

# Survey of parasites of Atlantic Chub Mackerel (*Scomber colias*) with economic and public health impact

Marianne Felgueiras Alves

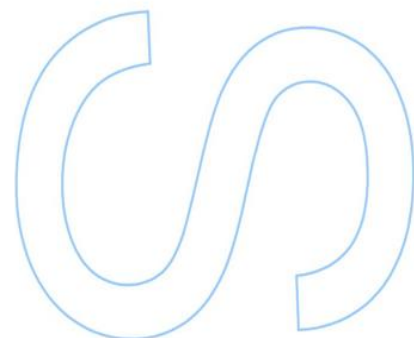
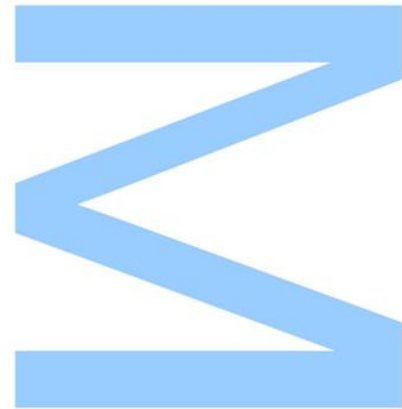
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## **Orientador**

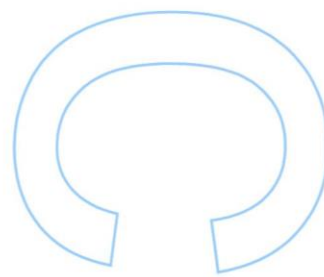
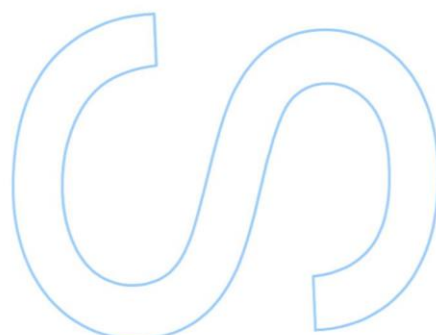
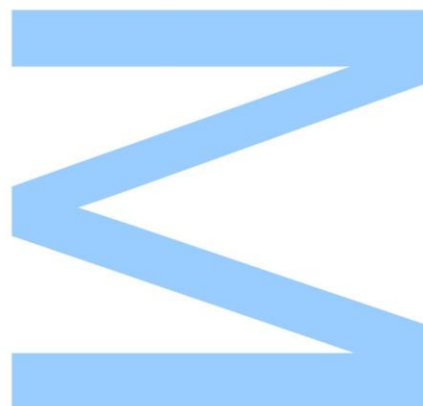
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Todas as correções determinadas pelo júri, e só essas, foram efetuadas

O Presidente do Júri

Porto, \_\_\_\_/\_\_\_\_/\_\_\_\_



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## Abstract

The Atlantic chub mackerel, *Scomber colias* Gmelin, 1789, is one of the main fisheries resources, been currently the most caught fish species in Portuguese ZEE.

The quality of fish is a growing concern and one of the factors that affect seafood quality and price are parasites. A multitude of parasites have been reported in fish. Some of them are economically important because decreases the fish flesh quality, others cause consumers rejection due to their appearance and some are zoonotic.

In the present study 64 specimens of the Atlantic chub mackerel (*Scomber colias*) from a local fish market in the northern part of the Portuguese mainland (Matosinhos), were analysed with the aim to determine the load of parasites with impact on economy and public health. Four parasite species; *Kudoa* sp. (prevalence=9.4%); didymozoid digeneans (prevalence=3.1%); *Anisakis* sensu lato (prevalence=48.8%) and *Rhadinorhynchus pristis* (prevalence=51.6%); were observed. *kudoa* detection was carried out by the method proposed by Burger and Adlard (2010) and that one proposed by Yokoyama et al (2014). No significant differences were observed between these two methods of detection ( $p=1.000$ ). Didymozoids digeneans were found in muscle of two examined fish causing myoliquefaction. The molecular and the phylogenetic analysis performed in these specimens include these parasites in the didymozoid clade that have been detected in the muscle of various fish species.

In conclusion, we can state that the parasites with impact on economy and public health from Atlantic chub Mackerel (*Scomber colias*) collected around Matosinhos were detected in relatively low levels and these fish are generally speaking, in very good and safe conditions to be consumed fresh or canned.

**Keywords:** *Scomber colias*, parasites, public health, Portuguese coast

## Resumo

A cavala, *Scomber colias* Gmelin, 1789, é um importante recurso pesqueiro sendo atualmente a espécie de peixe mais capturada na zona económica exclusiva portuguesa.

O controle da qualidade do pescado é um requisito fundamental para o consumidor e um dos fatores que a afecta são os parasitas. Existe uma enorme diversidade de parasitas de peixes sendo de especial importância aqueles que alteram a qualidade organoléptica do pescado nomeadamente a consistência da sua carne e aqueles que têm capacidade zoonótica, isto é, têm capacidade de infetar o Homem.

Com o objetivo de determinar a qualidade da cavala capturada na região norte de Portugal foi efetuada a pesquisa de parasitas com potencial zoonótico ou com capacidade de diminuir o seu valor comercial em 64 exemplares adquiridos na Lota de Matosinhos.

As espécies de parasitas detetadas com potencial zoonótico ou comercial foram mixosporídeos do género *Kudoa* (prevalência=9.4%); digenéticos didymozoides (prevalência = 3.1%); nematodes do género *Anisakis* (prevalência = 48.8%) e exemplares de acantocéfalos identificados como *Rhadinorhyncus pristis* (prevalência = 51.6%). A deteção de *Kudoa* foi levada a cabo por dois métodos; o proposto por Burger and Adlard (2010) e o proposto por Yokoyama et al (2014) não se tendo verificado diferenças significativas entre os dois métodos de deteção ( $p=1.000$ ) que foram muito baixos. Os exemplares de didymozoides que foram detetados no músculo causam mioliquefação. As análises moleculares e filogenéticas realizadas em amostras destes exemplares incluem estes parasitas no clado de didymozoides que têm vindo a ser detetados no músculo de várias espécies de peixes. Os níveis de infeção por *Anisakis* detetados, sobretudo no que se refere à intensidade da infeção foram baixos.

Deste estudo concluiu-se que a população de cavala (*Scomber colias*) capturada na região do Porto apresenta, no ponto de vista parasitológico, muito boas condições para ser consumida fresca ou ser utilizada na industria conserveira.

**Palavras-chave** *Scomber colias*, parasitas, saúde publica, costa portuguesa

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## List of abbreviations

<b>A</b>	Abundance
<b>AIC</b>	Akaike information criterion
<b>CECAF</b>	Fishery Committee for the Eastern Central Atlantic
<b>CoxI</b>	Mitochondrial cytochrome oxidase I gene
<b>DIC</b>	Differential Interference Contrast
<b>DGRM</b>	Direção-geral de recursos naturais segurança e serviços marítimos
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>I</b>	Intensity
<b>ICES</b>	International Council for the Exploration of the Sea
<b>IPTG</b>	Isopropyl $\beta$ -D-1-thiogalactopyranoside
<b>K</b>	Fulton's condition
<b>LB</b>	Luria Bertrani medium
<b>LT</b>	Total length
<b>MI</b>	Mean intensity
<b>ML</b>	Maximum-Likelihood
<b>P</b>	Prevalence
<b>PBS</b>	Phosphate buffered saline
<b>PCR</b>	Polymerase Chain Reaction
<b>PO</b>	Producer's Organizations
<b>SD</b>	Standard deviation
<b>TEM</b>	Transmission electron microscopy
<b>WT</b>	Total weight
<b>XGAI</b>	5-Bromo-4-Chloro-3-Indolyl $\beta$ -D-Galactopyranoside
<b>ZEE</b>	Exclusive economic zone

# 1. Introduction

## 1.1. Atlantic chub mackerel (*Scomber colias*) taxonomy

According to Integrated Taxonomic Information System (itis.gov), Atlantic chub mackerel taxonomic position is the following.

<b>Kingdom:</b>	Animalia
<b>Phylum:</b>	Chordata
<b>Subphylum:</b>	Vertebrata
<b>Superclass:</b>	Osteichthyes
<b>Class:</b>	Actinopterygii
<b>Subclass:</b>	Neopterygii
<b>Infraclass:</b>	Teleostei
<b>Superorder:</b>	Acanthopterygii
<b>Order:</b>	Perciformes
<b>Suborder:</b>	Scombroidei
<b>Family:</b>	Scombridae
<b>Genus:</b>	<i>Scomber</i> Linnaeus, 1758
<b>Species:</b>	<i>Scomber colias</i> (Gmelin, 1789)

The genus *Scomber* comprises the species, *Scomber scombrus* Linnaeus, 1758 (Atlantic mackerel), *Scomber japonicus* Houttuyn, 1782 (chub mackerel), *Scomber colias* Gmelin, 1789 (Atlantic chub mackerel), and *Scomber australasicus* Cuvier, 1832 (blue mackerel). Traditionally, *S. japonicus* has been considered the most geographically widespread species in the genus being found in Atlantic, Indian and Pacific Oceans. Matsui (1967) noted a considerable morphological variability between Pacific and Atlantic specimens, and recently high level of genetic divergence at mitochondrial and nuclear DNA have been reported (Scoles et al., 1998; Collette, 2003; Infante et al.,

2007). These observations lead to the acceptance of *S. japonicus* in the Indo-Pacific, and *S. colias* in the Atlantic.

## 1.2. General features

Atlantic chub mackerel is a fast growing early maturing species that can attain 50 cm total length and 13 years of age. Larva growth is slow and almost in a linear way over the first 10 to 15 day and, subsequently they have a relatively high metabolic rate (Castro-Hernández and Santana-Ortega, 2000). In the Portuguese waters, it grows up to 20 cm in the first year of life and matures at 1-2 years of age (Martins, 2007).

Atlantic chub mackerel (Fig.1) have a typical bluish to greenish dorsal and silvery white ventral colour marked by spotting or wavy broken lines (McEachran and Fechhelm, 2005; Collete and Nauen, 1983). Pectoral fins are short and has 19 or 21 rays. First dorsal fin originates behind pectoral fin bases and has 9 or 10 spines and the second dorsal fin has 11 or 12 rays followed by five finlets. Caudal peduncle is very slender and lacks lateral keel between upper and lower oblique keels, the lateral line is single and slightly arched anteriorly. The scales are small and easily lost, although they are larger and more conspicuous around the gular region (Collete and Nauen, 1983).



Figure1 General external features of *Scomber colias* specimen  
(Source:<http://www.fishbase.se/Photos/PicturesSummary.php?StartRow=1&ID=54736&what=species&TotRec=3>).

## 1.3. Geographic distribution and habitat

*S. colias* is a cosmopolitan costal-pelagic species distributed in warm and temperate waters of the Atlantic Ocean, Mediterranean and Black Sea (Collette ,1986)

(Fig. 2) It is found over the continental slope from the surface to a depth of 300 m. In the eastern Atlantic chub mackerel occurs from the Bay of Biscay to South Africa, including the Canary, Madeira, Azores and Saint Helena Islands. In the coast of America this species is frequent from Massachusetts to Florida, Bahamas, Gulf of Mexico and southern Venezuela, Brazil, Uruguay and Argentina. (Castro-Hernández and Santana-Ortega 2000).

Higher temperature often related to weak upwelling seems to play an important role in the recruitment success, spawning, and productivity of mackerel. This species prefers temperatures between 14 °C to 15.2°C, salinities between 35.1 to 35.4‰ and a typical content of dissolved oxygen of 6 ml/l. (Valderrama, 1995; Castro-Hernández and Santana-Ortega 2000). Similarly, to most scombrids, mackerels are highly migratory species, schools moving northward with vernal warming and southward with autumnal cooling (McEachran and Fechhelm, 2005). Transition from the juvenile stage to the adult immature stage is accompanied by an offshore migration from shallow coastal waters to the slope area and an inverse migration is observed during the spawning season (Castro, 1993; Castro-Hernández and Santana-Ortega 2000).

