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Light and electron microscopic observations of *Leptotheca koreana* n. sp. (Myxosporea) in the kidney of cultured rockfish *Sebastes schlegeli*

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ABSTRACT: The structure and sporogenesis of *Leptotheca koreana* n. sp. from cultured rockfish *Sebastes schlegeli* from South Korea were studied by light and transmission electron microscopy. Broadly oval spores and disporous pseudoplasmodia were observed in the lumen of renal tubules. Spores were 8.59 ± 1.25 µm in length, 13.42 ± 1.0 µm in width in sutural view and 8.13 ± 0.52 µm in thickness in the plane perpendicular to the suture. The width of each valve was always smaller than spore length. Two spherical polar capsules were equal in size (3.86 ± 0.45 µm in diameter) containing a polar filament with 6 to 7 turns, opening at the anterior end of the spore. Two uninucleate sporoplasms filled the spore cavity. The asynchronous division of secondary and tertiary cells and asynchronous development in spore formation of the present *Leptotheca koreana* resembled the disporous sphaerosporids. Cytoplasmic projections of pseudoplamodia were considered to be rhizoids, as they seem to strengthen the attachment to the epithelial cells of the renal tubules. The capsulogenic cells in early sporoblast had large amounts of rough endoplasmic reticulum but had a few Golgi apparatus.

KEY WORDS: *Leptotheca koreana* n. sp. · Cultured marine fish · South Korea

INTRODUCTION

Fish-parasitic myxosporeans comprise an extraordinarily large number of species. The host specificity of the myxosporean species differs; however, it is always restricted to a well-definable circle of related hosts (Molnár 1994). Many species of the genus *Leptotheca* have been described in various fish hosts, and to our knowledge 7 species of the genus *Leptotheca* are known from wild marine fish of the genus *Sebastes*.

Ultrastructural characteristics of various developmental stages of *Leptotheca elongata* in the gall bladder of the hake *Merluccius merluccius* were reported by Desportes & Théodoridès (1982). Recently, Tun et al. (2000) described developmental stages of *L. fugu* in the intestine of cultured tiger puffer *Takifugu rubripes* based on the observations of Diff-Quick stained specimens.

The rockfish *Sebastes schlegeli* is an important cultured marine fish in Korea, and no myxosporean parasites have been reported from this fish species. In the present study, we found a *Leptotheca* species in the renal tubules of cultured rockfish for the first time, and classified it as a new species, *Leptotheca koreana*. Light microscopy and TEM were used to describe the spores and sporogenesis.

MATERIALS AND METHODS

Juvenile rockfish *Sebastes schlegeli* (10 to 15 cm in body length) were taken from commerical netcages in South Korea. Squash preparations of fresh tissues from kidney were examined by light microscope. Spores were described and measured according to the guide-

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lines for species description of myxosporeans by Lom & Arthur (1989), and by using a light microscope equipped with an ocular micrometer and an image analysis software (ImageTool v2.0, UTHSCSA, San Antonio, TX, USA). Mean and standard deviations of each spore characteristic were obtained from 175 fresh mature spores.

For histological study, the kidney tissues were fixed in Bouin's solution and embedded in paraplast. Sections 5 µm thick were stained with hematoxylin and eosin. For TEM study, portions of the kidney tissue were fixed in 2% glutaraldehyde at 4°C overnight and postfixed 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, Tokyo, Japan).

RESULTS

Spore characteristics of *Leptotheca koreana* n. sp.

Mature spores (Figs 1 & 2) were broadly oval (sutural view), measuring 8.59 ± 1.25 µm in length, 13.42 ± 1.0 µm in width in sutural view and 8.13 ± 0.52 µm in thickness in the plane perpendicular to the suture. Valves were smooth and equal, and had an anterior notch just on the opening end of the polar capsule in each valve. The width of each valve was always smaller than spore length. Two spherical polar capsules were equal in size (3.86 ± 0.45 µm in diameter) containing a polar filament with 6 to 7 turns, opening at the anterior end of the spore. Two uninucleate sporoplasms filled the spore cavity.

