

New report of *Anisakis* larvae from Blunthead Puffer, *Sphoeroides pachygaster* caught off Strait of Sicily

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ABSTRACTS

Aim of the present paper was to report and identify by morphological and molecular methods the presence of anisakid L3 larvae found in 7 specimens of *Sphoeroides pachygaster* caught off Strait of Sicily from 2012 and 2015. Nematode larvae (n=9) were collected from three fish samples: the larvae were morphologically identified as belonging to the genus *Anisakis* Type I and, stored in 70% ethanol, were underwent molecular identification at species level by PCR- RFLP analysis of the rDNA (ITS-1, 5.8S gene, and ITS-2) region. Sequencing of ITS regions and comparison with sequences in GenBank were also performed. The parasites were molecular identified as belonging to *A. pegreffii* that is the predominant species in Mediterranean Sea. This is a new report of *Anisakis* sp. in *S. pachygaster*. The blunthead puffer *Sphoeroides pachygaster* is a fish species of Atlantic origin: in the last years there are indeed numerous reports of this alien fish species in the Mediterranean included Italian Seas. The presence of the species *Anisakis pegreffii* may support the hypothesis of complete adaptation of *S. pachygaster* in the Mediterranean Sea.

KEY WORDS

Anisakis; PCR-RFLP; nematodes.

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INTRODUCTION

The blunthead puffer *Sphoeroides pachygaster* (Müller & Troschel, 1848) is widely distributed in tropical and temperate waters on both sides of the Atlantic Ocean (Shipp, 1990). The species is known in the Mediterranean Sea where in the last years several records were reported (Cherif et al., 2010; Hemida et al., 2009; Relini et al., 1995) included Italian Seas (Adriatic Sea, Tyrrhenian Sea, Ionian Sea, Sicilian Channel) (Vacchi & Cau, 1986; Arculeo et al., 1994; Giordano et al., 2012; Ragonese

et al., 1997; Tursi et al., 1992; Ligas et al., 2006; Visentin & Borg, 2014). The presence of this alien species in the Mediterranean has been ascribed to the migration or casual transport through the Strait of Gibraltar and the Suez Canal; the spread is caused and favored by the ongoing phenomenon of global warming (Massuti et al., 2010). Actually it is believed that *S. pachygaster* form a well established population in the Mediterranean Sea, with local reproduction, because of the discovery of young and adult specimens (Lipej et al., 2013; Ragonese et al., 2001).

There are not reported in the literature reports of parasitic nematodes of the Anisakidae family in blunthead puffer. This paper reports a parasitological survey of n. 7 specimens of this fish species, caught off Strait of Sicily, indicating at the same time for the first time the presence of nematode larvae of *Anisakis* sp.

MATERIAL AND METHODS

Fish samples were collected in the Strait of Sicily, in front of the Agrigento coast, during trawl surveys, from 2012 and 2015: 7 specimens of *S. pachygaster* were caught accidentally by fishing vessels. The specimens were transported refrigerated at the C.I.S.S. of the University of Messina and were identified, measured and weighted (Fig. 1).

On the studied fish, a parasitological exam was performed examining the coelomic cavity for metazoan parasites, by visual and stereoscopic inspection. Nematode larvae were collected, washed in saline solution, fixed in 70% ethanol and cleared with glycerol for morphological identification by light microscopy (Berland, 1961) (Fig. 3). The larvae, identified according to morphological characters as belonging to the genus *Anisakis*, were stored in 70% ethanol for underwent molecular identification at species level by PCR- RFLP analysis of the rDNA (ITS-1, 5.8S gene, and ITS-2) region using two restriction enzymes, *Hinf*I and *Hha*I, for the identification of *Anisakis* spp. (D'Amelio et al., 2000; Pontes et al., 2005) (Fig. 4). Purification of ITS gene amplification products was carried out with Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare) following the manufacturer's instructions. The purified products were sent to Macrogen company (Amsterdam, Holland) for Sanger sequencing. The morphological and molecular identification of the parasites were performed by the C.Re.N.A. of IZS of Palermo (Italy).

RESULTS AND DISCUSSION

The results are reported in Table 1. Nematoda larvae (n= 9) were isolated in the coelomic cavity of 3 samples of *S. pachygaster*. Analysis of the morphological characteristics, observed by light microscopy, allowed to identify the parasites as *Anisakis* larvae Type I (sensu Berland, 1961) (Fig. 3). In the molec-



Figure 1. *Sphoeroides pachygaster*.



