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# Co-Infection of Two Myxosporean Parasites - *Parvicapsula anisocaudata* and an Unidentified Myxosporean - in the Kidney of Cultured Olive Flounder, *Paralichthys olivaceus*

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Two species of myxosporean parasites - *Parvicapsula anisocaudata* and an unidentified myxosporean - were found in the lumina of renal tubules and the tubular epithelium, respectively, from cultured olive flounder, *Paralichthys olivaceus* in Korea. The latter was also seen in interstitial tissue of spleen and inter-renal gland of the head kidney. Group of pseudoplasmodia of *P. anisocaudata* were firmly attached on the epithelium of renal tubules through pseudopodia. In the renal tubule epithelium, a group of unidentified myxosporean trophozoites, which were 2-3 times larger than intraluminal trophozoites of *P. anisocaudata*, was observed. The parasites being burst out into the lumen was occasionally encountered with partial break of the epithelium. Although infection of *P. anisocaudata* and unidentified myxosporean parasites did not induce any cellular reaction of the host, occlusion of renal tubules and rupture of renal epithelium would impact negatively on the renal functions of severely infected fish.

*Key words* : Myxosporea, *Parvicapsula*, Olive flounder, Renal tubule, Ultrastructure

## Introduction

Myxosporean parasites of the genus *Parvicapsula* infect mostly urinary system (renal tubule or urinary bladder) of marine and anadromous fishes (Lom and Dyková, 1992), and 10 species belonging to this genus have been described thus far (Køie, 2003). Some species of this genus have been reported as potential pathogens of both wild and farmed fish. *Parvicapsula* sp. infects the kidneys and pseudobranchs of coho salmon *Oncorhynchus kisutch*, causing inflammation and significant mortality (Yasutake and Elliot, 2003). *P. minibicornis*, described as a new species by Kent et al. (1997), infects the kidneys of adult sockeye salmon *O. nerka* returning to a tributary of the Fraser River in British Columbia, consequently causing prespawn mortality (Raverty *et al*., 2000; St-Hilaire *et al*., 2002). *Parvicapsula pseudobranchicola* recently described in farmed Atlantic salmon *Salmo salar* in Norway, infects the pseudobranchs, gill, liver and kidney, causing mortalities ranging from low-grade to significant (Karlsbakk *et al*., 2002; Sterud *et al*., 2003).

Olive flounder, *Paralichthys olivaceus*, is a successfully cultured, commercially valuable species in Korea. Recently, we reported various developmental stages of *Parvicapsula anisocaudata* in the renal tubular lumina of olive flounder (Cho and Kim, 2004). In the present study, we found other unidentified myxosporean trophozoites from the renal tubular epithelia of olive flounder, and described

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histopathological effects elicited by coinfection with the 2 myxosporeans (*P. anisocaudata* and an unidentified myxosporean).

## Materials and Methods

Olive flounder, *Paralichthys olivaceus*, (500-600 g in body weight) were obtained from a commercial farm in Jeju Island of Korea. The fish were taken alive to the laboratory, killed by overexposed to MS-222 (Sigma Chemical Co., St Louis, MO, USA), and all organs were excised for fresh and histological examinations.

For histological study, each organ was fixed in Bouin's solution and embedded in paraffin. The blocks were sectioned with 5 ㎛ thickness and stained with haematoxylin and eosin. For transmission electron microscopy (TEM) study, a small portion of the kidney was fixed in 2% v/v glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4℃ overnight and postfixed in 1% w/v cacodylic $OsO_4$ for 2 h. The specimens were dehydrated, embedded in epoxy resin (Spurr) and ultrathin-sectioned, stained with uranyl acetate and lead citrate, and examined by a JEOL JEM1200 transmission electron microscope (JEOL LTD., Japan).

## Results

Examination of the freshly excised kidney preparations revealed various stages of *Parvicapsula anisocaudata* in the lumen of renal tubules (Fig. 1). In H&E-stained preparations, spores and plasmodia of *P. anisocaudata* were found in the renal tubules with a various degrees of infestation (Fig. 2A). The parasites were not noted in the glomeruli, interstitial haemopoietic tissue and other tissues examined. Although large mass of plasmodia nearly occupied the tubular lumina, no significant histological changes were observed in the tubular epithelium. In ultrastructural view, long or short pseudopodia of *P. anisocaudata* trophozoites deeply penetrated into the epithelium of renal tubules (Fig. 3A,B & C). Most luminal space of the renal tubule was occupied by numerous *P. anisocaudata* trophozoites (Fig. 3D).

