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Sinuolinea tetraodoni n. sp., a myxosporean parasite of freshwater pufferfish *Tetraodon palembangensis* from Southeast Asia – light and electron microscope observations

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ABSTRACT: *Sinuolinea tetraodoni* n. sp. from the lumen of the kidney tubules and renal corpuscles of pufferfish *Tetraodon palembangensis* imported from Southeast Asia (Thailand, Sumatra) is described and illustrated. It is distinguished from all previously described members of the genus by the shape and the small size of the mature spore (length 9.56 ± 0.51 µm; width 10.11 ± 0.52 µm), the presence of a desmosome-like junction connecting the 2 spores in the pseudoplasmodium, and its geographical distribution. Prevalence of infection was 100 % in examined fish. Two triangular spores perpendicular in plane to the suture, developed within the surrounding pseudoplasmodium. Thin valves surrounded the binucleate sporoplasm cell and 2 spherical polar capsules (3.19 × 3.21 µm). Data concerning ultrastructure and sporogenesis are also presented.

KEY WORDS: *Sinoulinea* spp. · *Myxosporea* · *Tetraodon palembangensis* · Southeast Asia

INTRODUCTION

Sinuolinea spp. (family: *Sinuolineidae*) have been described from different species of fish. To our knowledge 10 species of this myxosporean have been described, all of them from marine fish (Table 1). J. Lom (pers. comm.) noted 3 further species (*S. contrariocapsularis* Evdokimova, 1972, *S. sakinachunumae* Ibragimov, 1988, and *S. markewitchi* Iskov, 1989), which were found in *Syngnathus nigrolineatus caspius* from the Caspian Sea. Unfortunately, no further details about these species have been available. Davis (1917) was the first to describe and name the genus *Sinuolinea* in fish from the Beaufort region of the USA. This author characterised the spores of this genus to be spherical or subspherical with 2 nearly spherical polar capsules. The sutural line forms a prominent sinuous ridge around the spore. In the present report, we describe *Sinuolinea tetraodoni* n. sp. from the kidney of pufferfish *Tetraodon palembangensis*.

MATERIAL AND METHODS

While checking the health condition of Asian pufferfish *Tetraodon palembangensis* imported from Southeast Asia (Thailand, Sumatra) by ornamental fish markets, we found a myxosporean infecting the kidney. Eight fish (2.4 to 3.0 g, 4.0 to 5.0 cm) were examined in March–July 1992. Fish were anaesthetised by chlorbutanol (1.1.1-trichlor-2-methylpropanol), 0.1 g l^{-1} water, necropsied and all parenchymatous organs studied for both fresh and histological examination. Parts of each organ were sampled and examined in fresh mounts by light microscopy. Morphology of spores and of developmental stages was observed in fresh mounts, dimensions were determined using a Leitz Dialux 20 light microscope equipped with an ocular micrometer and assisted by a computer (model PET 300 I; Leitz). Blood smears and kidney impressions were air-dried, fixed in methanol and stained with Giemsa's solution. For histological examination speci-

Table 1: *Sinuolinea tetraodoni* n. sp. Characteristics of spores found in pufferfish *Tetraodon palembangensis* compared with other *Sinuolinea* spp. L: length; W: width; D: dimension (all in µm)

