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## Pathological and Ultrastructural Findings of *Sphaerospora dicentrarchi* (Myxosporaea: Bivalvulida) infection in European seabass (*Dicentrarchus labrax*) farmed in Turkey

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**ABSTRACT:** *Sphaerospora dicentrarchi* is typically histozoic parasite of the wild and cultured European sea bass (*Dicentrarchus labrax*) of the Mediterranean coastal countries. In this study, pathological and ultrastructural findings of *Sphaerospora dicentrarchi* infection in the European sea bass were firstly investigated in Turkey. For this purpose, 862 European seabass (*Dicentrarchus labrax* L) were collected and evaluated from eight cultivation fish farms (offshore floating cages) in four different locations of Aegean Sea of Turkey (three farms from Kazıklı, one farm from Çandarlı, two farms from Taşburun and two farms from Güllük Regions). It was observed that this parasite was especially intense in adult fish and in the period of June-October when the water temperature increased. Microscopically, the parasites were seen as the most intensive in stomach, intestines, gall bladder, pancreas, heart and testis, respectively. Stomach was the most commonly infected organ. In transmission electron microscopy examination, a bag-like group of mature couple spores were seen surrounded by polar capsules. As a result, it was determined that *Sphaerospora dicentrarchi* infection is very common in adult sea bass in the Aegean region of Turkey, as in Greece, Spain, Portugal and Italy.

**Keywords:** *Sphaerospora dicentrarchi*, Pathology, Transmission electron microscopy, European sea bass.

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## INTRODUCTION

Parasites and related diseases are responsible for considerable economic losses in the mariculture. Among them, protozoan parasites, particularly Myxosporeans are important pathogens and cause a serious threat to marine culture and environment (Mladineo, 2003; Roberts, 2012). *Sphaerospora dicentrarchi* is a Myxozoan and typically histozoic parasite of the wild and cultured European seabass. It reported firstly in Spain and after the first report, it has become one of the most common parasites of the European seabass (Sitjà-Bobadilla and Alvarez-Pellitero, 1990; Sitjà-Bobadilla and Alvarez-Pellitero, 1992; Le Breton, 1999). The most important damage caused by this parasite in fish is chronic and systemic infection of many organs such as gut, kidney, intestines and gall bladder (Xavier et al., 2013; Sitjà-Bobadilla and Alvarez-Pellitero, 1993). In addition to these effects, it is suspected that the parasite is also cause immunopromising in host immune system and predisposition for formation of opportunistic infections (Rigos et al., 1999). The prevalence of infection seems to increase steadily especially in the Mediterranean coastal countries such as Italy, Spain and Portugal (Sitjà-Bobadilla and Alvarez-Pellitero, 1993; Santos, 1996; Fioravanti et al., 2004; Mladineo, 2006).

In the present study, pathological and ultrastructural findings of *Sphaerospora dicentrarchi* infection in the European seabass were firstly investigated in Turkey as another Mediterranean coastal country.

## MATERIALS AND METHODS

### Study area and animals

862 European seabass (*Dicentrarchus labrax*) in different ages (or biomass) were collected from eight cultivation fish farms (offshore floating cages) in four locations of the Aegean Sea of Turkey (three farms from Kazıklı, one farm from Çandarlı, two farms from Taşburun and two farms from Güllük Regions) from October 2016 to August 2017. The fish were divided into 3 groups according to their body weight (biomass). Numbers and biomass of fish with presence of parasite and regions according to groups were pre-

sented in Table 1.

### Pathology

Following necropsy, tissue samples from stomach, intestines, liver, gall bladder, kidney, heart and gonads were fixed in 10% neutral buffer formalin solution for 48 hours, processed through graded alcohols and xylene, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin-eosin (HE) and periodic acid schiff (PAS) staining methods and examined under a light microscope (Culling et al., 1985).

