


RESEARCH ARTICLE

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Functional insights into the infective larval stage of *Anisakis simplex s.s.*, *Anisakis pegreffii* and their hybrids based on gene expression patterns

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Abstract

Background: *Anisakis simplex* sensu stricto and *Anisakis pegreffii* are sibling species of nematodes parasitic on marine mammals. Zoonotic human infection with third stage infective larvae causes anisakiasis, a debilitating and potentially fatal disease. These 2 species show evidence of hybridisation in geographical areas where they are sympatric. How the species and their hybrids differ is still poorly understood.

Results: Third stage larvae of *Anisakis simplex s.s.*, *Anisakis pegreffii* and hybrids were sampled from *Merluccius merluccius* (Teleostei) hosts captured in waters of the FAO 27 geographical area. Specimens of each species and hybrids were distinguished with a diagnostic genetic marker (ITS). RNA was extracted from pools of 10 individuals of each taxon. Transcriptomes were generated using Illumina RNA-Seq, and assembled de novo. A joint assembly (here called merged transcriptome) of all 3 samples was also generated. The inferred transcript sets were functionally annotated and compared globally and also on subsets of secreted proteins and putative allergen families. While intermediary metabolism appeared to be typical for nematodes in the 3 evaluated taxa, their transcriptomes present strong levels of differential expression and enrichment, mainly of transcripts related to metabolic pathways and gene ontologies associated to energy metabolism and other pathways, with significant presence of excreted/secreted proteins, most of them allergens. The allergome of the 2 species and their hybrids has also been thoroughly studied; at least 74 different allergen families were identified in the transcriptomes.

Conclusions: *A. simplex s.s.*, *A. pegreffii* and their hybrids differ in gene expression patterns in the L3 stage. Strong parent-of-origin effects were observed: *A. pegreffii* alleles dominate in the expression patterns of hybrids albeit the latter, and *A. pegreffii* also display significant differences indicating that hybrids are intermediate biological entities among their parental species, and thus of outstanding interest in the study of speciation in nematodes. Analyses of differential expression based on genes coding for secreted proteins suggests that co-infections presents different repertoires of released protein to the host environment. Both species and their hybrids, share more allergen genes than previously thought and are likely to induce overlapping disease responses.

Keywords: *Anisakis simplex*, *A. Pegreffii*, Transcriptome, Allergens, Gene expression

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