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**Review of the PhD thesis of Chunlian Li, MSc**  
*Use of molecular tools (Multi-Gene Approach) in building a cladistic  
framework of selected families of araphid diatoms*

prepared under the scientific supervision  
of Prof. dr hab. Andrzej Witkowski and co-supervision of Dr. Matt Peter Ashworth

I find that the thesis presents a set of new and important results and it is up to the standards that one should uphold for a PhD thesis. My detailed report is below.

The basis for issuing the opinion are the conditions set out in Article 13 of act. 1 of March 14, 2003 on Academic Degrees and Academic Title, and on Degrees and Title in the field of art (Dz. U. 2013, nr. 65 poz.595 z póź. zm). I was asked to report on the doctoral thesis, which was prepared in the Faculty of Geosciences of University of Szczecin, Poland, by Chunlian Li, MSc. The doctoral thesis, entitled – *Use of molecular tools (Multi-Gene Approach) in building a cladistic framework of selected families of araphid diatoms* consists of two publications, was made under the supervision of Professor Dr. hab. Andrzej Witkowski (Palaeoceanology Unit of University of Szczecin, Poland) and co-supervision of Dr. Matt Peter Ashworth (University of Texas at Austin, USA).

The doctoral thesis presented for the review includes authors statements, acknowledgement, summary, and three chapters – introduction, material and methods and two publications. The additional bibliography comprises over 90 items.

The results obtained so far have seen publication as an article (1) or was submitted to prestigious journals (2):

- 1) Li, C.L., Ashworth, M.P., Witkowski, A., Lobban, C.S., Zgłobicka, I., Kurzydłowski, K.J. & Qin, S. (2016). Ultrastructural and molecular characterization of diversity among small araphid diatoms all lacking rimoportulae. I. five new genera, eight new species. *Journal of Phycology* 52(6): 1018–1036.
- 2) Li, C.L., Witkowski, A., Ashworth, M.P., Dąbek, P., Sato, S., Zgłobicka, I., Witak, M., Khim, J.S. & Jung, C.J. (2018). The morphology and molecular phylogenetics of some marine diatom taxa within the Fragilariaceae, including twenty undescribed species and their relationship to *Nanofrustulum*, *Opephora* and *Pseudostaurosira*. *Phytotaxa*, (accepted for publication).

The entire doctoral thesis covering the proper substantive part and the complementary part is over 240 pages. In accordance with the statutory requirements for doctoral theses, a thesis should “constitute an original solution to the scientific problem (...) and demonstrate general theoretical knowledge of a candidate in a given discipline”. With this in mind, when assessing the doctoral thesis of Chunlian Li, MSc, the following criteria were adopted: relevance of the subject matter, correctness in formulating the research goals and hypotheses and methodology of the research.

The importance of the subject matter

The work carried out by Li, MSc covers an interesting area of contemporary research in phycology - the study of morphological ultrastructure and molecular phylogenetics of selected, very small-celled araphid diatoms. Diatoms (Bacillariophyta) constitute one of the most diverse and ecologically important group of algae. Amongst them, the smallest (c.a. 10-20  $\mu\text{m}$  long) are believed to be the most species-rich group of diatoms. Furthermore, although diatom distributions have been explored extensively in numerous morphological studies, they have predominantly focused on the freshwater of the Northern Hemisphere. This has caused confusion among the published results of various works. For example, intensive research of marine flora for the past 30 years has clearly indicated the high level of underestimated diversity of the small-celled diatoms associated with this environment. However, the

characterisation of diatom diversity and ecology requires accurate and consistent taxonomic identification.

The importance and diversity of diatoms are clearly presented in the introduction of the work. Their origin, classification system and phylogenetic systematics are based on the latest publications. The phylogenetic relationships of populations representing *Opephora*, *Pseudostaurosira* and *Nanofrustulum* were studied using a combination of nucleotide sequence data (SSU rDNA, *rbcL* and *psbC*) and qualitative and quantitative morphological characteristics of the silica cell wall gathered primarily from observation by LM and SEM. There is a growing interest in these topics of broadly understood phylogenetic and taxonomic relationships among diatoms. Due to the DNA sequence data well-recognition, as well as perception of different characteristics of diatomaceous shells, several new taxa were distinguished by Li MSc (eight new species in the publication in *Journal of Phycology* and twenty species in *Phytotaxa*). The new for science species belong largely to five established new genera - *Castoridens*, *Cratericulifera*, *Gedaniella*, *Hendeyella* and *Serratifera*. Chunlian Li, MSc is the co-author of other genera e.g. *Orizaformis* in other publications. Based on morphological studies, dichotomic keys for similar genera and species were proposed within *Gedaniella* and *Serratifera*. It is worth noting that the publications present the most complete picture of the Fragilariaceae phylogenesis to date, and in particular of small cellular phylogeny. This is the first effort to solve the taxonomic problem of staurosiroid diatoms, in particular in *Nanofrustulum*, *Pseudostaurosira* and *Opephora*, proposing descriptive signs for each of the above-mentioned types.