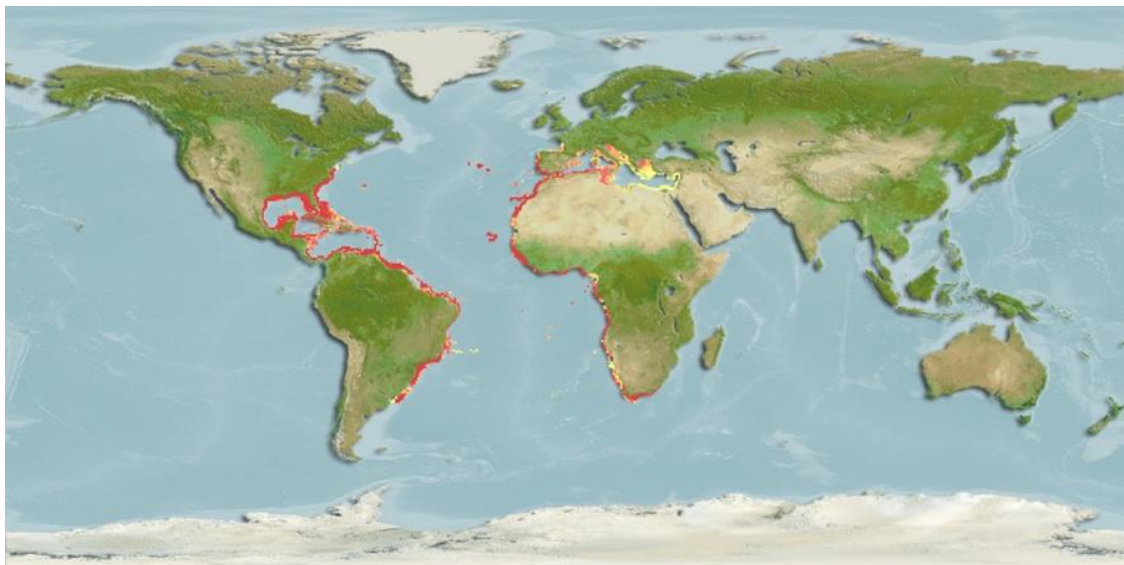


Figure 2 Map with the geographical distribution of *Scomber colias*  
 (Source: <http://www.fishbase.se/summary/Scomber-colias.html>)

#### 1.4. Feeding habits

The Atlantic chub mackerel feeds mainly small fish and zooplankton, been copepods the most important items, followed by appendicularians and mysids

euphausiids season (Castro, 1993; Castro-Hernández and Santana-Ortega 2000). However, there are differences between the diets of juvenile, immature and adult chub mackerel. Larvae feed mainly on zooplankton, and juveniles and adults mostly on fish species such as *Sardina pilchardus* and *Atherina presbyter* (Cabral and Murta, 2002).

### 1.5. Economic importance

Atlantic chub mackerel is an important species for Portuguese fisheries. Since 80's, Portuguese landings of chub mackerel ranged between 4000 and 23000 t reaching the lowest level until the early 2000s and increased subsequently (Martins et al., 2013).

Historically, Atlantic chub mackerel has always occupied the second position in the ranking of the landed species, but from 2012 forward Atlantic chub mackerel won the first position. At the same time sardine decreased by 14.3% due to the application of EU directives transposed to national laws (directives 2179-A/2015 and 5119-H/2015), that determined catch limits for sardine fishing in Portuguese ZEE. The national ports with larger Atlantic chub mackerel landings are Sesimbra, Olhão, Sines, Porto and Figueira da Foz. (DGRM, 2016)

According, to the DGRM in 2015 there were more marine fish catches compared to 2014, due to increased catch of Atlantic chub mackerel and horse mackerel, which currently are the most caught species in Portugal (Figure 3).

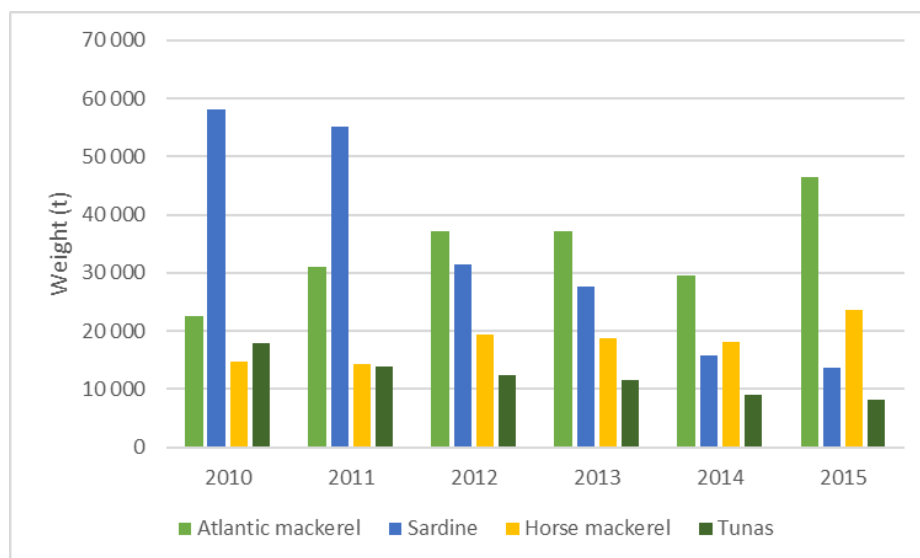


Figure 3 The top fish species catches in Portugal, in tonnes (Source: DGRM, 2016)

In Portugal, the most important canned fish are tuna and sardine. However, canned Atlantic chub mackerel and mackerel recorded an increase of 4.4% from 2013 forward (DGRM, 2016).

### 1.6. Atlantic and Pacific chub mackerel parasites and their economic and public health impact

Fish and fish products are crucial components of the human diet. Almost 17 % of human protein intake come from fish and this value reaches 70 % in some coastal countries (FAO, 2014). Portugal is the largest fish consumer per capita in the European Union and in the third worldwide (FAO, 2011).

Currently, the quality of fish is a growing concern for industries that sell fresh or canned meat products, and for public health authorities.

One of the factors that affect seafood quality and price are parasites (Alvarez-Pellitero and Sitja-Bobadilla, 1993). A checklist compiled from peer reviewed journals of the metazoan parasites of Atlantic and Pacific Mackerel and its geographical distribution is reported in table 1.

Table 1 Atlantic and Pacific chub mackerel metazoan parasites and its geographical distribution.

Parasite	Host	N	P (%)	MI	MA	Location	References
<b>Myxozoa/ Myxosporea</b>							
<i>Kudoa caudata</i>	<i>Scomber japonicus</i>	ND	ND	ND	ND	Pacific Ocean (Peru)	Kovaleva and Gaevskay, 1983
<i>Kudoa histolytica</i>	<i>S. colias</i> *	76	40	ND	ND	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
<i>Kudoa scomberi</i>	<i>S. japonicus</i>	1	ND	ND	ND	Pacific Ocean (Japan)	Li et al., 2013
<i>Myxobolus acanthogobii</i>	<i>S. japonicus</i>	10	40	ND	ND	Pacific Ocean (Japan)	Yokoyama et al., 2005
<b>Monogenea</b>							
<i>Grubea cochlear</i>	<i>S. colias</i> *	76	5,3	ND	0,08	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
		182	1,1	ND	0,02	Atlantic Ocean 34.1.1 CECAF division (Morocco)	Shukhgalter, 2004
		29	10,3	ND	0,1	Atlantic Ocean X	Shukhgalter, 2004

<i>Kuhnia abarica</i>	<i>S. colias</i> *	151	3,3	1	0,03	ICES division (Azores islands) Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Costa et al., 2007
		151	3,3	ND	0,03	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
		100	18	ND	0,29	Atlantic ocean (Brazil)	Oliva et al., 2008
		30	10	1	0,1	Mediterranean Sea (Gulf of Asinara)	Mele et al., 2014
		29	10,3	ND	0,21	Atlantic Ocean X	Shukhgalter, 2004
<i>K. scombercolias</i>	<i>S. colias</i> *	151	10,3	1,8	0,71	ICES division (Azores islands) Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Costa et al., 2007
		151	39,1	ND	0,71	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
		100	46	ND	3,77	Atlantic ocean (Brazil)	Oliva et al., 2008
		68	1,5	ND	0,01	Atlantic Ocean 34.1.2 CECAF division (Canary Islands)	Costa et al., 2011
		30	59	2,2	1,3	Mediterranean Sea (Gulf of Asinara)	Mele et al., 2014
<i>K. scombri</i>	<i>S. colias</i> *	90	1,1	ND	0,01	Atlantic ocean (Argentina)	Cremonte and Sardella, 1997
		29	24,1	ND	0,28	Atlantic Ocean X ICES division (Azores islands)	Shukhgalter, 2004
		182	19,2	ND	0,36	Atlantic Ocean 34.1.1 CECAF division (Morocco)	Shukhgalter, 2004
		151	43,7	2,24	0,98	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Costa et al., 2007
		100	11	ND	0,23	Atlantic Ocean (Brazil)	Oliva et al., 2008
		151	43,7	ND	0,98	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
		60	91,7	5,6	ND	Pacific Ocean (Peru)	Oliva et al., 2008
		62	45,2	2,3	ND	Pacific Ocean (Chile)	Oliva et al., 2008
<i>K. sporostonae</i>	<i>S. colias</i>	68	1,5	ND	0,01	Atlantic Ocean 34.1.2 CECAF division (Canary Islands)	Costa et al., 2011
		30	52	2,7	1,4	Mediterranean Sea (Gulf of Asinara)	Mele et al., 2014
		ND	ND	ND	ND	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Rohde, 1989
		151	0,66	1	0,007	Atlantic Ocean 34.1.2	Costa et al., 2007

<i>Pseudokuhnia minor</i>	<i>S. japonicus</i>	60	43.3	5.2	ND	CECAF division (Madeira islands)	Oliva et al., 2008
		62	25.8	2.3	ND	Pacific Ocean (Peru)	
	<i>S. colias</i> *	76	94,7	ND	10,86	Pacific Ocean (Chile)	Oliva et al., 2008
						Atlantic Ocean 34.1.3	Shukhgalter, 2004
						CECAF division (Mauritania)	
		182	78	ND	14,74	Atlantic Ocean 34.1.1	Shukhgalter, 2004
						CECAF division (Morocco)	
		29	17,2	ND	0,24	Atlantic Ocean X	Shukhgalter, 2004
	<i>S. colias</i>					ICES division (Azores islands)	
		151	98,7	13,9	13,7	Atlantic Ocean 34.1.2	Costa et al., 2007
					CECAF division (Madeira islands)		
151		98,7	ND	13,72	Atlantic Ocean 34.1.2	Oliva et al., 2008	
<i>S. colias</i>					CECAF division (Madeira islands)		
	68	54,4	ND	1,35	Atlantic Ocean 34.1.2	Costa et al., 2011	
					CECAF division (Canary Islands)		
<b>Digenea</b>							
<i>Bacciger sp.</i>	<i>S. colias</i> *	76	1,3	ND	0,03	Atlantic Ocean 34.1.3	Shukhgalter, 2004
<i>Clavogalea trachinoti</i>	<i>S. colias</i> *	151	6,6	ND	0,12	CECAF division (Mauritania)	Oliva et al., 2008
						Atlantic Ocean 34.1.2	
<i>Didymozoidae gen.</i>	<i>S. colias</i> *	46	6,5	ND	0,09	CECAF division (Madeira islands)	Shukhgalter, 2004
						Atlantic Ocean X	
						ICES division (Azores islands)	
						Atlantic Ocean 34.1.1	
Didymozoids in visceras	<i>S. colias</i>	68	4,4	ND	0,07	Atlantic Ocean 34.1.2	Costa et al., 2011
						CECAF division (Canary Islands)	
Didymozoids in muscle	<i>S. japonicus</i>	ND	ND	ND	ND	Pacific Ocean (Japan)	Abe and Okamoto, 2015
<i>Halvorsenius exilis</i>	<i>S. colias</i> *	182	0	ND	1,48	Atlantic Ocean 34.1.1	Shukhgalter, 2004
						CECAF division (Morocco)	
<i>Lecithocladium harpodontis</i>	<i>S. colias</i> *	151	6,6	ND	0,09	Atlantic Ocean 34.1.2	Oliva et al., 2008
						CECAF division (Madeira islands)	
		100	80	12,7	10,2	Atlantic ocean (Brazil)	Alves et al., 2003

