

České Budějovice, 18th of January, 2019

Examiner report of Diploma thesis by Bc. Michal Stoklasa

Thesis title: **Mitochondrion-related organelles in diplomonads**

It was my pleasure to read and review the Diploma thesis of Bc. Michal Stoklasa. This thesis is written in English, is of appropriate length (110 pages), contains references to 169 publications, and its structure is correct and possess all expected parts.

This thesis is focused on the characterization of the mitochondria-related organelles of two members of the order Diplomonadida – *Spironuceus vortens* and *Hexamita* sp. These organisms are of immense evolutionary interest as the diplomonad *Giardia intestinalis* contains mitosomes – one of the most reduced mitochondrial organelles – while the diplomonad *Spironucleus salmonicida* is known to contain hydrogenosomes. Adding data from other closely related species will help our understanding of the evolutionary transitions between these two types of organelles.

Overall, the presented thesis is well written and, in my opinion, fulfills all the requirements and expectations for granting Bc. Michal Stoklasa the title of Mgr. It is clear that Michal has learned many techniques of wet lab biology and bioinformatics and has deep knowledge of the studied field. I would also like to point out, as someone whose laboratory experience ends with RNA/DNA work, that Michal has done a ample amount of experimental work including transmission electron microscopy, fluorescence microscopy, and transfections. This is all the more impressive, given that *Spironucleus vortens* is not a model organism. The discussion is detailed and on par with current literature. Michal also demonstrated that he is capable of careful interpretation of his work, as the discussion generally does not overstate the presented results. It also presents an interesting analysis and hypothesis about the evolution of ATP generation in diplomonads.

I have some critical points and questions. The most significant criticism is related to the bioinformatics tools used for some of the analyses.

Critical points

1. During analyses of the *Hexamita* organelle, the author searches for proteins of interest using blast with an e-value threshold of e^{-5} . This is a very “permissive” e-value threshold, which might lead to false positive results – i.e.: we think that a particular protein is there based on these blast results, but in actuality we have found some differentially functioning paralog instead. In the materials and methods it is stated that alignments were made, but the author does not explain if any more checks were done to ensure correct proteins were found.
2. Using “similarity between proteins” is a very weak (maybe even useless) tool to identify “relationships”. For example, on page 73 the author stated:

“Three different hydrogenases were found in the genome of *Hexamita* sp. Percentage identity of amino acid residues indicate that Hydrogenase 1 from *Hexamita* is most related to the cytosolic Hydrogenase 1 from *S. salmonicida* (Table 4).”

And then on page 75:

“The three hydrogenases from *S. vortens* were the most similar to the cytosolic hydrogenase 1 of *S. salmonicida*.”

It would be a lot more convincing and clear if a small phylogenetic tree were made to show which of the seven paralogs of hydrogenases of *S. salmonicida* are most closely related to the discussed sequences from *Hexamita* sp. and *S. vortens*.

3. The author states that alignments were made in Geneious and BioEdit – as far as I understand, these programs largely use other known alignment programs and algorithms. It should be explained exactly which of these were used in the Materials and Methods section.

4. A phylogenetic tree of ACS2, showing that the *Hexamita* ACS2 and the ACS2 of *S. salmonicida* are of the same evolutionary origin, would strengthen the author’s hypothesis about the evolution of ATP synthesis in diplomonads.

The above criticisms are not in any way meant to downgrade the thesis, rather they are meant as suggestions for the author’s future research or the publication of these results.

In conclusion, this is a well written thesis based on a sizable amount of experimental and bioinformatic work, which has very interesting and important results. **I am therefore pleased to give my recommendation that this thesis be accepted as a partial fulfilment of the Mgr degree and I believe it should be evaluate with the highest mark.**



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Questions:

1. Did the author perform an alignment and manual check for any of the proteins identified in *Hexamita* sp. or was the e-value threshold only a selection mechanism.
2. The localization of Hydrogenase to the cytosol and Hydrogenase Maturases to the organelle is intriguing. What is the author's interpretation or working hypothesis to explain such a pattern.
3. Given funding and time, what would be the next step/question the author would work on?