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#### PhD Report for Mr. Vojtěch Žárský

**Title: Analysis of the genome of a free-living amoeba *Mastigamoeba balamuthi* and its comparison with pathogenic *Entamoeba histolytica***

In his work, Mr. Žárský has analyzed in detail the genome of the anaerobic, free-living amoeba *Mastigamoeba balamuthi*. This included sequencing of the genome, comparative analyses with other species belonging to the group of parasitic Entamoebidae, development of adaptation mechanisms, analysis of the evolution of the Entamoebidae with respect to parasitic lifestyle, and the importance of peroxins also with respect to parasitic lifestyle.

By sequencing the genome of *M. balamuthi* and subsequent detailed analysis, Mr. Žárský has identified some peculiarities of the genome in comparison to its closest parasitic relative *Entamoeba histolytica*. This is for example the high GC content or the larger number of genes. Also, some molecules important for the lifestyle of *E. histolytica* like the Gal/GalNac lectin and members of the BspA family could not be found. In addition, chitin synthesis and degradation seems to be different from *E. histolytica*.

In contrast to the Entamoebidae *M. balamuthi* still forms flagella. It was therefore not surprising to identify genes coding for proteins that are necessary for the formation and function of the flagella. However, *M. balamuthi* does not appear to form external dynein arms, which was also shown by earlier ultrastructural analyses.

Some great surprise was the identification of *peroxin* genes, since peroxiredoxins have never been found in these organisms before. *Pex* genes (although fewer) could be detected in subsequent studies in various *Entamoeba* species. Based on these data, the hypothesis is made that functional peroxisomes must still be present in reduced form in these organisms.

A very interesting observation was also that there are members of the Neodermata who have peroxins while others have lost them.

In summary, it can be said that the present work of Mr. Žárský is an outstanding dissertation. I find the publication performance of Mr. Žárský particularly remarkable. This includes not only the four publications on which the dissertation is based (2 x first author, 1 x second author, 1 x co-author), but also ten other publications (1x first author, 9 x co-author).

### Questions

1. Compared to Entamoebidae, the genome of *M. balamuthi* contains between 4000-8000 more protein-coding genes. What kind of genes are these? Are the genomes of the Entamoebidae e.g. reduced due to lifestyle?
2. How was it possible to identify the *pex* genes? How many open reading frames coding for proteins of unknown function were found? Is there evidence for larger gene families that are unique to *M. balamuthi*?
3. How can one explain that the increase in GC content correlates with the free-living lifestyle? For *Plasmodium falciparum* (low GC content) and *P. vivax* (high GC content) this would not be true. Furthermore, I am of the opinion that *E. invadens* also has a high GC content, whereas *E. histolytica* has a high A/T content.
4. Is there a difference in the type and number of genes between *M. balamuthi* and *E. histolytica* acquired via lateral gene transfer?
5. Many gene families known from *E. histolytica* could not be found in *M. balamuthi*? Is there any information on the ATG family of *E. histolytica*? This is a large protein family about whose function almost nothing is known. It would therefore be interesting to check whether these genes are also present in the genome of *M. balamuthi*.
6. What is the function of the cresol synthesis pathway?
7. What could be the function of PEX19, but also of the reduced set of peroxins found in *E. histolytica*, *E. dispar* and *E. mashkovski*? It is astonishing that peroxins are found in *E. histolytica*, but not in *E. invadens*, although both have a very similar lifestyle. Is there a possible explanation for this?
8. The question posed in point 7 also applies to Neodermata. Do Neodermata with peroxins differ in their lifestyle from those without peroxins, or is there another explanation?

In summary, the PhD thesis fulfils all requirements to confer the PhD degree on Mr. Žárský.