### Electron microscopy

Tissue sections prepared from stomach and intestines taken in 1 mm thickness were processed for transmission electron microscopy investigations. These sections were fixed for 24-48 hours in 2.5% glutaraldehyde solution (pH 7.3 and 4 °C). Between fixation processes and after fixation, tissues were washed with sodium phosphate buffer. Following first fixation process, the tissues were fixed for a second time in 1 hour at 4 °C in a dark environment with 1% OsO<sub>4</sub>. Subsequently, the tissues were kept in graded acetone series (30%, 50%, 70%, 90%, 96%, 100%) and acetone: Epon (1: 1 and 1: 3) solutions. After all these procedures, tissues embedded in pure Epon in capsules were incubated for 18 hours in a 60 °C oven. Semi-thin sections were taken from prepared blocks and stained with Toluidine blue. After staining, the target regions were determined. Ultra-thin sections were then taken from these regions and they were stained with uranyl acetate and lead nitrate (Culling et al., 1985). Stained sections were examined in Transmission Electron Microscope (TEM).

## RESULTS

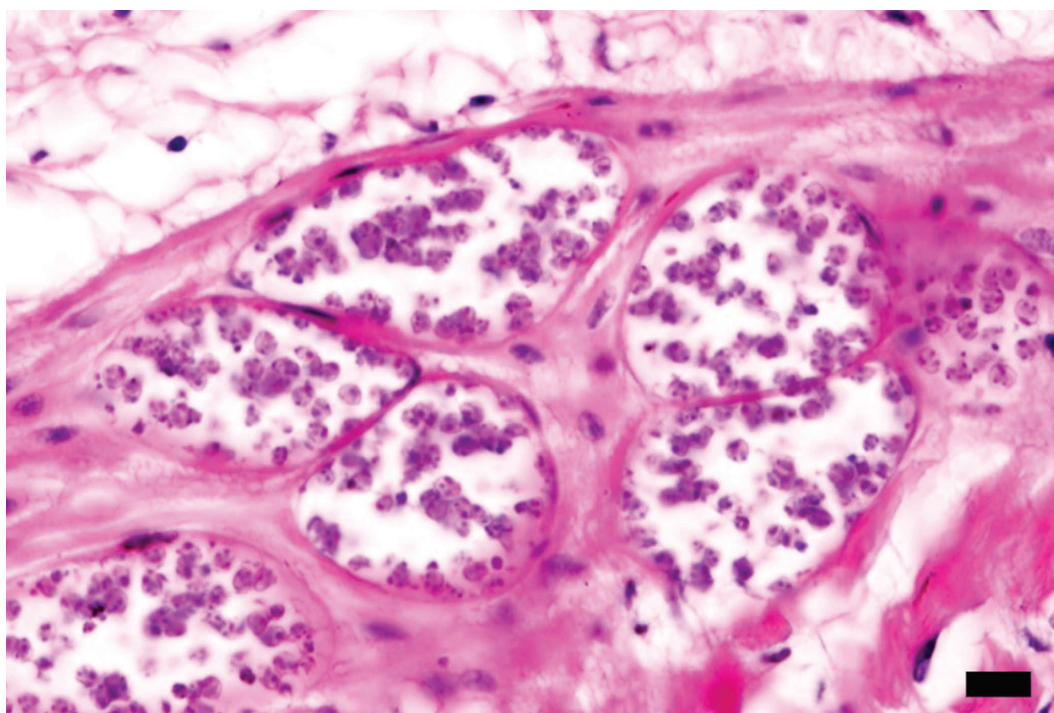
The parasite was especially intense in adult fish and in the period of June-October when the water temperature is risen. Grossly, in groups 1 and 2, serous and aqueous exudate with white clotted content in the lumen of gut and intestines was seen. Gastro-intestinal mucosa was hyperemic and grayish-yellow mucous exudate was found in the lumen (group 3) (Figure 1). In addition, in group 2, mucous (catarrhal)

**Table 1.** Total fish numbers and biomass of fish with presence of parasite and regions according to groups.

	Group 1	Group 2	Group 3
<b>Biomass</b>	25-30 g	80-100 g	250-480 g
<b>Total fish numbers</b>	120	162	580
<b>Infected fish numbers</b>	41/120 (34%)	115/162 (71%)	522/580 (90%)
<b>Region</b>	Kazıklı	Kazıklı, Taşburun	Kazıklı, Taşburun, Güllük, Çandarlı



**Figure 1.** Mucous (catarrhal) exudate in the mucosa of stomachs (Group III).



**Figure 2.** Bag-like groups of spores of *Sphaerospora dicentrarchi* in stomach, H.E., Bar: 10  $\mu$ m

exudate was observed in some cases. Testis was gray-black colored in only one case. Intestinal enlargement was detected in all three groups.

