

New species of the genera *Zschokkella* and *Ortholinea* (Myxozoa) from the Southeast Asian teleost fish, *Tetraodon fluviatilis*

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Key words: Myxosporea, *Zschokkella*, *Ortholinea*, *Kentmoseria*, *Tetraodon fluviatilis*

Abstract. Three new myxosporean species are described from *Tetraodon fluviatilis* (Osteichthyes: Tetraodontidae) imported from Southeast Asia to the Czech Republic. *Zschokkella tetrafluvi* sp. n. lives in the gall bladder. Di- or monosporic plasmodia produce ellipsoidal spores averaging $11.3 \times 7.2 \mu\text{m}$. *Zschokkella pleomorpha* sp. n. infects renal tubules and renal corpuscles; mono- to polysporic plasmodia produce spores averaging $15.7 \times 7.1 \mu\text{m}$. In the process of maturation, immature subspherical spores assume elongated shape. In both species, extremely curved suture line does not bisect poles of the spore. *Ortholinea tetrafluvi* sp. n. occurs as a rule in mixed infections with *Z. pleomorpha* sp. n. in the renal tubules. Mono- to polysporic plasmodia produce spores with a wide anterior and a narrow posterior end, averaging $8.3 \times 7.8 \mu\text{m}$. Both *Z. pleomorpha* sp. n. and *O. tetrafluvi* sp. n. have also limited number of stages located within the renal tubule epithelium, where they can complete sporogony. In one of the fish specimens, a myxosporean tentatively identified with *Sinuolinea tetraodon* El-Matbouli et Hoffmann, 1994 was found. A new genus is proposed for *Ortholinea alata* Kent et Moser, 1990 – *Kentmoseria* gen. n., and its diagnosis is presented.

Protozoan parasites of Southeast Asian fish have been paid very little attention until now, although enormous development of commercial fish farming and ornamental fish breeding takes place in this region. Parasitic protozoa are mentioned in reviews dealing with fish parasitoses in general (e.g., Davy and Graham 1979, Shariff 1980, Arthur 1987) and papers dealing with certain protozoan parasite species or groups (e.g., Bondad-Reantaso and Arthur 1989, Shaharom-Harrison et al. 1990, Lom et al. 1991, El-Matbouli and Hoffmann 1994) are still not numerous. In view of the recognized pathogenicity inflicted by many protozoan fish parasites on commercial and aquarium fish, it is desirable to obtain data as complete as possible on the protozoans infecting fish in this region. This communication brings description of three new myxosporean species of the genera *Ortholinea* and *Zschokkella* from an ornamental fish, *Tetraodon fluviatilis* (Hamilton-Buchanan, 1822).

MATERIALS AND METHODS

Specimens of *Tetraodon fluviatilis* were obtained from pet fish shops in České Budějovice. Allegedly, they were directly imported from aquarium fish breeders from Southeast Asia. Some of them were in moribund condition and as soon as they died, they were examined for protozoan fish parasites. Additional material was obtained from fish from the same source purposely killed by an overdose of MS 222. All body

organs were examined in fresh mounts, and myxosporeans were observed, measured and photographed in fresh state. Samples of tissues were fixed in Bouin's and embedded in paraffin. Histological sections were stained with hematoxylin-eosin and Giemsa. Other samples were fixed in cacodylate buffered 2% osmic acid and embedded in Epon-Araldite. Semithin sections were stained with toluidine blue.

RESULTS

Zschokkella tetrafluvi sp. n.

