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Light and electron microscopic observations of *Ceratomyxa sparusaurati* (Myxosporea: Bivalvulida) from the gall bladder of rock bream (*Oplegnathus fasciatus*)

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In a previous study on the parasites of cultured rock bream (*Oplegnathus fasciatus*), we reported the discovery of a new species, *Ceratomyxa oplegnathus*, obtained from the gallbladder. In the present study, we found another *Ceratomyxa* species, *C. sparusaurati*, also from the gallbladder of rock bream. The morphological and ultrastructural characteristics of *C. sparusaurati* were investigated using light and transmission electron microscopy (TEM).

Key words: *Ceratomyxa sparusaurati*, Rock bream (*Oplegnathus fasciatus*), Gall bladder

Members of the genus *Ceratomyxa* Thélohan, 1892 are coelozoic parasites in marine teleosts, and most of them are known to infect the gall bladders (Lom and Dykova, 2006). To date, approximately 300 species belonging to *Ceratomyxa* have been reported (Rocha et al., 2015). Although most *Ceratomyxa* species are relatively less harmful to their host than histozoic myxosporeans (Alvarez-Pellitero and Sitjà-Bobadilla, 1993), several *Ceratomyxa* species have been reported to cause severe pathological changes on their parasitizing location of the host (Gunter et al., 2009).

In a previous study on the parasites of cultured rock bream (*Oplegnathus fasciatus*), we reported a new species *Ceratomyxa oplegnathus* from the gallbladder (Cho et al., 2006). In the present study, we found another *Ceratomyxa* species, *C. sparusaurati*, from the gallbladder of rock bream. *C. sparusaurati* was first reported from gilthead sea bream (*Sparus aurata*) in the Mediterranean by Sitjà-Bobadilla et al. (1995), and is known to be able to provoke inflammatory reactions and histopathological damage such as vacuolization, sloughing, and detachment of epithelial cells (Palenzuela et al. 1997). In the present study, we investigated the morphological and ultrastructural characteristics of *C. sparusaurati* using light and transmission electron microscopy (TEM).

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Materials and Methods

Rock bream (50 fish; body length 15-20 cm) were obtained from commercial fish farms situated at southern coastal area of Korea. Fish were transported to the laboratory, and killed by overexposure to the anaesthetic MS222 (Sigma Co. Ltd.). They were then necropsied to examine for the presence of parasites in major organs; brain, gill, intestine, kidney, liver, spleen, heart, gonad, urinary bladder, skin and muscle etc. Bile juice from the gallbladder was collected with 1 ml syringes and centrifuged at 1500 rpm at room temperature to get myxosporean parasites. The resulted pellet was observed in a fresh material or fixed

in 2% glutaraldehyde in phosphate buffer (pH 7.0) for transmission electron microscopic (TEM) study. Descriptions and measurements of spores were performed according to the guidelines for species description of myxosporeans by Lom and Arthur (1989), and by using a light microscope equipped with an ocular micrometer and image analysis software (ImageTool ver 2.0, UTHSCSA, USA). Mean and standard deviations of each spore characteristics were obtained from fresh 150 mature spores.

For TEM study, portions of the tissue infected by myxosporeans were fixed in 2% glutaraldehyde at 4°C overnight and post-fixed with OsO_4 in the same buffer for 2 h. The specimens were dehydrated, embedded in resin and ultrathin-sectioned, stained with uranyl acetate and lead citrate and examined by JEM1200 transmission electron microscope (JEOL LTD., Japan).