The research problem, main goal of the thesis and research hypotheses of the PhD student are presented in the introduction. The research problem that emerged was formulated: "one major aim of this thesis is intended to look for clear, shared derived ('synapomorphic') characters to distinguish small-celled diatoms and make them more easily recognized and interpreted by ecologists". The specific research problem assigned to the study conducted by Li, MSc is based on the hypothesis that following potentially synapomorphic characteristics might help in distinguishing some difficult to identify small diatoms: stria morphology (areolae and their position on the valve face and mantle), the presence of 'warts' on the valve face, and marginal spines and the shape of the sternum (*Opephora*, *Pseudostaurosira*, *Nanofrustulum*). Additionally, DNA sequence data were used to try to resolve the phylogenetic relationships of small-celled genera. Detailed reading of the thesis by Chunlian Li, MSc authorises me to state that the main goal was formulated correctly, clearly defining the direction of the studies. I believe that in the context of the research problem and the

objectives of the thesis, the hypothesis was correctly made. The PhD student clearly defined the direction and scope of the research, and her empirical verification enabled her to accomplish the assumed research goals.

As Chunlian Li, MSc, correctly points out, morphology and molecular phylogeny seem to be the only way forward in present day systematic taxonomy. Moreover, they form a detailed autecological basis that is in great need for applied works e.g. estimation of indicative diatom values. Considering the above, the choice of the subject of the doctoral thesis by C. Li, MSc should be considered to be a correct one. Due to the insight and multifaceted recognition issues, this work provides a host of valuable information about key phylogenetic and taxonomic values of the studied diatom group.

The research methodology is clearly described and illustrated. Working methods have been divided into subsections describing the material collection (from around the world), taxa cultivation, DNA extraction, PCR amplification and LM and SEM examination. Next, the phylogenetic analyses are presented along with the several software programs used for them. This section concludes with a very short subunit concerning terminology (only nine basic characters are explained).

The published works provide answers to the questions posed by C. Li, MSc. The publications contain data from the methodology for studied diatoms to information resulting from DNA sequence data analysis and phylogenetic relationships of small-celled, araphid diatom genera. I do not review the publications here because the articles were evaluated by both journal editors and peer reviewers. The fact that the manuscripts were accepted and published confirms that they were highly rated. The quality and importance of these publications by C. Li, MSc are positively appreciated by scientists from different fields - taxonomists and individuals interested in marine biodiversity, as well as phylogenetics and individuals involved in the difficult taxonomy of diatoms, e.g. in bioindication. I hope that the mistake in the References of the second publication was corrected [Schumann (1867) and four other publications are before 'Sabbe'].

There are certainly open questions raised by the thesis, for example the use of a net of 20  $\mu\text{m}$  size (why not 10  $\mu\text{m}$ ?) for the material collection, lack of data in the presented work about the diversity of the small-celled araphid diatoms in the material collected (are they all identifiable by means of LM?) and change (or not) of the frustules morphology during keeping them in a culture. I regret that so little has been said about cryptic species. Can we expect morphological traits that allow distinguishing (even with the help of SEM) taxa that appear as sister clades? I mean here about, for example, *Pseudostaurosira brevistriata* in the

first publication (p. 1033, fig. 10). But it is natural for such questions to arise given the amount of new material presented, and further work will clarify such issues. The presentation of only two publications as a doctoral thesis may not seem to be a significant achievement. However, the journals in which they appear add a great importance to them in the diatomological world. In my opinion, the publications by C. Li, MSc have made important contributions to our knowledge of small araphid diatoms from littoral marine waters of the world. Moreover, her works of taxonomy, phylogeny and ecology are a 'marriage' of necessity.

All things considered, the results presented in the publications are impressive due to the breadth of the topics covered and the range of problems addressed, from classic taxonomy through phylogeny to autecology and biomonitoring, each of which needs an excellent knowledge of diatoms. All of these considerations conspicuously point to the same conclusion: Chunlian Li, MSc has clearly demonstrated an ability for creative and interesting scientific work. The presented results are up to the standards that one should uphold for a PhD thesis.

Sincerely,



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