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Autoreferát disertační práce
Summary of the doctoral thesis

Analýza genomu volně žijící améby *Mastigamoeba balamuthi* a porovnání s patogenní amébou *Entamoeba histolytica*

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

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Abstract

Examination and comparison of organisms have been tremendously important for the study of life's history on earth. The progress of our understanding of the genetic basis of heredity and the recent boom of sequencing technologies allows us to continue in this exciting field of research from the perspective of genes and genomes. In this work, I focus on the study of an anaerobic amoeba *Mastigamoeba balamuthi*, which is related to an important human pathogen *Entamoeba histolytica*. Comparative analysis allows us to draw some conclusions about the nature of the common ancestor of *Mastigamoeba* and *E. histolytica*, how it adapted to the anaerobic lifestyle, and about the way the *Entamoeba* lineage evolved to become a successful parasite. Surprisingly we also noticed that besides hydrogenosomes (hydrogen-producing organelles related to mitochondria), *M. balamuthi* also harbors peroxisomes – organelles thought to be absent in anaerobic organisms. This finding motivated us to inquire more about peroxisomes in other eukaryotic lineages. We found out that there is a reduced set of peroxisomal markers in certain *Entamoeba* species. Moreover, we showed that peroxisomes were independently lost in several lineages of parasitic helminths and a free-living tunicate *Oikopleura dioica*.

Abstrakt

Výzkum a srovnávání organismů byly nesmírně významné pro studium historie života na Zemi. Pokrok v porozumění genetickému základu dědičnosti a nebývalý rozvoj sekvenačních technologií nám umožňuje pokračovat na tomto vzrušujícím poli výzkumu z perspektivy genů a genomů. V této práci se zaměřuji na studium anaerobní améby *Mastigamoeba balamuthi*, která je příbuzná významnému lidskému parazitu *Entamoeba histolytica*. Srovnávací analýza nám umožnila učinit některé závěry o vlastnostech společného předka *M. balamuthi* a *E. histolytica*, jak se přizpůsobil životu bez kyslíku, a jak se linie *Entamoeba* vyvinula v úspěšného parazita. Zjistili jsme přitom překvapivě, že vedle hydrogenosomů (odvozené mitochondrie produkující vodík) má *M. balamuthi* také peroxisomy - organely o kterých se soudilo, že je anaerobní organismy ztratily. Tento objev nás podnítil ke studiu distribuce peroxisomů u dalších eukaryotických linií. Zjistili jsme, že i u některých druhů *Entamoeba* nalézáme redukovaný soubor proteinů typických pro peroxisomy. Dále jsme ukázali, že peroxisomy byly nezávisle ztraceny u některých skupin parazitických červů a u volně žijícího pláštěnce *Oikopleura dioica*.

1 Introduction

Mastigamoeba balamuthi is a free-living anaerobic amoeboid flagellate (Chavez, Balamuth, and Gong 1986), which is of particular interest to us because it is among the closest free-living relatives of the parasitic *Entamoeba histolytica* (Pánek et al. 2016). *E. histolytica* has a simple life cycle: infectious cyst is ingested, amoeba excysts in the small intestine, and travels to the large intestine where it multiplies and produces cysts. However, in some cases, the amoeba can perforate the intestinal wall, enter the bloodstream, and invade the liver, causing a life-threatening amoebic liver abscess (Haque et al. 2003). Both *M. balamuthi* and *E. histolytica* belong to a group of anaerobic amoebozoa called Archamoebae. While thorough comparative studies of some parasites and their free-living relatives have been conducted (Janouškovec et al. 2015; Mathur et al. 2019; Lukeš et al. 2014; Jackson et al. 2016), such analysis is still missing for any group of anaerobic eukaryotes, including Archamoebae.

One of the most significant changes during the adaptation to anaerobiosis is the loss of oxidative phosphorylation and reduction or modification of mitochondrial pathways which gave rise to hydrogen-producing hydrogenosomes of *M. balamuthi* (Gill et al. 2007) and highly reduced mitosomes of *E. histolytica* (Tovar, Fischer, and Clark 1999). Also, peroxisomes, eukaryotic organelles which usually

carry oxygen-dependent pathways, were never found in any of the anaerobic eukaryotes (Gabaldón 2010; Gabaldón, Ginger, and Michels 2016).