Species	Spore size	Polarcapsule size	No. of coils	Pseudoplasmodium size	Infection locus	Host	Locality	Source
Sinuolinea tetraodoni	L: 9.0–10.9 W: 9.2–11.1	L: 3.0–3.5 W: 2.9–3.7	7–8	L: 12.9–19.3 W: 11.3–18.6	Urinary bladder, kidney	*Tetraodon palembangensis*	Southeast Asia	This study
S. capsularis	D: 12.0–14.0	D: 4.5	–	D: 27.0	Urinary bladder	*Paralichthys albiguttus*, *P. dentatus*, *Spheroides maculatus*	Beaufort region (USA)	Davis (1917)
S. arborescens	L: 15.0 W: 12.0	D: 5.0	–	D: 75.0	Urinary bladder	*Siphostoma floridae*	Beaufort region (USA)	Davis (1917)
S. dimorpha	D: 15	D: 4.5	–	L: 57.0 W: 90.0	Urinary bladder, ureter	*Cynoscion regalis*	Beaufort region (USA)	Davis (1917)
S. rebae	D: 10.0–13.2	D: 2.5–3.5	7–8	L: 42 W: 14.0–18.0	Urinary bladder	*Solea solea*, *Coelorinchus coelorinchus*	Plymouth (England)	Tripathi (1948)
S. triangulata	D: 14.0–15.0	D: 5.0–6.5	7–10	D: 20.0	Urinary bladder	*Sphaeroides vermicularis*	Sea of Japan	Shulman (1966)
S. magna	D: 19.0–30.0	D: 5.0–7.5	6–12	–	Urinary bladder	*Coryphaenoides acrolepis* *C. pectoralis*	Southern California (USA)	Yoshino & Noble (1973)
S. cyclopterina	L: 14.0–16.0 W: 13.0–14.0	D: 4.0–5.0	–	D: 50.0–60.0	Urinary bladder	*Cyclopterus lumpus*	White Sea	Bashkatova (1932)
S. murmanica	D: 12.0–14.0	D: 2.0	–	D: 20.0–30.0	Kidney, urinary bladder	*Ammodites tobianus*	Barents Sea	Bashkatova (1932)
S. sinuosa	D: 9.0–12.0	D: 3.0	–	–	Urinary bladder	*Boreogadus saida*	White Sea	Shulman (1953)
S. lesteri	D: 15.0–19.0	D: 3.0–4.0	3–5	D: 100.0	Gall bladder	*Hemiscyllium ocellatum*	Heron Island (Australia)	Moser et al. (1989)

mens were fixed in 5 % buffered formaldehyde and embedded in Historesin®. Sections were stained with H&E, Giemsa's solution and using the periodic acid-Schiff (PAS) reaction.

Kidney tissues were also fixed in 6.25 % Sörensen phosphate buffered glutaraldehyde (PH 7.4) for 3 h and postfixed with 1 % OsO_4 in the same buffer for 2 h. After dehydration, they were embedded in Epon-812. Semithin sections were stained with toluidine blue and safranin, ultrathin sections were contrasted with uranyl acetate and lead citrate, and then examined by electron microscope (Zeiss EM 109).

DESCRIPTION

Sinuolinea tetraodoni n. sp. (Fig. 1)

Host: Pufferfish *Tetraodon palembangensis*.

Locality: Southeast Asia.

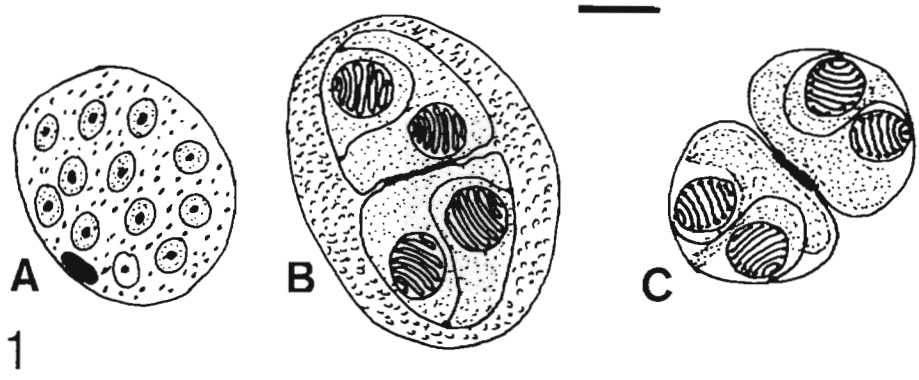
Site of infection: Lumen of the kidney tubules, distal and proximal tubules, collecting ducts, renal corpuscles.

Prevalence: 8 of 8 host specimens examined.

Sporogonic stages: These were composed of 12 cells within a pseudoplasmodium in the lumen of the kidney tubules. Two spores developed within each pseudoplasmodium.

