

# Proteomic investigation of *Pseudoterranova decipiens* and *Contracaecum osculatum* third stage larvae reveals their possible allergenic potential

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*Pseudoterranova decipiens* and *Contracaecum osculatum* are important zoonotic food-borne parasites. Nonetheless, knowledge about the proteomic profiles of *P. decipiens* and *C. osculatum* third stage larvae (L3) is very limited. Therefore, we performed proteomic investigation of *P. decipiens* and *C. osculatum* L3 larvae using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

In total, 397 and 261 proteins were detected in *P. decipiens* and *C. osculatum* L3 larvae, respectively. Amongst all detected proteins, 85 were identified in both anisakids. Sequences of heat shock proteins (hsps), spindle- and centrosome- associated proteins and fructose biphosphate aldolase were the most frequently detected shared proteins. Furthermore, 8 allergens were detected in *P. decipiens*: Ani s 2, Ani s 3.0101, Ani s 3.0102, Ani s 5, Ani s 8, Ani s 9, Ani s PEPB and Ani s Troponin. The following allergens were identified in *C. osculatum*: Ani s 2, Ani s 5, Ani s 13, and Asc l 3. Bioinformatics analysis allows predicting 28 probably allergens of *P. decipiens*, and 25 probably allergens *C. osculatum*. Among possible allergens most frequently were detected sequences of hsps, followed by paramyosin, peptidyl-prolyl cis-trans isomerase, enolase, and tropomyosin. Additionally, western blot analysis confirmed the cross-reactivity of rabbit anti-*A. simplex* IgG antibodies with protein extracts from *P. decipiens* and *C. osculatum* L3 larvae. Most of all detected proteins were phylogenetically related to *Anisakis simplex*, *Toxocara canis*, and *Ascaris suum*. Label-free quantification analysis showed that hemoglobin and disorganized muscle protein 1 were the most abundant in *P. decipiens* proteome, while enolase proteins were the most abundant in *C. osculatum*. Gene ontology (GO) analysis allows assigning GO terms for 297 proteins of *P. decipiens* and 205 proteins of *C. osculatum*. For *P. decipiens*, and *C. osculatum* proteins 1171 and 871 annotations were found, respectively. Nevertheless, the structures of GO terms between the both proteomes were quite similar. Encyclopedia of Genes and Genomes (KEGG) analysis was conducted. Forty three KEGG pathways of *P. decipiens*, and 32 pathways of *C. osculatum* were assigned. The most represented pathways of both proteomes were: biosynthesis of antibiotics, purine metabolism, glycolysis/gluconeogenesis, citrate cycle, and pyruvate metabolism. Carbohydrate metabolism pathways were the major group among all identified KEGG pathways.

Our study provides scientific community novel data regarding proteomic investigations of *P. decipiens*, and *C. osculatum* L3 larvae. Based on these results we can conclude that *P. decipiens* and *C. osculatum* should be considered as a potential source of allergens which could lead to IgE-mediated hypersensitivity. Nevertheless, further studies on allergenic potential of *P. decipiens* and *C. osculatum* L3 larvae are needed.

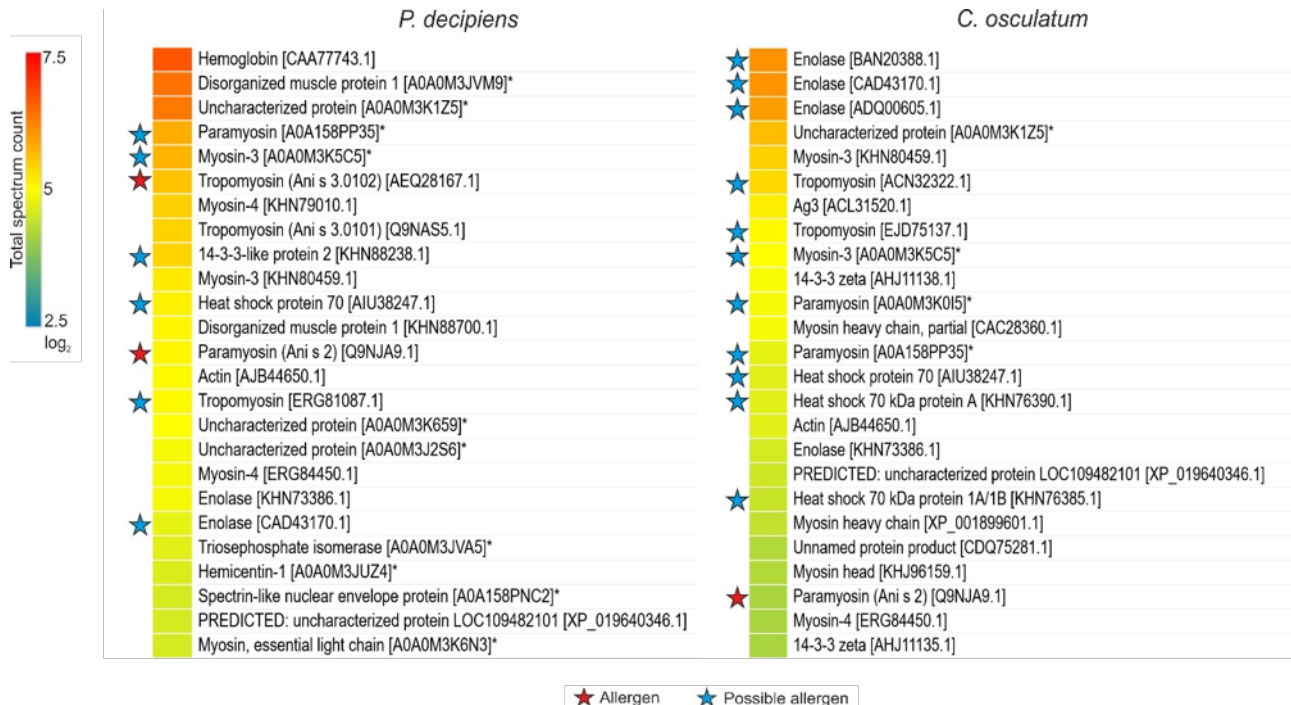


Fig. 1. Heat map of top 25 most abundant proteins of *P. decipiens* and *C. osculatum* determined by label-free quantification mass spectrometry. Average total spectrum count of proteins identified in all three replicates was log<sub>2</sub>-transformed and visualized using Morpheus (<https://software.broadinstitute.org/morpheus/>). Proteins identified as allergens and possible allergens are additionally marked in the heat map. Accession nos. of proteins were obtained from NCBI-nr protein database or UniProtKB (\*).