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Evaluation of the thesis "Anaerobic peroxisomes in Archamoebae", submitted by Mgr. Tien Le for obtaining a PhD degree.

The scope of the PhD project of Mgr. Tien Le is broad and the scientific quality of the work performed is high.
Research was performed on three different anaerobic species of the protistan Archamoebae group, two free-living organisms (*Mastigamoeba balamuthi* and *Pelomyxa schiedti*) and one parasitic one (*Entamoeba histolytica*). The work has been executed in a very thorough, systematic way with appropriate, often the most up-to date methods. The conclusions drawn from the results of the experiments are warranted. The work is highly original and provides new knowledge; the results demonstrate for the first time the presence of peroxisomes in each of these anaerobic organisms, thus breaking the paradigm that peroxisomes are absent in such organisms.
This specific research project started when the genomes of *M. balamuthi* and *E. histolytica* were analysed. Surprisingly, genes coding for homologs of well-known peroxisome biogenesis factors – so-called peroxins – were identified in the genome of these organisms. The presence of peroxisomes was subsequently experimentally proved, and aspects of the biogenesis and possible metabolic roles of the organelles characterised in considerable detail. The experimental approach involved methods from a variety of scientific disciplines: bioinformatics, parasitology, biochemistry, molecular and cell biology, and molecular evolution.
In case of *P schiedti*, an organism that apparently is not yet cultivable, a bioinformatics analysis was performed on single-cell derived genome and transcriptome. Based on the results of this analysis, indications for the presence of mitochondrion-related organelles and peroxisomes were obtained. The predicted proteomes of the organelles were determined and used to reconstruct *in silico* their probable metabolic capacities. Electron microscopy was used to demonstrate the presence of the organelles.
The work showed that the biogenesis machinery and enzymatic content of the peroxisomes in the three different anaerobic protists are reduced compared to those of peroxisomes in most other, aerobic organisms, although the extent of this reduction is different between the three species. In contrast, the presence of some other proteins, notably enzymes of myo-inositol metabolism, was demonstrated. The possible role of this metabolism is discussed but remains to be established.
The chapters describing the research on *M. balamuthi* and *E. histolytica* have been published in two high-impact international journals, PNAS and PLoS Pathogens, respectively, while the chapter about *P. schiedti* has been deposited as a draft manuscript on a preprint server.
These three chapters represent work performed by a team of researchers, but the contribution made by Mgr. Tien Le, who is the first author on the PNAS paper and co-author on the two other ones, has been clearly described and is considerable.

The three experimental chapters of the thesis are preceded by an Abstract and an Introduction (Chapters 1 to 7) in which particularly the biogenesis and metabolic functions of peroxisomes and their interaction with other organelles have been described in a detailed and largely accurate manner. I spotted only a few minor errors in scientific description, references and formulation in the Introduction and the Summary

accompanying the thesis (After the defence, I will provide the student with the pdf's in which I indicated the mistakes and the corrections to make using 'sticky notes').
The presentation of the thesis is in accurate English.

To my opinion, the quality of the experimental work described in this thesis, and the way in which the work and background knowledge have been presented in the thesis, meet the criteria to proceed with the oral presentation and defence for obtaining the PhD degree.

Prof. Paul A.M. Michels

Possible questions for the PhD thesis defence of Mgr. Tien Le

1. In the analysis of *Mastigamoeba balamuthi* and *Entamoeba histolytica*, there is a discrepancy between the number of proteins identified as potential peroxisomal, based on the presence of a PTS, and the number of proteins experimentally identified as present in the peroxisomes.
   (i) Do you know if this has also been reported for peroxisomes of other organisms?
   (ii) Does it mean that PTS sequences have only limited predictive value for peroxisomal localisation?
   (iii) Do you have explanations for the discrepancy?

2. Protein transport through the peroxisomal membrane mediated by peroxins have received a lot of attention in each chapter of the thesis. Metabolite transport in the mitochondrion-related organelles of *Pelomyxa schiedti* received also attention, as shown in Figure 2 of the chapter about this protist. However, nowhere in the thesis (not in the Introduction neither in the chapters describing the research performed on the anaerobic peroxisomes) is discussed how metabolites are exchanged across the membrane of peroxisomes. What is known about solute transporters of peroxisomes? Did the bioinformatics analysis of the three anaerobic protists provide any clues?

3. In the list of 'questions for future studies' of the section 'Perspective' of the thesis, you mentioned: "Are anaerobic peroxisomes essential for the life of anaerobes?"
   Does the result for *Entamoeba invadens* not already answer this question? Is it not true that no peroxins have been identified in the genomes of well-studied anaerobes like *Giardia* and *Trichomonas*?
   Could you speculate if the reduction of the peroxisomes observed in *Mastigamoeba, Entamoeba* and *Pelomyxa* species – and notably the loss of β-oxidation, H_2O_2-producing oxidases and catalase – is a stage in losing the organelles altogether?

4. You consider that, in *E. histolytica*, Pex5 is recycled without a ubiquitination step, because of the absence of a conserved Cys residue near the N-terminus of Pex5 and the absence of Pex10, Pex12 and Pex4. Could you consider an alternative way of Pex5 ubiquitination? Are there examples in the literature?