Spore characteristics: In the sutural plane spores appeared ellipsoid in shape but in plane perpendicular to the suture they appeared triangular. The suture line was quite sinuate. There were 2 spherical polar capsules near the narrow end of the spore, opening near the suture but in oppo-

Figs. 1 to 4. *Sinuolinea tetraodoni* infecting *Tetraodon palembangensis*. Fig. 1. Line drawings of various sporogonic stages. (A) Early pseudoplasmodium; (B) disporic pseudoplasmodium; (C) 2 mature spores connected via desmosome-like junction. Scale bar = 4 µm. Fig. 2. *S. tetraodoni* in the lumen of a kidney tubule. Note disporic pseudoplasmodia with mature spores (arrows). Fresh preparation, phase contrast, ×1000. Fig. 3. Mature spores in the lumen of renal tubules. Giemsa, ×300. Fig. 4. Infected distal tubule showing mature spores (arrows). Giemsa, ×740



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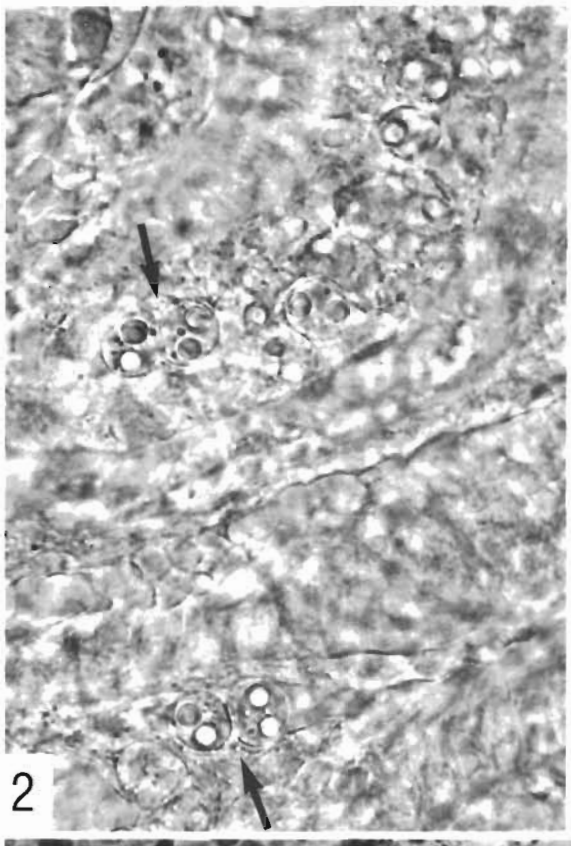

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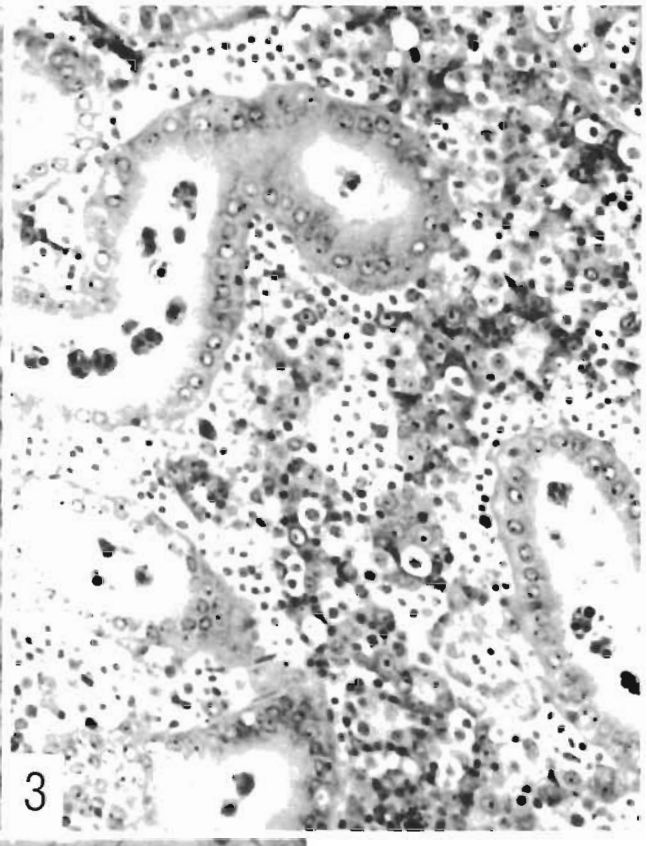

