

Professor Eliza Wyszko

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Laboratory of Subcellular Structures Analysis

**Review of the Doctoral Dissertation of MSc Amit Kumar Nagwani entitled:
"Searching on aging markers of the tardigrade *Paramacrobiotus experimentalis*"**

Amit Kumar Nagwani's doctoral thesis presented for review was prepared in the Department of Bioenergetics, Institute of Molecular Biology and Biotechnology, Faculty of Biology of Adam Mickiewicz University in Poznan under the supervision of Professor Hanna Kmita and Professor UAM Łukasz Kaczmarek

The doctoral dissertation was prepared as a collection of three published and thematically related scientific articles, which were preceded by a broad discussion.

List of scientific articles included in the dissertation

Review article:

- 1 Nagwani AK., Kaczmarek Ł., Kmita H. (2022) Applicable life-history and molecular traits for studying the effects of anhydrobiosis on aging in tardigrades.

Diversity, 14(8): 664.

MEiN (July 2023) points -70, Journal Impact factor (2023) - 3.031

Research articles:

2. Nagwani AK., Melosik I., Kaczmarek Ł., Kmita H. (2023) Recovery from anhydrobiosis in the tardigrade *Paramacrobiotus experimentalis*: better to be young than old and in a group than alone. *bioRxiv* (Preprint). Under review in *Heliyon*

MEiN (July 2023) - 40 , Journal Impact factor (2023) - 4.0

3. Nagwani AK., Budka A., Łacka A., Kaczmarek Ł., Kmita H. (2023) The effect of hypomagnetic field on survival and mitochondrial functionality of active *Paramacrobiotus experimentalis* females and males of different age.

Frontiers in Physiology, 14: 1253483.

MEiN (July 2023) - 100, Journal Impact factor (2023) - 4.0

It should be noted that the candidate took an active part in the scientific life of the university, presenting his results at numerous scientific conferences.

The research work conducted by the PhD student was financially supported by four grants:

1. The Polish National Science Centre grant, NCN OPUS 2016/21/B/NZ4/00131 (PI Prof. Hanna Kmita).
2. The Polish National Science Centre grant, NCN PRELUDIUM 2021/41/N/NZ3/01165 (PI Amit Kumar Nagwani).
3. The European Union: Passport to the future - Interdisciplinary doctoral studies at the Faculty of Biology, Adam Mickiewicz University. POWR.03.02.00-00-I022/16-17.
4. ID_UB grant number 017/02/SNP/0006. (PI Amit Kumar Nagwani).

The presented collection of 3 publications was elaborated by well-prepared description and constitutes a comprehensive introduction to the research issue; i.e. searching on aging markers of the tardigrade.

The statements presented in the dissertation and the relevant sections included in the scientific articles show that MSc Amit Kumar Nagwani had an important and creative contribution to the realization of scientific articles included in the collection of the doctoral dissertation. Moreover, he is the first author in all of the three presented publications.

The Ph.D student is a co-author of two other scientific articles and therefore I assess the candidate's publication achievements as very good.

PhD thesis consists of one review paper and two experimental papers which focus on potential tardigrade multilevel aging markers.

Tardigrades are known as the most stress-tolerant animals because of the ability of some taxa within the phylum to survive some of the most challenging environmental stress conditions for living organisms, such as desiccation, freezing, and radiation.

The ability to withstand such environmental extremes is provided by cryptobiosis. Specifically, five major types of cryptobiosis is distinguished, namely anhydrobiosis (caused by desiccation), anoxybiosis (caused by oxygen depletion), chemobiosis (caused by high level of toxicant concentration), cryobiosis (caused by extremely low temperature) and osmobiosis (caused by high solute concentration).

Tardigrades' lifespan varies from species to species. In terms of their natural lifespan, some live a few months, while others can live for up to 2 years. But due to cryptobiosis they can go into a dormant state which stretches their lifespan even further. For example, by undergoing anhydrobiosis, they can live more than 30 years without any food or water.

Anhydrobiosis appears to be the most known form of cryptobiosis (also in tardigrades) and is described as the ability to resume activity after complete dehydration (desiccation). The aging markers are inevitable for estimation of the effect of tardigrade anhydrobiosis on aging which is explained differently by the "Sleeping Beauty" and "Picture of Dorian Grey" hypotheses, and could have applicative consequences. Therefore, the aim of the thesis was to indicate potential tardigrade aging markers.

To realize the aim several multilevel markers were analyzed which correlate with lifespan of tardigrades. They include: vitality rate, average number of laid eggs per female, survival of the extreme conditions (i.e., anhydrobiosis and exposure on hypomagnetic field - HMF), the level of the mitochondrial inner membrane potential ($\Delta\psi$) and the level of intracellular reactive oxygen species (ROS).

The markers' indication and verification is the main achievement of the Ph D. thesis. The other research analyzing tardigrade diversity in terms of their life-history traits and anhydrobiosis performance as well as the *Pam. experimentalis* model are important achievement.

These research results may be an introduction to developing new anti-aging strategies.

Question:

Do you have any idea how to understand mechanisms that underlie the ability to stabilize biological structures, from macromolecules across cellular, tissue and organ levels to the whole animal and how is possible restart life after years of metabolic suspension?

To summarize, I rate Amit Kumar Nagwani's doctoral dissertation highly. This work is an original analysis of a scientific problem, which may be a valuable source of information for further work in this research topic.

The dissertation being the subject of the review fulfills the conditions laid down in the Act of 20 July 2018. The Law on Higher Education and Science (Journal of Laws of 2022, item 574, as amended), the Act of 3 July 2018 on Introductory Provision of the Act - Law on Higher Education and Science (Journal of Laws of 2018, item 1669 as amended) and the Resolution No. 133/2020/2021 of the Senate of Adam Mickiewicz University, Poznań of 28 June 2021. Therefore I recommend the admission of Candidate to next steps of Ph.D. title conferment procedure.

