Republic. Brno, Czech Republic.
- April 2009 Phytobenthos (Czech Algological Society) – oral presentation: Red algae and green filamentous algae – their occurrence in the Czech Republic. Vyškovec, Czech Republic.