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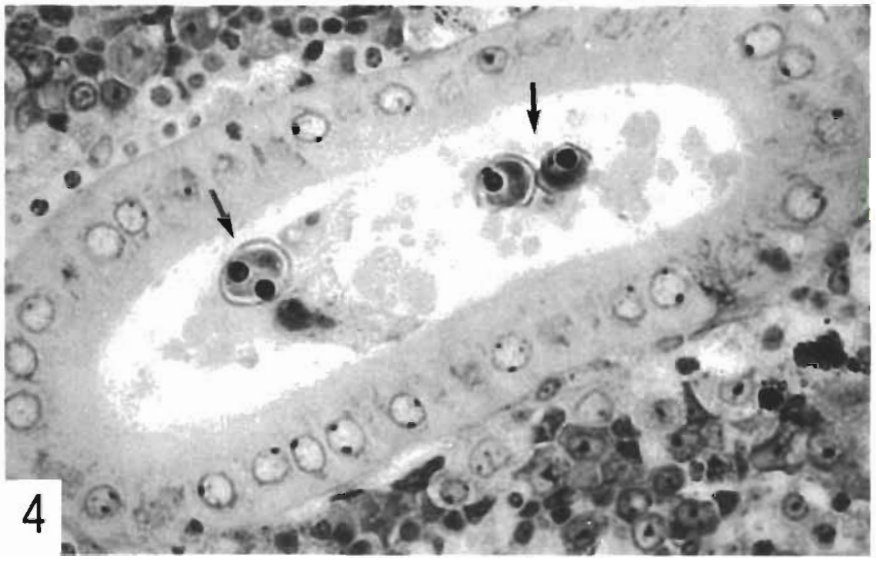

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site directions. The sporoplasm had 2 nuclei. Mean length of the spore (n = 50) was 9.56 ± 0.51 µm, mean width 10.11 ± 0.52 µm. Mean length of polar capsules was 3.19 ± 0.15 µm, mean width 3.21 ± 0.22 µm.

RESULTS

Light microscope observations

In all 8 examined pufferfish, developmental stages and mature spores of *Sinuolinea tetraodoni* n. sp. (Figs. 2, 3 & 4) were found in fresh preparations and tissue sections of the kidney. In fresh imprints from the kidney, every 2 mature spores were usually connected on their flattened side (Fig. 5A, B). Pseudoplasmodia found in fresh preparations of the kidney were round to elongate, contained 2 spores and many refractile granules in the cytoplasma of the pseudoplasmodia cells. Examination of histological sections revealed early stages and mature spores of *S. tetraodoni* primarily in the distal and proximal tubules of the kidney (Figs. 3 & 4). In some cases, spores and developmental stages were also detected in Bowman's space and glomerular capillaries of the renal corpuscles (Fig. 6). Developmental stages appeared in the same location as spores, but rarely also in the tubular epithelia. In PAS-stained sections, spores exhibited PAS-positive bodies in the sporoplasm and in the shell valves. The cell cytoplasm of the developmental stages was also PAS-positive. Spores were not found in other organs examined. In blood smears no myxosporean developmental stages were observed.

Transmission electron microscope observations

The pseudoplasmodia in the lumen of the kidney tubules were in close contact with the microvilli of epithelial cells (Fig. 7). At all junctions of tubular epithelial cells, the pseudoplasmodia sent out projections between the microvilli. These projections were about the same length as the microvilli (Figs. 7 & 8; see also Fig. 10). At the point of contact with the electron-dense junctions (desmosomes), the tip of the pseudoplasmodia projection also become dense (Fig. 8). The tips of the microvilli however did not form any cell junction with the plasmalemma of the pseudoplasmodia.

