



**CHARLES UNIVERSITY IN PRAGUE**

**FACULTY OF SCIENCE**

**DEPARTMENT OF BOTANY**

**Address for correspondence:** Benátská 2, CZ-128 01 Praha 2, Czech Republic

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Review report on PhD thesis entitled

‘SYSTEMATICS OF COCCAL GREEN ALGAE OF THE CLASSES CHLOROPHYCEAE AND TREBOUXIOPHYCEAE’

by

Lenka Štenclová

Lenka Štenclová submitted a PhD thesis which represents an important step towards clarification of the taxonomy of selected green coccoid algae. The thesis consists of the introduction summarizing the green algal systematics, and four manuscripts being either published or submitted for publication. All four papers are scientifically sound and relevant to an international audience. Therefore, I recommend the thesis for the defense.

Below I summarize my specific comments to particular chapters:

The introduction presents a historical overview of green algal systematics, well summarizing the actual problems in the concept of genera and species. It also summarizes the most important results of four scientific papers. Unfortunately, the language is rather poor, with a number of mistakes (e.g., wrong sentence structure, subject-verb disagreement, missing prepositions, typing errors, wrong verb form). In some cases, it is even hard to follow the exact meaning of certain sentences, and I have a feeling the Introduction was written in a hurry without a careful reading.

The chapter two represents a nice taxonomic study improving the systematics of the family Oocystaceae, combining morphology, ultrastructure and genetic data of more than 50 cultured strains. The only reservation I have concerns the selection of phylogenetic tree presented in the main text (concatenated SSU rDNA + rbcL phylogram), lacking a number of taxa discussed in the paper (e.g., the genus *Elongatocystis*). I am just wondering why the much complete phylogram is not shown (e.g., the phylogram inferred from the concatenated data set of complete SSU rDNA data and partially filled rbcL data).

The third chapter (a submitted manuscript) deals with the phylogenetic position of the genus *Nephrocytium*, forming a new family within Sphaeropleales. The results and conclusions are well supported by presented data. I fully trust the authors concerning the distinction of a newly described species from all previously described taxa. Nevertheless, it would be great to see a figure plate presenting the drawings of all *Nephrocytium* taxa, since their delimitation is not so simple keeping in mind quite extensive morphological plasticity. In addition, I found several minor mistakes in the manuscript, e.g., missing support values in the phylogenetic trees or specifying a basionym in *N. lunatum* (page 113).

The fourth chapter (a recently submitted manuscript) is a thorough taxonomical study dealing with the systematics of selected crucigenioid algae. Again, the presented conclusions are well supported by morphological, ultrastructural and genetic data. On the other hand, the discussion is confusing in some parts. For example, in Results *Crucigeniella apiculata* is moved to the genus *Crucigenia*, proposing a new combination *Crucigenia apiculata* (page 167). However, in discussion the strain CCAP 218/4 is proposed to be labelled as *Crucigeniella apiculata* (page 173), which is in obvious contradiction with the proposed taxonomic revision. In addition, it would be nice and very beneficial to summarize morphological differences among particular crucigenioid genera by a table

or a figure plate. These information are scattered throughout the text. Several minor mistakes include decimal commas in the phylograms or keeping identical sequences for Bayesian inferences resulting in false branch lengths. Finally, it is strange to write “we” in a single-author paper. Accordingly, it is curious to see the first sentence in Acknowledges stating “We owe my thanks to...”.

The final chapter is a well-written paper clarifying the phylogenetic placement of *Dispora speciosa*. I highly value the authors not only discuss the taxonomical issues, but present their findings in a broad evolutionary context, focusing on a morphological simplification in a number of green algal lineages.

#### Questions:

- 1) What is the type species of *Oocystis*? Though Řeháková (1969) proposed *O. lacustris* as a type, her proposal appears to have been largely ignored. Subsequent studies, incl. Algaebase and INA databases, mention *O. nägelii* as a type.
- 2) The chapter four deals with the taxonomy and phylogenetic position of the genus *Komarekia*, which is recognized as a valid, well-defined genus in Chlorellaceae. The genus was established in 1981 by Fott, with a type species *K. appendiculata*, formerly described as *Hofmania appendiculata*. However, the genus *Hofmania*, with a type *H. appendiculata*, was described already in 1900 and has therefore a priority over *Komarekia*. If the type of *Hofmania* is a member of *Komarekia*, why *Komarekia* is not a junior synonym of *Hofmania*? Unfortunately, this issue is not discussed in the manuscript.
- 3) On page 16, Lenka writes “Problematic situation occurs when several markers give contradictory hypothesis, which is usually caused by separated evolution of nuclear and chloroplast genomes”. How the nuclear and chloroplast genomes can undergo separate evolution in green algae? These genomes are tightly linked together since the primary endosymbiosis event.
- 4) I fully agree with Lenka’s statement on page 15 mentioning limitations of molecular phylogenetics: “Molecular phylogeny is useful tool to describe the natural variability, which is more or less continual. Borders of species, genera and higher taxonomic unites are still quite arbitrary.” However, on page 169 and so on, she repeatedly writes that “for a more proper species concept (...) it would be suitable to (...) apply the ITS2 CBC approach.” In fact, this concept is highly arbitrary, as well, specifying species borders by just the presence or absence of compensatory bases in the ITS2 molecule, which is unlinked to any speciation processes. How can the CBC approach help us to better define species boundaries?

PSD

Pavel Škaloud  
Charles University, Department of Botany  
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