

Opponent's review of the Ph.D. Thesis

**by Eva Nývltová: Evolution of hydrogenosomes: adaptation of free-living protists
Mastigamoeba balamuthi and *Naegleria gruberi* to oxygen-poor environment**

submitted at the Charles University in Prague, Faculty of Science, 2015

Reviewer: Anton Markoš

The core of the thesis represent three papers, in two of which E. Nývltová is the first author. They present – in top journals – results of an excellent teamwork performed essentially at the Lab of Biochemical and Molecular Parasitology; as testified by the Supervisor, the author of the thesis contributed substantially to the output.

The kernel is preceded by (1) short Introduction; (2) a state-of-the art of the classification of mitochondrial-type organelles; (3) characterization of the model organism *M. balamuthi*. It is followed (4) by a short summary of aims of the work, and (5) and extensive list of references documenting a recent paradigm-shift in the fascinating area of eukaryogenesis and organelle evolution.

The papers are followed by a short Conclusion. As to the scientific contents I have no doubts about the qualities of the materials submitted; yet I must point to two formal deficits.

1. My first comments concern the review on mitochondria and derived organelles. I put aside lapses in English (e.g. agrammatical sentences) and a great amount of acronyms that make reading quite difficult. I put asidr lapsi lie Stramenophila (insted Stramenopila, p.18), or membrane bound (instead membrane-bounded p.20 and elsewhere). All this could have been avoided by more careful editing.

I take, however, for nonsensical the statement about acetyl-CoA serving as a “terminal electron acceptor” (p.13): would the author explain the sentence?

My last comment concerns the Figures. First, the author show in many schemes a pathway

glycolysis → pyruvate → anaerobic fermentation

In my understanding, the scheme is void: fermentation is the pathway from glucose to pyruvate, i.e. glycolysis in this case; what follows after pyruvate is a trick how to get rid of both pyruvate and “thrash” reducing equivalents – a more proper depicting should look like

glucose fermentation → pyruvate → electron sink

If the sink is hydrogen or succinate, there is an extra bonus in form of ATP, but strictly speaking the process is not fermentation. The second (and minor) comment concerns picturing the respiratory chain (in aerobic mitochondria) as 4 complexes scattered independently across the inner membrane: to picture them as a single bunch would be more proper to their functioning.

2. The second point is more serious and concerns my role as a reviewer. The author gives me no guidance for evaluation in a situation when all core papers submitted have multiple authors. I have therefore no other cues to know her contribution except her being the first author of two papers, and the written testimony of the Supervisor. I don't take this for satisfactory: such papers often serve as a support for more theses, hence more external information is of utmost importance, should the role of the reviewer not be formal. Therefore I strictly demand that the author, in her presentation at her thesis defense, gives a detailed enumeration of the project history. I have no doubts that she will contribute a satisfactory account of what I consider to be a lacking chapter in her thesis. (Possible testimony of here co-authors present at the defense will be welcomed as well.)

After this, I will be happy supporting her thesis as acceptable.

Prague, April 20, 2015

Anton Markoš