<i>L. excisum</i>	<i>S. colias</i> *	100	80	12,8	10,24	Atlantic ocean (Brazil)	Oliva et al., 2008
		46	2,2	ND	0,04	Atlantic Ocean X	Shukhgalter, 2004
		182	41,2	ND	1,48	ICES division (Azores islands)	Shukhgalter, 2004
						Atlantic Ocean 34.1.1	
76	55,3	ND	4,96	Atlantic Ocean 34.1.3	Shukhgalter, 2004		
				CECAF division (Morocco)			
<i>Nematobothrium scombri</i>	<i>S. colias</i> *	100	55	4,6	2,5	Atlantic ocean (Brazil)	Alves et al., 2003
		151	27,8	ND	0,62	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
	<i>S. japonicus</i>	62	4.8	1.3	ND	Pacific Ocean (Chile)	Oliva et al., 2008
	<i>S. colias</i> *	100	55	2,1	2,53	Atlantic Ocean (Brazil)	Oliva et al., 2008
	<i>S. colias</i>	68	1,5	ND	0,03	Atlantic Ocean 34.1.2	Costa et al., 2011
						CECAF division (Canary Islands)	
<i>N. filiforme</i>	<i>S. colias</i>	30	7	1	0,1	Mediterranean Sea (Gulf of Asinara)	Mele et al., 2014
		30	21	2,2	0,5	Mediterranean Sea (Gulf of Asinara)	Mele et al., 2014
<i>Nematobothrium sp.</i>	<i>S. colias</i> *	90	8,6	ND	0,14	Atlantic Ocean (Argentina)	Cremonte and Sardella, 1997
		46	2,2	ND	0,02	Atlantic Ocean X	Shukhgalter, 2004
	<i>S. colias</i>	68	7,4	ND	0,13	ICES division (Azores islands) Atlantic Ocean 34.1.2 CECAF division (Canary Islands)	Costa et al., 2011
<i>Opechona bacillaris</i>	<i>S.colias</i> *	46	2,2	ND	0,02	Atlantic Ocean X	Shukhgalter, 2004
		76	14,5	ND	0,32	ICES division (Azores islands) Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
<i>O. orientalis</i>	<i>S.colias</i> *	182	19,2	ND	1,39	Atlantic Ocean 34.1.1	Shukhgalter, 2004
		76	48,7	ND	2,58	CECAF division (Morocco) Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
<i>Opechona sp.</i>	<i>S.colias</i> *	100	85	43,6	37,1	Atlantic Ocean (Brazil)	Alves et al., 2003
		90	77,8	ND	40,6	Atlantic Ocean (Argentina)	Cremonte and Sardella,1997
		100	85	43,7	37,15	Atlantic Ocean (Brazil)	Oliva et al., 2008
<i>Prodistomum orientalis</i>	<i>S.colias</i> *	151	19,9	6,8	1,35	Atlantic Ocean 34.1.2	Oliva et al., 2008
		68	1,5	ND	0,01	CECAF division (Madeira islands)	Costa et al., 2011
Atlantic Ocean 34.1.2							

						CECAF division (Canary Islands)		
<b>Cestoda</b>								
<i>Callitetrarhynchus gracilis</i> (L.)	<i>S. colias</i> *	76	2,6	ND	0,05	Atlantic Ocean 34.1.3		Shukhgalter, 2004
<i>Nybelinia</i> sp.	<i>S. colias</i> *	29	34,5	ND	8,14	CECAF division (Mauritania) Atlantic Ocean X		Shukhgalter, 2004
		151	22,5	1,6	0,35	ICES division (Azores islands) Atlantic Ocean 34.1.2		Oliva et al., 2008
						CECAF division (Madeira islands)		
<i>Phyllobothrium</i> sp.	<i>S. colias</i> *	182	3,8	ND	1,04	Atlantic Ocean 34.1.1		Shukhgalter, 2004
		90	8,8	ND	1	CECAF division (Morocco) Atlantic Ocean (Argentina)		Cremonete and Sardella, 1997
		29	3,4	ND	0,03	Atlantic Ocean X		Shukhgalter, 2004
						ICES division (Azores islands)		
<i>Unidentified</i>	<i>S. colias</i>	68	8,8	ND	0,09	Atlantic Ocean 34.1.2		Costa et al., 2011
<i>Trypanorhyncha larvae</i>						CECAF division (Canary Islands)		
<i>Scolex pleuronectis</i> (L.)	<i>S. colias</i> *	90	5,6	ND	0,7	Atlantic Ocean (Argentina)		Cremonete and Sardella, 1997
		76	5,3	ND	0,07	Atlantic Ocean 34.1.3		Shukhgalter, 2004
						CECAF division (Mauritania)		
		100	30	5,1	1,53	Atlantic Ocean (Brazil)		Oliva et al., 2008
		151	15,2	18,1	2,76	Atlantic Ocean 34.1.2		Oliva et al., 2008
						CECAF division (Madeira islands)		
	<i>S. colias</i>	68	8,8	ND	1,18	Atlantic Ocean 34.1.2		Costa et al., 2011
						CECAF division (Canary Islands)		
<i>Tentacularia coryphaenae</i> (L.)	<i>S. colias</i> *	151	2,25	1,4	0,31	Atlantic Ocean 34.1.2		Oliva et al., 2008
						CECAF division (Madeira islands)		
	<i>S. japonicus</i>	60	8.3	1.0	ND	Pacific Ocean (Peru)		Oliva et al., 2008
		62	1.6	1.0	ND	Pacific Ocean (Chile)		Oliva et al., 2008
<b>Nematoda</b>								
<i>Anisakis</i> sp	<i>S.colias</i> *	90	36,7	ND	0,7	Atlantic Ocean (Argentina)		Cremonete and Sardella,1997
		10	ND	ND	ND	Atlantic Ocean 34.1.2		Mattiucci et al., 2002
						CECAF division (Madeira islands)		
		154	69,5	ND	ND	Atlantic Ocean 34.1.2		Costa et al., 2003

		29	100	ND	16,6	CECAF division (Madeira islands)	
		182	55,5	ND	2,83	Atlantic Ocean X ICES division (Azores islands)	Shukhgalter, 2004
		76	11,8	ND	0,2	Atlantic Ocean 34.1.1 CECAF division (Morocco)	Shukhgalter, 2004
		151	53,6	2	1,01	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
		100	4	3,5	0,14	Atlantic Ocean 34.1.2	Oliva et al., 2008
		60	10,0	1,2	ND	CECAF division (Madeira islands)	Oliva et al., 2008
		62	43,5	2,7	ND	Atlantic Ocean (Brazil)	Oliva et al., 2008
	<i>S. japonicus</i>	218	74,3	ND	ND	Pacific Ocean (Peru)	Oliva et al., 2008
	<i>S. colias</i>	68	11,8	ND	0,25	Pacific Ocean (Chile)	Oliva et al., 2008
						Pacific Ocean (Japan)	Suzuki et al., 2010
						Atlantic Ocean 34.1.2	Costa et al., 2011
<i>A. simplex</i>	<i>S. colias*</i>	44	77,27	4,59	3,55	CECAF division (Canary Islands)	
		ND	ND	ND	ND	Mediterranean sea (Aegean sea)	Akmirza, 2003
		266	62,4	2,9	1,8	Atlantic Ocean 34.1.2	Pontes et al., 2005
<i>A. pegreffi</i>	<i>S. colias*</i>	17	83,3	ND	ND	CECAF division (Madeira islands)	
		ND	ND	ND	ND	Atlantic Ocean 34.1.1	Abattoy et al., 2011
		13	ND	ND	0,38	CECAF division (Morocco)	
	<i>S. japonicus</i>	ND	ND	ND	ND	Mediterranean sea (Alboran sea)	Abollo et al., 2003
	<i>S. colias</i>	32	75	13,8	10,3	Atlantic Ocean 34.1.2	Pontes et al., 2005
		29	96,6	15,1	ND	CECAF division (Madeira islands)	
	<i>S. japonicus</i>	417	ND	ND	ND	Mediterranean sea (Tunisia)	Farjallah et al., 2008
<i>A. physeteris</i>	<i>S. colias*</i>	ND	ND	ND	ND	Pacific Ocean (China)	Quiazon et al., 2008
						Mediterranean (Aegean sea)	Chaligiannis et al., 2012
						Mediterranean sea (Sardinia)	Piras et al., 2014
						Pacific Ocean (Korean)	Bak et al., 2014
						Atlantic Ocean 34.1.2	Pontes et al., 2005
<i>A. ziphidarum</i>	<i>S. colias*</i>	ND	ND	ND	ND	CECAF division (Madeira islands)	
						Atlantic Ocean 34.1.2	Pontes et al., 2005
<i>Camallanus sp.</i>	<i>S. colias*</i>	46	15,2	ND	0,26	CECAF division (Madeira islands)	
						Atlantic Ocean X	Shukhgalter, 2004
						ICES division (Azores islands)	

<i>Capillariidae gen.</i>	<i>S. colias</i> *	76	1,3	ND	0,01	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
<i>Contraecaecum sp.</i>	<i>S. colias</i> *	90	26,7	ND	0,6	Atlantic Ocean (Argentina)	Cremont and Sardella, 1997
		29	24,1	ND	2,38	Atlantic Ocean X ICES division (Azores islands)	Shukhgalter, 2004
		182	5,5	ND	0,06	Atlantic Ocean 34.1.1 CECAF division (Morocco)	Shukhgalter, 2004
		76	3,9	ND	0,11	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
		100	2	2,5	0,05	Atlantic Ocean (Brazil)	Oliva et al., 2008
<i>Hysterotylacium sp.</i>	<i>S. colias</i> *	90	50	ND	1,9	Atlantic Ocean (Argentina)	Cremont and Sardella, 1997
		76	19,7	ND	0,64	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
		182	22,5	ND	0,85	Atlantic Ocean 34.1.1 CECAF division (Morocco)	Shukhgalter, 2004
		151	22,5	ND	0,58	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
	<i>S. japonicus</i>	417	ND	ND	ND	Pacific Ocean (Korean)	Bak et al., 2014
<i>H. aduncum</i>	<i>S. colias</i> *	173	77,1	4,63	ND	Atlantic Ocean (Argentina)	Navone and Sardella, 1998
<i>H. fabri (L.)</i>	<i>S. colias</i> *	151	7,3	1,6	0,12	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
<i>Oncophora melanocephala</i>	<i>S. colias</i> *	151	6	ND	0,03	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
<i>Pseudoterrnova sp.</i>	<i>S. colias</i> *	90	6,7	ND	0,1	Atlantic Ocean (Argentina)	Cremont and Sardella, 1997
	<i>S. colias</i>	68	1,5	ND	0,01	Atlantic Ocean 34.1.2 CECAF division (Canary Islands)	Costa et al., 2011
<i>Raphidascaaris sp.</i>	<i>S. colias</i> *	100	52	27,4	14,25	Atlantic Ocean (Brazil)	Oliva et al., 2008
<b>Acanthocephala</b>							
<i>Corynosoma sp. (L.)</i>	<i>S. colias</i> *	100	10	2,1	0,2	Atlantic Ocean (Brazil)	Alves et al., 2003
<i>Bolbosoma sp.</i>	<i>S. colias</i> *	50	16	ND	ND	Atlantic Ocean (Brazil)	Rego and Santos, 1983
		29	6,9	ND	0,07	Atlantic Ocean X ICES division (Azores islands)	Shukhgalter, 2004