The earliest developmental stages observed were 1-cell stages found in the lumen of the kidney tubules (Fig. 9). The first division of this stage resulted in 1 secondary cell enclosed in a primary cell (Fig. 10). The cytoplasm of both these cells contained mitochondria and free ribosomes. These early stages most probably divide to increase the parasite number resulting in primary cells which contained 4 or more secondary cells (Fig. 11). These have to be regarded as initial sporogonic stages. The sporoblast cells started their differentation while in close contact with each other. Each pseudoplasmodium produced 2 spores, usually connected by desmosome-like junctions (Fig. 12). The morphogenesis of the polar capsule followed the same pattern as in species of the genus *Sphaerospora*. The primordium had a long external tube so that more than 4 transverse sections of the tube could be made out. As assessed from the sections the tube can make 7 or 8 windings inside the polar capsule (Fig. 13). At the stage when the globular capsular primordium had been formed, the valvogenic cells already enclosed the capsulogenic and sporoplasmic cells and adhered together by desmosome-like junctions (Figs. 13 & 14). At the stage of a nearly mature spore, large electron-dense inclusions appeared in the cytoplasm of the primary cells, thus showing a degeneration process. In the resulting binucleate sporoplasm (Fig. 14), electron-dense inclusions (sporoplasmosomes) – characteristics of sporoplasms in most myxosporeans – were lacking. In the tubular lumen of kidneys of infected fish all stages of sporogony occurred simultaneously.