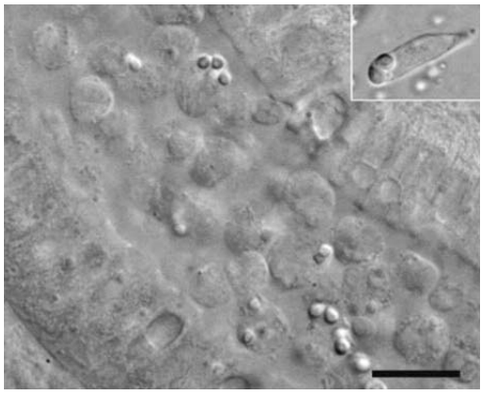

Fig. 1. Freshly squashed preparation of renal tubule of olive flounder, *Paralichthys olivaceus*, infected with various developmental stages of *Parvicapsula anisocaudata*. Bar= 15 ㎛. Inset shows the mature spore.

In the renal tubule epithelium, a group of unidentified myxosporean trophozoites, which were 2-3 times larger than intraluminal trophozoites of *P. anisocaudata*, was observed (Fig. 2B), but mature spores were not found. The parasites being burst out into the lumen was occasionally encountered with partial break of the epithelium (Fig. 2C). They were often detected in interstitial haemopoietic tissue (Fig. 2D), luminal space of interrenal gland (Fig. 2E), and splenic parenchyma (Fig. 2F). However, we could not find any evidences that the parasites could evoke the cellular reaction of the host. In ultrastructural observations, several primary cells of plasmodia were closely associated (Fig. 4 A&B), and 3-5 secondary cells were found within a plasmodium (Fig. 4 C&D).