<i>Bolbosoma vasculosum</i> (L.)	<i>S. colias</i> *	151	2	1	0,02	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
<i>Corynosoma</i> sp.	<i>S. colias</i> *	100	10	2,1	0,2	Atlantic Ocean (Brazil)	Alves et al., 2003
<i>Corynosoma australe</i> (L.)	<i>S. colias</i> *	90	64,4	ND	10,6	Atlantic Ocean (Argentina)	Cremont and Sardella, 1997
		100	12	1,4	0,17	Atlantic Ocean (Brazil)	Alves et al., 2003
		100	12	1,4	0,1	Atlantic Ocean (Brazil)	Oliva et al., 2008
		62	38,7	9,3	ND	Pacific ocean	Oliva et al., 2008
<i>Rhadinorhynchus cadenati</i>	<i>S. colias</i> *	29	65,5	ND	1,83	Atlantic Ocean X ICES division (Azores islands)	Shukhgalter, 2004
		182	59,9	ND	4,43	Atlantic Ocean 34.1.1 CECAF division (Morocco)	Shukhgalter, 2004
		76	15,8	ND	0,2	Atlantic Ocean 34.1.3 CECAF division (Mauritania)	Shukhgalter, 2004
<i>Rhadinorhynchus pristis</i>	<i>S. colias</i> *	154	50,6	3,19	ND	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Costa et al., 2004
	<i>S. colias</i> *	100	7	1,9	0,13	Atlantic Ocean (Brazil)	Oliva et al., 2008
	<i>S. colias</i>	68	4,4	ND	0,04	Atlantic Ocean 34.1.2 CECAF division (Canary Islands)	Costa et al., 2011
<b>Crustacea/ Copepoda</b>							
<i>Caligus pelamydis</i>	<i>S. colias</i> *	ND	ND	ND	ND	Black sea	Nikolaeva, 1980
		100	2	1,5	0,02	Atlantic Ocean (Brazil)	Oliva et al., 2008
	<i>S. japonicus</i>	60	1,7	1,0	ND	Pacific Ocean (Peru)	Oliva et al., 2008
		62	17,7	1,1	ND	Pacific Ocean (Chile)	Oliva et al., 2008
<i>Clavellisa scombri</i>	<i>S. colias</i> *	ND	ND	ND	ND	Atlantic Ocean	Solonchenko and Bodyanitski, 1968
		100	2	1	0,02	Atlantic Ocean (Brazil)	Oliva et al., 2008
		151	7,9	1,5	0,12	Atlantic Ocean 34.1.2 CECAF division (Madeira islands)	Oliva et al., 2008
	<i>S. japonicus</i>	60	20,0	1,7	ND	Pacific Ocean (Peru)	Oliva et al., 2008
		62	6,5	1,8	ND	Pacific Ocean (Chile)	Oliva et al., 2008
	<i>S. colias</i>	30	7	0,1	0,1	Mediterranean Sea (Gulf of Asinara)	Mele et al., 2014
<i>Hatschekia</i> sp.	<i>S. colias</i> *	66	1,5	1	0,02	Mediterranean sea	Amirzha, 2003
<i>Lernaea</i> sp.	<i>S. colias</i> *	66	18,2	1,08	0,2	Mediterranean sea	Amirzha, 2003

\* In the references identified as *S.japonicus*, but are now recognize as *S. colias*

Some of these parasites are of economic or public health concern.

The genus *Kudoa* Meglitsch, 1947 is an important histozoic myxosporean parasite of marine fish (Eiras et al., 2014). These parasites can develop white pseudocysts in muscle fibers and other organs and, in muscle many species cause myofibrillar degeneration, sometimes referred as soft-flesh disease (Langdon, 1991). Due to the appearance and texture of the flesh infected fish are often rejected by consumers, resulting in significant losses to industries. Additionally, potential public health risks of this parasite have been reported by several authors. The symptoms of fresh fish infected with some species of *Kudoa* include diarrhoea and emesis, symptoms are sometimes severe, although they usually disappear within 24 h (Velasco et al., 2008; Suzuki et al. 2015; Sugita-Konishi et al., 2015).

Digenean trematodes of the family Didymozoidae are tissue parasites of marine fishes and are especially common in oceanic pelagic fish. In addition to parasitized atypical sites, they may be found encapsulated, sometimes in pairs and may exhibit varying degrees of sexual dimorphism sometimes being hardly recognizable as digeneans (Pozdnyakov and Gibson, 2008). These parasites do not seem to pose a public health problem but they can reduce the commercial value of fish mostly that ones that occurs in muscle and cause unappetizing aspect of fish (Pascual et al., 2006).

Larval stages of Anisakidae are nematodes commonly found in the viscera and musculature of many species of teleost fish throughout the world's oceans and seas (Mattiucci et al., 2007). Humans can become accidentally infected by consuming uncooked or poorly cooked infected fish. Anisakiasis produced by L3 larvae of the genus *Anisakis*, is considered a worldwide human health problem. Approximately 20,000 cases of anisakiasis has been reported worldwide (Umehara et al., 2007; Qin et al., 2013). Clinical signs of anisakiasis include edema in gastric mucosa, epigastric pain, vascular occlusion and pseudo-tumoral formation (Takabe, et al., 1998). These parasites are also of great importance in the canning industry as they are visible macroscopically. Some anisakid larvae have been occasionally found in tins, negatively affecting the value of the product (Madrid et al., 2016).

Acanthocephala Rudolphi, 1802 are intestinal parasites of vertebrate definitive hosts, and most species belonging to this group occurs in fish (Amin and Omar, 1987). The morphology of the adults is very similar between species, especially the internal organs, but the proboscis hooks, their shape, number and arrangement differ between species and genera (Kennedy, 2012). These parasites are not a public health concern

but during the evisceration of fish they are visible to the naked eye and this can cause rejection by consumers.

## 2. Objectives

As mentioned above the fishing quotas, the fresh consumption, and the use of Atlantic mackerel in the canning industry have been rising in Portugal. For all these reasons the quality and safety of Atlantic chub mackerel fished in Portuguese ZEE is of growing concern.

The aim of the present study was to do a survey in the Atlantic chub mackerel quality concerning parasites that can cause public health or economic concern.

## 3. Materials and Methods

### 3.1. Fish sample

Between October and November 2015, specimens of the Atlantic chub mackerel, *Scomber colias* (n=64) were obtained from a local fish market in the northern part of the Portuguese mainland (Matosinhos). The fish were stored at -20°C until examination. After fish defrosting, total length (Lt), total weighed (Wt) and liver weight were measured. Fulton's condition factor was determined using the equation  $K = 100 Wt/Lt^3$  and the hepatossomatic index (ratio between the liver weight and the total weight expressed in percent) determined.

### 3.2. Parasites detection, identification and, quantification

A macroscopic observation with special attention to muscle softening was performed. The entire musculature of each fish was removed, filleted and examined macroscopically and under a stereomicroscope.

For detection of *Kudoa* spores two samples (1 gram each) of dorsal muscle (just behind the head) of each fish were collected and processed by the method proposed by Burger and Adlard (2010) and by the method proposed by Yokoyama et al., (2014). Briefly, in the method of Burger and Adlard the muscle was placed in the lip of a petri dish, moistened with 5 ml of phosphate buffered saline (PBS), macerated with scalpel blades squashed with the basis of petri dish and the liquid squeezed and allowing to settle into centrifuge tube. In the method proposed by Yokoyama et al (2014), muscle was squashed with 5 ml of PBS in a small glass mortar, passed through a steel mesh, filtered with a 100 µm nylon mesh strainer centrifuge at 400xg for 15 min, re-suspended in PBS, placed onto a 15% to 25% Percoll gradient solution, centrifuged at 2190xg for 30 min. and re-suspended in PBS. In both cases an aliquot (15 ml) was observed under a Differential Interference Contrast (DIC) microscope (400X). Kudoid spores were photographed and measurements according to Burger and Adlard (2010).

For detection of anisakids larvae the body cavity and the viscera were examined macroscopically and under a stereomicroscope. All detected nematodes were collected, washed in PBS and fixed in alcohol 70% (v/v). Identification at generic level was based on the morphological characteristics as describe by Berland (1961) after clearing and mounting the parasites in glycerine.

All other macroscopically detected parasites found were collected, counted and stored in alcohol 70% (v/v) for further processing. Small metazoan parasites from the digestive tract were not search during this study.

For ultrastructural studies conducted in didymozoid parasitized muscle, approximately 3mm<sup>3</sup> of fresh tissue was cut and immediately fixed in 5% glutaraldehyde plus paraformaldehyde 4% in 0.5 M sodium cacodylate buffer (pH 7.2). Subsequently the samples were washed three times with sodium cacodylate buffer and post-fixed with 2% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2) for 1 h with stirring followed by overnight at 4° C with slow stirring. The dehydration was performed using an ethanol series (25–100%; v/v), and once using propylene oxide. Samples were embedded in mixtures of propylene oxide and resin (EMBed-812), followed by EMBED-812 for at least 24 h, before being placed in embedding moulds with resin, and being allowed to polymerize at 55°C. Thin sections were cut with an ultramicrotome (Bromma Ultratome Nova, LKB, Sweden) and mounted in copper grids. The sections were contrasted with a saturated solution of uranyl acetate for 7 min and with lead citrate (Reynolds, 1963) for 7 min, before being visualized using an electron microscope JEM-1400Plus (Jeol Ltd., Inc., MA, USA) operating at 85 kV.

For all groups of detected parasites, the prevalence of the infection was determined. The mean intensity and the mean abundance were determined for all parasites excluding myxosporidean. All parameters were calculated according to Bush et al., (1997).

### 3.3. Molecular and phylogenetic analysis

It was not possible to do a molecular analysis of kudoids do to the very low levels of infection.

To performed the molecular analysis of the detected didymozoid, DNA was extracted by crushing the parasites with a sterile pestle in an Eppendorf tube containing DNA extraction buffer (50 mM Tris-HCl, pH 8.0, with 10 mM EDTA) and using the QIAamp DNA MiniKit (QIAGEN, Germany) following the protocol for DNA extraction from tissues. The extracted DNA was eluted with 30 µl of water and quantified in a BioPhotometer (Eppendorf AG, Hamburg, Germany).

The 18S ribosomal RNA the 28S ribosomal RNA and the mitochondrial cytochrome oxidase I (coxI) genes were amplified by Polymerase Chain Reaction (PCR) using the primers and the profiles described on Table 2.

Table 2 Targeted gene, sequence of the oligonucleotide primers used in the PCRs and PCR profiles utilized

Gene	Sequence (5´-3´)	Reference	PCR program
<b>18S</b>	forward AAC CTG GTT GAT CCT GCC ATG	Pascual et al., 2006	94°C (5min)
	reverse TGA TCC TTC TGC AGG TCC ACC TAC		[35x 94°C (1min) 55°C(1min) 72°C (2min)] 72°C (10min)
<b>28S</b>	forward GTC CGA TAG CGA ACA AGT ACCGT	Mladineo et al., 2009	94°C (5min)
	reverse AGC ATA GTT CAC CAT CTT TCG GGT CTC AA		[35x 94°C (1min) 54°C(1min) 72°C (2min)] 72°C (10min)
<b>coxI</b>	foward TTT TTT GGG CATC CTG AGG TTT AT	Mladineo et al., 2009	94°C (30s)
	reverse CAA CAA ATC ATG ATG CAA AAGG		[35x 94°C (30s) 52°C(90s) 72°C(90s)] 72°C(10min)

PCRs were carried out in a MyCycler™ thermal cycler (Bio-Rad laboratories Inc., Hercules, CA, USA) using 5 U of Taq polymerase, 1x reaction buffer supplemented with 25 Mm MgCl<sub>2</sub> (Promega, WI, USA), 5 mM deoxynucleoside triphosphates, 10 mM of each primer and 20 to 70 ng of DNA. The PCR products were analyzed by agarose gel electrophoresis [1% (w/v)] using standard protocols (Sambrook and Russell 2001[1x TAE buffer, 1 µl of GreenSafe (NZYTech, Lisbon, Portugal) and GeneRuler™ DNA Ladder Mix (ThermoScientific, Carlsbad, CA, USA) as molecular ruler]. Gels were visualized using a UV transilluminator device (GelDoc Biorad). The DNA fragments were isolated from agarose gels using NZYGelpure kit (NZYTech, Lisbon, Portugal).

Purified PCR products were cloned into pGEM®-T Easy vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* DH5α competent cells following the instructions of the manufacturer

The white colonies obtained were grown overnight at 37°C, in 5 ml of liquid LB medium supplemented with 5 ul of ampicillin, with shaking. Plasmid DNA was isolated from *E. coli* cultures using the NZYMiniprep kit (NZYTech, Lisbon, Portugal). to confirm transformation restriction analysis was performed using EcoRI (Thermo Fisher Scientific, Inc., MA, USA). The insert was sequenced at STAB Vida (Lisbon, Portugal). Sequences

obtained were compared to the GenBank database NCBI using Blast (July 2016). From now on our isolate was named *Didymozoidae* sp. Sc-2016.

The 18S and 28S rRNA gene sequences, as well as the *CoxI* sequences obtained here used as a query at NCBI (Blastn) to recover all Platyhelminthes sequences showing high similarity. Sequences with reduced length, that will compromise phylogenetic analyses were not used. Sequences were aligned using MUSCLE (Edgar, 2004), and phylogenetic relationships inferred using Maximum-Likelihood (ML) and 1000 resamples [Fasttree; (Price et al., 2009, 2010)]. The model used was the general time reversible model with a proportion of invariant sites and a gamma distribution (GTR+I+G; the implemented model in Fasttree). This model is appropriate since the Akaike information criterion (AIC), as implemented in Modeltest (Darriba et al., 2012; Guindon and Gascuel, 2003), suggests the use of such model (for *CoxI* dataset) or much simpler models (TIM2ef+I for 18S rRNA and TVM+G for 28S rRNA datasets). There are 261, 328 and 326 ungapped positions in the 18S, 28S and *CoxI* datasets respectively.