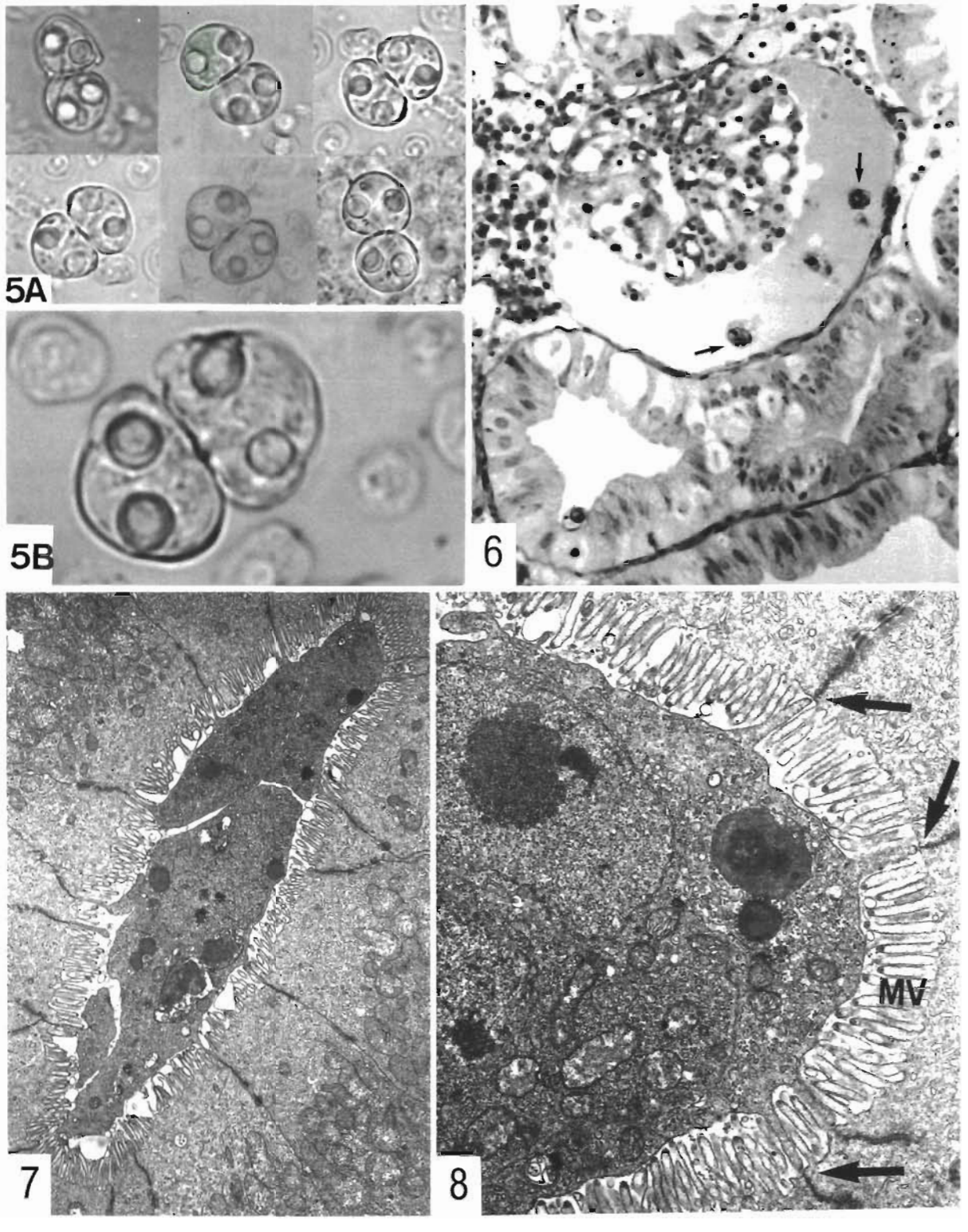
DISCUSSION

Sinuolinea tetraodoni n. sp. detected in the kidneys of pufferfish in this study are the first to be reported from freshwater fish. All *Sinuolinea* species listed in Table 1 have different hosts and geographical distribution to those of *S. tetraodoni*. The shape of *S. tetraodoni* quite resembles that of *S. triangulata* (Shulman 1984) in the urinary bladder of *Sphaeroides vermicularis* from the former USSR. However, the dimensions of *S. triangulata* spores and their polar capsules as described by Shulman (1984) are evidently greater (Table 1).

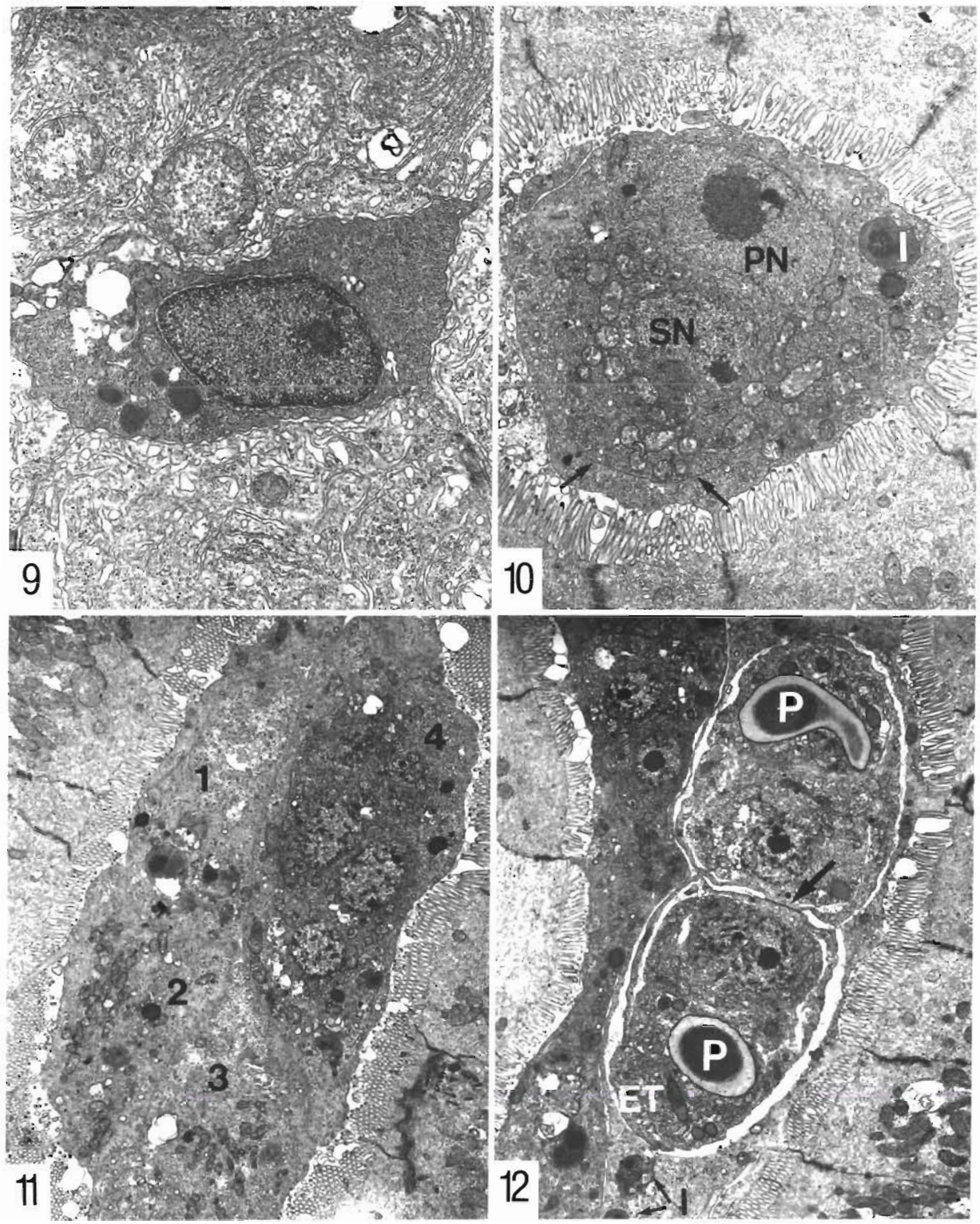
Among the many papers on myxosporean fine structure, there are none on *Sinuolinea*. The present findings show that the structure and morphogenesis of the spores of this *Sinuolinea* species match the characteristic myxosporean pattern. Mature spores of *S. tetraodoni* have a binucleated sporoplasm. This feature has been reported in only 2 light microscopical (LM) descriptions of *Sinuolinea*: *S. magna* (Yoshino & Noble 1973) and *S. rebae* (Tripathi 1948).