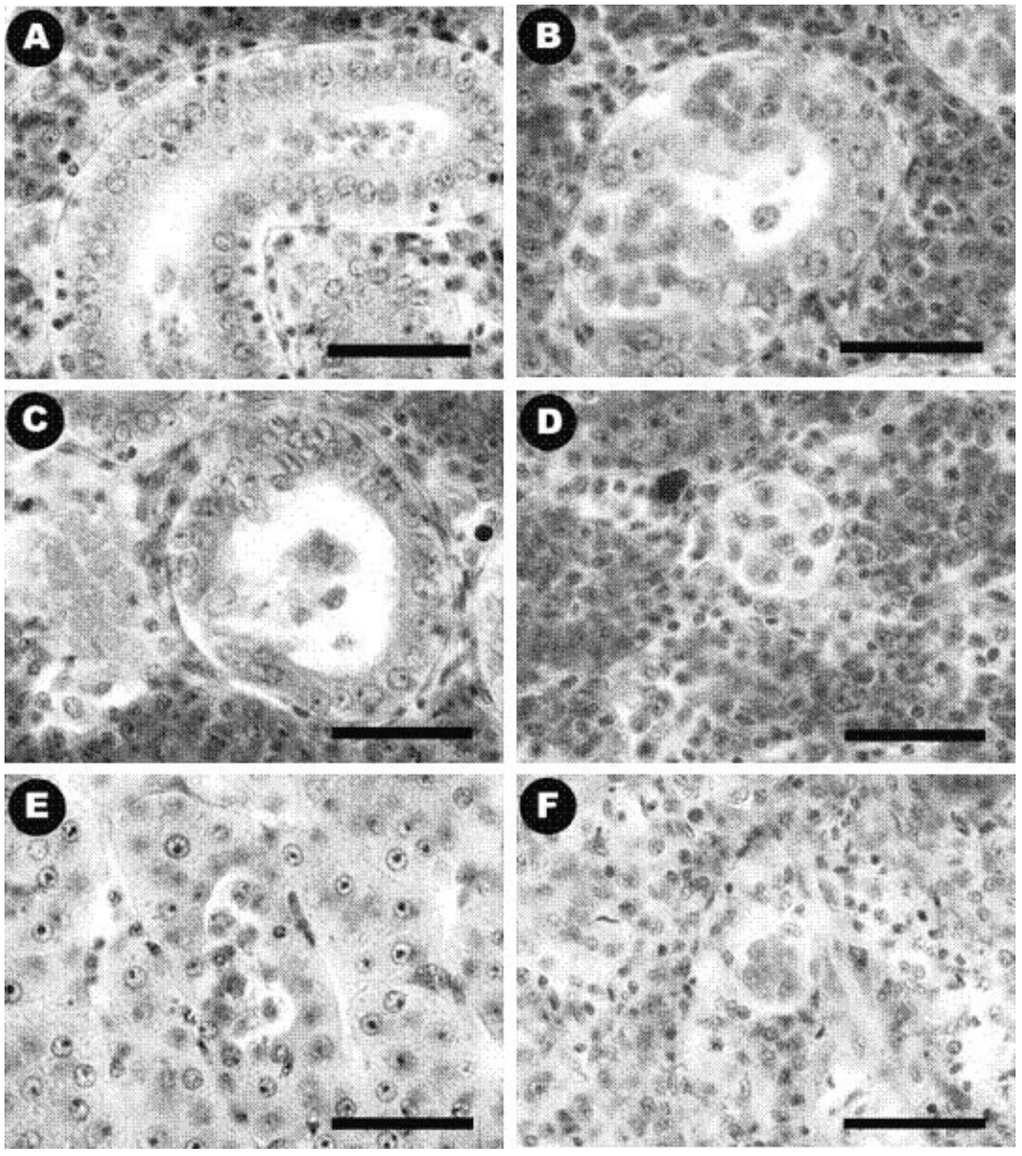


Fig. 2. Micrographs of histological sections from infected flounder *Paralichthys olivaceus* with *Parvicapsula anisocaudata* and an unidentified myxosporean vegetative stages. All bars= 18 ㎛. (A) Intraluminal stages of *P. anisocaudata* in the renal tubule (B, C) Intraepithelial stages of unidentified myxosporean stages in the renal tubule. The epithelium were disrupts. (D) Unidentified myxosporean in interstitial tissue of head kidney. (E) Unidentified myxosporean in interrenal gland tissue. (F) Unidentified myxosporean in interstitial tissue of spleen.

## Discussion

In the present results, long pseudopodia or finger like projections of the plasmodium of *P. anisocaudata*, which makes parasite firmly attached host tissues, deeply penetrated into microvilli of epithelium of renal tubule. This appearance commonly encountered in the ultrastructural descriptions of other coelozoic myxosporeans (El-Matbouli and Hoffmann, 1994; Lom *et al*., 1986; Paperna *et al*.,

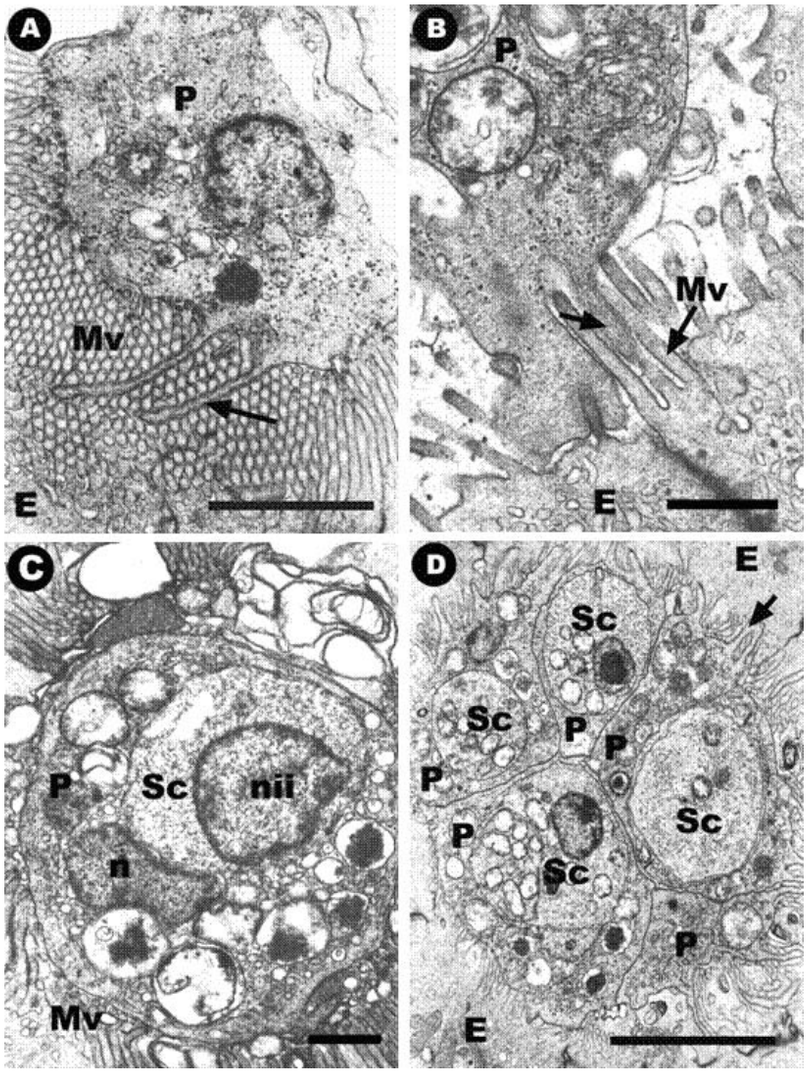


Fig. 3. Electron micrographs of *Parvicapsula anisocaudata* from the renal tubule of olive flounder, *Paralichthys olivaceus*. (A, B) Attachment of trophozoites on the epithelium through pseudopodia (arrowed). Mv, microvilli of epithelial cell of renal tubule. Bar=1 ㎛ (Fig. A) and 500 nm (Fig. B). (C) A plasmodia (P) containing a vegetative nucleus (n) and a secondary cell (Sc). nii, nucleus of secondary cell. Bar=800 nm. (D) Obstruction of the luminal space of the renal tubule by 5 plasmodium (P) containing secondary cell (Sc). E, epithelial cell of renal tubule. An arrow indicates pseudopodia. Bar=2 ㎛.