### 3.4. Data analysis

Data analysis was carried out using IBM SPSS Statistics 22 statistic software.

The two methods used for detection of *Kudoa* were compared using the McNemar test, a non-parametric test for dependent samples.

Relationships between fish parameters (weight, length, Fulton's condition index and hepato-somatic index) and occurrence (*Kudoa*; *Didymozoides*; Anisakids and Acantocephala) or abundance of parasites (*Didymozoides*; Anisakids and Acantocephala) were analysed using non-parametric Spearman rank correlation test.

For all tests, statistical significance was accepted when  $p < 0.05$ .

## 4. Results

Were examined sixty-four table size Atlantic chub mackerels with a mean weight of  $210.9 \pm 33.5$  g (range: 157-285) and a mean total length of  $28.2 \pm 1.37$  cm (range = 26-32). Sex determination was not possible due to the low developmental stage of the gonads. The mean condition factor was  $0.94 \pm 0.05$  (range: 0.81-1.09) and the hepatossomatic index was  $0.80 \pm 0.13$  (range: 0.54 – 1.17).

Four parasite species (*Kudoa* sp, didymozoid digeneans, *Anisakis* type I and the acantocephalan *Rhadinorhynchus pristis*) with potential to cause economic or public health impact were detected. The infection levels of these parasites were reported in Table 3.

Table 3 Mean infection levels  $\pm$  SD (range) of parasites of economic and/or public health importance detected in Atlantic chub mackerel

Parasites	Prevalence%	Intensity		Abundance	
		Mean $\pm$ SD (min–max)	Median	Mean $\pm$ SD (min–max)	Median
<i>Kudoa</i> sp.	9,4	-	-	-	-
Didymozoidea gen. sp.	3,1	1,00 $\pm$ 0,00 (1-1)	1,0	0,03 $\pm$ 0,18 (0,1)	0
<i>Anisakis</i> type I	48,4	1,84 $\pm$ 1,19 (1-5)	1	0,89 $\pm$ 1,24 (0-5)	0
<i>Rhadinorhynchus pristis</i>	51,6	1,94 $\pm$ 1,46 (1-6)	1	1,00 $\pm$ 1,43 (0-6)	1

*Kudoa* sp. spores were observe in skeletal muscle of 6 *S colias*. The levels of infection in all parasite fish were very low. A maximum of 4 spores were detected in each slide. Significant differences were not observed for the two methods used for *Kudoa* detection (p=1.000).

Spores were stellate in apical view (fig.4). The four pyriform polar capsules were of different size; one large, two intermediate and one small. The dimensions of spores are recorded in table 4. Do to the very low level of infection only few spores were measured (n=11) and *Kudoa* spores in lateral view were not observed.



Figure 4 Mature spores in apical view of *Kudoa* sp. in *S. colias*

Table 4 Spore measurements of *Kudoa* sp. from muscle of *S. colias*, with mean± standard deviation and range in parentheses, in µm

<u>Spore</u>		<u>Large polar capsule</u>	<u>Small polar capsule</u>	
<u>Width</u>	<u>Thickness</u>	<u>Length</u>	<u>Width</u>	<u>Width</u>
11,74±2,46	6,91±1,64	4,90±0,68	3,80±0,92	3,26±0
(9,78-16,30)	(4,89-9,78)	(3,35-6,52)	(2,68-4,89)	(3,26-3,26)

In two examined fish a localized muscle softening was observed. A dissection was done and a yellowish mass surrounding by destroyed muscle was observed (Figure.5 A, B). A more detailed observation revealed several parallel yellowish threads about 7 mm long by 200-300 µm wide. An optical microscopy examination revealed that they are uterus filled with yellow eggs (Figure 6) different developmental stages (Figure 7). The eggs showed internal masses of undifferentiated cellular material or first embryonic stages (Figure 8). The eggs measured 15-20 µm long and had a thick (0.30 µm) (Figure 9). These features lead to suspected to be didymozoids digeneans.



Figure 5 Didymozoids in trunk musculature of *S. colias*. A) It is possible to observed the degeneration of muscular tissue around the parasites (arrow) B) Detail of parasite were it is visible the uterus completely filled with yellow eggs.



Figure 6 Didymozoid eggs in trunk musculature of *S. colias*

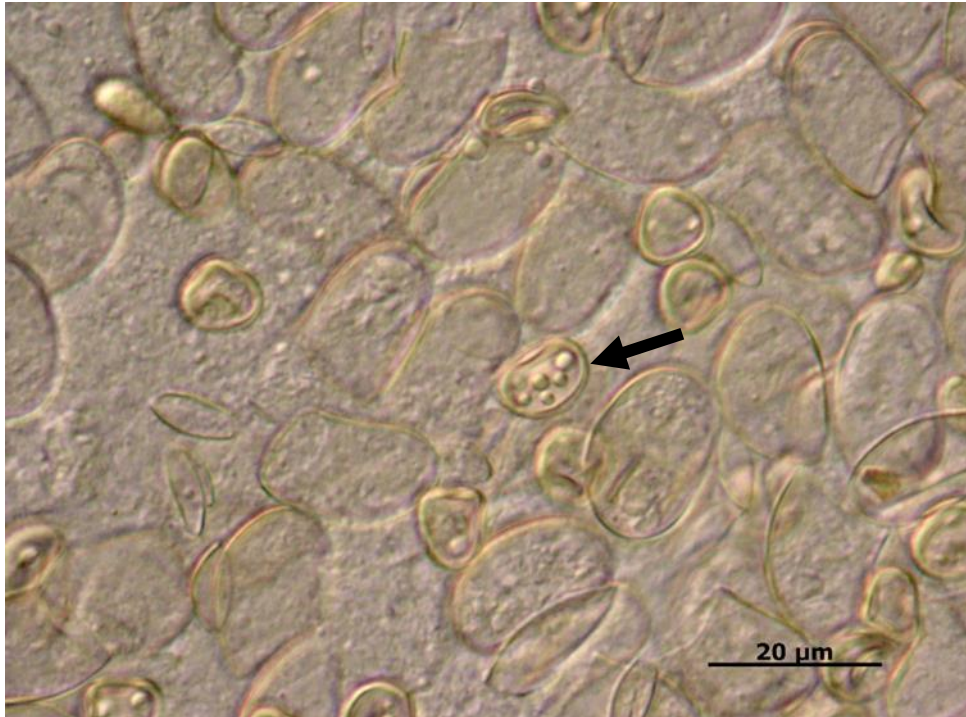


Figure 7 Eggs of didymozoid in several developing stages, including pre-vitellogenic oocyte (arrow)

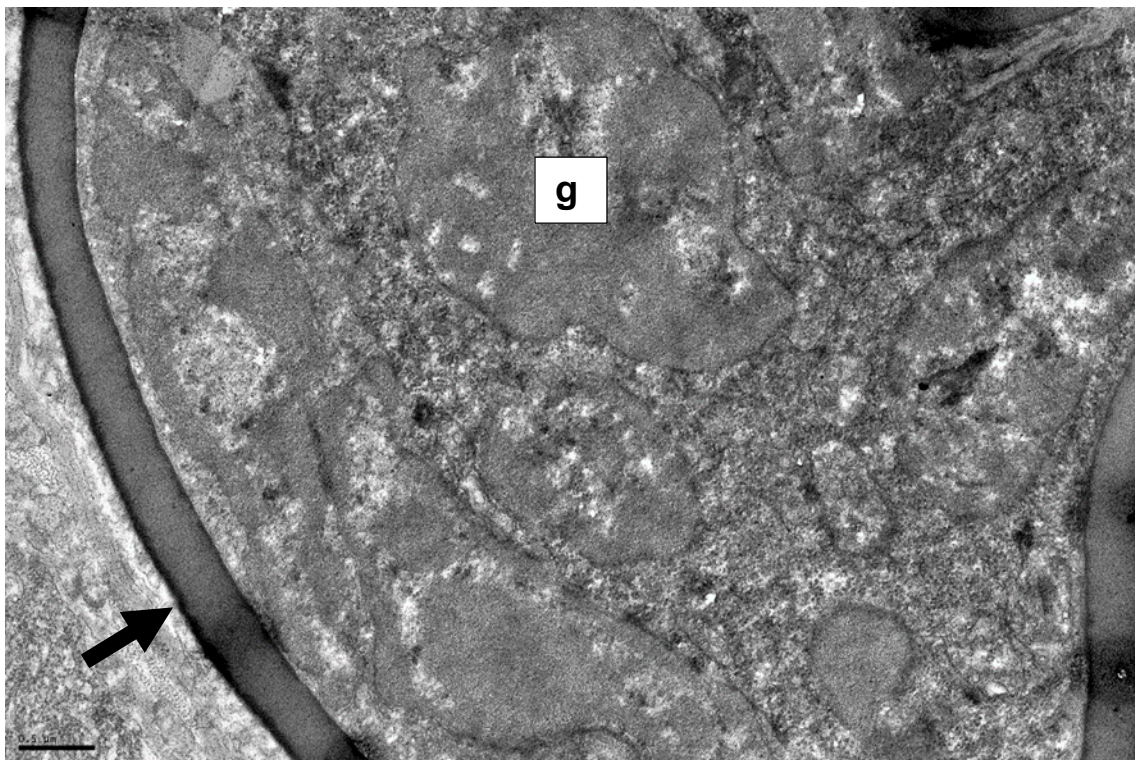


Figure 8 TEM micrograph showing the thick-walled (arrow) and the internal germ balls (g) of a didymozoid egg. Scale bar= 0.5 µm

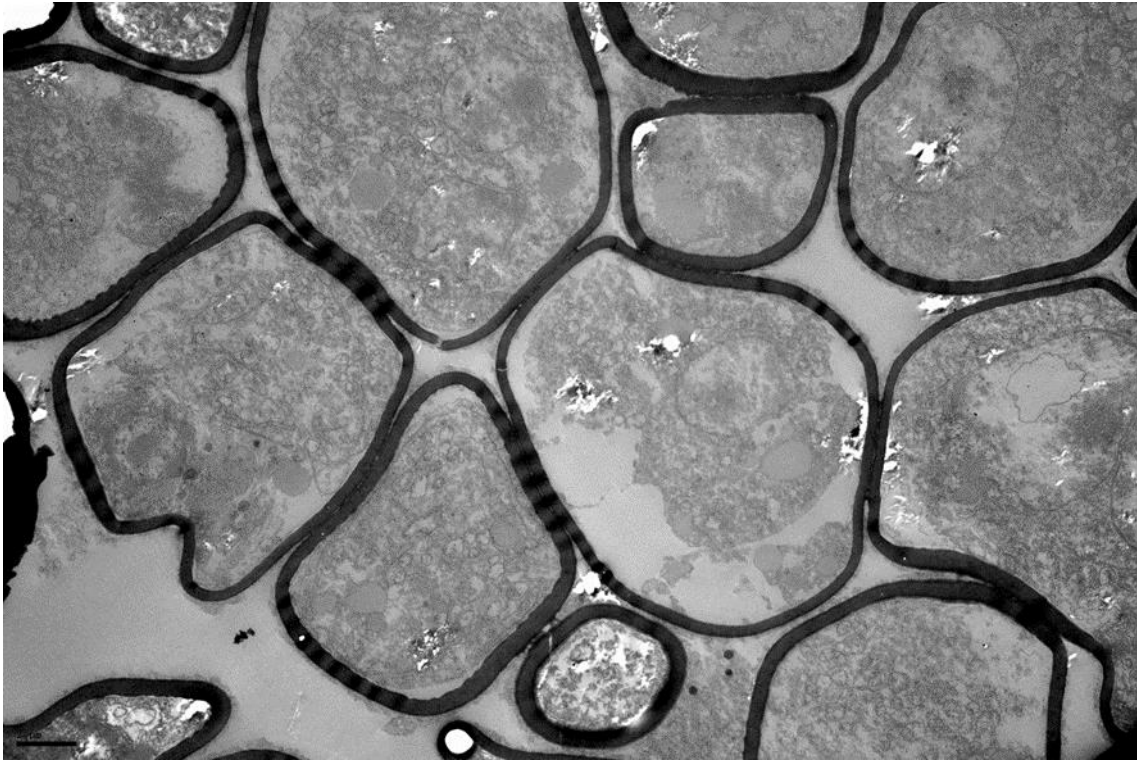


Figure 9 TEM micrograph showing several thick-walled didymozoid eggs. Scale bar =0.5  $\mu$ m

In the phylogenetic analysis of 18S rDNA gene sequences (Figure 10), *Didymozoidae* sp. SC-2016 sequence was closely related to the *Didymozoidae* sp SJ-350 (LC003495.1) and *Didymozoidae* sp SP-2005 (DQ 87251.1), with a high bootstrap (0.99) support. The phylogenetic analysis of 28S rDNA gene sequences (Figure 11) and *cox1* (Figure12) showed a high similarity between the SC-2016 and SJ-350 sequences with a bootstrap (0.99) and (1.00) respectively. In all phylogenetic trees SC-2016 sequence was included in the clade comprising the muscle-parasitizing didymozoids.