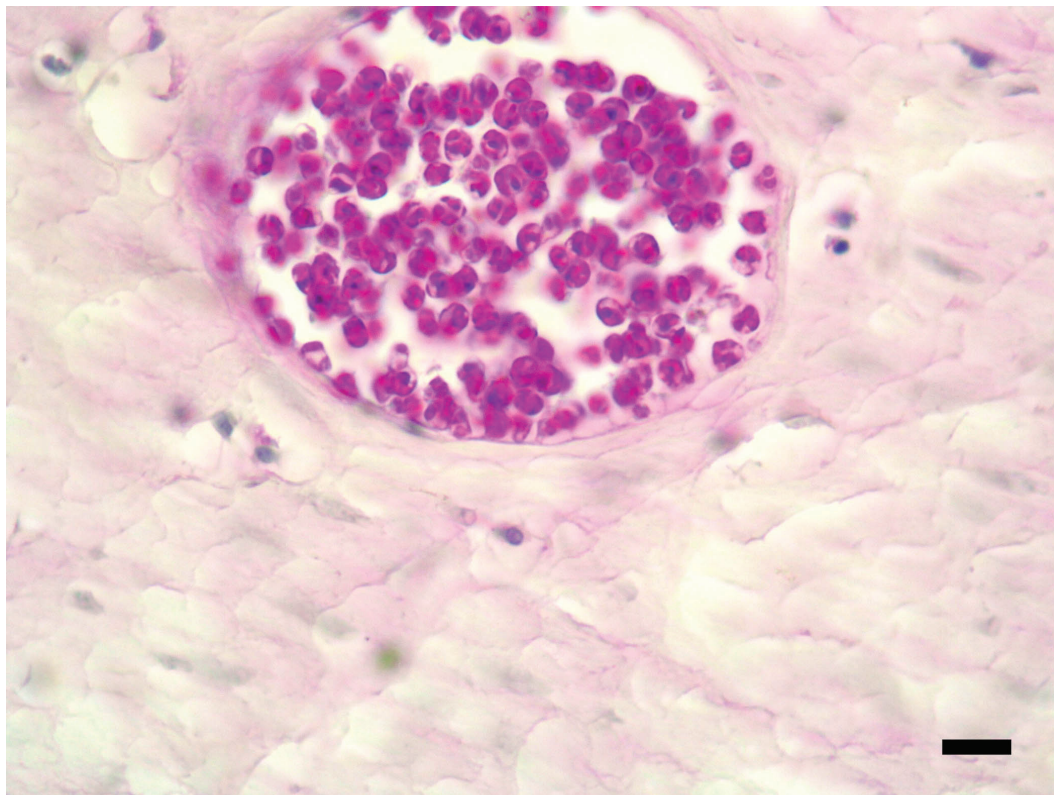
In histopathological studies, presence of *Sphaerospora dicentrarchi* in the European seabass was detected 34% in group 1, 71% in group 2, and 90% in group 3. They were determined as the most intensive in stomach, intestines, gall bladder, pancreas, heart and testis, respectively. Stomach was the most com-

monly infected organ. *Sphaerospora dicentrarchi* spores were seen as bag-like groups on the walls of these organs (Figure 2). These bag-like groups were thin-walled and seen as cystic structures. Very spores in them were identified by strongly positive PAS stain (Figure 3). The size of cysts and the number of parasites were variable. Cysts were mostly seen in single groups and sometimes in groups of 3-6. Distribution and intensity of *Sphaerospora dicentrarchi* in organs

were presented in Table 2. No inflammatory reaction to the parasite was detected in most of the cases. However, in the summer period, some fish in groups 2 and 3 had an inflammatory reaction around myxospores especially in stomach and intestines. The cellular response consisted a small number of lymphocytes, fibroplasia, and significant increase in eosinophilic granular cells (EGCs). This cellular response indicated subacute gastroenteritis. In addition, hyperplasia was seen in goblet cells of mucosa. Epicarditis was

remarkable in group 1 together with myxospores in three cases. In these fish, there were marked inflammatory cell infiltration and fibrous exudate around the parasite.