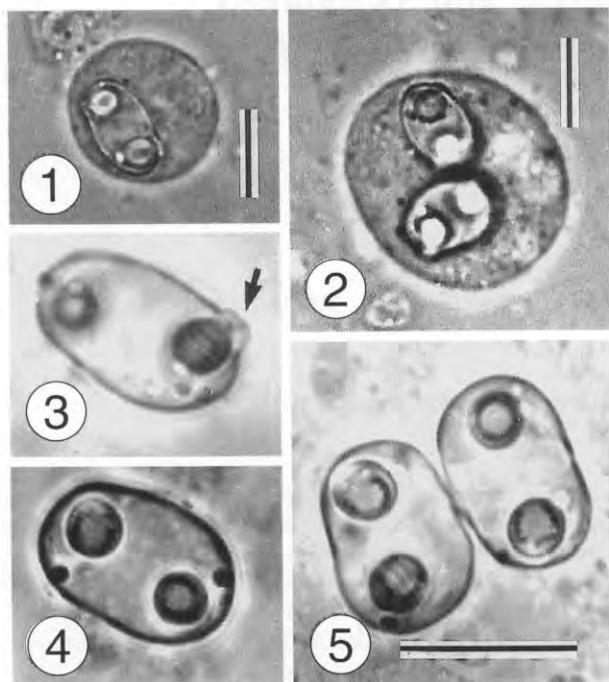
Figs. 1–6

S i t e : gall bladder.

P r e v a l e n c e : four of seven fish examined were infected.

Light microscopy. Sporogonic pseudoplasmodia are rounded, with a hyaline ectoplasmic layer, ranging from 13 to 23 μm . They produce one or two spores (Figs. 1–2).

Spores (Figs. 3–5) are ellipsoidal with a smooth surface and a fine suture line of irregular course which never crosses the rounded spore ends (Fig. 6). Mostly the suture appears slanted in a curve across the sides of the spore, delimiting two unequal shell valves. Sometimes the suture runs quite close to the mid-spore length, reminiscent of *Leptotheca*. Spore size is 11.3 (10.5 – 11.3) $\times 7.2$ (6.8 – 7.8) μm . Rounded polar capsules, 3.4 (3.2 – 3.5) $\times 2.3$ (2.5 – 3.1) μm in size taper to



Figs. 1-5. Fresh mounts of *Zschokkella tetrafluvi*. Figs. 1-2. Mono- and disporic pseudoplasmodia; bars = 5 µm. Figs. 3-5. Spores; arrow points at the spore shell vaulted over the polar capsule apex. Bar = 10 µm.

their laterally positioned discharge ends. This end is inserted into a distinct, about 1.8 µm wide flat elevation of the shell valve surface (Fig. 3). Polar filament is coiled in 4 to 5 rather loose, transverse turns.

Taxonomic affinities. Since *Tetraodon fluviatilis* inhabits freshwater and slightly brackish habitats (Sterba 1968) in Southeast Asia and since the species of *Zschokkella* are found both in freshwater and marine hosts we have compared this and the following species with all *Zschokkella* records from gall bladders. We excluded from comparison, however, species with spores having surface ridges (such as *Z. nova* Klokacheva, 1914; *Z. carassii* Nie et Lee, 1964; *Z. sturionis* Tripathi, 1948 or *Z. ganapatii* Padma Dorothy et Kalavati, 1992) and also species from hosts inhabiting northern regions such as *Z. orientalis* Konovalov et Shulman, 1966 from salmonids inhabiting northern Asian part of Russia. In addition, morphology of all such species was different from *Zschokkella tetrafluvi* n. sp.

Among *Zschokkella* species from truly marine fish, there are some species clearly different in all respects, such as *Z. atlantica* Gayevskaya, Kovaleva et Krasin, 1985 with spores up to 21 µm long, infecting *Coryphaenoides rupestris* near the Mid-Atlantic ridge. A few species reveal some similar features; *Z. microcapsula* Moser et Noble, 1977 has spores of similar size with