Results and Discussion

Light microscopical observation

Among 50 examined fish, *C. sparusaurati* was found from 20 fish. Mature spores (Figs. 1A & B) were crescent-shape in front view with a convex anterior end and a slightly or highly concave posterior one, and measuring 5.4 ± 0.33 (4.74-6.09) μm in length, 17.1 ± 1.4 (15.3-21.1) μm in width in sutural view. Two subequal valves were together along sutural line. Two equal polar capsules were subspherical or ovoid, 2.63 ± 0.15 (2.24-2.89) × 2.27 ± 0.14 (2.03-2.53) μm in diameter (Figs. 1A & B). They situated near the sutural line. A sporoplasm occupied most of spore cavity (Figs. 1A & B). Trophozoites of *C. sparusaurati* were round (Fig. 1C) or comet (Fig. 1D) shape with a short pseudopodial tail, sometimes branched. Numerous dark granules were observed in the fresh trophozoites (Fig. 1D). Disporic trophozoites (Fig. 1E) and, occasionally, aberrant spores with 3 polar capsules and 3 valves were found (Fig. 1F).

Shape and size of spores of the present specimen is almost identical to that of *C. sparusaurati* reported from the gallbladder of gilthead seabream, *Sparus aurata* (Sitjà-Bobadilla et al. 1995) and sharpsnout sea bream, *Puntazzo puntazzo* (Rigos et al. 1999) in Mediterranean sea. Rock bream and sharpsnout sea bream are assigned to the family Oplegnathidae, Order Perciformes. According to Molnár (1994), most myxosporean species sharing related host species. Thus, we classified present specimen as previously described species, *C. sparusaurati*, based on morphological characteristics and infection site (Table 1).

Ultrastructural characteristics

Presporogonic and sporogonic stages of *C. sparusaurati* from the gallbladder of *O. fasciatus* were ex-

Table 1. Comparison of spore characteristics between original description of *Ceratomyxa sparusaurati* (Sitjà-Bobadilla et al. 1995) and the present specimens

	Sitjà-Bobadilla et al. 1995	Present
Spore	Crescent-shape with a convex anterior end and a flattened to curved posterior one. Two smooth valves meet at the suture.	
Length (㎛)	5.65 ± 0.74 (4.5～7.5)	5.4±1.56 (4.47～6.09)
Thickness (㎛)	15.76 ± 1.01 (14.0～17.5)	17.1±0.2 (15.3～21.1)
Polar capsule	Two, equal subspherical	
Length (㎛)	2.79 ± 0.27 (2.2～3.4)	2.63±0.2 (2.24～2.89)
Polar filament	6	–
Sporoplasm	Binucleated sporoplasm	
Host	*Sparus aurata*	*Oplegnathus fasciatus*
Organ	Gallbladder	

