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Review of the doctoral thesis "Genome size variation in microalgae and its evolutionary consequences" by Mgr. Dora Čertnerová

The thesis investigates genome size variation and methods for measuring genome size in microalgae. It is organized into a general summarizing part providing an overview on the research topic and background, the methodology and key results and a second part consisting of publications arising from the research.

The thesis comprises five publications which are already published (one in press) in good quality peer-reviewed journals (impact factors between 3,5 and 5). For all five publications the contribution of the candidate was 80% or higher and particularly included study design and literature survey as well as manuscript preparation.

Two of the papers (paper 1 and 2) are review-like and focus on methodical aspects, i.e. protocols for measuring genome size using flow cytometry including best practice recommendations.

The third paper again focusses on protocols for genome size estimation. Here different protocols are applied for several strains of microalgae and the results are compared. The discussion does not illuminate reasons behind the differential success of the different protocols tested. This paper therefore presents interesting results and reports genome size estimates for different microalgae. A generalization regarding the preferential methodology in future studies on other algae is, however, not addressed

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26.08.2022

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in detail. I could also not find comments of replication of the different experiments/protocols.

Paper 4 reports variation of intraspecific genome size variation for *Synura petersenii* and paper 5 deals with genome size variation over time within distinct strains. The analyses presented in paper 4 are extensive and the number of investigated strains (131 strains) exceeds by far the depth of investigation for intraspecific variation in genome size in microalgae in the literature. This contribution and the findings of considerable intraspecific variation therefore significantly adds to our understanding of this topic. Similarly, paper 5 deals with changes of genome size within strains over time. The main observation in this study is a doubling / having of genome size which is interpreted as indication for a synchronizes generation cycle in the cultures, i.e. of meiosis and zygote formation respectively. The analyses are again sound and the data point to the presence of a generation cycle in unicellular microalgae which again is a novel in interesting finding.

All the different contributions are scientifically sound and provide a substantial progress in microalgal genome size analysis and genome size evolution. The review and test of protocols for genome size analysis and its application for microalgae summarizes the current knowledge, identifies knowledge gaps and provides a robust basis for further development of methods for future studies on microalgal diversity and genome evolutions. This is one of the first studies that successfully analyses genome size variation within and among microalgal strains and thereby paves the ground for a successful application of genome size analyses to research topics such as species delimitation, (cryptic) generation cycles in microalgae and genome size evolution. The thesis clearly demonstrates that Dora Certnerová is developing into an international competitive scientist in the field. The scientific quality of the thesis is high and the thesis is well written with respect to phrasing and stylistic issues. Statements are very clear and comprehensive. The literature review is comprehensive.

I therefore consider the extent and the quality of the thesis as extensive and fully adequate as thesis for the award of doctoral degree.

I consider several aspects worth of discussion during the defence:

At a first glance, results of these two papers seem in part to contradict each other. In particular, paper 5 states that "with the exception of a single species (S. macropora, see below), DNA content variation within and among conspecific strains was not random but corresponded to different ploidy levels". In contrast, paper 4 states that "genome size ranged continuously from 0.97 pg to 2.02 pg of DNA across the investigated strains" within one species. The contribution of potential methodical inaccuracies, of intraspecific genome size variation and of ploidy shifts to overall observed variation in genome size measurements within species is certainly worth discussing in more detail.

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Further, potential reasons for the failure of different extraction protocols for distinct species are not entirely clear, i.e. to what extent the results allow for predicting suitable protocols for further strains.

The findings of considerable intraspecific genome size variation further question the validity of species boundaries, possibly also to the applied species concept in protists. This latter issue has numerous implications related to the practice of species delimitation and the species category and their congruence with the basic conceptual ideas of species.

/(Prof. Dr. Jens Boenigk)