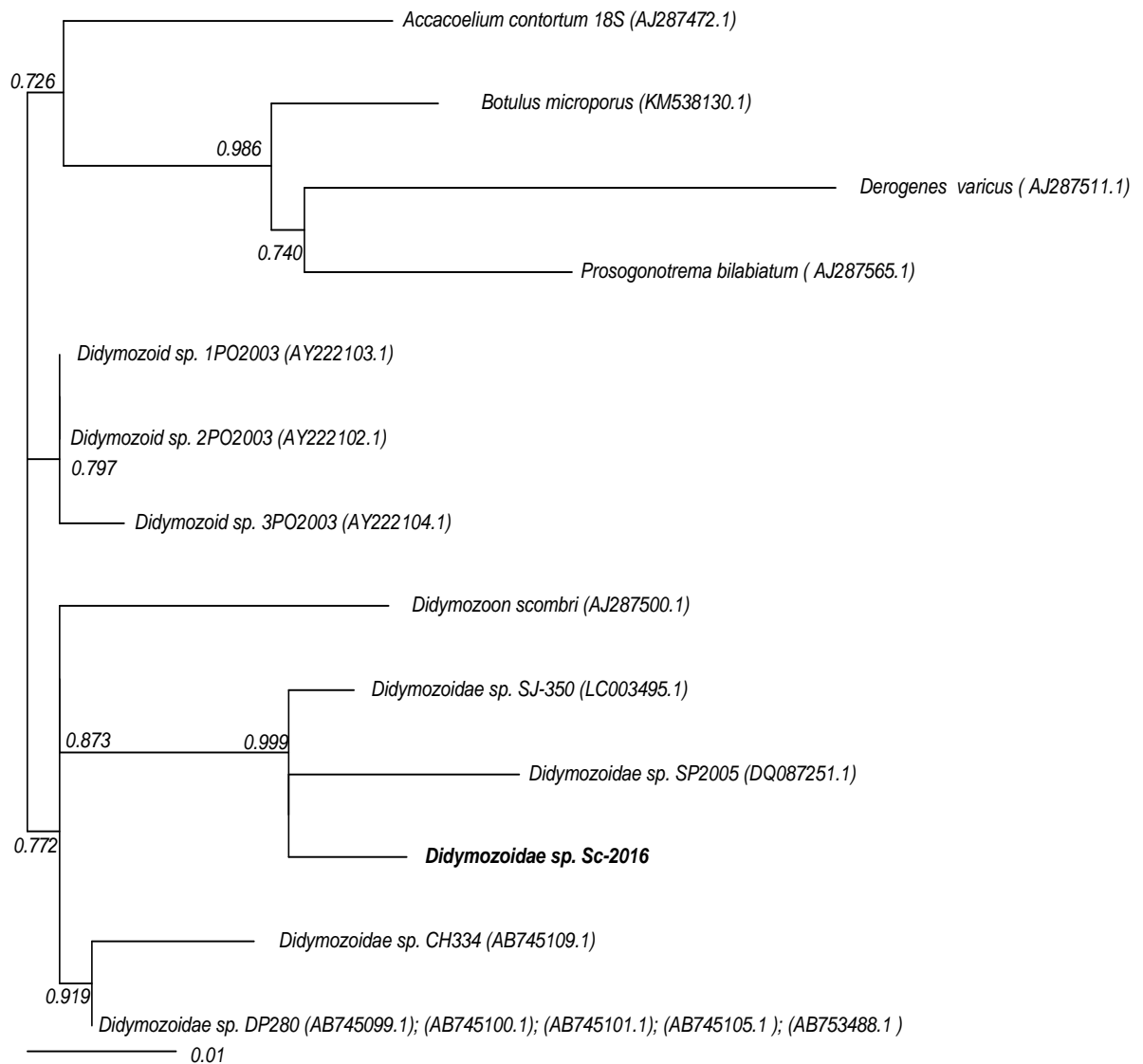


Figure 10- Maximum likelihood (ML) phylogenetic tree based on partial 18S rRNA gene sequences. GenBank accession numbers are indicated in brackets. Numbers along branches indicate the bootstrap values and only those equal or above 0.5 are indicated.

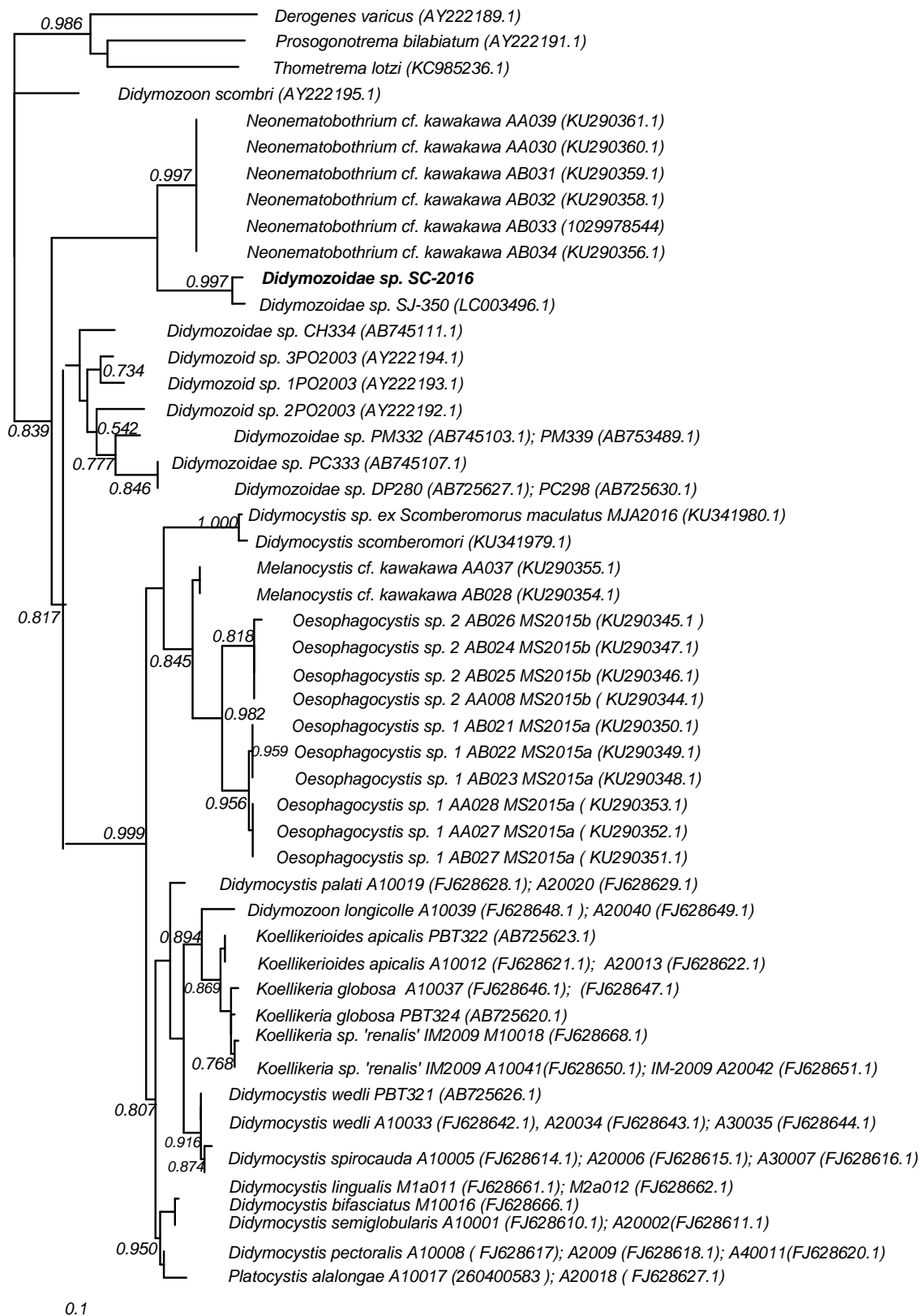


Figure-11- Maximum likelihood (ML) phylogenetic tree based on partial 28S rRNA gene sequences. GenBank accession numbers are indicated in brackets. Numbers along branches indicate the bootstrap values and only those equal or above 0.5 are indicated



Figure-12- Maximum likelihood (ML) provisory phylogenetic tree based on partial *Coxl* gene sequences. GenBank accession numbers are indicated in brackets. Numbers along branches indicate the bootstrap values and only those equal or above 0.5 are indicated

*Anisakis* type I larvae (L3) spirally coiled in a thin capsule adhering to the wall of the host or free in the body cavity were detected in almost half of fish examined (P = 48%). The intensity of infection was always low (maximum intensity 5).

The specimens of Acanthocephala detected were identify as *Radinorhynchus pristis*. These parasites were also detected in approximately one half of the observed fish (P = 52%). It was stated that Acanthocephala abundance were negatively correlated with fish size (weight and length;  $r = -0.247$ ;  $p = 0.049$  and  $r = -0.253$ ;  $p = 0.044$ , respectively).

## 5. Discussion

This is the first report of the occurrence of the genus *Kudoa* in *S. colias* on the continental Portuguese coast. In the genus *Scomber* only *Kudoa caudata*, Kovaleva and Gayevskaya, 1983, in Pacific Ocean, *Kudoa hystolytica* (Pérard, 1928) Meglitsch 1947 in Atlantic Ocean and Mediterranean Sea (Mauritania) and more recently *Kudoa scomberi* Li, Sato, Tanaka, Ohnishi, Kamata, and Sugita-Konishi, 2013, in Pacific Ocean where been detected (Kovaleva and Gaevskay, 1983; Shukgalter, 2004; Li et al., 2013; Eiras et al., 2014). According, to Whipps and Kent (2006) *K. hystolytica* is morphologically similar to *Kudoa thyrsites*. The spores detected in the present study were very dissimilar to *K. caudata* and *K. scomberi* but though a little bigger in width, they are morphometric similar with *K. hystolytica* and *K. thyrsites*. (Langdon, 1991; Shaw et al., 1997; Yokoyama et al., 2004; Yokoyama and Itoh 2005; Whipps and Kent, 2006; Levsen et al., 2008; Eiras et al., 2014). *K. thyrsites* infections levels seems to be quite variable geographically and with host species. Prevalence values from 0.8 % in *Scomber scombrus* from North Sea to 93% in farmed *Salmo salar* from British Columbia has been reported (Langdon et al., 1992; Dawson-Coates et al., 2003; Levsen et al., 2008; Cruz et al., 2011). However, the only reference of *Kudoa* infection levels in Atlantic chub mackerel is that one of, Shukgalter, (2004) which refers a prevalence of 40 % of *K. hystolytica* in *S. colias* (referred as *S. japonicus*) off Mauritania. In the present study detected infection levels were much lower (less than 10%).

The presence of muscle-parasitizing didymozoid in *S. colias* from the continental Portuguese coast is also reported for the first time. The eggs size and the sequence of the 18S rDNA gene of the specimens detected in the present study (SC-2016) are similar to that one's reported for muscle-parasitizing didymozoid from *S. scombrus* off Spain (GeneBank ref. DQ087251) detected by Pascual et al., (2006) and specimens detected off Japan in *S. japonicus* (SJ-350, GeneBank refLC003497.1) by Abe and Okamoto (2015). The analysis of 28S rDNA and coxI SC-2016 sequences match 100% with SJ-350. According, to these results there is no evidence to consider SC-2016 as a different species. According, to the phylogenetic analysis performed it was readily apparent that SC-2016 was included in the muscle-parasitizing didymozoids clade. These results support the idea of Abe et al. (2014) suggesting the muscle-parasitizing didymozoids differ phylogenetically from all the other didymozoids detected in other host-organ.