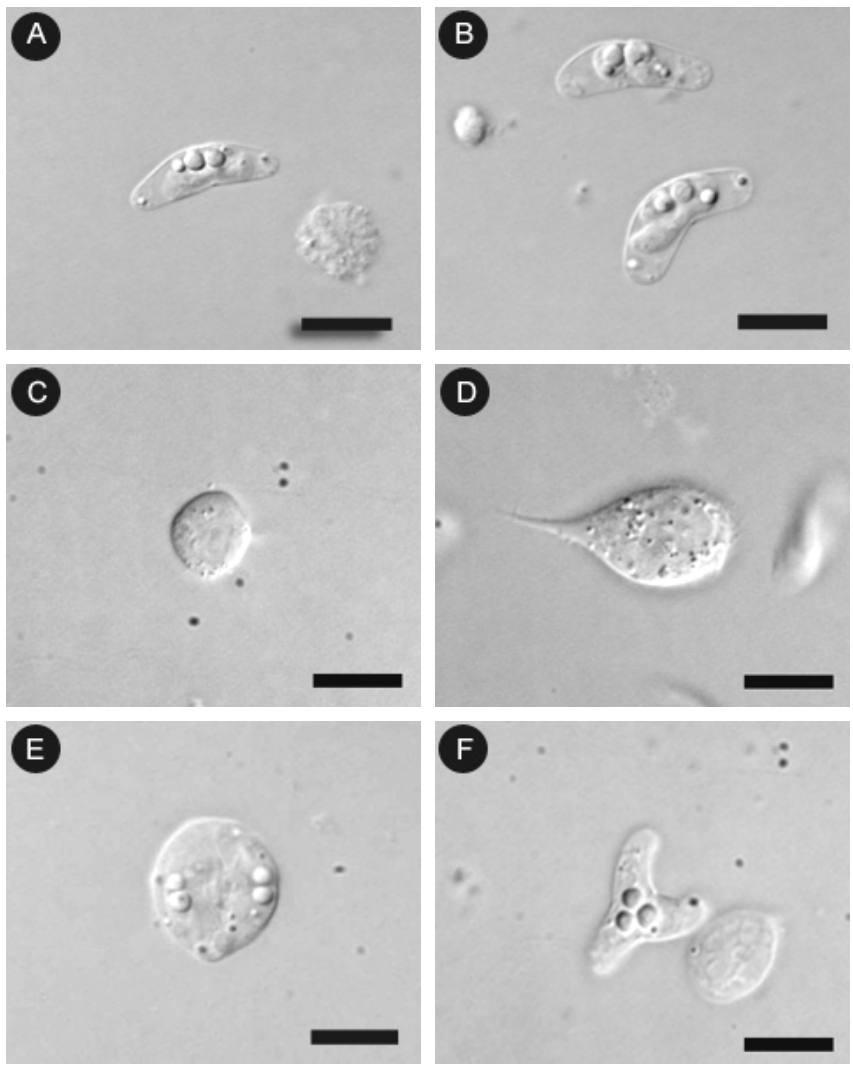

Fig. 1. Photomicrographs of *Ceratomyxa sparusaurati* from the gallbladder of *Oplegnathus fasciatus*. Bar=10 μm. (A, B) Mature spores. (C) Round trophozoites. (D) Comet shaped trophozoite. (E) Disporic trophozoite. (F) Tri-capsular abnormal spore.

amined using TEM. The earliest, uninucleate primary cell (Fig. 2B) developed into bi-nucleate stage (Fig. 2C) with a vegetative nucleus (nucleus of primary cell) and a generative cell (secondary cell). Subsequent endogenous divisions of a secondary cell resulted in tri-nucleate stage (Fig. 2D), in which 2 secondary cell with a vegetative nucleus. In more advanced stage, an inner tertiary cell appeared in the cytoplasm of secondary one (Fig. 3A). This secondary cell with inner tertiary one was going to further divisions. Ultrastructural view of early stages with inner generative cell (secondary and tertiary cell) revealed that pinocytic pit, papillae and well developed mitochondria located at their peripheral regions (Fig. 2A). Nuclear appearances of inner generative cell were also similar to that of primary cell with peripheral heterochromatin and eccentric nucleolus (Fig. 3A). Electron density of cytoplasm of inner gen-

