

Reviewer's report on Ph.D. thesis of Pragya Tripathi

Thesis title: **“Investigation of the Subunit Composition of Mitochondrial Dehydrogenase Complexes, Putative Kinetochores, and Localization of Paraflagellar Rod Proteins in Marine Diplonemids”**

Ostrava, June 16th, 2023

The thesis under consideration is focused on the study of the most abundant and diverse marine microeukaryotes – diplomonads which for this reason with a high probability play a role in marine ecosystems. Phylum Diplomonadida are part of euglenozoans group, which also includes euglenids and kinetoplastids containing the human-pathogenic *Trypanosoma* spp. and *Leishmania* spp. The overarching topic of the thesis is to provide the functional and cell-biology studies of the type species *Paradiplomonas papillatum* (originally designated *Diplomonas papillatum*). The work combines several diverse methodological approaches starting from the first diplomonads nuclear genome and transcriptome sequencing and comprehensive analysis to the functional analysis of dehydrogenase complexes, as well as paraflagellar rod proteins and homologs of kinetochore proteins. I would like to state here that presented data significantly extended our understanding of marine Diplomonads biology.

The thesis itself is comprised of two main parts. The first part contains an introduction to the Phylum Diplomonadida which followed by the second part of 4 Chapters - 1 published, 1 submitted and 2 “in preparation” publications (the last



two publications are supplemented by an introduction, material and methods, results and discussion). Here I would like to comment on the formal aspect of the thesis.

1) Annotation is written from the first person while contribution in the third person - minor thing but grate on the ears.

2) Introduction has a number of shortcomings.

Content. I am lacking the background information about paraflagellar rod proteins and kinetochore proteins.

Overview of the known information is mixed up with the received results. For example, part "Genomic view of *P. papillatum*" includes the results of the first publication.

Figures. Figure 4 is not relevant to the text while Figure 5 is presented but not cited in the main body of the Introduction part. Figure 6 could be more informative.

References. Name of the species must be Italicized. 2020a and 2020b in citations (Škodová-Sveráková et al., 2020b) and (Škodová-Sveráková et al., 2020a) as well as (Tashyreva et al., 2022b) and (Tashyreva et al., 2022a) are not indicated in reference section the way with "a" or "b".

Improper citation. For example, the part started with “In *P. papillatum*, paramylon appears to be synthesized under nutrient-poor conditions...” should cite (Tashyreva et al., 2022).

Consistency in citation format should be kept.

3) I believe that the text should have been carefully proofread. The table content doesn't correspond the body of the thesis. Add the space between headings, remove the repetition. Name of the species must be *Italicized* in Conclusions. The first part of conclusion written as a story-telling and it doesn't sound as the achieved data. Figure 3 in Conclusions looks meaningless and improper cited.

The rest of my comments and questions will focus on unpublished works (Chapter 3 and 4). Discussion parts in these Chapters make a good impression: explain the results, pointed out unanswered questions and give the future direction. However, most of the figure need improvement except IFA results. The text says “The mechanisms by which proteins are targeted to the flagella are complex, as they do not employ N-terminal target peptides, but instead rely on small, specific associations with the intraflagellar transport apparatus”. Could you please elaborate more why only C-tagging was performed for all proteins?

What is known about Intraflagellar Transport signal sequences? Would it be useful to present alignment with homologous Euglenozoa proteins?



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All in all, the work makes a substantial contribution to our knowledge of Diplonemids and I recommend the committee to award Pragya Tripathi the title of Ph.D. provided that oral defense is successful.

Ing. Natalya Kraeva, Ph.D.

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