Host: Rockfish *Sebastes schlegeli*

Locality: Hadong, Kyongsangnam-Do, South Korea

Site of infection: Lumen of the renal tubules

Etymology: The specific name refers to the nation, Korea

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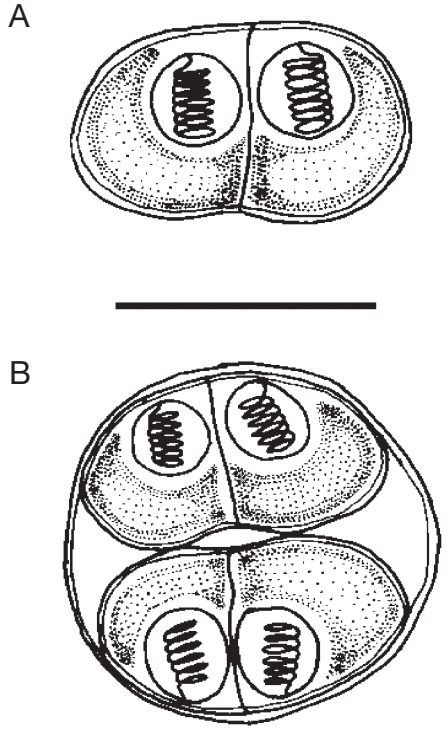

Fig. 1. *Leptotheca koreana* n. sp. infecting *Sebastes schlegeli*. Diagram of (A) mature spore and (B) disporous pseudoplasmodium. Scale bar = 10 µm

Histology

Host tissue response to infection with *Leptotheca* sp. was minimal, although large numbers of parasites were presented in the renal tubules of fish (Fig. 3).

Transmission electron microscopy

The earliest stage observed was an oval primary cell (pseudoplasmodium) containing 2 secondary cells (Fig. 4A). Each secondary cell produced a tertiary daughter cell (Fig. 4B,C), and subsequent divisions of the daughter cell gave rise to 6 tertiary cells (Fig. 4D). The primary cell in the lumen of the kidney tubules was in close contact with the micovilli of epithelial cells (Figs 4B & 5A,B). At all junctions of tubular epithelial cells, the pseudoplasmodia sent out long finger-like pseudopodial projections between the microvilli.

Sporogenesis was asynchronous, and 2 spores were formed inside the same primary cell. In immature spores, 2 valvogenic cells, 2 capsulogenic cells and 2 uninucleated sporoplasms were observed (Fig. 5B). Valvogenic cells occupied an external position in relation to other sporogonic cells. They became elongated and flattened as the development of spores progressed and formed finely striated valves (Figs 5C,D & 6B). Large capsulogenic cells occupied most of the spore volume (Fig. 5D). The cytoplasm of the capsulogenic cells contained high amounts of rough endoplasmic reticulum, several mitochondria and lipid droplets (Fig. 5B,D). Polar capsules were spherical and had a projection at the apical end (Fig. 5D). Three layers of different electron density were present in the capsule. Each mature polar capsule contained a 6 to 7 turns of the polar filament (Fig. 6A,B).