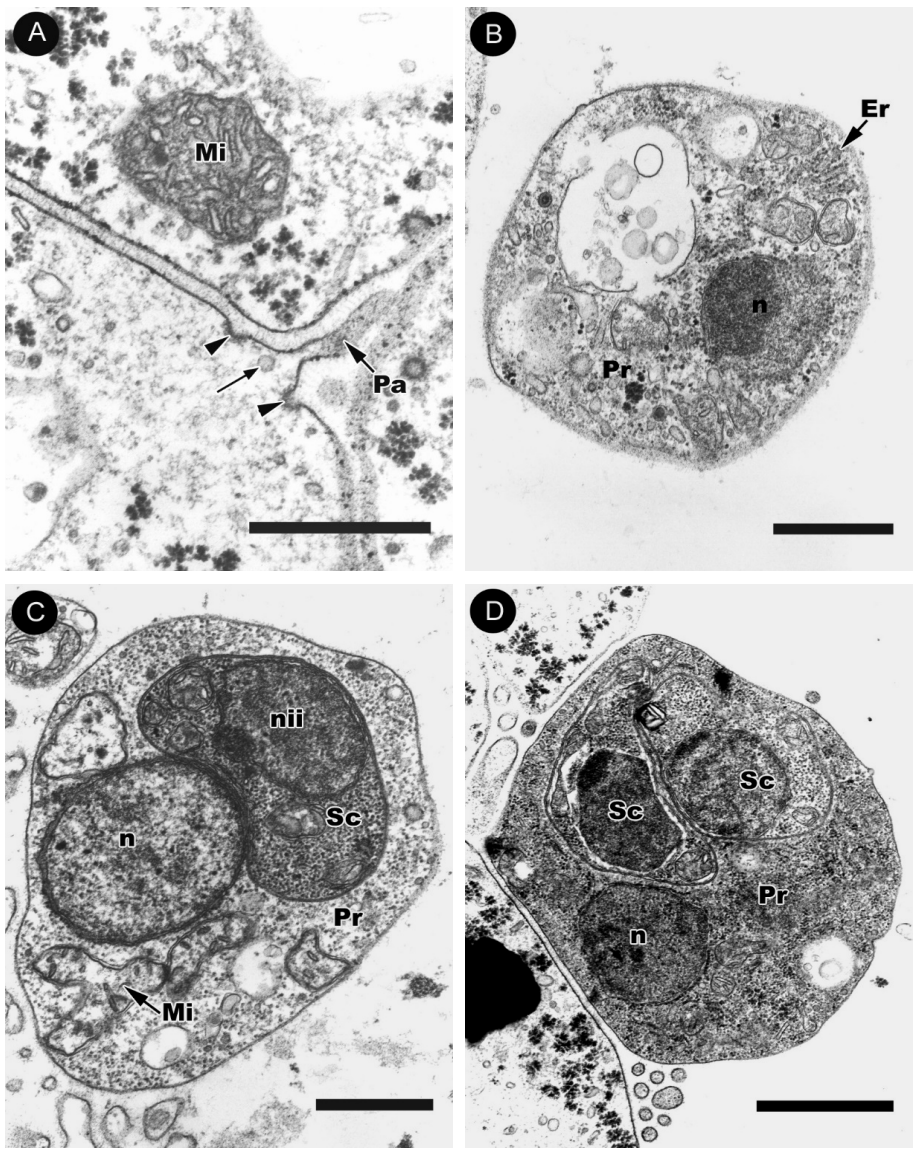

Fig. 2. Electron-micrographs of early stages of *Ceratomyxa sparusaurati* from the gallbladder of *Oplegnathus fasciatus*. (A) Peripheral structure of the trophozoite. Mi, mitochondria; Pa, papillae. Arrows indicate vesicles and arrowheads note pinocytic pits. Bar=600 nm. (B) Early primary cell (Pr) with a nucleus (n). Bar= 600 nm. (C, D) Primary cell contains one or two secondary cell (Sc). Mi, mitochondria. All bars = 1 μm.

erative cells was various. Electron density of inner generative cells was denser than that of primary cell (Figs. 2C, 3A) or similar (Fig. 2D). The density of tertiary cells was similar to that of secondary ones (Fig. 2A).

Sporogenesis and spore formation was asynchronous and disporous (Fig. 3A). Two groups of generative cells formed two sporoblast. Sporoblast was consisting of two capsulogenic cells, 2 valvogenic cells and a binucleate sporoplasmic cell (Fig. 3A). Maturation of capsulogenic cell was also asynchronous (Figs. 3B & C). One capsulogenic cell exhibited