Transmission electron microscopic examination revealed a bag-like group of mature couple spores surrounded by polar capsules. They were surrounded by electron-dense glycogen granules and lipid inclusions in the sporoplasm (Figures 4, 5, 6).



**Figure 3.** A bag-like group of spores of *Sphaerospora dicentrarchi* in gall bladder, PAS, Bar: 10  $\mu$ m

**Table 2.** Distribution and intensity of *Sphaerospora dicentrarchi* in organs.

	Group 1	Group 2	Group 3
<b>Stomach</b>			
Lamina epithelialis	-	+	-
Lamina propria	++	+++	+++
Tunica muscularis	+++	++	+++
Tunica serosa	++	++	++
<b>Intestines</b>			
Lamina propria	-	+++	++
Lamina muscularis	+	+	++
Tunica serosa	++	++	+
Gall bladder	+	+++	+++
Hepatopancreas and external pancreas	-	+	++
Mesenterium	+	++	+
Heart, epicardium (in 3 seabass)	++	-	-

-:no parasite, +: mild; ++: moderate, +++: severe

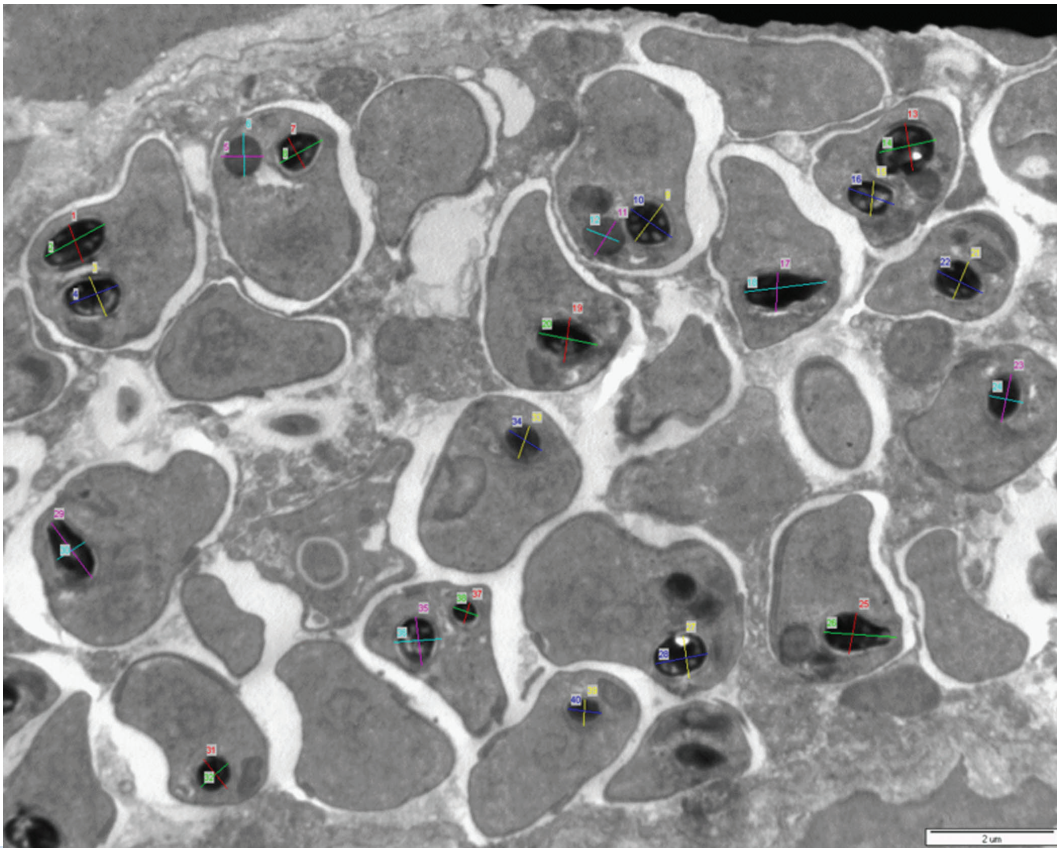
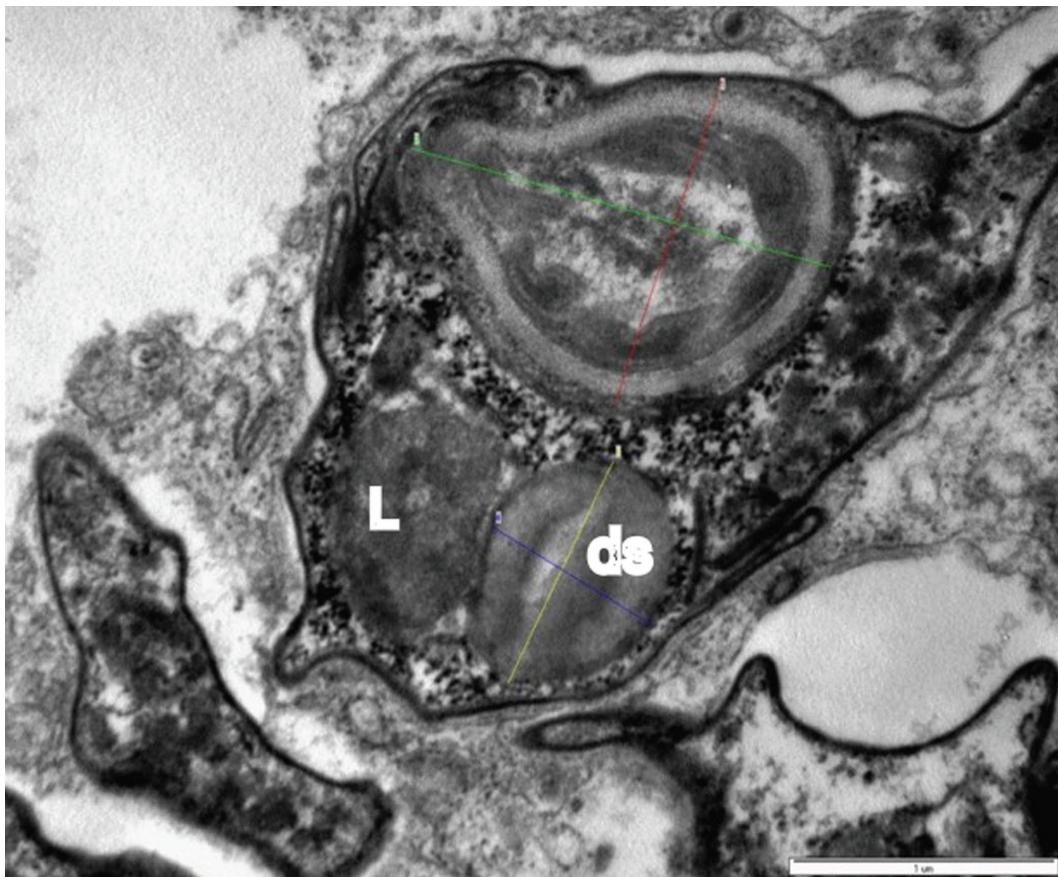


Figure 4. TEM image of *Sphaerospora dicentrarchi* mature spores.



Figure 5. TEM image, A bag-like group of mature spores (ms) of *Sphaerospora dicentrarchi* and lipid inclusion (L), frontal view.



**Figure 6.** TEM image, a bag-like group of developing stage (ds) of *Sphaerospora dicentrarchi* and lipid inclusions (L), frontal view.

## DISCUSSION

In seabass, *Sphaerospora dicentrarchi* first identified in Spain in 1992. Then it is stated that the prevalence is 95% in natural environment fish in Portugal, 52.2% in cultured fish of Italy and 54.13% in cultured fish of the Adriatic sea (Sitjà-Bobadilla and Alvarez-Pellitero, 1992; Alvarez-Pellitero and Sitjà-Bobadilla, 1993; Fioravanti et al., 2004; Mladieno, 2006; Xavier et al., 2013). However, it has not been previously reported in Turkey, despite being another Mediterranean country. The presence of *Sphaerospora dicentrarchi* in cultured seabass was reported for the first time in Turkey with this study. Parasite prevalence rate was determined as 34% in 25-30 g fish, 71% in 80-100 gr fish and 90% in 250-480 gr fish. These rates are considerably higher, and considering this high rate, it suggested that *Sphaerospora dicentrarchi* has been a significant unknown problem in seabass aquaculture in the Aegean region of Turkey for many years.

