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Short Communication

Isolation and identification of *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) from gilthead sea bream (*Sparus aurata* L.) in the Mediterranean Sea, Greece

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The gilthead sea bream is one of the main aquacultured fish species in the Mediterranean Sea. *Sparicotyle chrysophrii* is an ectoparasite specific for sea bream and able to cause mortality in the Mediterranean aquaculture system. In this study, a total of 20 gilthead sea bream were sampled for parasitological research at Heraklion Bay, Crete, Greece. Gills of the fish were dissected and examined for parasites using a light microscope. *S. chrysophrii* was identified by field emission scanning electron microscopy, PCR and sequencing.

Key words: Gilthead sea bream, Mediterranean Sea, *Sparicotyle chrysophrii*, field emission scanning electron microscopy, PCR, sequencing.

INTRODUCTION

Monogeneans are known to be mostly host-specific through the life cycle (Desdevises et al., 2002). The monophyly of the Monogenea comprises two groups: the Monopisthocotylea and the Polyopisthocotylea (Mladineo and Maršić-Lučić, 2007). Polypisthocotyleans are pathogenic to economically important fish around the world (Ogawa, 2002). There have been reports about mortalities due to polyopisthocotyleans in several cultured fish species (Silan et al., 1985; Ogawa, 2002). Among them, *Sparicotyle chrysophrii* is a gill monogenean specific for sea bream and able to cause mortality in aquaculture systems (Sanz, 1992). *S. chrysophrii* was originally called *Microcotyle chrysophrii*, belonging to genus *Microcotyle* (Microcotylidae, Polypisthocotylea); however, it is now placed in the monospecific genus *Sparicotyle* (Mladineo and Maršić-Lučić, 2007). Its clinical signs include lethargy due to hypoxia, emaciation, histopathological damage, and severe anemia (Cruz e Silva et al., 1997).

The gilthead sea bream, *Sparus aurata* L., is one of the main cultured fish species in the Mediterranean Sea (Melis et al., 2014). European Union (EU) is the largest producer of gilthead sea bream worldwide; Greece dominates aquaculture production of this species (FAO,

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2012). *S. chrysophrii* has been regarded as one of the most threatening ectoparasites for the Mediterranean aquaculture (Antonelli et al., 2010). The aim of this study was to isolate and identify *S. chrysophrii* from gilthead sea bream in the Mediterranean Sea, Greece based on morphological and molecular analysis.

MATERIALS AND METHODS

Sampling

From March to May 2015, a total of 20 gilthead sea bream (237 ± 83.1 g) were sampled for parasitological examinations. Fish were purchased from fishermen at Heraklion Bay (35°20′N, 25°08′E), Crete (South Greece). Sampled fish were packed on ice in separate plastic bags and then transported to the diagnostic facility within 1 h. Examination was carried out immediately after arrival. Skin, fins, and gills of the fish were examined. Gill arches were removed and placed on a glass microscope slide. The slides were examined for parasites using a light microscope. Infected samples were immediately fixed in 2.5% Glutaraldehyde for scanning electron microscopy (SEM).

Field emission scanning electron microscopy (FE-SEM)

Specimens were fixed in modified Karnovsky's fixative, post fixed in 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2), dehydrated through an ethanol serial solutions of 50, 60, 70, 80, 90, 95, 100, 100% for 1 h per each concentration. Specimen drying was performed at room temperature overnight and sputter coated with gold/palladium. Samples were examined using a JSM-6700F field emission scanning electron microscope (JEOL, Japan).

Molecular analysis of parasites

DNA extraction was conducted using the DNeasy® Blood and Tissue Kit (QIAGEN) following the manufacturer's instruction. For amplification of the 28S rDNA, PCR was performed using C1/D2 primer pair: C1: 5'-ACC CGC TGA ATT TAA GCA T-3', D2: 5'-TGG TCC GTG TTT CAA GAC-3'. The amplification was carried out as previously reported (Chisholm et al., 2001). The amplified PCR product was sequenced using the BigDyeTM terminator cycle sequencing kit (Macrogen Genomic Division). The sequence obtained in this study was aligned with that of the *S. chrysophrii* 28S ribosomal RNA gene (AF311719) available in the GenBank database, and the percentage sequence similarity was determined.

RESULTS AND DISCUSSION

Samplings in the present study were conducted from March to May 2015 as *S. chrysophrii* exhibited seasonal changes in its prevalence (Antonelli et al., 2010). Previous reports show that cyclic peaks in the abundance of parasite have been mainly recorded in Mediterranean Sea during spring and summer (Reversat et al., 1992; Sanz, 1992). In general, high water temperature is regarded to promote hatching of monogenean eggs and propagation of parasites (Gannicott and Tinsley, 1998). Morphological examinations by FE-SEM showed elongated and sharp narrowing shape (Figure 1A). The enlarged picture of head area of *S. chrysophrii* was taken to observe a characteristic structure such as pit-like invagination although the trial failed to observe it (Figure 1B). It is known that pit-like invaginations contain taxonomic and functional significance: pit-like invaginations have been observed on other Microcotylidae such as *P. luquei* and *Microcotyle labracis* (Antonelli et al., 2010); pit-like invaginations play a role in absorption of organic nutrients directly from seawater, excretion, and osmoregulation (Halton, 1978; Oliver, 1981; Willams and McKenzie, 1995). *S. chrysophrii* infecting gills (Figure 1C, D) was found while examining gills of the gilthead seabream sampled during the study. Pathological effects are mainly related to its attachment to gills: blood loss and reduction of breathing surface (Repullés-Albelda et al., 2011).

