

On the morphology of *Anisakis pegreffii*: a comparative analysis of three microscopic techniques used to build a new parasite atlas

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BACKGROUND: Human anisakidosis is a parasitic anthroponosis caused by larval nematodes of the family *Anisakidae*. Here, we report a detailed description of the morphology of *Anisakis pegreffii* third-stage larva performed using a conventional light and confocal microscopy, and scanning electron microscopy (SEM) that provide a basis for both phenotypic studies and genetic mutations.

METHODS: The collected larvae from fish were morphologically identified as *Anisakis* larvae Type I, and they were characterized by PCR-RFLP to identify the *Anisakis pegreffii* specie. Using NC5/NC2 primers, ribosomal genomic regions ITS1, 5.8 SrRNA and ITS2 of DNA were amplified and PCR products were sequenced. Fifteen larvae belonging to *Anisakis pegreffii* were fixed, sectioned, and examined with a light and confocal microscope and by SEM.

RESULTS: In our studies, have been acquired detailed ultrastructural images, which have been integrated with those derived from the dissection of the parasite, obtained with light and confocal microscopy. The structural and ultrastructural images concerning the third stage larvae of *Anisakis pegreffii* have been studied, analyzed and compared among them. The derived overall view has allowed detecting new interesting details of a well-known parasite and has been schematically showed.

CONCLUSIONS: The aim of this study is to furnish an updated atlas of *Anisakis pegreffii*. Confocal microscopy, as well as the light and electron microscopy have played a pivotal role in the accumulation of new scientific data regarding the anatomical structures of this nematode. This work is the result of one year of engagement by the Authors and the outcome is a comprehensive atlas on *Anisakis pegreffii* microscopy.