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Anisakis spp. larvae in three mesopelagic and bathypelagic fish species of the central Mediterranean Sea

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ABSTRACT

In this work 437 fish samples of species belonging to the families Myctophidae (*Electrona risso* and *Diaphus metopoclampus*) and Phosichthyidae (*Vinciguerria attenuata*) were examined for the presence of Anisakidae larvae. The study was performed with fishes in the central Mediterranean Sea, particularly in the Strait of Sicily and in the Strait of Messina. The visual inspection and chloro-peptic analysis revealed the presence of nematode parasites with prevalence values between 2.9% in *Electrona risso* samples and 5.4% in *Vinciguerria attenuata* samples. A positive correlation was found between standard length (SL) and prevalence of infestation in *D. metopoclampus* samples ($p < 0.05$). The larvae examined were morphologically ascribed, at genus level, to *Anisakis* morphotypes I and II and molecularly identified as *Anisakis pegreffii*, *Anisakis ziphidarum* and *Anisakis physeteris*, in 67%, 9% and 24% of the fish samples examined. Overall, *A. pegreffii* and *A. ziphidarum* larvae were isolated in 14 and 2 specimens of *D. metopoclampus* respectively, *A. physeteris* larvae were found in 3 *E. risso* and 2 *V. attenuata*. A positive correlation was found between standard length and prevalence of infestation in *D. metopoclampus* samples ($p < 0.05$). First information is provided on the presence of *Anisakis* spp. larvae of the myctophid fish species *E. risso*, *D. metopoclampus* and *V. attenuata* from the Central Mediterranean. It is also confirmed the role of lanternfishes (Myctophidae) as paratenic hosts for *Anisakis* spp.

1. Introduction

Anisakidae is a family of parasitic nematodes, including *Anisakis* genus, which performing their life cycle in marine environment. Anisakids have a worldwide distribution and a life-cycle involving invertebrates, fishes, and marine mammals [1–3]. The accurate identification of anisakid nematodes at any life cycle stage is important both to deepen the knowledge on their taxonomy, ecology, epidemiology and for diagnosis and control, as larval stages cause a clinical disease in humans known as Anisakidosis [4,5].

In the Mediterranean area, *Anisakis* spp. larvae can be considered the most important hazard for human consumers due to ingestion of raw, undercooked, or improperly processed marine fish and cephalopods [3,5–7]. Among the nine species of *Anisakis* so far characterized genetically, *A. pegreffii* has been identified as agents of human anisakiasis in the Mediterranean area, confirmed by molecular markers [8,9]; the parasites of this species can be found in different Mediterranean fishes belonging to different ecological distribution [1].

Mesopelagic and bathypelagic fish species are widely distributed in all oceans and characterized by high biomass [10,11], representing significant components in the marine pelagic food web. Their ecological importance is linked to their particular migratory behavior: most mesopelagic species make extensive daily vertical migrations to the epipelagic zone at night, where they feed on plankton and other prey, and thereafter they come back to deep waters during daytime [10]. This kind of migration assures a continue transport of energy between different water layers. Several studies [12–15] underlined the role of mesopelagic fish in the life cycles of parasites including those of the *A. simplex* complex in different areas, benefiting from extensive diurnal vertical migrations of their fish hosts. Mateu et al. [15] suggested, for the first time, that myctophids may play a role as paratenic hosts in the life-cycle of *A. pegreffii* and *A. physeteris* in the Spanish waters of the Mediterranean Sea. However, due to the few parasitological studies that have been carried out on bathypelagic fish, the knowledge on their parasite fauna remains scarce. The aim of this work is to explore the presence of *Anisakis* spp. larvae in *E. risso*, *V. attenuata* and *D.*