The main characteristic of *S. chrysophrii* is the haptor with its clamps (Repullés-Albelda et al., 2011), which was the main purpose of the morphological examination by FE-SEM in this study. However, there was no morphological characteristic. It may be because the samples were destroyed when they were removed from gills, which occurs quite often during the on-site disease diagnostic process. It emphasizes the usefulness of molecular analysis although molecular analysis based on morphological examinations is considered as the most desirable method for parasite identification (Perkins et al., 2010). For molecular identification, the 28S rRNA gene of *S. chrysophrii* was sequenced and compared with the 28S rRNA gene of another *S. chrysophrii*. The sequence obtained in this study showed 99% identity with that of another *S. chrysophrii*.

The present study revealed that the percentage of fish infected by *S. chrysophrii*, prevalence value was 40%, mean intensity was 3.6 ± 1.4, and maximum abundance was 6. According to the previous report, *S. chrysophrii* has produced mortalities in farmed fish and even higher mortalities in sea cages due to its favor of high densities and net biofouling (Sanz, 1992; Sitjà-Bobadilla et al., 2006). However, *S. chrysophrii* has been rarely isolated in wild sea bream from Mediterranean Sea (Paperna et al., 1977; Mladineo and Marsic-Lucic, 2007). It is known that *S. chrysophrii* is commonly related to mixed infections with other parasites and secondary bacterial infections (Cruz e Silva et al., 1997). In addition, further studies of *S. chrysophrii* are required as it can switch easily to the new host although Monogeneans are known to be dependent to their hosts (Mladineo and Maršić-Lučić, 2007).

Conflict of interests

The authors did not declare any conflict of interest.

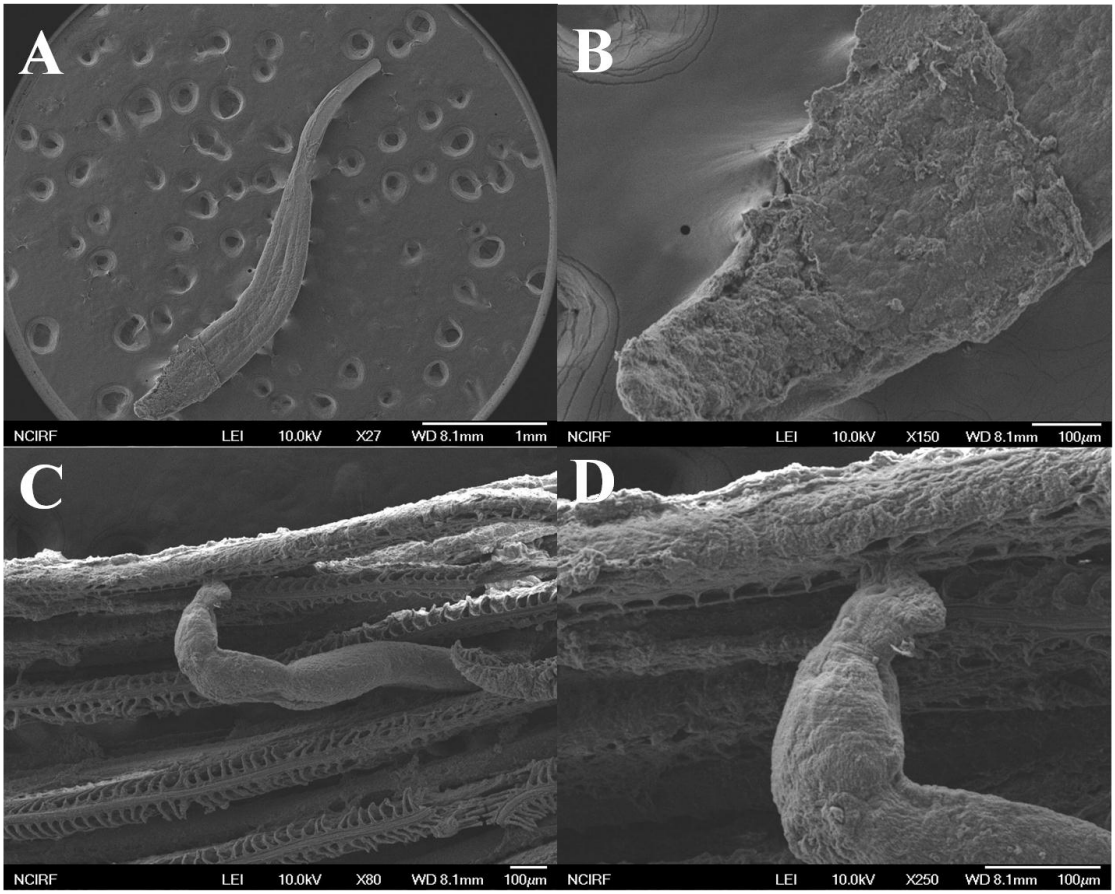

Figure 1. Field emission scanning electron micrograph (FE-SEM) of *S. chrysophrii* from gilthead sea bream. **A,** FE-SEM of an entire specimen. **B,** Head area of the parasite. **C,** The parasite attached to the gills of affected fish. D, The enlarged picture of the parasite attachment. Scale bars: A = 1 mm; B, C, D = 100 µm.

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