MUNI
SCI

Review on the Ph.D. thesis "Genome size variation in microalgae and its evolutionary consequences " submitted by Dora Čertnerová

PhD thesis by Dora Čertnerová consists of five published papers in well-established WoS-indexed journals. In all these papers the candidate is the first or only author. These five main chapters are put in the general methodical and evolutionary context by the introductory part also written by the candidate.

The thesis is well written and clearly organized in a logical sequence. Its principal strength is in a wide spectrum of relevant methods, ranging from field sampling – plant identification based on a good knowledge of the studied model group - microalgae, sample preparation for molecular and cytometric analyses, laboratory techniques of DNA isolation, purification and amplification, algae cultivation techniques, chlorophyll fluorescence measurement, statistical analyses and, particularly, sophisticated flow cytometric analyses.

The thesis (i) brought many new analytical data on genome sizes in a group of microalgae, (ii) summarized the currently used protocols in flow cytometry of algae, (iii) developed and tested new own flow cytometric protocols, (iv) described substantial intraspecific genome size variation in *Synura petersenii*, and (v) identified a haploid-diploid life cycle with isomorphic stages in unicellular algae for the first time.

In summary, the candidate has demonstrated very good theoretical knowledge, practical skills, independent critical thought, and high motivation for research work. All specific aims of the thesis have clearly been accomplished. The thesis of Dora Čertnerová meets or exceeds the standard criteria necessary for obtaining the University Ph.D. degree in botany. I consider it eligible for the defense without any reservation.

To clarify some aspects related to the subject of the PhD Thesis, I have the following **questions or comments for the candidate**:

(1) In Paper 1 as well as in Paper 4, you have mentioned a range of variation in genome size across microalgae --- more than 28 thousand-fold (page 44 or 81, respectively). I was surprised by such a broad range in algae genome size variation, much broader than that in land plants or animals. However, in the algae genome size database (https://cvalues.science.kew.org/search/algae), the reported range is

Masarykova univerzita, Přírodovědecká fakulta, Ústav botaniky a zoologie

Kotlářská 2, 611 37 Brno, Česká republika
T: +420 549 49 3351, E: bures@sci.muni.cz, botzool.sci.muni.cz

more than ten-times narrower. Similarly, the genus with the putatively largest genome reported in your Paper 1 – *Valonia* – has a genome more than hundred-times smaller than the 286 pg reported in your paper, as can be seen from the original paper by Karaun (2005) you cited here. Can you explain this discrepancy?

(2) Among the methodological recommendations in the final notes in Paper 1, you also stated that: „In case of any uncertainty regarding life cycle stage of the analysed microalgal samples, report in publications genome size estimates in absolute units (e.g. pg · cell-1) rather than attempt to assign it to 1C/2C-value.“ (p. 47). It is slightly inaccurate because 1C/2C-value is also in absolute units. On the other hand, the opposite term "relative or arbitrary units" has a specific meaning in flow cytometric community. It would be more correct to write, "In case of any uncertainty regarding life cycle stage, don't forget to emphasize this information in tables and everywhere in the text where you report DNA content."

(3) Flow cytometry allows us not only to measure nuclei but also to count them. In your study on intraspecific genome size variability in *Synura petersenii* (Paper 4), you inferred a potentially adaptive evolution of genome size based on correlative analyzes of the karyoplasmic ratio and the inverse relationship between the genome size and the growth rate. Unlike animals or plants, where such correlative analyzes are often performed, the combination of (i) single cell, (2) short life cycle, and (3) suspension cultivation enables us to test the adaptive potential of genomic changes experimentally in microalgae (or at least in some of them). Have you considered competition experiments with some other single-cell species of this group in which you would mix strains with different genome sizes and expose them to conditions that might favor larger/smaller genomes (e. g. higher concentrations of nitrogen and phosphorus in the cultivation medium)? In real-time, you could observe whether the ratio in the number of nuclei changes in the peaks representing the genome size fraction in such a suspension in real time or how this ratio responds to ongoing changes in these nutrients.

(4) As a negligible issue, I consider using the term “polyphyletic origin” because it sounds to me like a *contradictio in adjecto* since any polyphyletic group is a human construct and thus does not have any own origin except that in the human brain that cannot be neither monophyletic, paraphyletic or polyphyletic. Unfortunately and despite this, the term "polyphyletic origin" is quite commonly used in the scientific community.

Brno 10/Sep/2022 Prof. RNDr. Petr Bureš, Ph.D.

Masarykova univerzita, Přírodovědecká fakulta, Ústav botaniky a zoologie

Kotlářská 2, 611 37 Brno, Česká republika
T: +420 549 49 3351, E: bures@sci.muni.cz, botzool.sci.muni.cz