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 bisphosphate 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 cistrans 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.

		P. decipiens		C. osculatum
7.5		Hemoglobin [CAA77743.1]	*	Enolase [BAN20388.1]
		Disorganized muscle protein 1 [A0A0M3JVM9]*	*	Enolase [CAD43170.1]
		Uncharacterized protein [A0A0M3K1Z5]*	*	Enolase [ADQ00605.1]
	*	Paramyosin [A0A158PP35]*		Uncharacterized protein [A0A0M3K1Z5]*
5	× ×	Myosin-3 [A0A0M3K5C5]*		Myosin-3 [KHN80459.1]
	*	Tropomyosin (Ani s 3.0102) [AEQ28167.1]	*	Tropomyosin [ACN32322.1]
Ŭ.		Myosin-4 [KHN79010.1]		Ag3 [ACL31520.1]
2.5		Tropomyosin (Ani s 3.0101) [Q9NAS5.1]	*	Tropomyosin [EJD75137.1]
	*	14-3-3-like protein 2 [KHN88238.1]	★ ★	Myosin-3 [A0A0M3K5C5]*
		Myosin-3 [KHN80459.1]		14-3-3 zeta [AHJ11138.1]
	*	Heat shock protein 70 [AIU38247.1]	*	Paramyosin [A0A0M3K0I5]*
		Disorganized muscle protein 1 [KHN88700.1]		Myosin heavy chain, partial [CAC28360.1]
log ₂	*	Paramyosin (Ani s 2) [Q9NJA9.1]	*	Paramyosin [A0A158PP35]*
		Actin [AJB44650.1]	★ ★	Heat shock protein 70 [AlU38247.1]
	*	Tropomyosin [ERG81087.1]	\bigstar	Heat shock 70 kDa protein A [KHN76390.1]
		Uncharacterized protein [A0A0M3K659]*		Actin [AJB44650.1]
		Uncharacterized protein [A0A0M3J2S6]*		Enolase [KHN73386.1]
		Myosin-4 [ERG84450.1]		PREDICTED: uncharacterized protein LOC109482101 [XP_019640346.1
		Enolase [KHN73386.1]	*	Heat shock 70 kDa protein 1A/1B [KHN76385.1]
	*	Enolase [CAD43170.1]		Myosin heavy chain [XP_001899601.1]
		Triosephosphate isomerase [A0A0M3JVA5]*		Unnamed protein product [CDQ75281.1]
		Hemicentin-1 [A0A0M3JUZ4]*		Myosin head [KHJ96159.1]
		Spectrin-like nuclear envelope protein [A0A158PNC2]*	*	Paramyosin (Ani s 2) [Q9NJA9.1]
		PREDICTED: uncharacterized protein LOC109482101 [XP_019640346.1]		Myosin-4 [ERG84450.1]
		Myosin, essential light chain [A0A0M3K6N3]*		14-3-3 zeta [AHJ11135.1]

★ Allergen 🛛 🛧 Possible allergen

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 log2-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 (*).