Euphausiids are well known intermediate host of Anisakids, and are important food source for the scombrids (Mazzocchi and Riberad`Alcalá, 1996). Moreover, the geographical distribution of anisakid nematodes is conditioned by the presence of appropriate definitive hosts in which the parasite can complete its life cycle as for example, the common dolphin, *Delphinus delphis*, that occurs in Atlantic Iberian waters (Silva, 1999). Prevalences of *Anisakis* sp. in *Scomber* sp. from Portuguese ZEE varied around 60 to 100% (Costa et al, 2003; Silva and Eiras, 2003; Shukhgalter, 2004) and several studies indicated that the parasitic burden increases with the age and the length of the fish (Platt, 1975; McGladery, 1986;). In this study anisakids infection levels should be consider low and were not correlated with fish length or weight, despite the tendency of higher values of infection in the upper length fish classes.

*Rhadinorhynchus pristis* infection levels reported in the present work were similar to the ones reported by Costa et al., (2011) in *S. colias* off Madeira islands. Although the fish observed in this study belongs only to table size fish (between 26 to 32 cm). A negative correlation was found between Acantocephala abundance and fish size (weight, length). This results are probably related with food items selected by Atlantic chub mackerel according to its size. Weib (1974) found that diet of *S. colias* 24 to 29 cm long was based on copepods, the main intermediate hosts of Acanthocephala, while fish between 30-37 cm long eat larger prey.

In conclusion, we can state that the parasites with impact on economy and public health from Atlantic chub Mackerel (*Scomber colias*) collected around Matosinhos were detected in relatively low levels so generally speaking, the fish were in very good and safe conditions to be consumed fresh or canned.

## 6. References

- Abattouy, N., Valero, A., Benajiba, M.H., Lozano, J., and Martín-Sánchez, J. (2011). *Anisakis simplex* s.l. parasitization in mackerel (*Scomber japonicus*) caught in the North of Morocco — Prevalence and analysis of risk factors. *International Journal of Food Microbiology* 150 , 136–139.
- Abaunza, P., Villamor, B., and Pérez, J. (1995). Infestation by larvae of *Anisakis simplex* (Nematoda: Ascaridata) in horse mackerel, *Trachurus trachurus*, and Atlantic mackerel, *Scomber scombrus*, in ICES Divisions VIIIb, VIIIc and Ixa (N-NW of Spain). *Sci. Mar.*, 59, : 223 – 233 .
- Abe, N., and Okamoto, M. (2015). Molecular characterization of muscle-parasitizing didymozoid from a chub mackerel, *Scomber japonicus*. *Acta Parasitologica* 3, 557–562.
- Abe, N., Okamoto, M., and Maehara, T. (2014). Molecular characterization of muscle-parasitizing didymozoids in marine fishes. *Acta Parasitologica*, 59, 354–358.
- Abolloa, E., Paggia, L., Pascual, S., and D'Amelioa, S. (2003). Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. *Infection, Genetics and Evolution* 3 , 175–181.
- Akmirza, A. (2003). Distribution of parasite fauna of chub mackerel in Aegean and Mediterranean Sea. *Turkish Journal of Marine Sciences* 9 , 187–195.
- Alvarez-Pellitero, P., and Sitja-Bobadilla, A. (1993). Pathology of Myxosporea in marine fish culture. *Diseases of Aquatic Organisms* 17, 229-238.
- Alves, D.R., Luque, J.L., and Abdallah, V.D. (2003). Metazoan parasites of chub mackerel, *Scomber japonicus* houttuyn (osteichthyes: scombridae), from the coastal zone of the state of rio de janeiro, brazil. *Brazil. J. Vet. Parasitol.* 12, 164-170 .
- Amin, and Omar, M. (1897). Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). *The Journal of parasitology* , 1216-1219.
- Bak, T.J., Jeon, C.H., and Kim, J.H. (2014). Occurrence of anisakid nematode larvae in chub mackerel (*Scomber japonicus*) caught off Korea. *International Journal of Food Microbiology* 191 , 149–156.
- Berland, B. (1961). Nematodes from some Norwegian marine fishes. *Sarsia*, 2, 1-50.
- Cabral, H.N., and Murta, A.G. (2002). The diet of blue whiting, hake, horse mackerel and mackerel on Portugal . *Appl. Ichthyol.* 18 , 14-23 .
- Castro, J. (1993). Feeding ecology of chub mackerel *Scomber japonicus* in the Canary islands area. *South African Journal of Marine Science*, 13, 323-328 .
- Chaligiannisa, I., Lalle, M., Pozio, E., and Sotiraki, S. (2012). Anisakidae infection in fish of the Aegean Sea. *Vet.Parasitology* 184 , 362– 366.

- Collete, B., and Nauen, C. (1983). Scombrids of the world. An annotated and illustrated catalog of tunas, mackerels, bonitos and related species known to date. *Fao Species Catalog 2*, 1983.
- Collette, B. (2003). Family Scombridae Rafinisque 1815—Mackerels, Tunas, and and bonitos. *Annotated Checklist of Fishes California Academy Sciences*, 28pp.
- Costa, G., Cavallero, S., D'Amelio, S., Paggi, L., Santamaria, M.T., Perera, C.B., and Khadem, M. (2011). Helminth parasites of the Atlantic chub mackerel, *Scomber colias* Gmelin, 1789 from Canary Islands, Central North Atlantic, with comments on their relations with other Atlantic regions. *Acta Parasitologica 56(1)*, 98–104;.
- Costa, G., Freitas, N., Dellinger, T.H., and MacKenzie K. (2007). Gill monogeneans of the chub mackerel, *Scomber japonicus* from Madeiran waters of the Atlantic Ocean, Portugal. *Journal of Helminthology 81*, 33-38.
- Costa, G., Pontes, T., Mattiucci, S., and D'Amelio, S. (2003). The occurrence and infection dynamics of *Anisakis* larvae in the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus*, and oceanic horse mackerel, *Trachurus picturatus* from Madeira, Portugal. *Journal of Helminthology 77*, 163-166.
- Cremonte, F., and Sardella, H.N. (1997). The parasite fauna of *Scomber japonicus* Houttuyn 1782 (Pisces: Scombridae) in two zones of the Argentine Sea. *Fisheries Research*, , 31, 1–9.
- Cruz, C., Silva, A., and Saraiva, A. (2011). Intensity of *Kudoa* sp. infection and alteration of muscle texture in *Sardina pilchardus* in Portugal. *Bull. Eur. Ass. Fish Pathol.*, 31, 205.
- Darriba, D., Guillermo L.T., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and high-performance computing. *Nat Methods*. ; 9(8), 772.
- Dawson-Coates, A.J., Chase, C.J., Funk, V., Booy, H.M., Haines, R.L., Falkenberg, L.C., and Whitkaker D.J., (2003). The relationship between flesh quality and numbers of *Kudoa thyrsites* plasmodia and spores in farmed Atlantic salmon *Salmo salar* L., *Journal of Fish Diseases 26*, 451-459. .
- DGRM. (2016). *Recursos da Pesca. Série Estatística Ano de 2015*. Lisboa: Direcção-Geral de recursos naturais, segurança e serviços marítimos.
- Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res*:32, 1792-1797.
- Eiras, J.C., Saraiva, A., and Cruz, C. (2014). Synopsis of the species of *Kudoa* Meglitsch, 1947 (Myxozoa: Myxosporea: Multivalvulida). *Syst Parasitol.* 87, 153–180 .
- FAO. (2011). *Food and Agriculture Organization Fishery and aquaculture statistics*. : .
- FAO. (2014). *The State of World Fisheries and Aquaculture*
- Farjallah, S., Busi, M., Mahjoub, M.O., Slimane, B.B., Paggi, L., Said, K., and D'Amelio, S. (2008). Molecular characterization of larval anisakid nematodes from marine fishes off the Moroccan and Mauritanian coasts. *Parasitology International 57*, 430–436.
- Guindon, S., and Gascuel, O. (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Syst Bio*:52, 696-704.

Herreras, V.M., Aznar, J.F., Balbuena, A.J., and Raga, A.J. (2000). Anisakid larvae in the musculature of the Argentinean hake, *Merluccius hubbsi*. *Journal of Food Protection* 63, 141–1143.

Infante, C.B. (2007). Phylogenetic differentiation between Atlantic *Scomber colias* and Pacific *Scomber japonicus* based on nuclear DNA sequences. *Genetica*, 130, 1-8.

Hernández J., and Ortega A. (2000). *Synopsis of biological data on the chub mackerel (Scomber japonicus, 1782)*. Rome: FAO Fish Synop. No. 157, 77 pp.

Dawson-Coates, J.A., Chase, J.C., Funk, V., Booy, H.M., Haines, R.L., Falkenberg, C.L., Whitkaker, J.D., Olafson, R.W., and Pearson, W.T. (2003). The relationship between flesh quality and numbers of *Kudoa thyrssites* plasmodia and spores in farmed Atlantic salmon *Salmo salar* L.,. *Journal of Fish Diseases* 26, 451-459. .

Kennedy, C.R. (2012). *Ecology of the Acanthocephala*. Cambridge: University of Exeter pp 12-26.

Kovaleva, A.A., and Gaevskaya, A.V. (1983). First data on fish myxosporidia of the southeastern Pacific open waters. *Vestnik Zoologii* , 17-611.

Langdon, S.J. (1991). Myoliquefaction postmortem (milky flesh) due to *Kudoa thyrssites* (Gilchrist) (Myxosporia: Multivalvulida) in mahi mahi, *Coryphaena hippurus* . *L. J Fish Dis* 14, :45–54.

Langdon, S.J., Thorne, T., and Fletcher, J.W. (1992). Reservoir hosts and new clupeoid host records for the myoliquefactive myxosporean parasite *Kudoa thyrssites* (Gilchrist). . *Journal of Fish Diseases* 15,, 459-471.

Levsen, A., Jorgensen, A., and Mo, T.A. (2008). Occurrence of postmortem myoliquefactive kudoosis in Atlantic mackerel, *Scomber scombrus* L., from the North Sea. *Journal of Fish Diseases* 31, 601-611. .

Li, Y.C., Sato, H., Tanaka, S., Ohnishi, T., Kamata, Y., and Sugita-Konishi, Y. (2013). Characterization of the ribosomal RNA gene of *Kudoa neothunni* (Myxosporia: Multivalvulida) in tunas (*Thunnus* spp.) and *Kudoa scomberi* n. sp. in a chub mackerel (*Scomber japonicus*). *Parasitol Res* 112, 1991–2003.

Lom, J., and Dyková, I. (1992). *Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science, Vol. 26*. Elsevier Science Publishers, 315 pp.

Madrid, E., Gil, F., García, M., Debenedetti, A.L., Trelis, M., and Fuentes, M.V. (2016). Potential risk analysis of human anisakiasis through the consumption of mackerel, *Scomber scombrus*, sold at Spanish supermarkets. *Food Control* 66:, 300-305.

Martins, M. (2007). Growth variability in Atlantic mackerel (*Scomber scombrus*). *ICES J. Mar. Sci.* 64, 1785-1790.

Martins, M., Skagen, D., Marques, V., Zwolinski, J., and Silva, A. (2013). Changes in the abundance and spatial distribution of the Atlantic chub mackerel (*Scomber colias*) in the pelagic ecosystem and fisheries off Portugal. *Scientia Marina* 77(4) , 551-563.

Matsui, T. (1967). Review of the mackerel genera *Scomber* and *Rastrelliger* with. *Copeia*, 71-83.

Mattiucci, S., and Nascetti, G. (2008). Chapter 2: Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite coevolutionary processes. *Advances in Parasitology*, 66, 47-148.

Mattiucci, S., Abaunza, P., Damiano, S., Garcia, A., Santos, N.M., and Nascetti, G. (2007). Distribution of Anisakis larvae, identified by generic markers, and their use for stock characterization of demersal and pelagic fish from European waters: An update. *Journal of Helminthology*, 81,, 117-127.

Mattiucci, S., Paggi, L., Nascetti, G., Santos, C.P., Costa, G., Benedetto, A.P., Ramos, R., Argyrou, M., Cianchi, R., and Bullini L., (2002). Genetic markers in the study of *Anisakis typica* (Diesing, 1860): larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). *Systematic Parasitology* 51, 159–170.