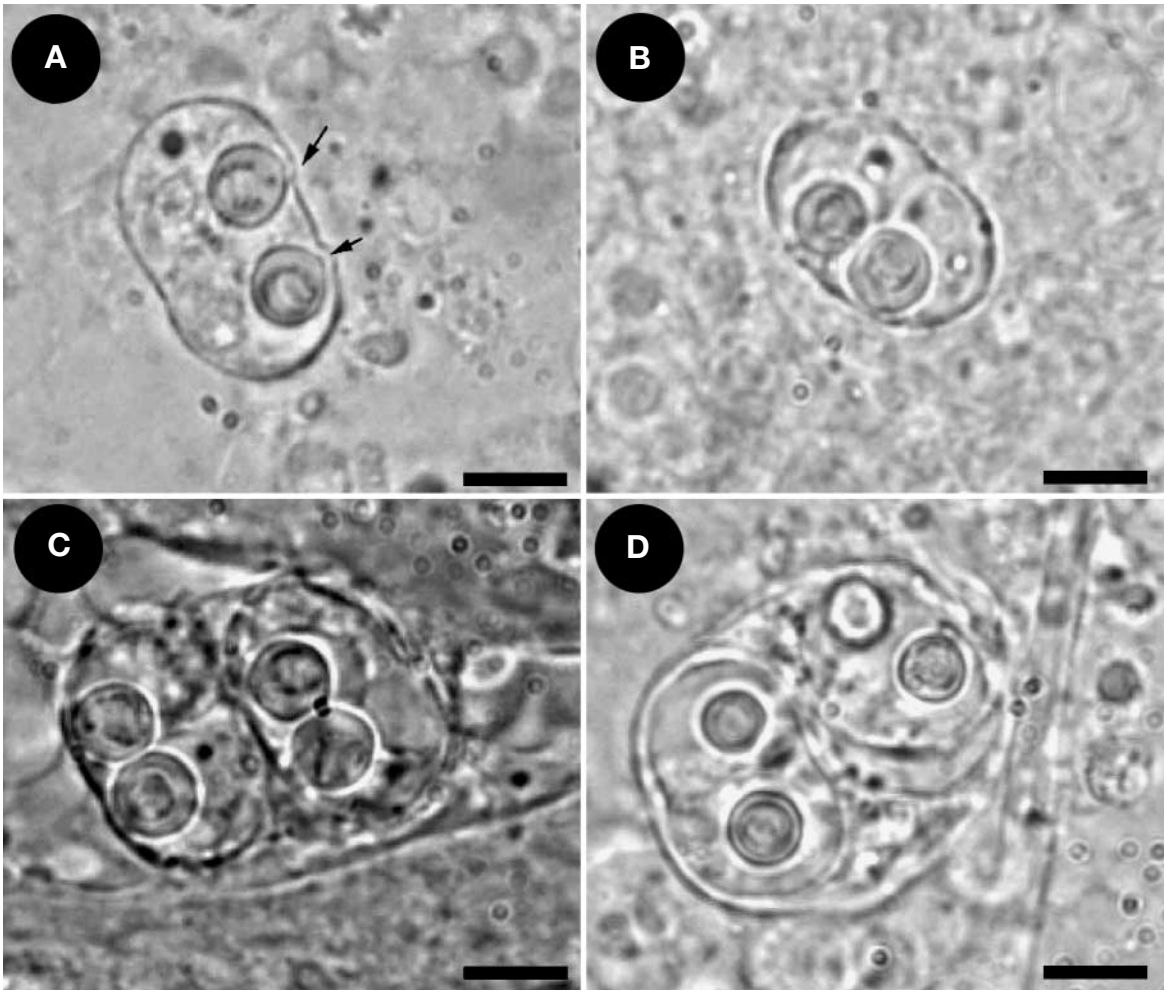

Fig. 2. *Leptotheca koreana* n. sp. infecting *Sebastes schlegeli*. (A,B) Mature spores from fresh preparations. Arrows indicate anterior notches. Scale bar = 5 µm. (C,D) Disporous pseudoplasmodia from fresh preparations. Scale bar = 5 µm

DISCUSSION

More than 40 species of myxosporeans in the genus *Leptotheca* have been described from fishes (Lom & Dyková 1992), and among them, 7 species—*L. macrospora*, *L. informis*, *L. longipes*, *L. sebasta*, *L. macroformis*, *L. kovaljovae* and *L. adeli*—were found from the wild fish species of the genus *Sebastes* (Moser et al. 1976, Gaevskaya & Kovaleva 1984, Love et al. 1984, Bakay & Grudnev 1998, Kalavati & MacKenzie 1999). *Leptotheca koreana* n. sp. in the present study was easily differentiated from the above *Leptotheca* species by combination of the following characters: spore size, spore shape, number of polar filament coils and sporoplasm condition. The length of the spore and the diameter of the polar capsule of *L. koreana* resembled that of *L. informis* in the gall bladder of several fish families including Anoplopomatidae, Gadidae,