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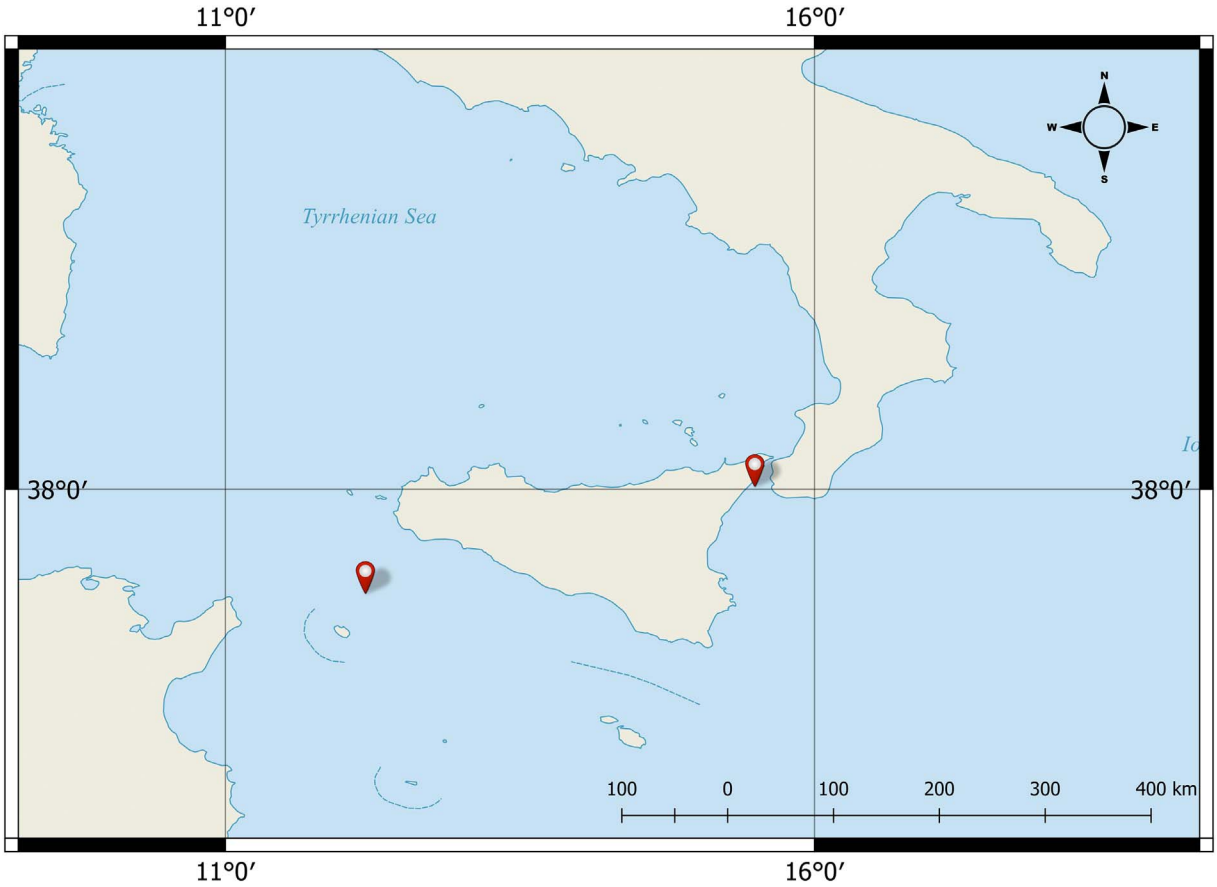

Fig. 1. Sampling areas.

metopoclampus collected from central Mediterranean Sea in order to broaden the knowledge of the ecological and biological aspects of the *Anisakis* spp. in these particular hosts.

2. Materials and methods

2.1. Study area and sampling method

A total of 437 fish samples were collected in two areas of the central Mediterranean Sea: the Strait of Sicily and the Strait of Messina (Fig. 1). In particular, 296 specimens of *Diaphus metopoclampus* (Cocco 1829), Family: Myctophidae, were caught in the Strait of Sicily during two trawl surveys carried out in October 2010 and May 2011, at a depth ranges of 571–724 m. In addition, 105 *Electrona risso* (Cocco 1829), Family: Myctophidae, and 36 *Vinciguerria attenuata* (Cocco 1838), Family: Phosichthyidae, were found stranded along the Sicilian coast of the Strait of Messina between the years 2012 and 2014. The strong hydrodynamism and the particular meteorological conditions (strong wind from the SE) are the main causes of deep fauna stranding in the Strait of Messina [16–18]. Most of the fishes collected were in good status, without visible signs of degradation. Only three specimens of *Vinciguerria attenuata* showed signs of desquamation. The specimens were identified following Whitehead et al. (1984), measured by calliper to the nearest 0.1 mm for the standard length assessment (SL) and weighed to the nearest 0.01 g.

2.2. Preliminary analysis and morphological assessment of the larvae

A parasitological exam was performed on fish samples examining the coelomic cavity and muscle by visual and stereoscopic inspection. Subsequently, a chloro-peptic digestion of viscera and muscles was carried out [19]. All the nematode larvae collected were washed in saline solution, fixed in 70% ethanol and cleared with glycerol for morphological identification by light microscopy Leica DM 2000, following the taxonomic keys [20].

2.3. Molecular analysis

All larvae collected were submitted to molecular identification at species level by PCR- based restriction fragment length polymorphism (PCR-RFLP) analysis of the rDNA comprising the internal transcribed spacers ITS (ITS-1, 5.8S gene, and ITS-2) region [21,22]. PCR amplification and sequencing of the ITS region and mitochondrial *cox*2 gene [23] were also carried out. For molecular identification, the larvae collected were washed, fragmented with a scalpel and frozen at − 20 °C for 24 h. Genomic DNA extraction were conducted by special kits based on the use of affinity columns (Sigma Aldrich), according to the manufacturer's instructions.

2.3.1. PCR-RFLP analysis

The nuclear rDNA containing ITS region was amplified using NC5 (5′-GTAGGTGAACCT GCGGAAGGATCATT-3′) and NC2 (5′-TTAGTTTCTTTTCCTCCGCT-3′) primers [24]. The PCR reactions, carried out in a final volume of 50 µl with the following PCR reactions: 2 mM MgCl2, 0,2 mM of each dNTP, 20 pmol/µl of each primer, buffer AmpliTaq Gold 1X, 3.0 U AmpliTaq Gold DNA Polymerase (AB) and 20–25 ng of genomic DNA. The PCR was performed using the following conditions: 10 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 58 °C and 75 s at 72 °C, followed by a final elongation of 15 min at 72 °C (Thermal Cycler 2720-Applied Biosystems). The PCR products were separated by electrophoresis in 1% agarose gel, stained with SYBR safe® (Invitrogen) in Tris- Borate- EDTA buffer and visualized by UV transilluminator