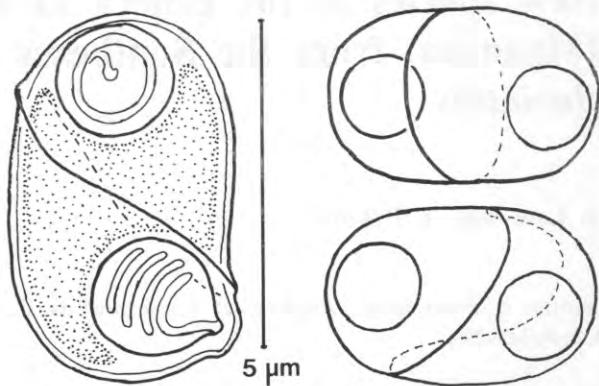


Fig. 6. Spores of *Zschokkella tetrafluvi*. Left, a typical spore; at right, diagrams of two spores to show variation in the course of the suture line. Bar = 5 µm.

similarly twisted suture line, but spores have different shape and polar capsules are smaller.

Considering species from estuarine hosts, *Z. mugilis* described by Sitja-Bobadilla and Alvarez-Pellitero (1993) from fishes of the genera *Mugil* and *Liza* has spores of similar size and comparable shape; however, they are more slender and suture line is "slightly curved" and the polar filament discharge pore is not elevated into a wide tubercle. *Z. magna* Chen et Hsieh, 1984 from *Mugil soiuy* differs, among other things, by its less twisted suture line. Since no *Zschokkella* species recorded thus far is identical, we propose to establish the present finding as *Zschokkella tetrafluvi* sp. n., the species name being derived from that of its host.

Zschokkella pleomorpha sp. n.

