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Review of the doctoral thesis of Pragya Tripathi '*Investigation of the subunit composition of mitochondrial dehydrogenase complexes, putative kinetochores, and localization of paraflagellar rod proteins in marine Diplonemids*'

The PhD thesis of Pragya Tripathi deals with several different aspects of the physiology of *Paradiplonema papillatum*, the model organism for diplonemids. These flagellate protists have been recently revealed as one of the most diverse and abundant groups of eukaryotes in the world oceans. The topic of the thesis is thus very timely and attractive. Although the thesis is a kind of compilation of different topics, all concern unique features of diplonemids making Pragya's work relatively well-focused.

The thesis comprises a literature review, one publication, two manuscripts, unpublished results and a conclusion/discussion chapter. The introduction summarizes the current knowledge of the diversity and morphology of diplonemids and provides a description of genomics and metabolism aspects of these organisms. It contains relevant and updated information but, taking into account the broad spectrums of topics the thesis is dealing with, the introduction is rather short (I guess that it has no more than 15 standard A4 pages). Specifically, the description of the unusual *P. papillatum* metabolism could be more detailed for non-experts and I would welcome a more informative scheme (or schemes) than is Fig. 6. This picture does not help much to understand the terms and enzyme names used in the text. No information is provided about the structure of kinetochores and the structure/function of paraflagellar rod although the thesis is aimed at these topics. The introduction contains quite a few typos and errors but not in a number disturbing the reading.

Pragya is a co-author of the paper published in BMC Biology, which provides a detailed analysis of the *P. papillatum* genome. I have no doubt this is an excellent work and it should be attached to the thesis. I would however recommend including only a link to the supplementary information file, as it has more than 100 pages. Pragya's contribution to this work was apparently not so significant and this huge supplement just makes the thesis very long (260 pages).

The manuscript of Záhonová et al, describing the subunit composition of dehydrogenase complexes, has been resubmitted to BBA-General Subjects. Pragya shares the first authorship with the other two authors. Although this publication is formally acceptable as the 'first-author publication', the general demand for the doctoral thesis is a significant and independent contribution to the field. According to the contribution statement,

Kristina Záhonová (the first author in authors' order) elaborated on the concept and wrote the original draft. Pragya's contribution sounds a bit more methodological. I don't want to question Pragya's credit to this publication, it is just not easy to assess how much this is 'Pragya's paper'. The quality of research is solid and convincing and I wish all authors to get it accepted soon. I would only point out that to consider the upper signal on the native gel (Fig. 5) as evidence for the incorporation of tagged proteins into a huge enzymatic complex, sounds a bit over-interpreted. Non-specifically aggregated proteins will most likely show such a signal.

The second Pragya's first author manuscript is a short localization study addressing the interesting fact that *P. papillatum* cells do not possess paraflagellar rod (PFR) but contain PFR protein components. Using protein tagging and immunofluorescence, PFR proteins were localized in cytosol and papilla. These data are interesting, the text is already well written and I'm sure it will be published later. The last research chapter is a fragment of manuscript aimed to clarify the organization of kinetochore. The authors are not stated but I guess it is mostly Pragya's work except for MS measurements. As CoIP experiments failed the results are not conclusive and the quality of writing and figures is relatively poor. It is indeed nothing wrong with including unpublished data (including negative results) in the thesis, however the presentation of results should meet the same criteria as for manuscripts. The last part of the thesis – the conclusion and future direction, mostly repeats what has discussed before and, unfortunately, it is the weakest part of the thesis. English is clumsy and a bit chaotic. It is pity because diplonemids are such an extraordinary group of organisms and all topic of the thesis could be discussed from a broad perspective. The future work on diplonemids would deserve a nice chapter.

To conclude, I cannot applaud this thesis too much as some parts fall short of the standards for a good doctoral thesis. But I see that Pragya has performed quality research and she should get a chance to defend her work. I wish her to do it very well, I will listen carefully.

Questions:

1) I found the metabolism of *P. papillatum* very interesting, particularly the proposed intensive gluconeogenesis. How common is this type of metabolism in Euglenozoa? Is it possible that the intensive degradation of amino-acids is responsible for the observed rearrangement of E1/2/3 subunits of three dehydrogenases? Isoleucine and valine catabolism via the branched-chain ketoacid dehydrogenase (BCKDH) will ultimately supplies succinyl-CoA and this is also the product of OGDH. Similarly, the degradation of leucine via BCKDH generates acetyl-CoA. Could it be that the activities of all three enzymes are somehow coordinated?

2) I do not understand the scheme (Fig. 1) in the conclusion part. According to its caption the scheme describes the subunit composition of the pyruvate dehydrogenase complex but shown an oxaloacetate -> succinyl-CoA conversion. Could this be explained?

3) The presence of PFR proteins in *P. papillatum* is intriguing. Is it really clear that this protist is not capable to form PFR, for instance under certain conditions? What about other structural components of PFR? Are they all missing in *P. papillatum*?

4) Can you speculate how the 'free' PFR proteins are structurally organized in *P. papillatum* (e.g. filaments)? PFR proteins are highly conserved. Are there domains/motifs unique or missing in PFR proteins of *P. papillatum*? Authors propose that the *P. papillatum* swims slowly due to the loss of PFR. How to address this hypothesis experimentally?

5) It is not clear how volcano plots, generated from CoIP data, have been calculated (Chapter 2, Chapter 4). How many biological repetitions? What parameters have been used?

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