2 Aims

- To analyze the genome of *M. balamuthi* and compare it with genomes of parasitic Entamoebidae.
- To describe adaptations of *M. balamuthi* to free-living anaerobic lifestyle.
- To identify putative ancestral and novel features that allowed the evolution of Entamoebidae into a successful parasitic group.
- To explore the distribution of peroxisomal markers in Archamoebae and other eukaryotes with respect to parasitism and anaerobic lifestyle.

3 List of publications and author contribution

Le, T., **Žárský, V**., Nývltová, E., Rada, P., Harant, K., Vancová, M., Verner, Z., Hrdý, I. and Tachezy, J., 2020. Anaerobic peroxisomes in *Mastigamoeba balamuthi*. *Proceedings of the National Academy of Sciences*.
Comment: This work presents the first evidence of peroxisomes in an anaerobic organism and outlines possible functions of peroxisomes in *M. balamuthi*.
Author contribution: Discovery of peroxisomal markers in the genome of *M. balamuthi*. Expression of several peroxisomal markers for antibody production.

Nývltová, E., Šut'ák, R., **Žárský, V**., Harant, K., Hrdý, I. and Tachezy, J., 2017. Lateral gene transfer of p-cresol- and indole-producing enzymes from environmental bacteria to *Mastigamoeba balamuthi*. *Environmental microbiology*, *19*(3), pp.1091-1102.
Comment: M. balamuthi acquired the cresol synthesis pathway via lateral gene transfer from prokaryotes. It may use the bacteriostatic property of cresol to compete with other microbiota.
Author contribution: A phylogenetic analysis of 4-hydroxyphenylacetate decarboxylase and tryptophanase.

Žárský, V. and Tachezy, J., 2015. Evolutionary loss of peroxisomes – not limited to parasites. *Biology direct*, *10*(1), p.74.
Comment: We show that peroxisomes were independently lost in several lineages of parasitic helminths and also in a free-living tunicate *Oikopleura dioica*.
Author contribution: Bioinformatics analysis of peroxisomal components in metazoans.

Žárský, V., Klimeš, V., Eliáš, M., Pačes, J., Vlček, Č., Nývltová, E., Hrdý, I., Barlow, L., Dacks, J., Hall, N., Roger, A., Tachezy, J., *Mastigamoeba balamuthi* genome and the nature of the free-living ancestor of *Entamoeba.* (unpublished manuscript)
Comment: In this manuscript, we present a draft genome sequence of *M. balamuthi* and compare it with genomes of Entamoebids and other amoebozoans. We show that some features crucial for the parasitism of *Entamoeba* were probably present in the common ancestor of *M. balamuthi* and *Entamoeba*,

while others may have been acquired in the *Entamoeba* lineage. We also describe flagellar components and plant cell wall-degradation pathways of *M. balamuthi*.
Author contribution: Bioinformatics analysis of the *M. balamuthi* genome, detection of transposable elements, and comparative analyses of amoebozoan genomes. Reconstruction of metabolic pathways, flagellar components, nuclear pore complex, and cyst wall components. Analysis of genes possibly transferred from prokaryotes.

4 Results

We sequenced and assembled the genome and transcriptome of the anaerobic amoeba *Mastigamoeba balamuthi* and compared these with the genomes of parasitic Entamoebidae and other amoebozoans (Figure 1). The draft genome sequence of *M. balamuthi* has 57.3 million base pairs (Mbp) (Entamoebidae have 20.8-40.9 Mbp) with an extremely high GC content of 61% (Entamoebidae have 23-30% GC). We used the actin gene to compare GC content also with species that don't have their genome sequenced (*Rhizomastix, Pelomyxa, Mastigella*), and we observed that within Archamoebae the GC content positively correlates with the free-living lifestyle. We predicted 16287 protein-coding genes (Entamoebidae have between 8300 and 12500 protein-coding genes) with an average of 3.4 introns per gene (less than one intron per gene in Entamoebidae). *M. balamuthi* has a rich set of transposable elements: DNA transposons (found in *E. moshkovskii* and *E. invadens*), LINE (long interspersed nuclear elements) retrotransposons (found in *E. histolytica* and *E. dispar*) and LTR (long terminal repeats) retrotransposons (Pritham, Feschotte, and Wessler 2005).

