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Artículo Original

Study of helminth parasites in the red mullet, *Mullus barbatus*, from the Mediterranean Sea and acquired in greater València, Spain

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ABSTRACT

The red mullet, *Mullus barbatus*, is a fish of great commercial value with a wide distribution in the North Atlantic and the Mediterranean Sea. However, the helminthfauna of the red mullet in Spain has been poorly studied and the potential parasitological risk for humans due to this fish remains almost unknown. With the aim to provide more information on the helminth community of this fish, 100 specimens caught in the Mediterranean Sea between January and May 2012 and acquired at various supermarkets near the city of València (Spain) were analysed. Helminths were identified based on morphological criteria. 50.0% of the specimens were parasitized by at least one of the six helminth species identified: three Digenea (*Opecoeloides furcatus*, *Proctoeces maculatus* and *Aponorus laguncula*); two Nematoda larvae (*Anisakis simplex* sensu lato and *Hysterothylacium* spp.) and one Acanthocephala (*Echinorhynchus gadi*). All helminths were present in viscera, while *Hysterothylacium* spp. were the only species found, always dead, in the flesh after artificial digestion. Thus, consumption of the red mullet does not imply a great risk of human parasitism. Although only a small number of helminth species was encountered, this is the first evidence of the presence of two of these species parasitizing *M. barbatus* from the Mediterranean.

Key words: *Mullus barbatus*, red mullet, helminthfauna, Mediterranean Sea.

RESUMEN

El salmonete de fango, *Mullus barbatus*, es un pescado de gran valor comercial y amplia distribución en el Atlántico Norte y en el Mar Mediterráneo. Sin embargo, su helmintofauna en España apenas ha sido estudiada y el riesgo potencial de parasitación para el ser humano a causa de su consumo es desconocido. Con el objetivo de obtener información acerca de la comunidad helmintiana del salmonete de fango, se analizaron

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100 especímenes capturados entre enero y mayo de 2012, de procedencia mediterránea y adquiridos en diferentes supermercados cercanos a la ciudad de València (España). Los helmintos fueron identificados en base a criterios morfológicos. El 50,0% de los salmonetes estaban parasitados por al menos una de las seis especies de helmintos identificadas: tres Digenea (*Opecoeloides furcatus*, *Proctoeces maculatus* y *Aponorus laguncula*); dos larvas de Nematoda (*Anisakis simplex* sensu lato e *Hysterothylacium* spp.) y un Acanthocephala (*Echinorhynchus gadi*). Todos los helmintos estaban presentes en las vísceras, mientras que *Hysterothylacium* spp. fueron las únicas especies halladas, siempre muertas, en la musculatura tras la digestión artificial. Por tanto, el consumo de salmonete no debería implicar un riesgo importante de parasitación humana. A pesar de hallar un bajo número de especies de helmintos, ésta es la primera evidencia de la presencia de dos de estas especies parasitando *M. barbatus* del Mediterráneo.

Palabras clave: *Mullus barbatus*, salmonete de fango, helmintofauna, Mar Mediterráneo.

INTRODUCTION

The red mullet, *Mullus barbatus*, a fish of great commercial value, with a wide distribution in the North Atlantic and the Mediterranean Sea, is not only one of the most consumed fish in Spain and other European countries but also a valid biological tag of the conditions of aquatic ecosystems. Consequently, the ecological interest in this species has increased in recent years (Corsi *et al*, 2002; Findik *et al*, 2011; Martínez-Gómez *et al*, 2012).

Numerous studies have evidenced the role of parasites as a valuable source of information on the biology of their hosts. They have also been used as important biological tags of the quality of ecosystems, environmental impacts, the presence of contaminating agents and the structuring of trophic chains (Marcogliese, 2005; Ferrer-Castelló *et al*, 2007). Therefore, the study of the parasite fauna of fish is a useful tool when assessing the conditions of aquatic ecosystems. Several authors have researched the parasite fauna of some species belonging to the genus *Mullus*, which includes other species of great commercial and ecological value such as the striped red mullet, *M. surmuletus*, studied at various points of the Mediterranean Sea (Arculeo *et al*, 1997; Ferrer-Castelló *et al*, 2007; Bayoumy *et al*, 2008; Klimpel *et al*, 2008; Nawel Amel *et al*, 2009). However, little light has so far been shed on the parasite fauna of the red mullet. In this context, the works by Carreras-Aubets *et al*. (2011a, 2012) carried out in some parts of the western Mediterranean stand out. Consequently, continuing the study of these species with the aim to reveal more aspects of their helminthfauna in other parts of the Mediterranean and the possible use of their helminths as biological tags will be of great interest.

The present work aims at the study of helminths parasites of the red mullet, *M. barbatus*, gather information on its helminth community in the Mediterranean, besides assessing the possible risk of human parasitation caused by the consumption of this fish sold in various supermarkets in greater València, Spain.