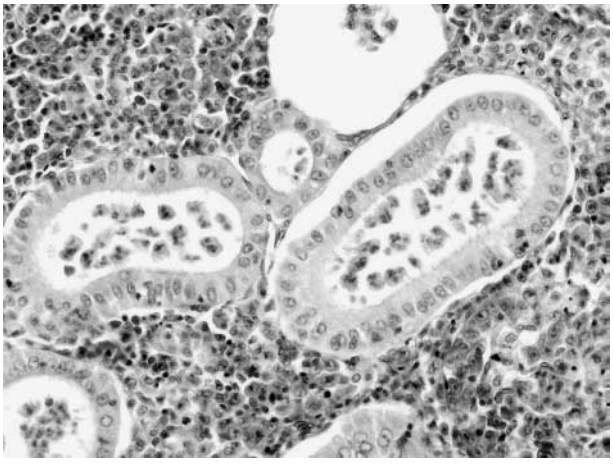

Fig. 3. *Leptotheca koreana* n. sp. infecting *Sebastes schlegeli*. Disporous plasmodia and mature spores have accumulated in the lumen of the kidney tubules

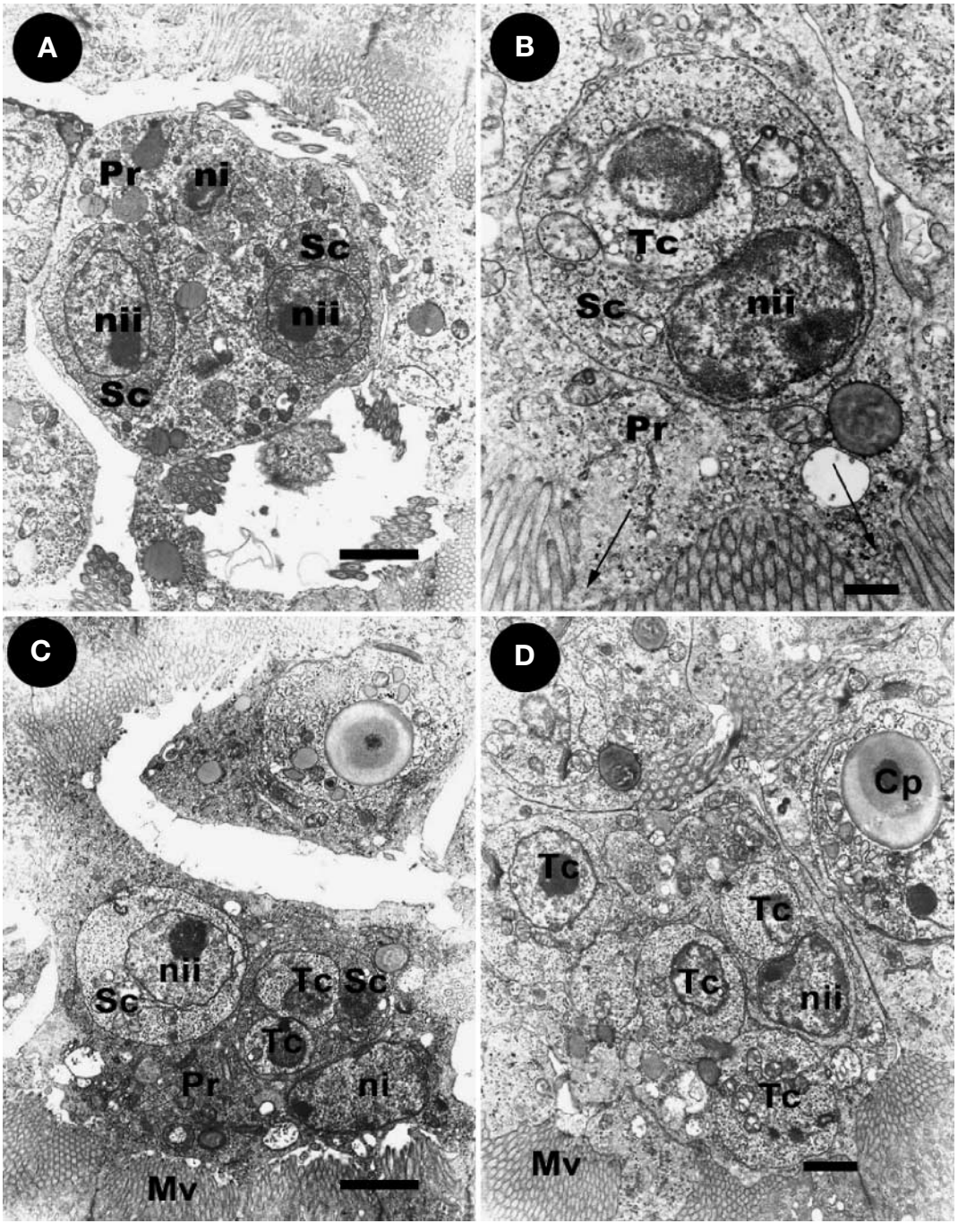

Fig. 4. *Leptotheca koreana* n. sp. infecting *Sebastes schlegeli*. (A) Primary cell (pseudoplasmodium, Pr) containing 2 secondary cells (Sc). ni: nucleus of primary cell; nii: nucleus of secondary cell. ×4000, scale bar = 2 µm. (B) A secondary cell with a tertiary cell (Tc). Arrows indicate finger-like projections of pseudoplasmodia. ×5000, scale bar = 0.5 µm. (C) Pseudoplasmodium containing 2 secondary cells with asynchronous development. Mv: microvilli of kidney tubule. ×4000, scale bar = 2 µm. (D) A secondary cell containing several tertiary cells. ×5000, scale bar = 12 µm

Macrouridae and Scorpaenidae (Moser & Noble 1976, Moser et al. 1976, Love et al. 1984, Kalavati & MacKenzie 1999). However, *L. koreana* was clearly differentiated from *L. informis* by having 2 uninucleate sporoplasms, which was confirmed by TEM observation and distinctly smaller spore thickness.

According to Lom & Dyková (1988), in myxosporean genera that have small mono- or disporic trophozoites,