We reconstructed evolutionary histories of amoebozoan genes and focused on the main events in the evolution of Archamoebae. During the transition to anaerobiosis of the common ancestor of Archamoebae, mitochondrial genome and respiration complexes have been lost, except for the succinate dehydrogenase complex (Complex II) which was found in *M. balamuthi* hydrogenosomes. Also, the detoxification system for ROS (reactive oxygen species) and RNS (reactive nitrogen species) has been modified in a way that is typical for anaerobic eukaryotes: the thioredoxin system, peroxiredoxins, and superoxide dismutase are present, while the glutathione synthesis and glutathione-dependent pathways have been lost. Also, flavodiiron protein, iron-sulfur flavoprotein, and rubrerythrin, enzymes typical for anaerobic prokaryotes, have been gained via LGT.

We found a complete pathway for chitin synthesis and degradation, and cell wall-binding components that were so far found only in Entamoebidae (lectins Jessie1/2/3 and Jacob). We, however, did not find homolog of the *Entamoeba* Gal/GalNAc lectin, which is attached to the plasma membrane by a C-terminal transmembrane domain and tethers the chitin-binding lectin Jacob via protein-protein interaction (Chatterjee et al. 2009). Instead, we discovered that in *M. balamuthi*, the chitin-binding proteins (chitinase, Jessie, Jacob) have each a C-terminal transmembrane domain. Thus they are possibly directly anchored to the plasma membrane. It has been observed that during excystation *E. histolytica* trophozoite first detaches from the cyst wall and then actively escapes through a small opening (Yorke and Adams 1926), while *M. balamuthi* instead degrades its cyst (J. Tachezy personal observation). Based on this, we propose that the indirect attachment of *Entamoeba* cyst wall proteins via Gal/GalNAc lectin allows for a quick release of the trophozoite from the cyst as soon as it reaches the small intestine.

M. balamuthi codes for an extensive repertoire of cysteine proteases, some of which are closely related to *E. histolytica* cysteine proteases essential for virulence and pathogenicity (Serrano-Luna et al. 2013). We also found a homolog of *E. histolytica* amoebapore (a cytolytic protein) in the genome of *M. balamuthi*. The *M. balamuthi* gene, however, resembles rather mammalian prosaposins, as it codes for a polyprotein composed of several saposin domains, each of which is homologous to the amoebapore

(Zhai and Saier 2000). Several gene families of *E. histolytica* cell surface (Gal/GalNAc lectin, BspA, Ariel1, EhSTIRPS) were probably acquired in the Entamoebidae lineage, and we did not find any homologs in *M. balamuthi* genome.

While Entamoebidae have completely lost flagellum, *M. balamuthi* forms flagellated cells. Because the genome sequencing projects of amoebozoans were mostly focused on species that lack flagella (*Acanthamoeba*, Dictyostelida, Entamoebidae), the description of genes associated with the amoebozoan flagellum has been missing. We found a rich set of proteins necessary for the assembly and function of the flagellum. We, however, did not find any genes for dyneins of the outer dynein arms, which is in accordance with ultrastructural observations that the outer dynein arms are absent in Archamoebae (Walker et al. 2001; Pánek et al. 2016).

We found a broad set of enzymes and metabolite transporters required for the degradation and utilization of polymers of the plant cell wall (cellulose, hemicellulose, and pectin) in the genome of *M. balamuthi*. This is a probable adaptation allowing to feed on plant debris in the anoxic sediments typical for *Mastigamoeba* and other free-living Archamoebae. It is also possible that these pathways were acquired by the common ancestor of Archamoebae and then secondarily lost in Entamoebidae.

We also discovered that *M. balamuthi* acquired a cresol synthesis pathway (cresol is an odorous phenolic compound) via lateral gene transfer from prokaryotes, and we showed that *M. balamuthi* produces cresol in concentrations that are bacteriostatic to non-cresol-producing bacteria (Nývltová et al. 2017). The acquisition of cresol synthesis may provide *M. balamuthi* a competitive advantage over other microbes in its habitat.