1987; Sitjà-Bobadilla and Alvarez-Pellitero, 1993).

Although intraepithelial developments were frequent feature of *Parvicapsula* sp. and *P. minibicornis* (Kent *et al*., 1997; Raverty *et al*., 2000; St-Hilaire *et al*., 2002; Yasutake and Elliott, 2003), the generative cells and nucleus of intraepithelial vegetative cells of the myxosporeans found in the present study were greatly larger than those of *P. anisocaudata*. Therefore, the present intraepithelial myxosporean parasite is a distinguishing species from *P. anisocaudata*.

In our study, although infection of *P. anisocauda-*

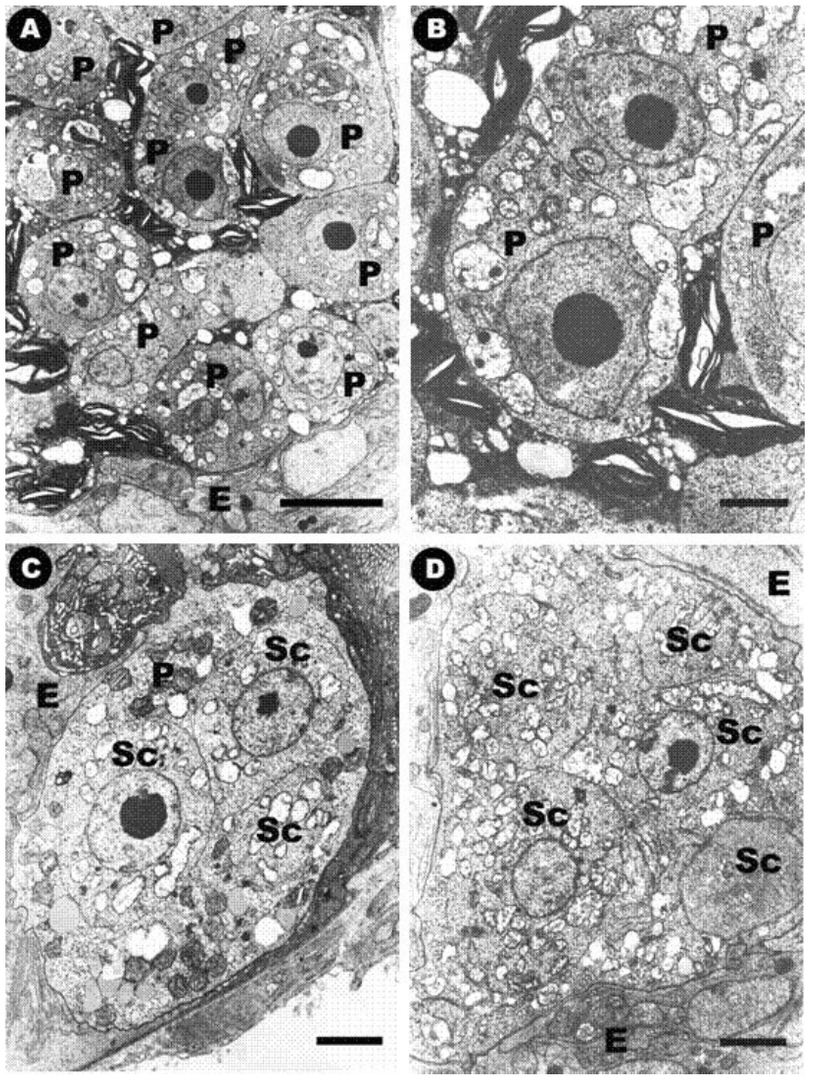


Fig. 4. Unidentified myxosporean vegetative stages within renal tubular epithelium of olive flounder, *Paralichthys olivaceus*. (A) Groups of plasmodium (P). E, epithelial cell of renal tubule. Bar=4 ㎛. (B) Association of two plasmodium or primary cells. Sc, secondary cell. Bar=1 ㎛. (C) Primary cell containing 3 secondary cells (Sc). Bar=2 ㎛. (D) Primary cell containing 5 secondary cells. E, epithelial cell of renal tubule. Bar=2 ㎛.

*ta* and unidentified myxosporean parasites did not induce any cellular reaction of the host, occlusion of renal tubules by *P. anisocaudata* and rupture of renal epithelium by unidentified myxosporean parasites would impact negatively on the renal functions of severely infected fish. Further research remains to be focused on the possible alteration of excretory or absorptive function of renal tubules in view of health monitoring.

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