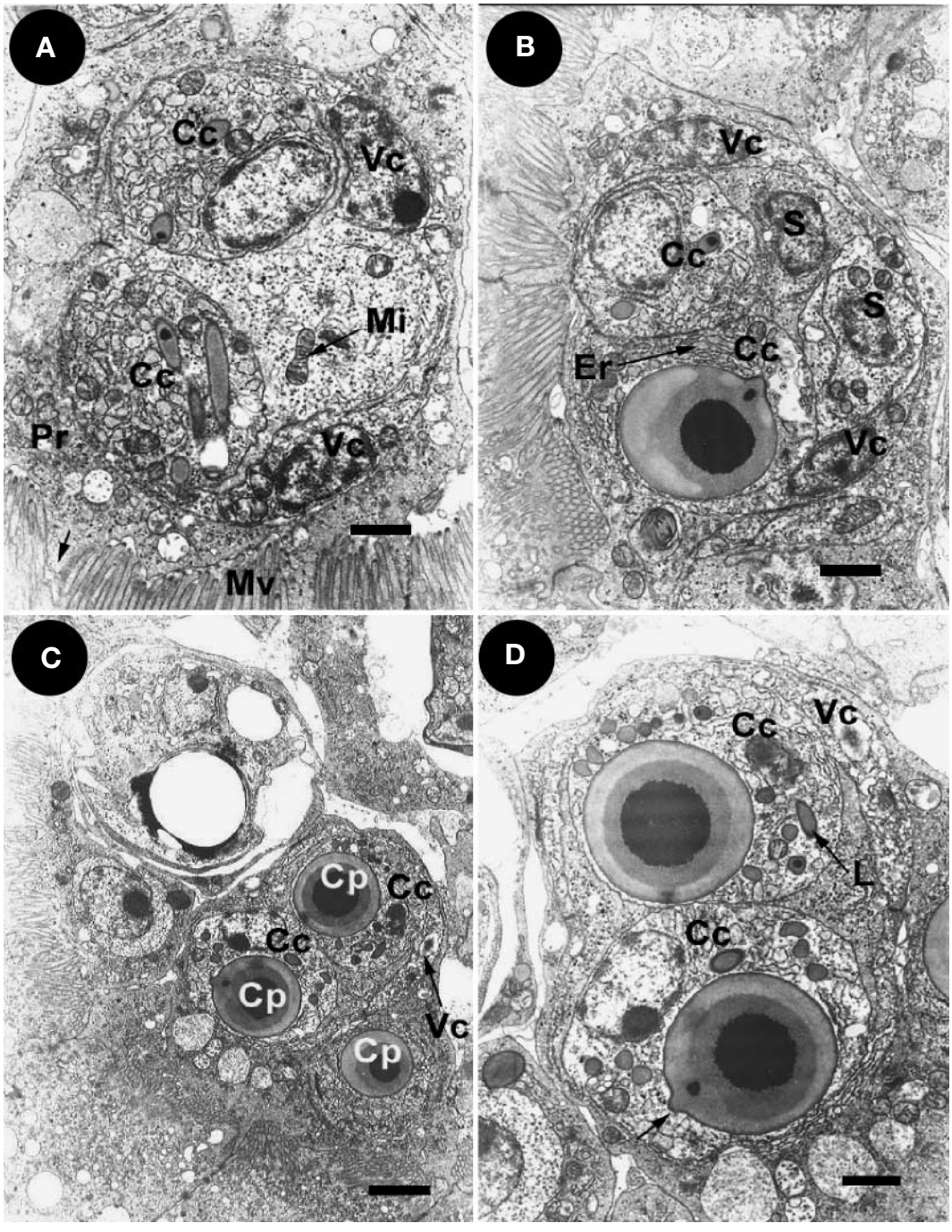

Fig. 5. *Leptotheca koreana* n. sp. infecting *Sebastes schlegeli*. (A) Two capsulogenic cells (Cc) and 2 valvogenic cells (Vc) in pseudoplasmodium (Pr). A short arrow indicates projection of pseudoplasmodium. Mi: mitochondria; Mv: microvilli of kidney tubule. ×6000, scale bar = 1 µm. (B) An immature spore containing 2 capsulogenic cells, 2 valvogenic cells and 2 uninucleate sporoplasms (S). Er: rough endoplasmic reticulum. ×6000, scale bar = 1 µm. (C) Disporous pseudoplasmodium. Each capsulogenic cell contains a capsular primordium (Cp). Valvogenic cells surround capsulogenic cells. ×3000, scale bar = 2 µm. (D) Capsular primordia consist of 3 layers differing in electron density. An arrow indicates apical projection of polar capsule primordium. L: lipid droplet. ×6000, scale bar = 1 µm

spores seem to be produced without pansporoblast formation. Rather, they are in the form of pseudoplasmodia with 1 vegetative nucleus, without the association of 2 generative cells. This has been well demonstrated in several species of *Sphaerospora* and *Leptotheca* (Desportes & Théodoridès 1982, Desser et al.