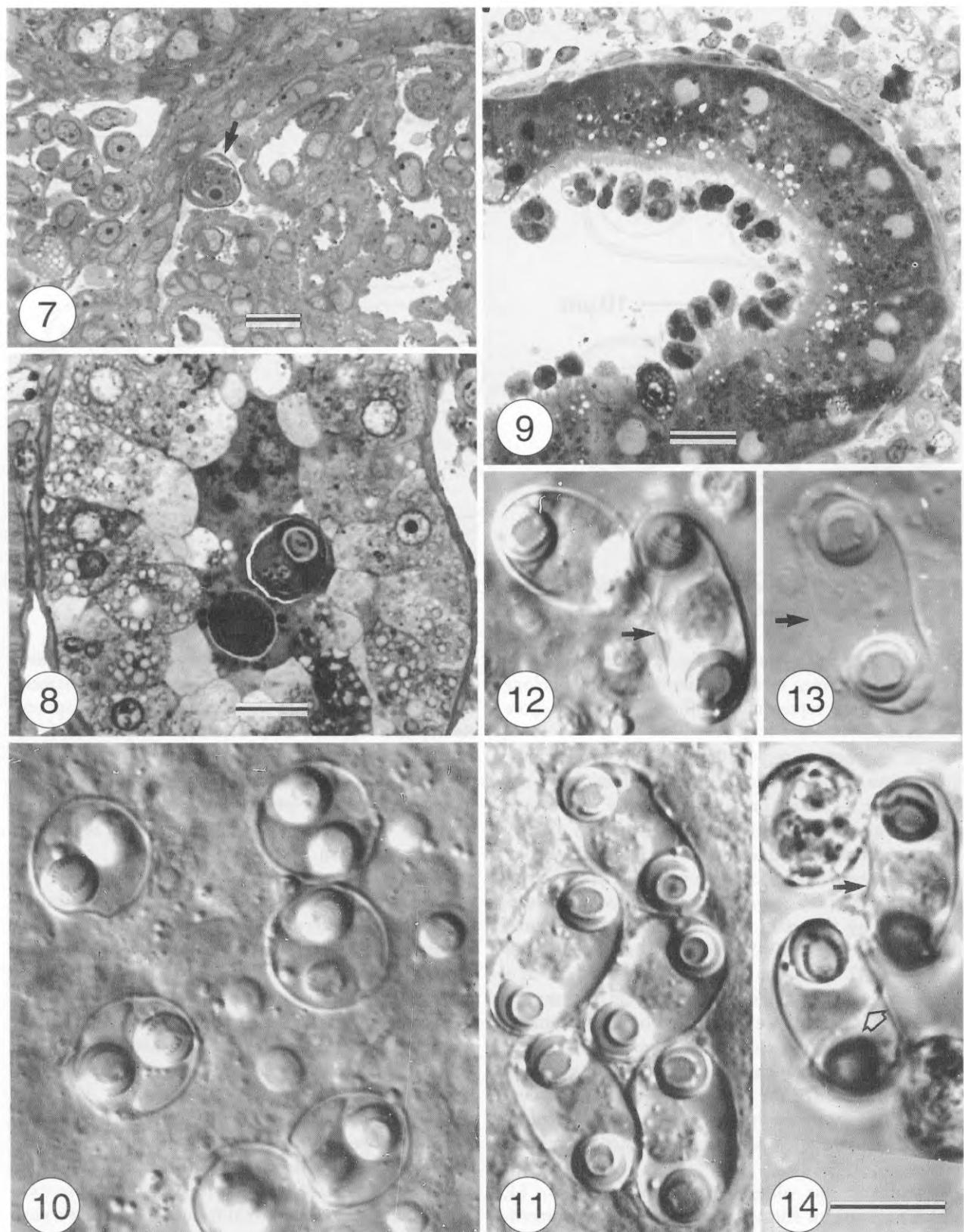
Figs. 7-18

S i t e : renal tubules and ducts.

P r e v a l e n c e : all seven fish examined were infected (mixed infection with *Ortholinea fluviatilis*).

Light microscopy. Sporogonic plasmodia have smooth surface and a wide range of sizes. Small mono- and disporic forms about 25 µm in size, are attached to the epithelial lining (Fig. 9) and some can be found in the renal corpuscles (Fig. 7). In heavily infected parts of renal tubules they fill the lumen. Larger polysporic forms are mostly elongated to fit the space of the tubule. They are attached to its epithelium by radiating, pointed extensions (Fig. 17). Their size may reach up to about 200 µm in length. Small trophozoites can also be found within the tubular epithelium; some of them complete sporogony there.

Spores as seen in fresh mounts are extremely variable. There are subspherical spores, then there are elongated spores with one side vaulted which appear almost



Figs. 7–14. *Zschokkella pleomorpha* sp. n. Figs. 7–9. Semithin sections, toluidine blue. Figs. 10–14. Fresh mounts. Fig. 7. Plasmodium in the renal corpuscle (arrow). Bar = 10 µm. Fig. 8. Two developing spores in a plasmodium within the lumen of the renal tubule. Bar = 10 µm. Fig. 9. Young plasmodia attached to the epithelium of renal duct. Bar = 15 µm. Fig. 10. Young, almost spherical spores. Fig. 11. Almost mature spores. Figs. 12–14. Arrows point at mature spores; hollow arrow points at the suture line. Bar to Figs. 10–14 = 5 µm.

