Assessment of the PhD thesis submitted by **Jan Michálek**: “**Genomes of Chromerid Algae”**

Jan Michálek‘s thesis contains 3 publications and an introductory and summarising part. All publications deal with different aspects of evolution and metabolism of Chromerids, recently discovered photosynthetic apicomplexans. Two publications were published already in 2015, the most recent is from this year. All publications were published in high quality journals. The earlier papers were already well accepted in the community and have been already cited more than 130 times. Jan Michálek has shared the first authorship of two papers. His contribution to each publication has been clearly stated and defined. During his studies, Jan Michálek has gained expertise in bioinformatics, biochemistry and evolutionary microbiology. He has reconstructed metabolic pathways from in silica data and computed their phylogenies.

The introduction chapter to the thesis is well written, in a condensed, but precise way. It provides overview of the research of Chromerids in the context of the evolutionary parasitology and algal photosynthesis. The research questions of the thesis are clearly formulated. I am missing more clear and stronger synthesis of the three research directions in the summary part and more ambitious outlook or perspective into future research and unresolved questions in this exciting field. The English of this chapter is very good, as far as I can judge. The unpublished, introductory part of the thesis is well edited, there are only few grammar errors. The literature is well taken into account, up to the most recent publications in the field.

Overall Jan Michálek has submitted a thesis of high standards. He has shown that during his studies he has gained abilities and knowledge in the field of evolutionary molecular biology and abilities to communicate and present the results in very competitive way. In summary I fully recommend this thesis for a public defence. And if the defence is successful, I can recommend the committee to grant Jan Michálek the title “Doctor of Philosophy”.

Here I have questions related to the thesis:

1. Do you expect that there are more, still unknown living Chromerid species in the nature? If yes, can you predict, based on the known genomic and evolutionary data of known Chromerids in which environment they might live? Will they be photosynthetic? Are there any attempts to find them?
2. In the plastid genome of Chromera, genes for subunits of Photosystem I and ATP synthase are split, but apparently this has no known effect on their performance. Are there any similar peculiarities in the structure of proteins involved in mitochondrial membrane complexes, e.g. mitochondrial ATP synthase? And what about the proteins of lipid synthesis?
3. It is not clear to me if Vitrella is capable (i.e. possesses apparatus) of myzocytosis. Can you speculate on the myzocytotic differences among Chromerids and their close relatives?
4. I am still puzzled by the reduced mitochondrial respiratory electron chain in Chromera (paper I) and mostly by its possible functional consequences. I have several questions related to this (see below) – but honestly, given the number of authors involved in the paper, I am not sure if Jan Michálek is the right person to ask… : i) In the figure 3 in the paper it looks like the branch starting with Complex II and ending with the alternative oxidase is completely futile, without any contribution to transmembrane proton gradient. If it is still preserved, can you speculate on its function? Why is it there if it only dissipates energy and does not contribute to ATP? Or are there any possible non-canonical proton transporters that could make some transmembrane potential? ii) If the scheme in Fig.3 is right, can you estimate the

efficiency of respiratory ATP production in Chromera mitochondria and compare it to ATP formation in standard respiratory chain? Can you predict any physiological consequences of the presumably less efficient respiration? iii) Do you have any data on the stoichiometry of the “futile” part (involving Complex II) and the active branch (Complex IV) in mitochondrial membranes of Chromera?

5. In the same area: if I understand it right, the donor of electrons to complex IV should be lactate? Can you speculate how is the branching of glycolysis at the level pyruvate regulated and achieved in Chromera? My understanding is that under aerobic conditions the major flow is to Acetyl-CoA and to the Krebs cycle before complexes I or II, while the pathway to lactate is active only when the respiration is jammed due to the lack of O2. But Chromera still respires, so the microanaerobic environment in/around mitochondria is not probable. How is the lactate formed? Is there any metabolic analysis that would detect significant lactate in Chromera?
6. In the paper you used SHAM to block the alternative oxidase. I am slightly confused here – in the text (p.1126 of the paper) it is stated that SHAM had no effect on oxygen consumption. But Fig.7B shows increase of respiration. There is no statistics, so it is difficult to judge if it is significant, but it looks like 50% increase. Why is it so? Another point - Chromera has very thick cell wall and some inhibitors do not penetrate it easily. Do you have any proof that SHAM really entered the cell in the short term experiments?

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