Disporous development and the presence of desmosome-like junctions between the shells of both spores in the pseudoplasmodium even after maturation observed using both LM and transmission electron microscopy (TEM) seem to be common characteristics of *S. tetraodoni*.

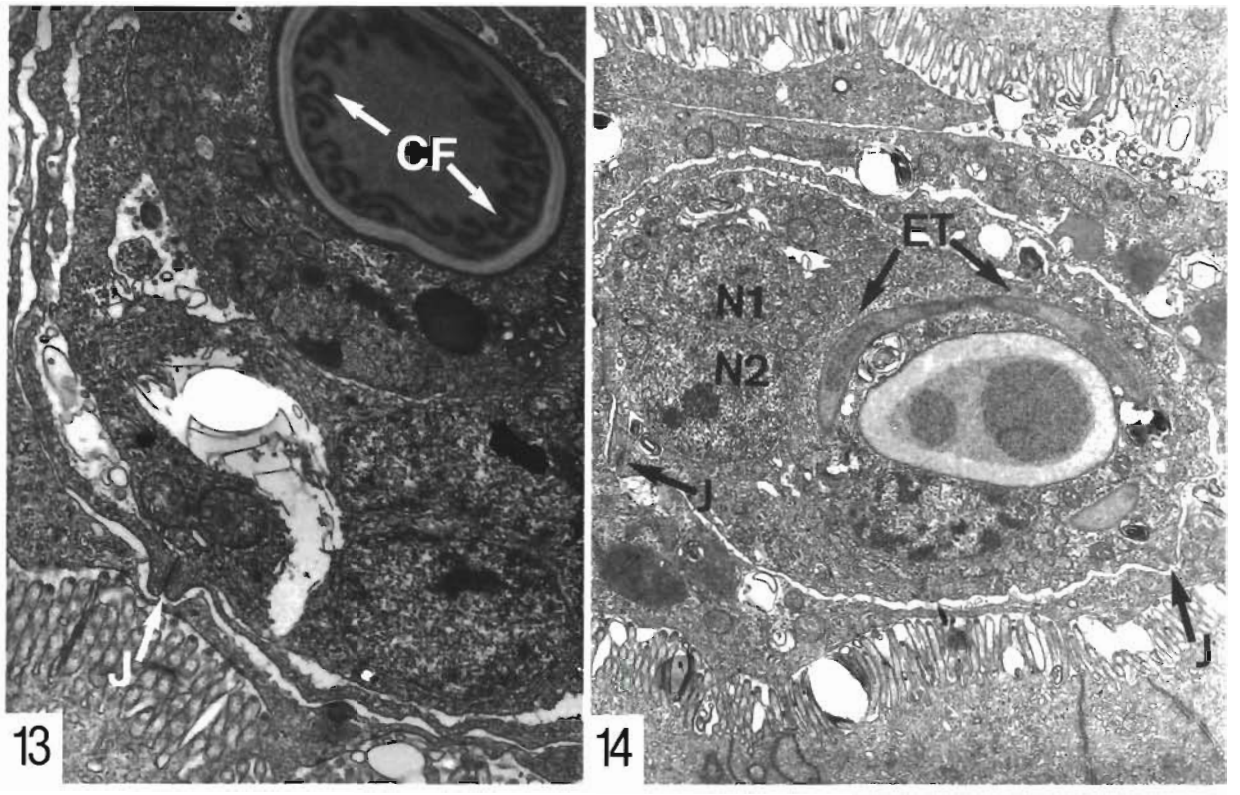
Capsulogenesis is supposed to proceed along the