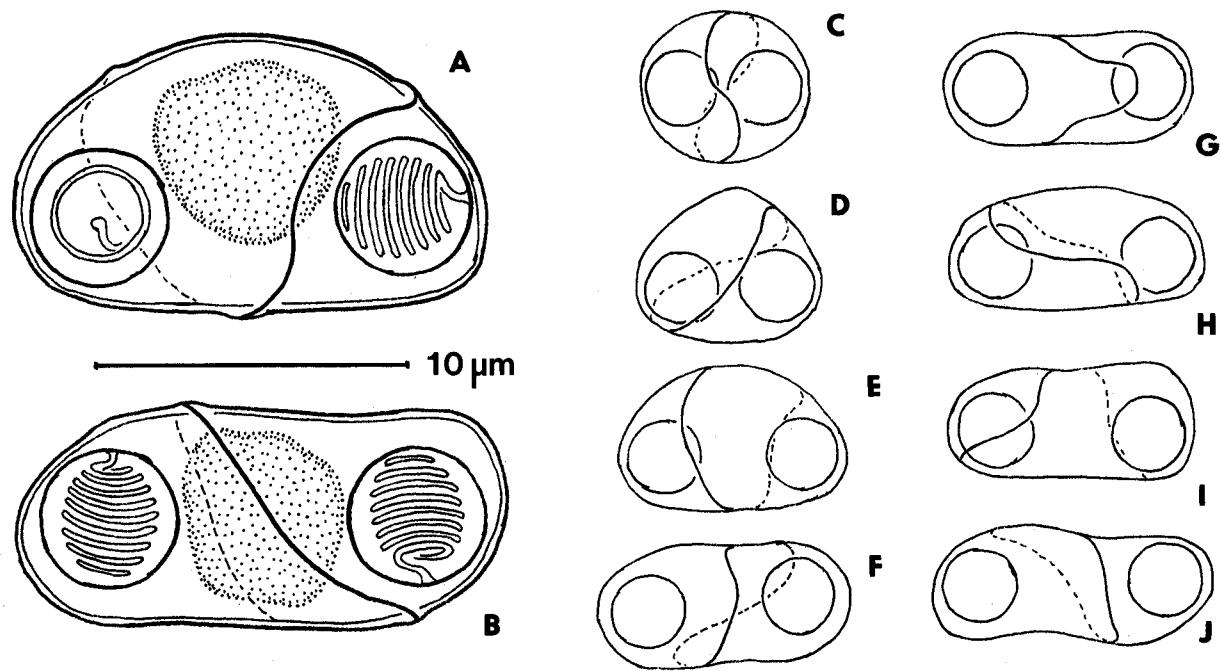


Fig. 15. A – almost mature spore of *Zschokkella pleomorpha*; B – mature spore; C–F – shape changes during spore maturation; G–J – shape variation in mature spores.

rounded triangular and, finally, ellipsoidal spores. Transitions between them indicate that they all belong to one species, representing different stages of spore maturation.

The subspherical spores (Figs. 10, 15C) appear to be non-mature, since even in the light microscope one can observe the shrinking capsulogenic nuclei in the shell valves. The suture line is inconspicuous with a wavy course, with no defined relation to the plane of the polar capsules. The diameter of these immature spores averages 10.7 μm . They contain subspherical polar capsules lying side by side with the size of 4.6 (4.2–5.2) μm . Number of turns of the polar filament coil is 8 (sometimes 7 or 9), the turns are tightly wound in a plane perpendicular to the point of filament discharge.

