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Host–parasite relationship of *Ceratomyxa puntazzi* n. sp. (Myxozoa: Myxosporea) and sharpsnout seabream *Diplodus puntazzo* (Walbaum, 1792) from the Mediterranean with first data on ceratomyxid host specificity in sparids

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ABSTRACT

Sparidae are economically important fishes to both, fisheries and aquaculture in the Mediterranean. Species diversification is an important strategy for the development of Mediterranean aquaculture. One of the species recently introduced is the sharpsnout seabream *Diplodus puntazzo* (Walbaum, 1792). During a parasitological study of fish from the Gulf of Valencia and the Mar Menor (Spain), myxozoan spores belonging to the genus *Ceratomyxa* were found in the gall bladder of *D. puntazzo*. A morphological description of the spores, which includes histology and transmission electron microscopy (TEM) as well as molecular (SSU ribosomal DNA) data resulted in the erection of a new species, *Ceratomyxa puntazzi* n. sp. A histopathological study of *C. puntazzi* n. sp. infection in *D. puntazzo* showed that the parasite causes necrosis and loss of epithelial cells in the gall bladder, and provokes a pericholangitis in the liver tissue surrounding the bile ducts. Furthermore, molecular data obtained from *C. puntazzi* n. sp. and three other ceratomyxids from the closely related fish species *Diplodus annularis* L. and *Sparus aurata* L. which share the same habitat suggest that the genus *Ceratomyxa* is host-specific in sparids, which agrees with data previously obtained from Serranidae, Labridae and Pomacentridae, and that ceratomyxid species from sparids in the Mediterranean originated from a common ancestor.

1. Introduction

The Sparidae are economically important teleosts with regard to fisheries and aquaculture in the Mediterranean. In 2007, gilthead seabream *Sparus aurata* L. aquaculture represented 67% of Mediterranean aquaculture production. Introduction of new species into Mediterranean aquaculture is an important strategy for the diversification of marketable products in the sector. Some examples for new, commercially important fish species are the sharpsnout seabream *Diplodus puntazzo* (Walbaum, 1792), the common dentex *Dentex dentex* L. and the blackspot seabream *Pagellus bogaraveo* (Brünnich, 1768) for which successful rearing and marketing has been achieved (Barazi-Yeroulanos, 2010).

Ceratomyxa Thélohan, 1982 is a genus of predominantly coelozoic myxozoan parasites. Over 270 species have been described (Eiras, 2006; Mladineo and Bočina, 2006; Ali et al., 2006; Reed et al., 2007; Abdel-Ghaffar et al., 2008; Heiniger et al., 2008; Gunter and Adlard, 2008, 2009, 2010; Gunter et al., 2009; Gunter et al., 2010a,b), most of them infecting the gall bladders of marine teleosts.

In general, *Ceratomyxa* species seem to cause little or no damage to their hosts, possibly due to their coelozoic

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development, however, some species have been shown to cause important pathogenic changes in their hosts. The best known pathogenic species is *Ceratomyxa shasta* (Noble, 1950), which occurs in salmonids in North America (Bartholomew et al., 1989b). *C. shasta* produces extensive haemorrhages and necrosis in the intestine of its hosts and causes substantial losses in wild and cultured salmonids (Bartholomew et al., 1989a, 2004). However, *C. shasta* is not a typical representative of the genus as it differentiates itself from all other *Ceratomyxa* spp. by various characters, most importantly, its histozoic occurrence. Its phylogenetic clustering with the otherwise monophyletic genus *Ceratomyxa* is also uncertain (Fiala, 2006; Fiala and Bartošová, 2010).

In the Mediterranean, bile-inhabiting species cause pathogenic changes in a number of sparids. *Ceratomyxa sparusaurati* Sitjà-Bobadilla et al., 1995 is a highly prevalent parasite of the gilthead seabream *S. aurata* in Mediterranean culture systems and produces notable histopathological damage, including swelling, vacuolization and sloughing of the epithelial cells, associated with mortalities (Palenzuela et al., 1997). *Ceratomyxa diplodae* (Lubat et al., 1989) has been related to mortalities as an opportunistic pathogen in potentially immunosuppressed, steroid treated sharpsnout seabream *D. puntazzo* (Katharios et al., 2007).