MATERIAL AND METHODS

A total of 100 specimens of the red mullet originating from the Mediterranean Sea were acquired at various supermarkets in greater València (Spain) between January and May 2012, and examined for parasites. Moreover, these fish specimens were caught by local fishermen off the Valencian coast.

In the laboratory, each specimen was measured, weighed and dissected. Visceral organs were removed and carefully checked for parasites under a stereoscopic microscope. The flesh of each specimen underwent artificial pepsic digestion, following the standardised methodology (Huang, 1990; Madrid *et al*, 2012). The resulting product was then examined under a stereoscopic microscope. The helminths detected were studied using standard helminthological techniques. Trematodes were fixed in Bouin's solution, whereas acanthocephalans were fixed in alcohol 70%. Both kinds of helminths were stained for 24 hours with Grenachers's boracic carmine and then differentiated with acidified alcohol, dehydrated in an alcohol series, cleared with xylene and mounted in Canada balsam

between slide and cover slip. Moreover, nematodes were fixed in 70% ethanol, cleared in Amann lactophenol and studied by direct examination between slide and cover slip. The parasites were identified based on morphological criteria, through the most relevant literature on each of the species involved (Berland, 1961; Grabda, 1991; Jones *et al*, 2002, 2005; Bayoumy *et al*, 2008). For the total of helminths found, as well as for each of the identified species and their microhabitat of occurrence (viscera and flesh), the following parameters were analysed: the number of parasitized hosts, the range of parasitation, prevalence, median intensity and mean abundance, according to Bush *et al*. (1997).

RESULTS

A total of 50 red mullets (50.0%) was found to be infected by at least one of the six helminths species identified (Table 1; Figure 1): three trematodes, namely *Opecoeloides furcatus* (19.0%), *Aponorus laguncula* (1.0%) and *Proctoeces maculatus* (1.0%); two nematodes, *Anisakis simplex* sensu lato larvae (2.0%) and *Hysterothylacium* spp. larvae (39.0%); and the acantocephalan *Echynorhynchus gadi* (3.0%). All of them were found in the viscera of the fish, while *Hysterothylacium* spp. were the only species found (10.0%), always dead, in the flesh after artificial digestion. The trematode *P. maculatus* and the acanthocephalan *E. gadi* are reported for the first time as helminth parasites of the red mullet.

DISCUSSION

The present work reports the finding of a total of six helminth species parasitizing the red mullet caught in the Mediterranean Sea and sold in supermarkets of greater València. However, the absence of larvae as well as adults belonging to the phylum Cestoda stands out. The studies of Carreras-Aubets *et al*. (2011a, 2012), focussing on the helminthfauna of the *M. barbatus* off the Catalan part of the Mediterranean Sea, reported up to 14 helminth species including Trematoda, Cestoda and Nematoda, although no Acanthocephala was found. Nevertheless, the low degree of biological diversity observed in the present study as well as the low helminth burden have to be emphasised, while the finding of new species, not having been reported until now as being present in the red mullet in this area, is surprising.

O. furcatus was the most prevalent and abundant trematode species, which agrees with other studies carried out on the red mullet as well as the striped red mullet in other parts of the Mediterranean Sea (Martínez-Vicaria *et al*, 2000; Ferrer-Castelló *et al*, 2007; Klimpel *et al*, 2008; Carreras-Aubets *et al*, 2011a, 2012). However, these authors reported prevalences and abundances of parasitation that are

Table 1. Number of parasitized hosts (No), prevalence (P) expressed as %, mean abundance (mA), median intensity (mI) and range of parasitation of the helminth species found in *Mullus barbatus*

Helminth species	No	P	mA	mI	range	Location
Trematoda						
Opecoeloides furcatus	19	19.00	0.52	2.74	1-11	Intestine
Aponorus laguncula	1	1.00	0.01	1	1	Stomach
Proctoeces maculatus	1	1.00	0.01	1	1	Stomach
Nematoda						
Anisakis simplex sensu lato	2	2.00	0.02	1	1	Mesenteries
Hysterothylacium spp.	39	39.00	0.90	2.31	1-27	Mesenteries and flesh
Acanthocephala						
Echinorhynchus gadi	3	3.00	0.03	1	1	Intestine