Mazzocchi, G.M., and Ribera-d'Alcalà, M. (1996). Recurrent patterns in zooplankton structure and succession in a variable coastal environment. *ICES Journal of Marine Science*, 52, 679-691.

McEachran, J., and Fechhelm, J. (2005). *Fishes of the Gulf of Mexico Volume 2: Scorpaeniformes to Tetraodontiformes*. Austin: University of texas press, pp 780.

McGladdery, E.S. (1986). *Anisakis simplex* (Nematoda: Anisakidae) infection of the musculature and body cavity of Atlantic herring (*Clupea harengus harengus*). *Can. J. Fish. Aquat Sc.* 43, 1312-1317.

Mele, S., Pennino, M.G., Piras, M.C., Bellido, J.M., Garippa, G., and Merella, P. (2014). Parasites of the head of *Scomber colias* (Osteichthyes: Scombridae) from the western Mediterranean Sea. *Acta Parasitologica* 59(1), 173–183.

Meloni, M., Angelucci, G., Merella, P., Siddi, R., Deiana, C., and Orrù, G. (2011). Molecular characterization of *Anisakis* larvae from fish caught off Sardinia. *Journal of Parasitology*, 97, 908-914.

Nikolaeva, V.M. (1980). On the Black Sea Didymozoidae, species composition, morphology and distribution. *Ekologiya Morya* 2, 81-85.

Oliva, M.E., Valdivia, M.I., Costa, G., Freitas, N., Carvalho, A.M., Sánchez, L., and Luque, L.J. (2008). What can metazoan parasites reveal about the taxonomy of *Scomber japonicus* Houttuyn in the coast of South America and Madeira islands? . *Journal of Fish Biology*, 72, 545–554.

Oliva, M., Luque, L.J., Valdivia, I., and Alves, D. (2002). Metazoos parasitos de *Scomber japonicus* (Pisces: Scombridae): una aproximacion anfiocanica. VII Enbrapoa & III Elapoa. *Foz do Iguaçu, Brasil*, 127.

Osanz-Mur, C. A. (2001). *Universidad Autoonoma de Barcelona*. Available on-line,at <http://www.tdx.cat/bitstream/handle/10803/5628/acom1de2.pdf?sequence¼1>, (Accessed on 10.08.16).

Pascual, S., Abollo, E., and Azevedo, C. (2006). Host-parasite interaction of a muscle-infecting didymozoid in the Atlantic mackerel *Scomber scombrus*. *L. ICES Journal of Marine Science*, 63, 169–175.

- Petrochenko, V.I. (1956). *Acanthocephala of domestic and wild animals. Vol. I (translated from Russian, 1971)*. Jerusalem, Israel: Keter Press.
- Piras, M.C., Tedde, T., Garippa, G., Virgilio, S., Sanna, D., Farjallah, S., and Merella, P. (2014). Molecular and epidemiological data on *Anisakis* spp (Nematoda: Anisakidae) in commercial fish caught off northern Sardinia (western Mediterranean Sea). *VET. PAR* 7118, 4.
- Platt, E.N. (1975). Infestation of cod (*Gadus morhua*) L. with larvae of codworm (*Terranova decipiens* Krabbe) and herringworm, *Anisakis* sp. (Nematoda, Ascaridata) in North Atlantic. *J. Appl. Ecol.* 12., 437-450.
- Pontes, T., D'Amelio, S., Costa, G., and Paggi, L. (2005). Molecular characterization of larval anisakid nematodes from marine fishes of madeira by a pcr-based approach, with evidence for a new species. *J. Parasitol* 91(6), 1430–1434.
- Pozdnyakov, S.E., and Gibson, D.I. (2008). *Family Didymozoidae Monticelli, 1888. Keys to the Trematoda, Volume 3, 631-643*.
- Price, M.N., Dehal, P.S., and Arkin, A.P. (2009). FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Mol Biol Evol*: 26, 1641-1650.
- Price, M.N., Dehal, P.S., and Arkin, A.P. (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* 5(3).
- Pulleiro-Potel, L., Barcala, E., Mayo-Hernandez, E., and Munoz, P. (2015). Survey of anisakids in commercial teleosts from the western Mediterranean Sea: Infection rates and possible effects of environmental and ecological factors. *Food Control*, 55, 12-17.
- Qin, Y., Zhao, Y., Ren, Y., Zheng, L., Dai, X., Li, Y., Mao, W., and Cui, Y. (2013). Anisakiasis in China: the first clinical case report. *Foodborne Pathog. Dis.* :10, 472–474.
- Quiazon, K.M., Yoshinaga, T., Ogawa, K., and Yukami, R. (2008). Morphological differences between larvae and in vitro-cultured adults of *Anisakis simplex* (sensu stricto) and *Anisakis pegreffii* (Nematoda: Anisakidae). *Parasitology International* 57 , 483–489.
- Rêgo, A. (1987). *Rhadinorhynchus pristis* (Rudolphi, 1802) acanthocephalan parasite of fishes, *Scomber scombrus* and *S. japonicus*. Some observations on the scanning electron microscope. *Memórias do Instituto Oswaldo Cruz*, 82, 287- 288.
- Rêgo, A., and Santos, C.P. (1983). Helminthofauna de cavalas, *Scomber japonicus* Houtt, do Rio de Janeiro. *Memórias do Instituto Oswaldo Cruz* 78.4 , 443-448.
- Renon, P., and Malandra, R. (1993). Frequenza di infestazione dell'anisakidosi in Teleostei marini pervenuti presso il mercato ittico di Milano nel triennio "91-93". *Archivio Veterinario Italiano*, 44, 118-130.
- Reynolds, E.S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *The Journal of cell biology*.
- Rohde, K. (1989). *Kuhnia sprostonae* Price, 1961 and *K. scombercolias* Nasir and Fuentes Zambrano, 1983 (Monogenea: Mazocraeidae) and their microhabitats on the gills of *Scomber* spp. (Teleostei: Scombridae), and geographical distribution of gill Monogenea of *Scomber* spp. . *Systematic Parasitology* 14, 93–100.

- Sambrook, J., and Russell, W.D. (2001). Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory. New York.
- Sanmartín, M.L., Quinteiro, P., Iglesias, R., Santamaría, T.M., Leiro, J., and Ubeira, M.F. (1994). *Nematodos Parasitos en Peces de las Costas Gallegas*. Madrid: Díaz de Santos.
- Scoles, D.C. (1998). Global phylogeography of mackerels of the genus *Scomber*. *Fish Bull*, 96, 823–842.
- Shaw, R.W., Hervio, L.M., Devlin, H.R., and Adamson, L.M. (1997). Infection of *Aulorhynchus flavidus* (Gill) (Osteichthyes: Gasterosteiformes) by *Kudoa thyrsites* (Gilchrist) Myxosporea: Multivalvulida. *Journal of Parasitology* 83, 810–814.
- Shukhgalter, A.O. (2004). The parasite fauna of the chub mackerel (Scombridae: *Scomber japonicus* Houttuyn 1782) in the central-eastern Atlantic (Atlantic coast of the Northern Africa and the Azores archipelago banks). *Parazitologiya* 38, 160-170.
- Silva, M. (1999). Diet of common dolphins, *Delphinus delphis*, off the Portuguese continental coast. *J. Mar. Biol. Ass. U.K* 79, 531–540.
- Silva, M., and Eiras, J. (2003). Occurrence of *Anisakis* sp. in fishes off the Portuguese West coast and evaluation of its zoonotic potential. *Bulletin of the European Association of Fish Pathologists*, 23, 13-17.
- Solonchenko, A.I., and Bodyanitski, A.V. (1968). *Parasite fauna of Scomber colias from the Atlantic Ocean near the South western coast of Africa*. "Naukova Dumka", *Biology of seas No. 14*. . Kiev : Parasites of marine animals pp. 90–95.
- Strømnes, E., and Andersen, K. (1998). Distribution of whaleworm (*Anisakis simplex* Nematoda, Ascaridoidea) L3 larvae in three species of marine fish: saithe (*Polachius virens* (L.)), cod (*Gadus morhua* (L.)) and redfish (*Sebastes marinus* (L.)) from Norwegian waters. . *Parasitology Research*, 84, 281-285.
- Sugita-Konishi, Y., Fukuda, Y., Mori, K.I., Mekata, T., Namba, T., Kuroda, M., Yamazaki, and A., Ohnishi, T. (2015). New validated rapid screening methods for identifying *Kudoa septempunctata* in olive flounder (*Paralichthys olivaceus*). *Japanese journal of infectious diseases*, 68(2), 145-147.
- Suzuki, J., Murata, R., Hosaka, M., and Araki, J. (2010). Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematodes. *International Journal of Food Microbiology* 137, 88–93.
- Suzuki, J., Murata, R., Yokoyama, H., Sadamasu, K., and Kai, A. (2015). Detection rate of diarrhoea-causing *Kudoa hexapunctata* in Pacific bluefin tuna *Thunnus orientalis* from Japanese waters. *International Journal of Food Microbiology* 194, 1-6.
- Takabe, K., Ohki, S., Kunihiro, O., Sakashita, T., Endo, I., Ichikawa, Sekido, H., Amano, T., Nakatani, Y., Suzuki, K., Shimada, H. (1998). Anisakidosis: a cause of intestinal obstruction from eating sushi. *The American journal of gastroenterology*, 93(7), 1172-1173.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol Biol Evol*, 30, 2725-2729.

- Tucker, C., and Hargreaves, J. (2004). *Biology and Culture of Channel Catfish* pp 416-417. U.S.A: Elsevier.
- Umehara, A., Kawakami, Y., Matsui, T., Araki, J., and Uchida, A. (2007). Molecular identification of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda:Anisakidae) from fish and cetacean in Japanese waters. *Parasitology International* 55:, 267–271.
- Valderrama, P. (1995). Distribución de los principales recursos pelágicos durante los veranos de 1992 a 1994. *Instituto del Mar del Perú*, 1995.
- Vardić, S., Valić, D., Kapetanović, D., Kurtović, B., and Teskeredžić, E. (2012). Molecular characterisation of Anisakidae larvae from fish in Adriatic Sea. *Parasitol. Res.* 111, 2385–2391.
- Velasco, G.M., Rodero, M., Cuéllar, C., Chivato, T., Mateos, M.J., and Laguna, R. (2008). Skin prick test of *Kudoa* sp. antigens in patients with gastrointestinal and/or allergic symptoms related to fish ingestion. *Parasitol Res.* 103, 713-5.
- Weib, R. (1974). Feeding behaviour and formation of fish concentrations in the chubmackerel (*Scomber colias*) in the northwest african fishing grounds. *ICES CM* , 15.
- Whipps, M.C., and Kent, L.M. (2006). Phylogeography of the cosmopolitan marine parasite *Kudoa thyrsites* (Myxozoa: Myxosporidia). *Journal of Eukaryotic Microbiology* 53, 364–373. .
- Williams, J.E., and Bunkley-Williams, L. (2000). Multicellular parasite (Macroparasite) problems in aquaculture. Encyclopedia of aquaculture. *Wiley, New York*, 565-569.
- Yamaguti, S. (1963). *Systema Helminthum. Vol. 5 Acanthocephala*. Interscience Publ.
- Yokoyama, H., and Itoh, N. (2005). Two multivalvulid myxozoans causing post-mortem myoliquefaction: *Kudoa megacapsula* n.sp. from red barracuda (*Sphyraena pinguis*) and *Kudoa thyrsites* from splendid alfonso (*Beryx splendens*). *Journal of Parasitology* 91, 1132–1137.
- Yokoyama, H., Abe, N., Maehara, T., and Suzuki, J. (2014.). First record of *Unicapsula seriola* (Myxozoa: Multivalvulida) from the muscle of Malabar grouper *Epinephelus malabaricus* in Japan. *Parasitology International* 63, 561–566.
- Yokoyama, H., Freeman, M.A., Itoh, N., and Fukuda, Y. (2005). Spinal curvature of cultured Japanese mackerel *Scomber japonicus* associated with a brain myxosporidian, *Myxobolus acanthogobii*. *Dis Aquat Org* 66, 1-7.
- Yokoyama, H., Whipps, M.C., Kent, L.M., Mizuno, K., and Kawakami, H. (2004). *Kudoa thyrsites* from Japanese flounder and *Kudoa lateolabracis* n.sp. from Chinese sea bass: causative myxozoans of post-mortem myoliquefaction. *Fish Pathology* 39, 79-85.