During a parasitological study of the newly established aquaculture species *D. puntazzo*, ceratomyxid spores were found in the bile. The aim of this study was to identify and morphologically describe the parasite found in *D. puntazzo* and to provide basic information on the host–parasite relationship by studying the pathology observed in relation to the location of the parasite in different organs. Furthermore, small subunit ribosomal DNA (SSU rDNA) sequences were obtained from the ceratomyxid in *D. puntazzo* as well as from closely related fish species from the same habitat in order to study the relationships between these and published species, to get a basic idea on host specificity of *Ceratomyxa* in sparids.

2. Materials and methods

2.1. Source of fish

Between 2007 and 2010, *D. puntazzo*, *Diplodus annularis* L. and *S. aurata* from different sites along the Spanish Mediterranean coast were studied for infection with myxozoans. Ninety-four specimens of sharpsnout seabream *D. puntazzo* (6–37 cm total length; 3.75–733.9 g) were obtained by local shore fishing in the Mediterranean off Jávea (Alicante) and off San Pedro del Pinatar (Murcia). They were transported live to the aquaculture facilities at the University of Valencia and analysed within 3 weeks of arrival. Forty-seven specimens of annular seabream *D. annularis* (12.5–20 cm total length; 39.22–158 g) were purchased at Valencia's fishing harbour (Valencia). These fish had been captured by commercial trawling and put on ice after capture. Twenty-one specimens of gilthead seabream *S. aurata* (11.5–28 cm total length; 18.43–385.4 g) were obtained from a fish farm, off San Pedro del Pinatar (Murcia). Fish were harvested and put on ice. All fish were examined within 24 h post mortem.

2.2. Morphological analysis of bile myxozoans

The gall bladder of each fish was isolated, held with forceps directly above the opening of an autoclavated 1.5 ml Eppendorf tube and ruptured. From the collected bile, 4 μl were placed on a microscopic slide, covered with a cover-slip and examined using light microscopy at 400× magnification. Spores detected in the bile of six *D. puntazzo*, three *S. aurata* and two *D. annularis* were measured and described. Apart from *Ceratomyxa* sp. 2 ex *S. aurata*, which was only detected in one fish, replicate samples from different individuals were used for DNA extraction and SSU rDNA sequencing of the different *Ceratomyxa* species. Digital photographs of spores in fresh smears were taken with a Leica DC300 (Leica Microsystems Ltd.) camera mounted on a Leica DMR microscope at 1000× magnification with Nomarski interference. Morphological measurements of spores followed the recommendations of Lom and Arthur (1989). The posterior spore angle of ceratomyxid spores was measured as described in Heiniger et al. (2008). Measurements were taken from digital images using the computer software UTHSCSA ImageTool Version 3.0 for Windows (The University of Texas Health Science Center at San Antonio, TX, USA). Measurements are given in μm as means ± standard deviation with range in parenthesis. Morphological descriptions were obtained from spores of the same bile as used for DNA extraction and SSU rDNA sequencing.

To determine morphological differences between the spores obtained from *D. puntazzo* and from *D. annularis*, spore measurements were analysed statistically. The Kolmogorov–Smirnov test was used for checking the normal distribution and the significance of the data. Differences between the spore measurements were tested using a *t*-test. Principal component analysis (PCA) was carried out for further data exploration. All statistical analyses were carried out using the computer program SPSS 15 (SPSS Inc.).

Fresh smears of spores on microscopic slides were air dried, stained with Diff-Quick® (Medion Diagnostics International) and mounted in a xylene-free mounting medium (Panreac Química S.A.U., Spain). 140 μl of infected bile of *D. puntazzo* was preserved in 100% ethanol. Syntype specimens and DNA samples were deposited in the Invertebrate Collection of the Natural History Museum (NHMUK), London, UK.

For TEM, infected gall bladders were fixed with 2.5% glutardaldehyde in 0.1 M PBS (pH 7.4), injecting the fixative slowly into the gall bladder, using a syringe with a fine needle (0.3 mm diameter) in order to fix trophozoites and spores and the epithelium of the gall bladder immediately and not await the slow penetration of the fixative through the gall bladder wall. Gall bladders were then placed in an Eppendorf with further fixative and left to fix for several days. After several washes with PBS, the gall bladders were post-fixed with 1% osmium tetraoxide in 0.1 M PBS and dehydrated in an ascending alcohol series from 30% to 100% ethanol. Gall bladders were transferred from 100% ethanol into epoxypropane. Embedding was performed by