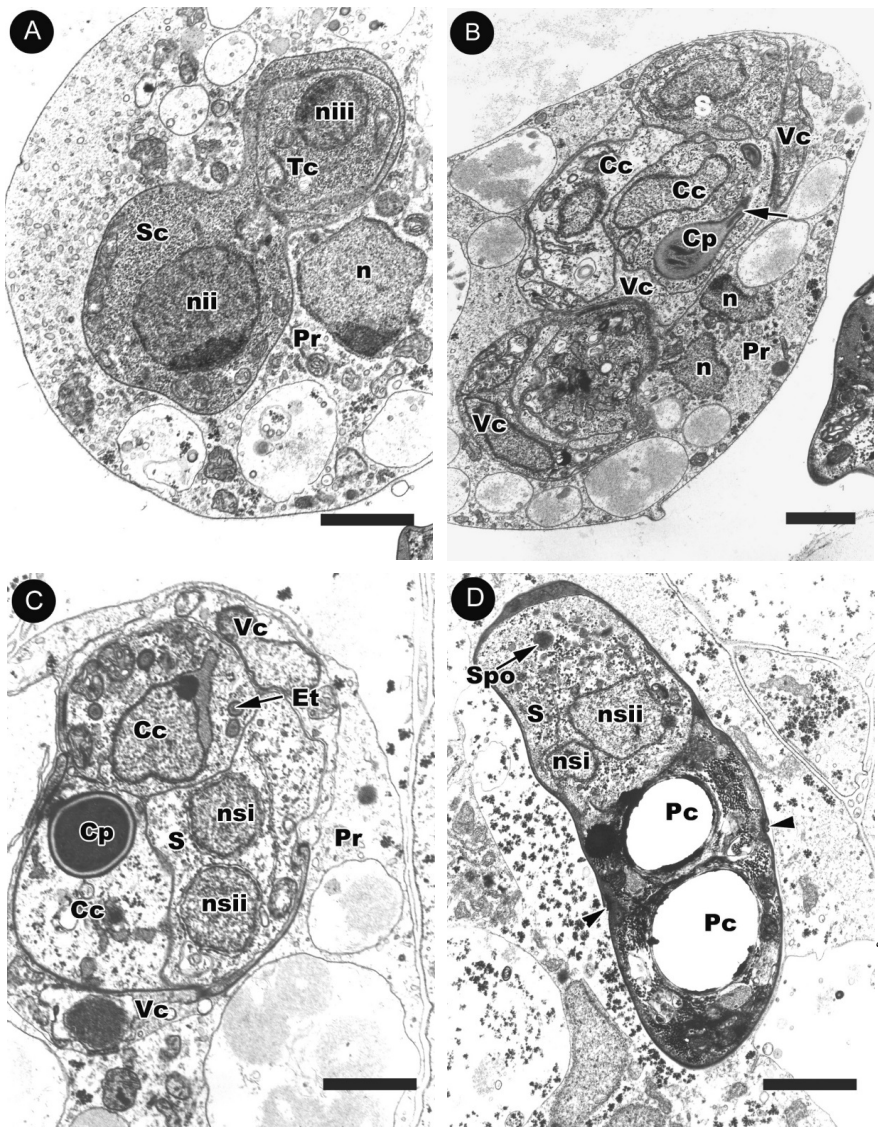

Fig. 3. Electron-micrographs of mature stages of *Ceratomyxa sparusaurati* from the gallbladder of *Oplegnathus fasciatus*. (A) A secondary cell (Sc) proliferating with enclosing an inner tertiary cell (Tc). n, nucleus of primary cell; Pr, primary cell. Bar=1 μm (B) Two sporoblasts within primary cell (Pr). A capsulogenic cell (Cc) contains capsular primordia (Cp) associated with external tubule (arrowed). Vc, valvogenic cell. Bar=1 μm. (C) Binucleate sporoplasmic cell (S). A capsulogenic cells contains capsular primordia or external tubules. nsi & nsii, nucleus of sporoplasmic cell. Bar=1 μm. (D) Almost mature spore with binucleate sporoplasm (S). nsi & nsii, nucleus of sporoplasm; Spo, sporoplasmosome. Arrowheads indicate valvular sutures. Bar=1 μm.

external tubule with capsular primordia while other one containing almost matured polar capsule. Two valvogenic cells became flattened to form the valvular sutures of the mature spore (Fig. 3C). Mature spore (Fig. 3D) had two valves tightly joined with suture, a binucleate sporoplasm and two polar capsules, situated near valvular suture.

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