Macroscopically, *Sphaerospora dicentrarchi* infection does not show any significant external lesions in the organs or skeletal system (Alvarez-Pellitero and

Sitjà-Bobadilla, 1993; Roberts, 2012). In this study, no any significant and specific macroscopic finding was found in all groups.

Myxosporean infections of the gall-bladder and urinary tract are very common and spores are easily detected on histological sections (Roberts, 2012). The parasite invasion of different organs such as kidney, gut, intestines, pancreas, gall-bladder and testes is noted in *Sphaerospora dicentrarchi* infection (Sitjà-Bobadilla and Alvarez-Pellitero, 1992; Alvarez-Pellitero and Sitjà-Bobadilla, 1993; Fioravanti et al., 2004). In these organs, the bag-like groups of spores surrounded by a fibrotic capsule are observed and spores are easily detected by their severe positive PAS reaction (Sitjà-Bobadilla and Alvarez-Pellitero, 1992; Sitjà-Bobadilla and Alvarez-Pellitero, 1993). In this study, parasites were found to be most intense in stomach, intestines, gall bladder, pancreas, heart and testicles, respectively. The spores were revealed as bag-like groups on the walls of these organs and were also identified by the strongly positive PAS stain.

In myxosporean infections, generally marked cel-

lular and subacute-chronic inflammatory infiltration is more specifically associated with myxosporean cells and this lesion consisted of a halo of inflammatory cells around the myxosporeans particularly in the kidney (Fioravanti et al., 2004; Roberts, 2012). In a study, it is noted that there is no significant cellular infiltration in most *Sphaerospora dicentrarchi* infection cases of seabass. In some cases of this study, leukocyte proliferations and spores surrounded by macrophages are seen in the infected organs (Alvarez-Pellitero and Sitjà-Bobadilla, 1993). In a different study, it is reported that hemorrhage and necrosis are observed in the intestinal submucosa and renal parenchyma in seabass with severe infected *Sphaerospora dicentrarchi* and no apparent finding in mildly infected seabass (Fioravanti et al., 2004). In the present study, in accordance with the literature, inflammatory infiltration was not observed in most of the cases. However, during the summer period, some fish in group 2 and group 3 showed an inflammatory reaction characterized by a small number of lymphocytes, fibroplasia, and marked increase in EGCs, especially around myxospores in the stomach and intestines.

Electron microscopy studies related to *Sphaerospora dicentrarchi* in the literature are not so many. In transmission electron microscopy examinations, mature spores showed a bag-like groups similar to light microscopic appearance or different forms from observed at light microscopy. Polar capsules are surrounded by densely packed glycogen granules and lipid inclusions (Sitjà-Bobadilla and Alvarez-Pellitero, 1992). Cells related to host response such as granulocytes, lymphocytes, and promyelocytes are frequent-

ly seen close to the sphaerospores (Alvarez-Pellitero and Sitjà-Bobadilla, 1993; Sitjà-Bobadilla and Alvarez-Pellitero, 1993). In this study, a bag-like group of mature couple spores was seen surrounded by polar capsules in transmission electron microscopic examinations.

## CONCLUSION

In conclusion, according to the results of the present study, it was revealed that *Sphaerospora dicentrarchi* infection is widespread in adult seabass in the Aegean region of Turkey with a very high rate as well as in Spain, Portugal and Italy. Appearance of infection in 25 g of fish indicated the intensity and early involvement of the parasite in the sea. To the author's knowledge, no data on fish of this size have been found in the literature. Multidisciplinary further studies are needed to develop effective treatment methods against this infection, which has become more important over time in the Mediterranean.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## FUNDING

This study was supported by Scientific Projects Commission of Aydın Adnan Menderes University (Project no. VTF-17016).

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures were performed on dead fish for diagnostic and research purposes, for this reason, the study did not require an ethical approval.

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