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4 January, 2024

Dr. Tomas Herben
Department of Botany
Faculty of Science
Charles University
Benatska 2
12800 Praha 2
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Dear Dr. Herben:

I here offer an opposition report on the Habilitation thesis for Dr. Jana Kulichová.

The Habilitation dossier for Dr. Kulichová for the thesis entitled "What can morphology tell us about evolution and ecology of diatoms?" includes 12 articles published and 1 submitted over a 13-year period. Groups of these articles have some logical organization, the majority of which are focused on morphometric approaches to documenting ontogenetic (size diminution) sequences of species and others apply molecular approaches to understanding variation in terms of ecology (which is likely a better term than 'biogeography' in the submitted book chapter). These papers are all published in reputable (rigorous, peer-reviewed), mostly international journals (except the aforementioned book chapter); some of them (those in *Protist*) are in higher impact journals. The papers have moderate citation rates (the candidate's H Index is 20 according to Google Scholar), appropriate for the discipline-focused nature of the papers. In some ways one can view the approach taken in these papers as a reaction to what the candidate has called, "A...subjective view of diatom species...". There is a search to increase objectivity in the delineation of taxa, their variation, and relating that variation to external, environmental conditions.

It should first be acknowledged that all but one of the submitted articles have been published in journals through a peer-review process. The review process in journals such as *Diatom Research*, *Protist, Nova Hedwigia, Fottea* and *Cryptogamie*, *Algologie* are rigorous. Through that process the reviewer community has endorsed the methods, approaches and discussion of these studies, whether on "model" taxa or community-based studies of various lineages of taxa. In these publications, the candidate has posed interesting questions, and worked with a variety of cutting-edge tools (morphometrics/statistics and molecular) to study questions of, in the candidate's words, "Microevolution of our species complexes" as well as "Ontogenetic and ecological signals in the

morphology of diatom cell walls". The publications are in a diverse set of journals, and their publication in these different venues argues for the candidate's ideas being seen and supported across a diversity of reviewers and readership. This is evidence of strong community support for the work by the candidate.

For the study of microevolution, the body of work presented here has been hampered, in my view, by two challenges. While we should acknowledge the amount of work that must have gone into developing various primers for the molecular work, in the end it might be argued that the wrong tools were used for the questions posed. Microevolutionary studies are usually focused on within-species phenomena, yet the molecular tools applied to these questions have been used to study species-level questions and higher categories / lineage evolutionary patterns. The candidate argues this approach helped to recognize species and lineages, but data to pursue finer level events (within species) was still based on these tools used to identify higher level patterns. Second, little effort has been made by the candidate to explore relationships within a genus broadly, to make sure the "lineages" being investigated (and compared) are indeed closely related. And when that has happened, say in the "Pinnularia borealis" paper, there has been no effort made to relate the huge diversity encountered in that "species" into a workable taxonomy. I count only 3 new species published in the work of the candidate across these 13 papers. More might be expected given the amount of variation identified across these studies. In general, this second criticism might be couched as a lack of acknowledgement about evolutionary history (with the word "history" emphasized). While that is not the focus of the candidate's questions, history provides a framework and a context for the within-species (lineage) of interest. There seems to be no appreciation of that context in the research program as demonstrated in the write up or attached publications.

While the candidate acknowledges the different results in studies based on the use of different molecular tools, they do not acknowledge the subjectivity inherent in those approaches or the in use of morphometry-based tools. If this candidate's research program is indeed attempting to move past the subjectivity of species-level work seen with descriptive morphology, it would be useful to acknowledge where there is the potential for subjectivity in their own approaches, the challenges in the use of these tools and how those issues can be mitigated.

I found the overarching introduction to the Habilitation to be relatively superficial, especially in the background materials related to diatoms. The scenario of "centrics led to araphids which led to raphids" is couched in 19th century views of diatom relationships (that have been maintained in classifications systems into the 21st century). Again, while these topics have not been the focus of the candidate's research, the broad-brush strokes used in this introduction might be taken as lacking an appreciation for the issues at these other levels of hierarchy.

So, where does this progress and trajectory of the candidate's work suggest a future program might be headed? The questions of microevolution, community assembly, and dispersal on the

diversity of diatoms in general, and in the communities they form and are a part of (having important roles in the development, resilience and turnover in freshwater ecosystems) are important (especially as we face human-induced challenges for the global freshwater supply) for scientific research and society in general. They require well-designed research programs, using a diversity of approaches. The candidate's closing sentence in the thesis may suggest challenges for that. In summarizing the results of the work in this Habilitation, they write, "...the lineages of our species complexes have evolved in their own way[s] ..." suggesting that, to date, no particular patterns have been found across these studies of freshwater raphid diatoms. It would seem hard to convince funding agencies to support future studies of these types if indeed these new tools have yielded no data that can be summarized into describable patterns. In paper 7, the most recent from the candidate, there seems to be a lot of hope put on use of metabarcoding to create large volumes of data that might be used to sort out such patterns. Such approaches will require new research tools and collaborations (mostly in the bioinformatics realm). The candidate has demonstrated a willingness and ability to forge strategic partnerships, based on the number and breadth of experiences of her co-authors, and that will put them in good stead for future research activities.

Sincerely,

J. Patrick Kociolek, Ph.D. Professor, Department of Ecology and Evolutionary Biology Curator, University of Colorado Museum of Natural History