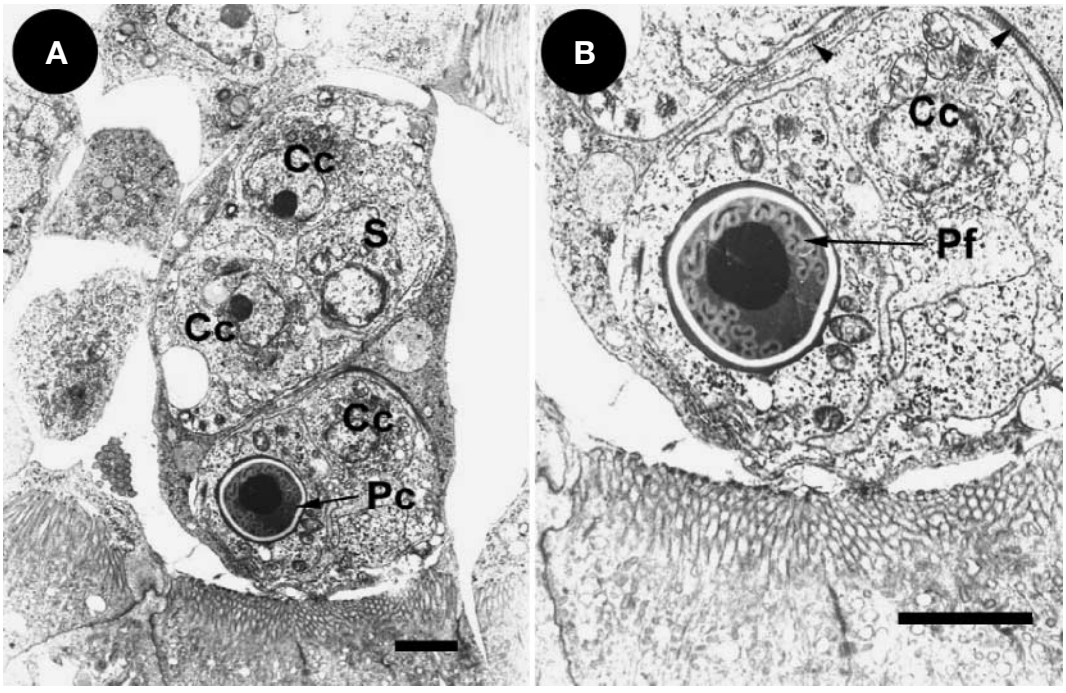

Fig. 6. *Leptotheca koreana* n. sp. infecting *Sebastes schlegeli*. (A) Disporous pseudoplasmodium showing asynchronous development. Pc: Polar capsule. ×3000, bar = 2 µm. (B) Coiled polar filament (Pf) in a transverse section of a polar capsule. Arrowheads indicate fine striation in spore valve. ×6000, scale bar = 2 µm

1983, Lom et al. 1982, 1985, Sitjà-Bobadilla & Alvarez-Pellitero 1993a, Tun et al. 2000). Sporogenesis of *Leptotheca koreana* in this study followed this general trend pattern. The asynchronous division of secondary and tertiary cells and asynchronous development in spore formation of the present *L. koreana* resembled the disporous sphaerosporids (Desser et al. 1983, Lom et al. 1985, Dyková et al. 1990, Sitjà-Bobadilla & Alvarez-Pellitero 1992, 1993a).

The cytoplasmic projections of pseudoplamodia formed in *Leptotheca koreana* were considered to be holdfast rhizoids, as they seem to strengthen the attachment to the epithelial cells of the renal tubules. These cytoplasmic outgrowths were also observed in *Zschokkella mugilis* in the lumen of the bile and gall bladder of mullets (Sitjà-Bobadilla & Alvarez-Pellitero 1993b), *Sinuolinea tetraodoni* in kidney tubules of pufferfish (El-Matbouli & Hoffmann 1994), *Ceratomyxa* spp. from the gall bladder of Mediterranean sea bass (Alvarez-Pellitero & Sitjà-Bobadilla 1993) and *Myxidium giardi* in the urinary bladder of eels (Paperna et al. 1987).

The capsulogenic cells in early sporoblast of *Leptotheca koreana* had large amounts of rough endoplasmic reticulum, but had a few Golgi apparatus. This is in accordance with similar observations in various other myxosporeans (Current & Janovy 1978, Desportes & Théodoridès 1982, Davies & Sienkowski 1988, Desser & Paterson 1978, Desser et al. 1983, Lom & Dyková 1988, Lom et al. 1985, Sitjà-Bobadilla & Alvarez-Pellitero 1993b, El-Matbouli & Hoffmann 1994).

Although infection with *Leptotheca koreana* had no apparent histological effects on the renal tubules, a finding consistent with other renal myxosporeans (Dyková & Lom 1982, Fischer-Scherl et al. 1986, El-Matbouli & Hoffmann 1992), occlusion of renal tubules by heavy infection with the parasites had detrimental effects on renal function.

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