Figure 2. One larva encapsulated on the liver.

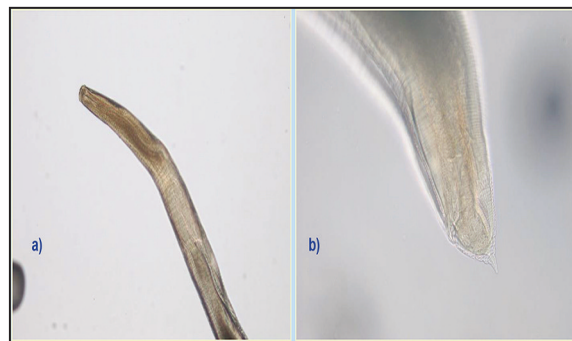


Figure 3. *Anisakis* larva Type I: a) anterior extremity b) tail showing mucron

year	Samples n.	Weight g	Locality	Presence of parasites	Larvae isolated	Morphological identification	Molecular identification (PCR-RFLP)
2012	1	1750	Strait of Sicily	1	2	<i>Anisakis</i> Tipo I	<i>A. pegreffii</i>
2015	2	560	Strait of Sicily	1	3	<i>Anisakis</i> Tipo I	<i>A. pegreffii</i>
2015	3	940	Strait of Sicily	1	4	<i>Anisakis</i> Tipo I	<i>A. pegreffii</i>
2015	4	730	Strait of Sicily	-	-	-	-
2015	5	420	Strait of Sicily	-	-	-	-
2015	6	385	Strait of Sicily	-	-	-	-
2015	7	130	Strait of Sicily	-	-	-	-
Totale	7			3	9		

Table 1. Results of fish examined, year, weight and fishing locality.

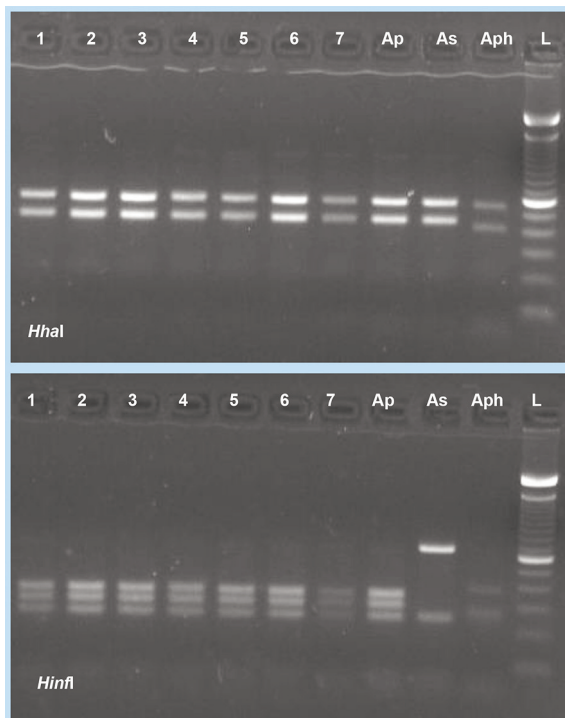


Figure 4. RFLP patterns obtained by digestion of the ITS region with the restriction enzymes Hha I and Hinf I. Lanes 1-7: *Anisakis pegreffii* (17), positive controls: Ap: *A. pegreffii*, *A. simplex* s.s., Aph: *A. physeteris* L: 100 bp ladder.

ular identification with PCR-RFLP analysis, the specific restriction profiles obtained were allowed to identify anisakid parasites as *Anisakis pegreffii*, according to the taxonomical keys available in literature (D'Amelio et al., 2000; Pontes et al., 2005). The sequences obtained were aligned and compared with the sequences deposited in GenBank, showing a 99% of identity with reference sequences of *A. pegreffii* (AY826720). *Anisakis pegreffii* is the predominant species in Mediterranean Sea while *A. simplex* s.s. is found in fish species of Atlantic Sea. The blunthead puffer *S. pachygaster* is a fish species of Atlantic origin, spread in all seas and oceans, warm and temperate. This alien species is known in the Mediterranean Sea where represent a definitively established population. The discovery of anisakid nematodes and identification of species such as *A. pegreffii*, may support the hypothesis of complete adaptation of *S. pachygaster* as well as of existence of a Mediterranean population.

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