

**A Review of the Doctoral Dissertation of Mr. Robert Stryński MSc
“Proteome profiling of the parasitic nematode *Anisakis simplex* s. s.”**

The doctoral dissertation submitted for review was completed under the supervision of Elżbieta Łopieńska-Biernat, DSc, PhD, prof. UWM, and Ms. Mónica Carrera Mouriño, PhD as auxiliary supervisor, at the Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland and at the Department of Food Technology, Institute of Marine Research, Spanish National Research Council in Vigo, Spain. Prof. Elżbieta Łopieńska-Biernat is a recognized specialist in parasite biochemistry and doctor Mónica Carrera Mouriño specializes in the field of proteomics. Both substantive care and research facilities created excellent conditions for the implementation of the planned research.

Formal evaluation

The doctoral dissertation of Mr. Robert Stryński, MSc, submitted for evaluation, comprises a total of 99 pages. It is prepared in a form in accordance with art. 187 para 3 of the Higher Education and Science Act dated 20 July 2018 and the guidelines of the Council of Scientific Excellence (Communiqué 19/2020, dated 09/11/2020), i.e., composed of published works and additional (unpublished) data. The basis of the dissertation is a thematically coherent set of scientific articles on the identification and characterization of the proteome of the L3 and L4 developmental stages of the parasitic nematode *Anisakis simplex*; it includes two original papers, published in international, reputable journals, in 2019 and 2022:

1. Stryński R., Mateos J., Pascual S., González Á. F., Gallardo J. M., Łopieńska-Biernat E., Medina I., Carrera M., 2019: Proteome profiling of L3 and L4 *Anisakis simplex* development stages by TMT-based quantitative proteomics. *Journal of Proteomics* 201: 1-11.
2. Stryński R., Mateos J., Carrera M., Jastrzębski J. P., Bogacka I., Łopieńska-Biernat E., 2022: 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 Sciences* 23(8): 4336.

Both publications are multi-authored; thus, the doctoral dissertation is an independent and separate part of a collective work, in which Mr. Robert Stryński is a leading contributor, either as the first author or one of the corresponding authors; he was also a co-author of the study concept, and collaborated in developing of the methodology, performing the experiments, analyzing the data and drafting the manuscript. In addition, the doctoral dissertation presented for evaluation contains a 59-page text (typescript), consisting of, among others, abstracts in Polish and English, a general overview of research on the biology, structure, systematics of nematodes, including the genus *Anisakis*; it also contains a characterization of proteomics, a bibliography, and co-author statements.

Substantive evaluation

Anisaki simplex is an Arctic Boreal species with a wide host range, and one of the more commonly-recorded nematodes in the marine environment. In its life cycle, the role of definitive host is played by marine mammals (Cetacea), which acquire parasites from intermediate or paratenic hosts (fish, cephalopods). However, the life cycle can also involve humans, who become infected by consuming raw or semi-dry fish or cephalopods. Hence, *A. simplex* has zoonotic significance, as both a cause of human anisakiosis and various allergies.

Despite many years of intensive research on various aspects of the biology of *A. simplex*, its precise nature remains obscure, especially in terms of the molecular mechanisms involved in its pathogenicity. However, a greater insight has recently been provided by methods from the field of proteomics, one of the pillars of modern Molecular Biology and Biotechnology. For several years now, proteomic analysis and protein identification have become very useful tools in both the pure and applied sciences. Hence, the topic undertaken by Mr. Robert Stryński on proteomics is fully justified.

Thus, the objective of the PhD student's research was to identify and characterize the proteome of the L3 and L4 larval stages of the nematode *A. simplex* at the systemic and tissue levels (cuticle, intestine). To this end, the study uses a range of modern techniques, including ultra high-performance liquid chromatography coupled with high resolution tandem mass spectrometry and isobaric tandem mass tagging; complex proteomic data analysis used various databases and servers, such as AllercatPro, HPIDB, and STRING. The L3 larvae were collected from European hake and L4 larvae from striped dolphins.

The identification and characterization of the systemic and the tissue-specific proteome of *A. simplex* L3 and L4 entail a number of significant scientific achievements by Mr. Robert Stryński, including:

- The comparison of the L3 and L4 stage proteome of *A. simplex* based on the 2443 identified proteins,