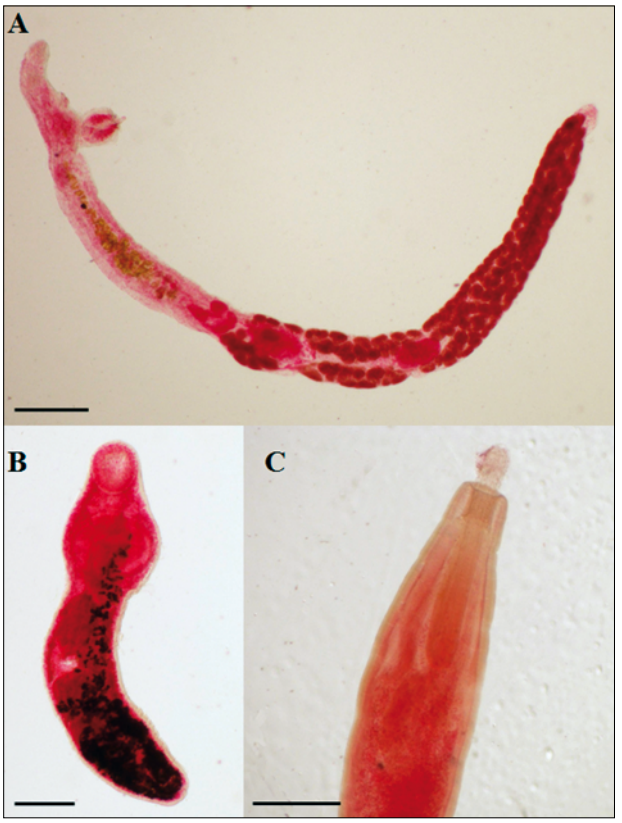

Figure 1. Some of the helminths found in *Mullus barbatus*. A) *Opecoeloides furcatus;* B) *Proctoeces maculatus*; C) *Echinorhynchus gadi*. Bar = 500 µm.

far higher in other Mediterranean parts, reaching in some seasons prevalences of nearly 90%. Therefore, the season of capture, body size and food habits of the fish were suggested to be the causal agents of these fluctuations (Martínez-Vicaria *et al*, 2000).

The low prevalence of *A. laguncula* corresponds with the results of other studies carried out by others in the Mediterranean Sea, reporting a low frequency (Nawel Amel *et al*, 2009; Carreras-Aubets *et al*, 2011a) or the absence of species of the genus *Aponorus* in *Mullus* spp., although these helminths are eurixenous and widely distributed (Carreras-Aubets *et al*, 2011b).

P. maculatus is the least known among the digenetic trematodes found, having been reported in some species of the genus *Mullus* in some studies carried out in the Mediterranean Sea (Nawel Amel *et al*, 2009), but this is first time it has been reported in *M. barbatus*.

With respect to nematodes, the detection of larvae of *A. simplex* sensu lato in this fish, although other authors had previously identified this anisakid as rare in the red mullet originating from the Mediterranean (Manfredi *et al*, 2000), stands out, in particular as aniskiasis is one of the most relevant human fish-borne diseases (Chai *et al*, 2005). Furthermore, all larvae of this nematode species were recovered only from the viscera of the fish and not from the flesh. Thus, the risk of human parasitation as a consequence of its consumption does not seem to be high.

Hysterothylacium spp. were found more often than *A. simplex*, a fact already evidenced by other studies (Carreras-Aubets *et al*, 2012; Serraca *et al*, 2013). Moreover, these helminths were the only ones found in the edible part of the fish, which could have reached the consumer. However, and taking into account that these nematodes were found dead after artificial digestion at 37ºC, the risk of human parasitation due to these species can be excluded. Nevertheless, the prevention measures against anisakidosis established by European and Spanish legislation for the consumption of fresh fish have to be followed as the role of anisakids as

causal agents of allergic syndromes in humans can be not fully ruled out (Valero *et al*, 2003).

The other helminth species, *E. gadi*, described as the most common acanthocephalan in salt water fish, has been found in more than 60 host species (Bayoumy *et al*, 2008). However, this helminth has hitherto not been reported in *M. barbatus* but in other species of the genus *Mullus* (Ferrer-Castelló *et al*, 2007; Bayoumy *et al*, 2008).

It was proposed that the parasite fauna of red mullets in the Mediterranean is the consequence of recent speciation and adaptation together with host-parasite co-evolution, possibly influenced by palaeogeographical and palaeoclimatic events (Klimpbel *et al*, 2008), which may explain that some of the helminth species indentified in this study correspond to those previously described in other hosts belonging to the same genus or to other phylogenetically related genera sharing the same ecosystem, although some of them, such as *P. maculatus* and *E. gadi*, have not yet been reported in *M. barbatus* in this area.

This study provides relevant information about the helminth parasites of *M. barbatus* in the Mediterranean Sea, having evidenced again the presence of *A. simplex* in this species but with a low risk of potential human parasitation as a consequence of its consumption. Moreover, the low prevalence of trematode species detected, often used as biological tags of the environmental quality (Lafferty, 1997; Blanar *et al*, 2009), as well as of the other helminthes could be interpreted as a symptom of poor water quality. Thus, the composition of this helminthfauna may be related to the water quality and the preservation of the aquatic ecosystem.

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