Figs. 5 to 8. *Sinuolinea tetraodoni* infecting *Tetraodon palembangensis* Fig. 5. Mature spores from fresh preparation. Note the desmosome-like junction between the mature spores (A) ×1000; (B) ×2300 Fig. 6. Mature spores (arrows) in the dilated Bowman's space of the renal corpuscles Giemsa, ×370 Fig. 7. Early developmental stages in close contact with the microvillar zone of epithelial cells of the renal tubule ×6300 Fig. 8. Pseudoplasmodium in the lumen of the renal tubule. Arrows indicate cytoplasma projections extending between microvilli (MV) at all junctions of tubular epithelial cells ×14 500



Figs 9 to 12 *Sinuolinea tetraodoni* infecting *Tetraodon palembangensis*. Fig. 9. One-cell developmental stage of *S. tetraodoni* in the lumen of the renal tubule ×14 500 Fig. 10 Pseudoplasmodium with 1 sporogonic cell in the lumen of the renal tubule Arrow: cell membrane of the sporogonic cell. PN: pseudoplasmodium nuclei, SN sporogonic nuclei, I: electron-dense inclusions in the cytoplasma of the primary cell ×8800 Fig. 11. Four pseudoplasmodia (1 to 4) of *S. tetraodoni* attached to the microvilli of epithelial cells of the renal tubule No 4 is darker in appearance with more ribosomes and harbors 3 visible sporogonic cells ×8800 Fig 12 Young disporoblast inside a pseudoplasmodium Only 2 capsulogonic cells can be seen P primordium, ET transverse sections of the external tube, I. opaque inclusions in the cytoplasma of the pseudoplasmodium cell. Note the desmosome-like junction between the 2 sporoblasts (arrow) ×6300



Figs. 13 & 14. *Sinuolinea tetraodoni* infecting *Tetraodon palembangensis*. Fig. 13. Section through a maturing spore showing coils of primordial polar filament (CF) within the primordium. J: junction of the shell valves. ×14 500. Fig. 14. Young sporoblast showing binucleate sporoplasm cell (N1, N2). ET: longitudinal section of the external tube; J: junctions of the shell valves. ×8800

pattern observed in most myxosporeans and described by Lom & Puytorac (1965). Concerning the origin of the polar capsule, present TEM observations of capsulogenic cells in early sporoblasts of *S. tetraodoni* did not reveal the presence of numerous Golgi complexes, but large amounts of rough and smooth endoplasmatic reticulum were observed. This is in accordance with similar observations in *Sphaerospora carassii* (Desser et al. 1983), *Sphaerospora galinae* (Lom et al. 1985) and *Myxobolus* sp. (Desser & Paterson 1978). In other myxosporeans, the Golgi apparatus seems also to be involved in the development of polar capsules (Lom 1969, Desser et al. 1983, El-Matbouli et al. 1990).

There were no apparent effects on the tubules during the sporogonic stages of development, a finding consistent with other renal myxosporeans (Dyková & Lom 1982, Molnár & Kovacs-Gayer 1986, El-Matbouli & Hoffmann 1992). However, infected renal corpuscles of pufferfish harboring mature and developmental stages of *Sinuolinea tetraodoni* showed some dilatation of glomerular capillaries and Bowman's space. During our observations no stages could be detected in the blood of infected pufferfish, but the low number of fish examined did not allow us to establish whether blood stages occur or not.

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