- The demonstration that more proteins were more abundant in L4 than in L3,
- The identification of 3435 proteins (1277 characterized) in *A. simplex* L4. Among these, 107 proteins were differentially regulated between the cuticle (49 proteins) and the rest of the larval body (58 proteins). In addition, 123 proteins were found to be differentially regulated between the intestine and the rest of the larval body, with 67 proteins being more abundant in the intestine and 56 in the rest of the body. Furthermore, 272 proteins were differentially regulated between the cuticle (133 proteins more abundant) and the intestine (139 proteins). Among the proteins identified in the intestine, many demonstrate catalytic activity, and a number involve both oxidative phosphorylation and the tricarboxylic acid cycle. In turn, the proteins identified in the cuticle, include those which take part in the immune response, those directly involved in cuticle formation or the extracellular matrix, and a number involved in glycolysis,
- The identification of tissue-specific enzymes from *A. simplex* L4 larvae. A total of 32 cuticular proteins were assigned to five enzyme classes, and 66 intestinal proteins were assigned to six enzyme classes. The most abundant in both the intestine and cuticle were hydrolases. The least abundant were oxidoreductases, translocases and transferases in the intestine, and oxidoreductases, transferases and lyases in the cuticle,
- The creation of models of predicted tertiary structures for *A. simplex* L4 oxoglutarate dehydrogenase and folliculin,
- The development of interaction network analysis for differentially-regulated proteins between the cuticle, the intestine and the rest of the body of *A. simplex* L4,
- The preliminary analysis of host-parasite interactions between *A. simplex* proteins and dolphin (definitive host) and human (incidental host) proteins,
- The identification/indication of dozens of proteins with potential allergenic properties in both L3 and L4 larvae.

It should be emphasized that the objective of the work has been achieved: the PhD student showed that he was able to plan the research, conduct experiments and interpret the results. It should be added that the choice of research objects (larval stages as well as individual elements of their structures – cuticle, intestine) was apt. Many studies have shown that the cuticle and intestine of nematodes have the greatest contact/interaction with the host, and in these, the excretory/secretory system plays a significant role. In contrast, unlike the L1–L3 stages, the L4 larvae in the cuticle and intestine are already fully developed and functional, as in the adult nematode stages. Moreover, they have a richer set of enzymes involved in a wide range of biological processes, which has now been confirmed for *A. simplex*.

The author has also demonstrated a very good knowledge of the literature in the field of his research; the correctly-selected references count 300 items.

As it is the reviewer's duty to spot any inaccuracies and formulate concerns and questions, I have a few remarks, with a request for relevant comments:

- In *A. simplex* eggs, it is possible that later larval stage (L3) can also develop, in addition to the first stages (L1, L2). In light of current knowledge, can such L3 larvae be invasive to subsequent hosts, especially the final host?
- Note – the lips or buccal cavity are not always present in the structure of nematodes.
- The names of the authors of descriptions of *A. simplex* and *A. typica* species should be in brackets: this is regulated by the International Code of Zoological Nomenclature.
- Standardized nomenclature for parasitic diseases (SNOPAD) recommends using the name "anisakiosis".
- For *Anisakis brevispiculata*, *A. paggiae* and *A. physeteris* there was a recent suggestion to move to the genus *Skrjabinisakis* (Safonova et al. 2021) – please comment accordingly.

In summary, the dissertation presented for evaluation provides a wealth of new and very important data on the *Anisakis simplex* proteome. The publication of the main theses in reputable international periodicals, ensuring a wide dissemination, is of great value. In my opinion, the additional data (typescript) attached in the dissertation contributes less to the overall achievement; however, it could be argued that their aim was to add the general theme of the issues studied and methodological assumptions. To some extent, this section lacked a broader connection with the results presented in the publications, and a summary, which was allowed for by the adopted formula of the dissertation. However, this remark refers only to the additional information and unpublished material and does not diminish the value of the dissertation as a whole.

The present study represents the most accurate and comprehensive collection of proteomic data of this parasite. Its findings demonstrate that the proteins in *A. simplex* larvae, especially those specific to the cuticle or intestine of the parasite, are involved in many biological processes, and can be important cellular components. Undoubtedly, further research is needed, however, the collected data, and the methods used, with their various proteomic analyses, can play a valuable role in the diagnosis and treatment of various parasitosis.

Summary and conclusion

In conclusion, I state that the doctoral dissertation of Mr. Robert Stryński MSc. contributes significant values to Science in the field of the biology of the parasitic nematode *Anisakis simplex* and meets all the requirements specified in the Higher Education and Science Act dated 20 July 2018 (Polish Journal of Laws of 2018 item 1668, as amended). Accordingly, I request that the

Scientific Council of the discipline of Biological Sciences of the University of Warmia and Mazury in Olsztyn admit Mr. Robert Stryński to further stages of the doctoral program.

In addition, taking into account the very good substantive and methodological level of the reviewed work and the great practical significance of the obtained results, I also submit a request that this dissertation be recognized with an award.

A handwritten signature in blue ink, reading "Leszek Rolbiecki". The signature is written in a cursive style with a large initial 'L'.

dr hab. Leszek Rolbiecki, prof. UG