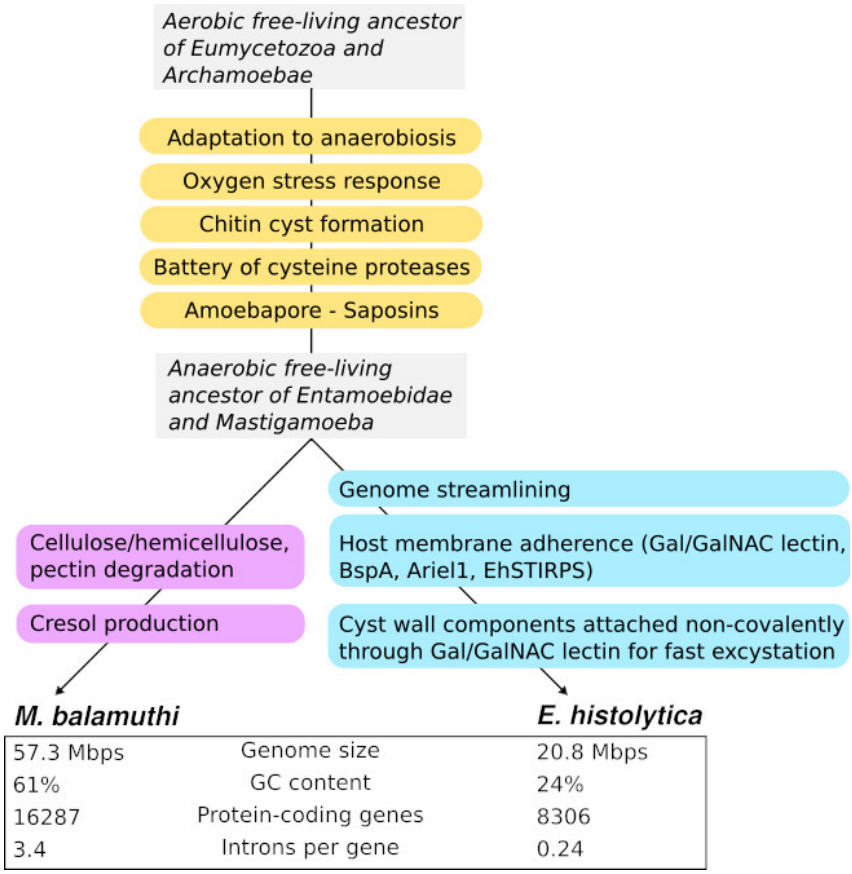

Figure 1. An overview of the preexisting features and adaptations during the evolution of *M. balamuthi* and *Entamoeba*.

During the analysis of the *M. balamuthi* genome, we found a complete set of conserved peroxins (PEX proteins) required for the peroxisomal biogenesis and protein import, which is surprising because no peroxisomes have been described in anaerobic organisms so far (Le et al. 2020). This encouraged us to inquire more about the distribution of peroxins in eukaryotic lineages (Figure 2). We found a reduced set of seven peroxins in the genomes of *E. histolytica*, *E. dispar*, and *E. moshkovskii*, while in *E. invadens*, we found only one peroxin - Pex19. We hypothesize that there are functional remnants of peroxisomes in some entamoebids (*E. histolytica*, *E. dispar*, *E. moshkovskii*) while in *E. invadens* peroxisomes were entirely lost (unpublished data). Independently we discovered Pex19 in the genome of *Monocercomonoides exilis*, which is an oxymonad that lacks both mitochondria and peroxisomes (Karnkowska et al. 2016). This suggests the possibility of an additional peroxisome-independent function of Pex19.

Furthermore, we discovered that parasitic roundworms of the order Trichocephalida and a large group of parasitic flatworms Neodermata (containing flukes, tapeworms, and monogeneans) had lost peroxisomes. Most surprisingly we found, that peroxisomes were lost in a free-living tunicate *Oikopleura dioica* that inhabits oxygen-rich surface sea waters (Žárský and Tachezy 2015).

