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September 15, 2017

Re: Ph.D. thesis "Diversity and biogeography of diplonemid and kinetoplastid protists in global marine plankton" by M.Sc. Olga Flegontova

The work under review ("Diversity and biogeography of diplonemid and kinetoplastid protists in global marine plankton" by M.Sc. Olga Flegontova) includes a comprehensive introduction to the topic, research objectives, a summary of the results and discussion, and a collection of 3 published (1 first-authored), and 2 unpublished (both first-authored) papers. The overall structure of the thesis is well organized and the work is presented in a concise and comprehensive manner.

The published papers represent a truly significant step forward in our exploration of diversity and biogeography of diplonemids. I personally consider it a very **rare paradigm-shifting scientific breakthrough** greatly expanding limits of our knowledge. This is further justified by high impact factor of both journals these papers were published in – 37.2 (!) for Science and 8.9 for Current Biology (2 papers). All data presented there are solid and very much bullet-proof.

The attached manuscript (in preparation) on EukRef is a bit harder to judge. The reason for that is simple - author has presented only 2 chapters of the manuscript stating that additional chapters were to be contributed by others. This makes it difficult to assess the "big picture". My specific questions regarding this work are following:

i) apparently not all the clades of trypanosomatids were present in your dataset and numerous taxa were clearly missing – e.g. *Kentomonas*, *Jaenimonas*, *Novymonas* and others. How can you explain that?

ii) I strongly disagree with a statement on page 113 "Due to extremely low divergence among their 18S rRNA genes, genera and species within Leishmaniinae cannot be distinguished based on this gene". Please comment.

iii) Clustering of *Phytomonas* with *Trypanosoma* spp. is very odd and, in my opinion, may reflect a problem in the alignment algorithm. Please comment.

In my opinion, the thesis would benefit from two additional things: i) two schematic trees illustrating phylogenetic relationships within kinetoplastids and diplonemids in the Introduction, and ii) a concise bullet-point summary (definitely not 4+ pages) of the main results presented in the thesis in relation to the research objectives.

The following 2 questions are of the general nature and intend to provoke some discussion.

1) Can you elaborate on potential experimental approaches (novel or old) which would allow studying diplonemids in the wet lab? I understand the cultivation of these creatures is still an issue.

2) What about species delimitation in diplonemids? One may consider current NGS-based approaches biased and not well-suited to answer this question.

In conclusion, the presented thesis is among the best I have ever reviewed and I strongly recommend M.Sc. Olga Flegontova to be awarded a Ph.D. title.

Sincerely,

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To
Prof. Miroslav Obornik m. p.
Head of the Committee for PhD studies
University of South Bohemia

Kaiserslautern 22. September 2017

Review for the PhD thesis presented by

Olga Flegontova, M. Sc.

in order to obtain

the degree of Ph.D. at the University of South Bohemia.

Diversity and biogeography of diplonemid and kinetoplastid protists in global marine plankton

The thesis presented by Olga Flegontova consists of three published papers, one submitted manuscript and one manuscript in preparation for publication. The overarching research goal was the analyses of biogeographic diversity patterns of planktonic marine diplonemids and kinetoplastids. Therefore, the Ph.D. candidate applied stat-of-the-art molecular biological techniques, data processing tools and statistical approaches. The data she has obtained in the framework of her thesis are of high quality and provided a significant advance of knowledge in the corresponding field of research. Not only the papers already published witness from the importance and quality of her contributions in this scientific discipline, but I am convinced that also the as yet unpublished chapters will find their ways into peer-reviewed journals with high impact factors.

The individual manuscripts and papers of this thesis are presented in section 5, and preceded by a nicely written introduction, which prepares the reader well for the subject and which provides all the relevant

information and literature to place this Ph.D. project in context to the current state-of-the-art in this field of research. The introduction provides the background to point out the significance of this study and it reveals our knowledge gaps regarding the investigated topic. It helps to understand the relevance of the obtained data and results. The goals of this study (section 2) emerge from the well-informed introduction section. In section 3, the most important and relevant findings are summarized and solidly discussed. It is obvious that the candidate knows her field of research 8and corresponding literature) very well and that she is able to interpret her obtained data solidly. She is also very capable of a critical discussion. The spectrum of methods used in this thesis was challenging and qualifies Olga Flegontova for a career in molecular ecology, but also in other related scientific disciplines.

The language style is superb, and the written thesis is well-prepared. The figures are of high quality and the way the data are described make it easy to understand these results. The methods are well-described and thus, easy to reproduce.

In summary, Olga Flegotova showed that she has everything, which is required of an independent young scientist with a promising career in this field (or related fields) of research. Therefore, I without hesitations recommend to accept this thesis of M.Sc. Olga Flegontova.

Prof. Dr. Thorsten Stoeck