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Review of the Ph.D. thesis by Michaela Czerneková entitled „**Storage cells and their role in tardigrade physiology**“

Tardigrades, the main object of the submitted dissertation, are one of the most interesting groups of organisms. They are capable of suspending their metabolism substantially (term cryptobiosis) in extreme conditions. While in this state, their metabolism and water content can drop to a minimum that is hardly detectable. They can survive in a dehydrated state (forming a characteristic tun) for several years. In relation to the environmental conditions, they may enter this state via anhydrobiosis, cryobiosis, osmobiosis, or anoxybiosis. But they also can survive other extreme conditions like pressure, radiation, and environmental toxins. Last, but not least, they are able to survive exposure to outer space. Everything mentioned offered Michaela Czerneková an extraordinary opportunity to study a very serious subject from scientific point of view.

The first part of the dissertation is based primarily on four publications in which the candidate is always the first author. Publications in full text are placed in the second part of the dissertation. These are the following publications:

The first paper “Mitosis in storage cells of the eutardigrade *Richtersius coronifer*” published in 2016 in Zoological Journal of the Linnean Society (IF=2.909). The main objectives of studies were to analyze the occurrence of mitosis in storage cells. Paper I.

The second paper “Experimentally Induced Repeated Anhydrobiosis in the Eutardigrade *Richtersius coronifer*” published in 2016 in Plos one (IF=2.776). Authors studied the factors constraining anhydrobiotic survival. Paper II.

In the third paper “The structure of the desiccated *Richtersius coronifer* (Richters, 1903)” published in 2016 in Protoplasma (IF=2.633). Authors described ultrastructure of tardigrade organs in desiccated state. Paper III.

In the fourth paper “A comparative ultrastructure study of storage cells in the eutardigrade *Richtersius coronifer* in the hydrated state and after desiccation and heating stress” published in 2018 in Plos one (IF=2.776). Authors described the ultrastructure of storage cells and their ultrastructure in relation to survival of stress conditions. Paper IV.

The above-mentioned publication activity of the candidate, which contains 4 papers published in well known peer-reviewed biological journals with a solid impact factor, all of which are the first author, is a very convincing proof of her scientific competence.

The second part of the dissertation discusses, generalizes and specifies on 42 pages (plus 18 pages of references) in a broader sense the results from the above mentioned publications. Candidate introduces tardigrades as a model organisms in cell biology and stress biology research. In section “Material and methods” the candidate states that all studies were performed on the tardigrade model species *Richtersius coronifer*. This was made possible by applying different microscopic techniques, such as transmission and scanning electron microscopy, fluorescent and confocal microscopy, histochemical and cytochemical techniques and stress related survival analyses. I want to emphasize that experiments were carried out mainly in Sweden and Poland. The following section “Results and discussion” summarizes and discusses the main results as mitosis in storage cells, experimentally-induced repeated anhydrobiosis, tun formation, ultrastructure of storage cells with regard to survival of dessication and heat stress. The main interest of the author was focused on storage cells (coelomocyte-type cells), which were enriched by new discoveries (eg the existence of 2 types of these cells). The main results of the dissertation are then briefly summarized in the “Conclusions and future perspectives” section. I noted that one side study in the form of poster presentation is included in dissertation as well .

In my opinion, the well done experimental work led to publishing four excellent papers in impacted journals. Dissertation represents very informed discussion of the topic and documents that Michaela Czerneková was really involved in the problem. Instead of description of particular parts of the thesis in detail, I have some questions, comments and recommendation for the candidate:

- Formal question: You are the first author of all four presented papers, however, several co-author are in the list as well (which I consider to be normal), nevertheless, could you briefly specify and describe your share and your responsibility in the papers III and IV, please?

- Why the model species *Richtersius coronifer* cannot be cultivated under laboratory conditions?

- Do you think another tardigrade extraction technique could be used, for example a slow centrifugation?

- What do you think are oval storage cells with “orange fibrous material”?

- In publication III you mention very briefly the existence of a discontinuous layer of "flocculent material or coat" in the cuticle of tardigrades. You state that the precise function and importance of this coat in anhydrobiosis of tardigrades remain to be studied. Does not this flocculent coat play a greater role than expected?

- 3D reconstructions based on serial sections (publication III) are excellent but very laborious. Perhaps it would be good for further research to use synchrotron tomography or microCT (more accessible) with the possibility of virtual sections of the object in all planes.

- Next methodological query. Tardigrades in anabiosis show the ability to survive very low temperatures. The field emission electron microscope (FESEM) works at temperatures around -195 degrees, which anabiotic tardigrades are likely to survive. In your opinion, could this microscopic technique, in this case, in particular, bring any advantage over conventional electron microscopy? Or perhaps a tissue image

somewhat different from the tissues of organisms unable to survive such low temperatures.

- Considering the characteristics of the tardigrades cuticle, I would recommend trying the old and now almost forgotten technique of fixation of objects by osmium vapors (while observing safety rules). For comparison with the methods used.

- I assume you will continue to deal with tardigrades. What direction will you focus in the future?

- Something beyond common questions: In your opinion, what impact will the Israeli spacecraft accident have on further studies of the ability of tardigrades to survive in space?

Summary - I can state that the submitted dissertation is complex and well prepared both formally and factually. I did not find any significant shortcomings or errors in that work. The introductory parts of the dissertation are so broad and well formulated that I recommend their publication in the form of a popular scientific article in a journal such as Živa or Vesmír. Appropriate methods, including sophisticated techniques of optical and electron microscopy, were used for the study. The experiments were qualified. The results of the dissertation are particularly important for the development of the scientific field. Based on her publications, Michaela Czerneková has already become one of the experts expanding knowledge of the important Tardigrada group. Several new information also have potential practical impact to the timeliness and importance of the dissertation for the development of the scientific field, and for practice, are fulfilled.

Conclusion - Candidate Michaela Czerneková elaborated an interesting, important and current topic and gained together with a team of co-authors a number of high-quality and new scientific results. In her work she used modern research methods, the application of which expanded the known facts. The author thus fulfilled the specified dissertation goal. The dissertation thus demonstrates the author's prerequisites for independent creative scientific work. The thesis fulfills the conditions for the defense. I therefore strongly recommend accepting the candidate’s thesis as the basis for obtaining the degree Ph.D.

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Reviewer