DIPARTIMENTO DI SANITÀ
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TO WHOM IT MAY CONCERN

The doctoral elaborate of Robert Stryiński **whose title is: "Proteome profiling of the parasitic nematode *Anisakis simplex* (s.s.)"** represents, overall, an important milestone in the study of the proteomic profile of one of the most important zoonotic parasite species, naturally infecting seafood in the Northern Boreal Region of the world, i.e. *Anisakis simplex* (s.s.). Indeed, the parasite provokes in humans the parasitic zoonosis, i.e. Anisakiasis, characterized by gastric - intestinal and gastroallergic diseases, characterised by different pathogenic features.

Therefore, the study carried out by Robert Stryiński on the proteome of the parasite species is of fundamental importance to understand mechanism of pathogenesis in humans as first, but also to detect possible molecules which could be in future useful to develop diagnostic method for the detection of the immune response in humans, as well as for the development of possible drugs to alleviate the symptoms correlated with the human disease. **These aspects represent original solutions to the scientific problems related to the zoonotic disease in humans**.

The doctoral elaborate of Robert Stryiński consists of 99 numbered pages, including copies of scientific articles with the research results and the statements of the co-authors. The dissertation begins with an abstract, a table of contents and a list of abbreviations used. In an abstract written in Polish and English, **the doctoral student synthesizes the main assumptions and results of the research.** Then the author lists the articles that make up the core of the dissertation. **This is followed by the authors' contribution in preparing for publication, research funding, and the scientific collaborations**. In the following chapters, the author presents in synthetic form the most important information **and a review of the literature on the research topic.** This part is complemented by carefully selected illustrations. After this synthetic introduction, he discusses the hypotheses. **Then the author moves seamlessly to the presentation of the results of the published research. In the presented articles, the doctoral student verifies the hypotheses established in the dissertation in a logical and coherent manner.** The whole dissertation ends with the chapter "Conclusions". which also proves the topicality of the subject. **The scientific literature on the argument** including 185 articles, most of which are publications from recent years, **is updated and highly informative for the specific contest of the study carried out by Robert Stryiński.**

To reach the objectives, clearly indicated in the elaborate, Robert Stryiński has used both updated and innovative methodological approaches (i.e the LC-MS/MS and the TMT of peptides for the relative quantification of the proteins), which have fully allowed to obtain interesting and expected findings. The data gathered are very well presented in his Thesis elaborate, clearly described and the achieved results support the

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discussion and conclusions, as resumed by the candidate in his elaborate. The statistical elaboration of the data was correct.

In particular, I would like **to pay attention to some interesting results,** among the others, achieved by Robert's Stryiński studies, such as: *i)* the characterization of the proteome profiles and its comparison of the developmental larval stages, i.e. 3rd and 4th stages, of the parasite species; *ii)* the identification of tissue proteins of the parasites having a contact with the accidental and natural hosts; *iii)* the characterization of the interactoma, i.e. the proteins global content involved in the interaction with natural hosts (a cetacean species) and accidental one (humans). At least, but not the last, the proteoma acquired by this analysis, when they will be available in dedicated of data Banks, **will constitute a very solid base for future research on this parasite species available for several scientists all over the world.** I consider this last aspect one on the main important characteristic in becoming an outstanding scientist.

The doctoral dissertation of Robert Stryiński **consists of two published original publications, in which the PhD student is the first author.** The results of Robert Stryiński's dissertation results are presented in the following original scientific articles:

- Stryiński R., Mateos J., Pascual S., González Á. F., Gallardo J. M., 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.org/10.1016/j.jprot.2019.04.006
- Stryiński R., Mateos J., Carrera M., Jastrzębski J. P., 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 Sciences. 2022; 23(8): 4336. https://doi.org/10.3390/ijms23084336

The above papers are included in *peer reviewed* journals with a high impact factor (IF), as for the research field represented by Robert Stryiński MSc. This proves that they belong to the group of leading journals in this field.

In conclusion, Robert Stryiński, because of his research work so far carried out during his PhD course**, is an excellent scientist, and his PhD elaborate, in its forms and content, is at the same level of excellence**. I would like to emphasize that **I have highly appreciated the dissertation by Robert Stryiński, MSc and state that it undoubtedly meets the requirements for doctoral dissertations required by the current rules.**

Therefore, I'm asking the Scientific Council of the Biological Sciences Discipline of the University of Warmia and Mazury in Olsztyn **to admit Mr Robert Stryiński, to further stages of the defence of his doctoral dissertation.**

Rome, 2nd of September, 2022 sincerely,

Prof. Simonetta Mattiucci

QUESTIONS:

I would also like to present **to Mr Robert Stryiński the following question**: What are, according to your hypotheses, the main important proteins derived from the interactome analysis between the parasite species and human host, an what is their possible role in the accidental-human host - parasite interaction.