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## Molecular Parasitology

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To whom it may concern

Jarmila Kliescikova, MUDr., has submitted a doctoral thesis on the cyst formation in *Acanthamoeba* and *Balamuthia* entitled "LIFE CYCLE OF THE FREE-LIVING AMOEBAS – DIFFERENTIATION OF AMPHIZOIC AMOEBAS OF THE GENERA ACANTHAMOEBA AND BALAMUTHIA" that also includes five original papers.

This is a clear, concise and highly comprehensive thesis. It comprises an introduction, in which Jarmila Kliescikova briefly introduces the topic of the thesis, followed by an extensive overview of the present state of knowledge on cyst wall composition and synthesis in different organisms and on free-living amoebae, their life-cycles and their medical relevance in particular. In compliance with the aims of her study, she specifically focuses on the biochemistry of cyst walls and on cellulose synthesis. Cellulose is one of the major components of *Acanthamoeba* cyst walls and it plays an important role in the resistance of *Acanthamoeba* cysts against treatment and disinfection. After the introduction, Jarmila Kliescikova lists the five studies that were performed within the frame of this thesis. Three of them have already been published in highly-ranked scientific journals, the other two are currently under preparation. The following chapter is dedicated to the results of her research, all major findings are explained in detail and are also demonstrated in several figures. The interpretation of her data, certainly helped by an excellent supervisor, is convincing and scientifically sound. The thesis is concluded with a very clear and compact summary of all results and their significance and a list of the entire cited literature. For her thesis, Jarmila Kliescikova has performed an exhaustive literature research and has incorporated numerous references into her work demonstrating that she is deeply familiar with the present state of knowledge on free-living amoebae on one hand, and with cyst formation and its biochemical background on the other hand.

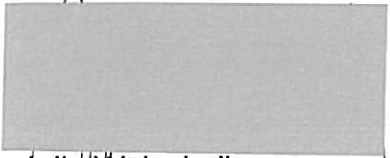
Concerning the style and writing, there are unfortunately numerous, however minor grammatical errors (e.g. missing articles or singular/plural mistakes). Also, the abstract could have been better structured and the introduction would have benefitted a lot from the incorporation of some figures demonstrating the partly complicated matters. One minor error with regard to the contents: there are not 6.1 billion contact lens wearers in the world, there are only around 7 billion people in the world, and even in highly industrialized countries only approximately 1/10<sup>th</sup> of the population are contact lens wearers. However, altogether the thesis is very well structured and was otherwise written with great diligence, moreover the literature was very well selected and all scientific findings are explained in a very clear and comprehensible way.

With her thesis, Jarmila Kliescikova gives a detailed overview on the current knowledge on cyst formation in free-living amoebae discussing all new data and also all open questions in detail. With

her studies she contributed significantly to the elucidation of the biochemical details of encystation in *Acanthamoeba* and *Balamuthia*. In *Acanthamoeba*, she clarified the details of pseudocyst formation and she could demonstrate the role of different carbohydrates during the assembly of the cyst wall and identify the details of cellulose synthesis. Thereby she detected mixed-linkage glucan, which was previously not known from protozoa. Also, she found that glycogen phosphorylase is of crucial importance for glycogen degradation to glucose during encystation. Moreover, she could identify the Golgi apparatus as one of the key players during cyst formation and cyst wall assembly and demonstrate the progress at different time points of the process. In *Balamuthia*, where basically nothing had been known before, she found that cyst formation is not induced by the same factors as it is in *Acanthamoeba* and that the process is altogether rather different. Also, she could demonstrate that the cyst wall is mainly composed of cysteine-rich proteins. The entire topic of encystation is of major medical importance, as *Acanthamoeba* and *Balamuthia* infections, although rare, usually have a severe progression and are both particularly difficult to treat. Cyst formation within the infected tissue is the major problem in treatment of *Acanthamoeba* infections. Moreover, cysts residing in the tissue may lead to re-infections even after successful treatment. In-depth knowledge on the biochemical details of cyst formation in these organisms will help to enable the development of a treatment scheme specifically targeting the cysts and thus efficiently treating these often dramatic infections. The thesis is innovative and concise and the five studies it is compiled of include new and convincing approaches. Jarmila Kliescikova employed sophisticated staining techniques, laborious microscopy such as transmission and scanning electron microscopy and novel techniques such as small interfering RNAs. Her results indisputably significantly add to the existing knowledge. The scientific impact of this thesis is also reflected by the fact that of the five studies performed, three have already been accepted as original publications in highly respected peer-reviewed international scientific journals.

Altogether, this is an excellent thesis that unequivocally qualifies Jarmila Kliescikova, MUDr., for being awarded the degree of a Ph.D.

Respectfully,



Julia Walochnik