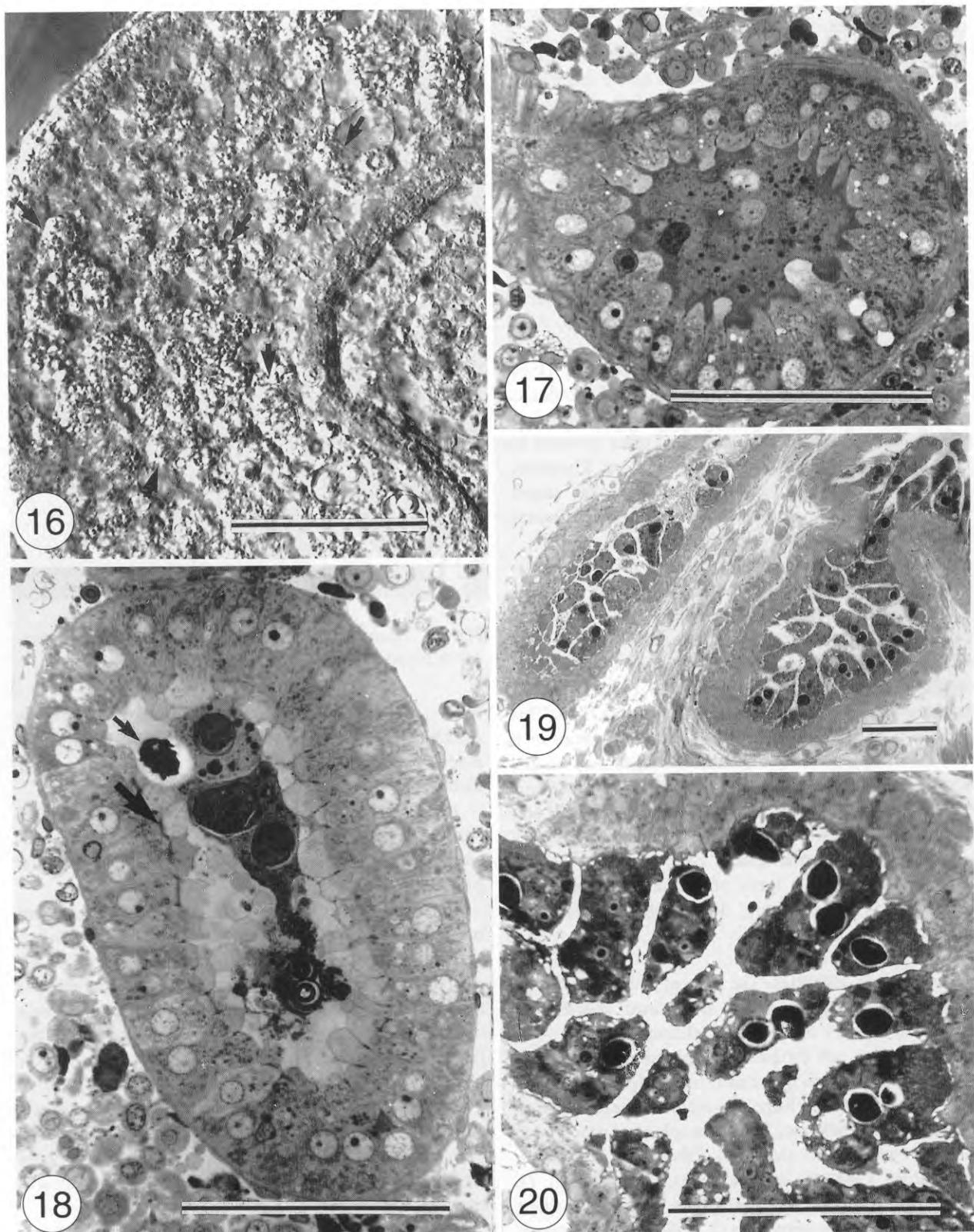
Next step in what we suppose to be the maturation process are slightly elongated, asymmetrical spores (Fig. 15D) with polar capsules situated side by side at the flattened side of the spore, while the more vaulted half is filled by the sporoplasm. At the opposing poles of the elongated spore appear the residual valvogenic nuclei.

Eventually, the almost mature spores (Figs. 11, 15A,E) assume an ellipsoidal shape mostly asymmetrical in that one side of the spore is vaulted. Their size is 15.6 (14.6–17.1) \times 8.9 (8.2–10.1) μm . The suture line seen as a delicate ridge has an irregularly oblique, wavy course, more transverse than longitudinal and the two shell valves are completely asymmetrical. Polar capsules are located in the spore ends. Their filament

discharge pores face opposite sides of the spore. Their average size (4.6 μm) and number of polar filament turns are the same as in the spherical immature spores. Sporoplasm appears as a rounded cytoplasmic body situated in the centre of the spore cavity.

Completely mature spores (Figs. 12–14, 15F–J) have more slender shape; while their length remains about the same – 15.7 (14.4–17.6) μm , their width is reduced to 7.1 (6.4–7.9) μm .

