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To
prof. dr hab. Nina Smolińska
the Chairman
of the Scientific Council
of the Discipline of Biological Sciences
at the University of Warmia and Mazury in Olsztyn, Poland

I am pleased to inform you about the successful completion of a doctoral thesis by Mr. Robert Stryiński, MSc.

The thesis entitled "Proteome profiling of the parasitic nematode *Anisakis simplex* s. s." was written by Robert Stryiński under the supervision of Elżbieta Łopieńska-Biernat, DSc, PhD, prof. UWM, and myself as an auxiliary supervisor. I was involved in designing and evaluating the progress of the PhD thesis, especially the proteomic part of the planned experiments.

The thesis presented by the doctoral student includes an abstract, an introduction with a literature review, the objective of the work, and a methodological section with the results and a summary. The literature section describes the problems associated with the *A. simplex* nematode, its morphology, anatomy, and developmental cycle in light of taxonomy and zoonotic threats to humans. The introduction is also a review of the literature on proteomic analyzes in light of research in parasitology, which well demonstrates the legitimacy and necessity of the research conducted in this thesis. It has been written with correct content and style to the extent necessary for proper understanding and interpretation of the results contained in the thesis.

In the presented doctoral thesis, Mr. R. Stryiński applied Advanced Proteomics Tools, i.e., high-throughput liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using TMT labelling on LTQ-Orbitrap Elite mass spectrometers to investigate the differences and similarities in proteomic profiles of the global proteome or selected tissues

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(intestine and cuticle) of L3 and L4 developmental stages of *Anisakis simplex*, a parasitic nematode, which will provide crucial data in the fight against anisakiasis in humans, of which this parasite is the causative agent. The results of the doctoral thesis are the basis for 2 original papers, which are included in the thesis submitted for evaluation. The mentioned papers were published in journals with a total Impact factor of 10.252 and 240 MEiN points. These are:

(1) Stryiński R, Mateos J, Pascual S, González AF, Gallardo JM, Łopieńska-Biernat E, Medina I, Carrera M. Proteome profiling of L3 and L4 Anisakis simplex development stages by TMT-based quantitative proteomics. Journal of Proteomics, 2019, 201, 1-11. https://doi.10.1016/j.jprot.2019.04.006

(2) Stryiński R, Mateos J, Carrera M, Jastrzębski JP, Bogacka I, Łopieńska-Biernat E. Tandem mass tagging (TMT) reveals tissue-specific proteome of L4 larvae of Anisakis simplex s. s.: enzymes of energy and/or carbohydrate metabolism as potential drug targets in anisakiasis. International Journal of Molecular Science. 2022, 23, 4336. https://doi.org/10.3390/ijms23084336.

The characterized global proteomes of *A. simplex* could be used in antiparasitic research and provide an answer to the question of how to neutralize parasitic larvae and thus help control anisakiasis. The identified tissue-specific proteins could serve as targets for new drugs against anisakiasis. Both, the global and tissue-specific proteome were identified, described, and characterized by Stryiński et al. for the first time. In addition, the results described in this work clearly demonstrate the potential of proteomics and TMT-based LC-MS/MS, especially as a universal tool for analyzing global proteomic dynamics in parasitic nematode stages and specific tissues.

My overall evaluation of Mr. Robert Stryiński's dissertation is very high. The doctoral student has proven that he is capable of planning and conducting research at a high level, choosing research techniques correctly, and drawing reliable conclusions based on the research results obtained. In evaluating the thesis, I conclude that it meets all the requirements for a doctoral thesis.

Due to all that, I ask the Council for the Discipline of Biological Sciences of the University of Warmia and Mazury in Olsztyn to accept the thesis and admit Mr. Robert Stryiński to further stages of for doctorial title conferment procedure.

Sincerely,
Dr. Mónica Carrera





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