

***Anisakis pegreffii* and *Anisakis simplex sensu strictu* in Mediterranean sea**

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BACKGROUND: Anisakiasis is a parasitic zoonosis caused by ingestion of nematode larvae belonging to *Anisakidae* family, following consumption of raw, undercooked or improperly processed fish. Mediterranean sea represent an aquatic ecosystem particularly suitable for the development of Anisakid larvae. The aim of this work is to found intra-species and inter-species nucleotide differences by phylogenetic analysis in this geographical area.

METHODS: In the period from January to November 2013, 584 fish from Mediterranean sea were screened, and they were found parasitised of 6318 type I anisakid larvae. Fish were eviscerated and observed by stereo microscope to collect larvae relived in the viscera, organs and muscles. After genus identification, the larvae were subjected to molecular analysis by extraction of DNA, amplification of ITS gene and restriction enzyme. PCR products were sequenced and the sequences were analyzed and aligned to examine the relationship of nucleotides.

RESULTS: The anisakid species we have identified were *Anisakis pegreffii* and *Anisakis simplex s.s.*. Phylogenetic analysis detects nucleotide differences between the two species. In the positions 251 and 267 was found a Cytosine in *Anisakis pegreffii* and a Thymine in *Anisakis simplex s.s.*, respectively. No difference was found in *Anisakis pegreffii* specie becoming from different fish of different areas.

CONCLUSIONS: The parasite DNA were amplified and sequenced to identify any nucleotide differences between the different species as well as within the same species. No intra-species sequence differences were found in *Anisakis pegreffii*. Two inter-species differences were found between *Anisakis pegreffii* and *Anisakis simplex*. Further studies will be conducted to confirm nucleotide differences in other target genes. This study was supported by the Italian National Reference Centre for Anisakiasis.