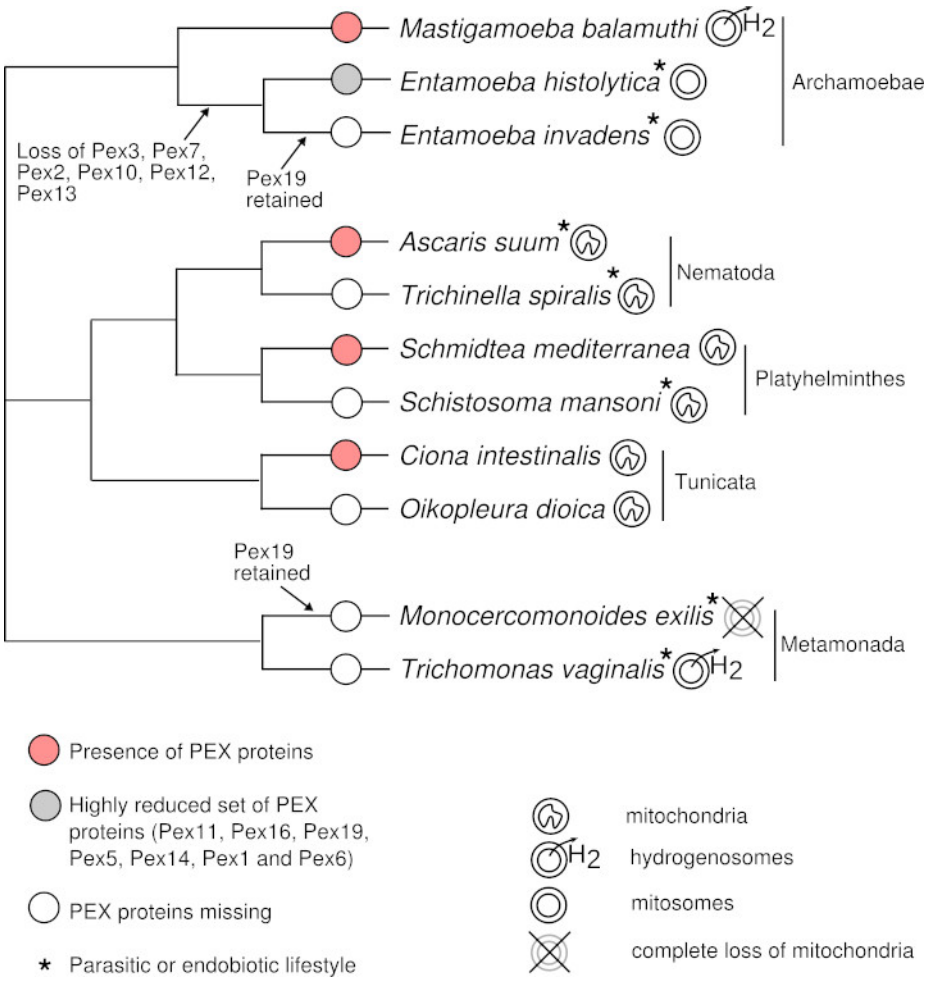

Figure 2. Novel insights into the distribution of peroxisomes in eukaryotes. Presence of mitochondria and mitochondrion-related organelles is highlighted.

5 Curriculum vitae

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Education:

- Ph.D., 2012-2020, Parasitology, Charles University, Thesis: Analysis of the genome of a free-living amoeba *Mastigamoeba balamuthi* and its comparison with pathogenic *Entamoeba histolytica*, Supervisor: Jan Tachezy

- Internship June – July 2015, Dalhausie University, Halifax, Canada, Supervisor: Andrew Roger

- Internship March 2015, Institute of Integrative Biology, Liverpool, UK, Supervisor: Neil Hall

- M.A., 2010-2012, Parasitology, Charles University, Thesis: Protein import into mitochondria and peroxisomes of parasitic protists, Supervisor: Jan Tachezy

- B.A., 2006-2010, Biology, Charles University, Thesis: Evolution of eukaryotic ABC transporters, Supervisor: Jan Tachezy

Grants and Awards:

- Principal investigator of a GAUK (Grant agency of Charles university) project “Cell localization and function of peroxisomal proteins in Archamoebae” (2012-2016)

- Charles university price of Bedřich Hrozný for “Discovery of eukaryotes without mitochondria” (co-author of a publication) (2016)

Publications:

Žárský V, Klimeš V, Eliáš M, Pačes J, Vlček Č, Nývltová E, Hrdý I, Dacks J, Barlow L, Roger A, Hall N, Tachezy J. *Mastigamoeba balamuthi* genome and the nature of the free-living ancestor of *Entamoeba*.

(unpublished manuscript)

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