Taxonomic affinities. Our attempts at identification of the present species followed the same considerations mentioned in the preceding species. *Zschokkella* species with smooth shell valves from the kidneys and/or urinary system of freshwater hosts are all different. Most species recorded from these organs in marine fish differ distinctly in spore shape – e.g., *Z. variabile* Kovaleva et Gayevskaya, 1982 has sharply pointed spores – or in size; *Z. pulchella* Kovaleva et Gayevskaya, 1982 has spores reaching 24 μm in length. *Z. meglitschi* Moser et Noble, 1977 has spores of comparable length and similarly oblique suture line; however, it is semicircular in one aspect. *Z. parasiluri* Fujita, 1927 as it was described by Yoshino and Noble (1973) from *Macrourus berglax* has comparable size and shape, but the obliquely wound suture line runs at least at one spore pole close to the polar filament discharge pore. Since our finding cannot be identified with any of the known named species, we propose to establish it as *Zschokkella pleomorpha* sp. n., the name indicating the changes of shape in the



Figs. 16–18. *Zschokkella pleomorpha* sp. n. **Fig. 16.** Fresh mount of a renal tubule filled with plasmodia (arrows). Bar = 50 µm. **Figs. 17–20.** Semithin sections, toluidine blue. **Fig. 17.** Large plasmodium attached with pseudopodial extensions to epithelial cells of the renal tubule. Bar = 50 µm. **Fig. 18.** An isolated spore (arrow) next to a plasmodium of *Zschokkella pleomorpha* with several sporoblasts in the renal tubule. Bar = 50 µm. **Fig. 19.** Plasmodia of *Ortholinea fluviatilis* attached to the walls of the urinary bladder. Bar = 50 µm. **Fig. 20.** Detail of the preceding figure. In Figs. 16–20, bar = 50 µm.

maturing spores.

Specimens of *Zschokkella pleomorpha* in semithin sections of the kidney are deposited in the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Protozool. Coll. No. PM - 024.

Ortholinea fluviatilis sp. n.

Figs. 19-22

Site: renal tubules and ducts.

Prevalence: all seven fishes examined were infected (mixed infection with *Zschokkella pleomorpha* sp. n.)

Light microscopy. Sporogonic plasmodia (Figs. 19-20) were attached to the epithelium of tubules or ducts, in heavily infected parts were also found unattached in the lumen. Their surface is often covered by thin, curved or sinuous, villi-like projections. Plasmodia containing spores ranged mostly from squat shapes about 20 μm in size to elongated specimens measuring about 11 \times 50 μm . They are polysporic.

Spores are ellipsoidal in frontal view, with wider anterior end (Figs. 21-22). Typically, this end is flattened. The suture is slightly undulated (Fig. 22C). The shell valves bear ridges arranged in variable patterns. The ridges may be regularly concentric around a small circular area, or around an elongated central field or they may be sharply bent at the ends of this field. This field may be in the longitudinal axis of the spore or it may be oblique to it. The pattern of ridges may be different on each of the two shell valves. Spore length is 8.3 (7.9 to 8.4) μm ($n = 20$), width is 7.8 (7.3-8.0) μm , thickness averages 6.8 μm . Subspherical polar capsules located in the sutural plane have the size of 3.1 (2.8-3.3) μm ; 4 to 6 turns of the polar filament are wound into a tight coil. The filaments discharge laterally.

Taxonomic affinities. The present finding is easy to differentiate from the seven *Ortholinea* species described to date by the set of its characters. Hosts – all marine fishes – are different. Of the many other differing features, one can quote in *O. australis* Lom, Rohde et Dyková, 1993 the huge plasmodia in hepatic ducts. *O. divergens* (Thélohan, 1895) has spherical spores with marked striation on the shell valves. In *O. gobiusi* Naidenova, 1968, the spores are hazel nut-shaped. *O. irregularis* Kabata, 1962 has spores irregular in shape and a more sinuous suture. In *O. orientalis* (Shulman et Shulman-Albova, 1953), the spores taper posteriorly into a sharp point. *O. polymorpha* (Davis, 1917) has spores with a smooth surface and elongated polar capsules. *O. undulans* (Meglitsch, 1970) has also elongated polar capsules in addition to undulated suture. *O.*

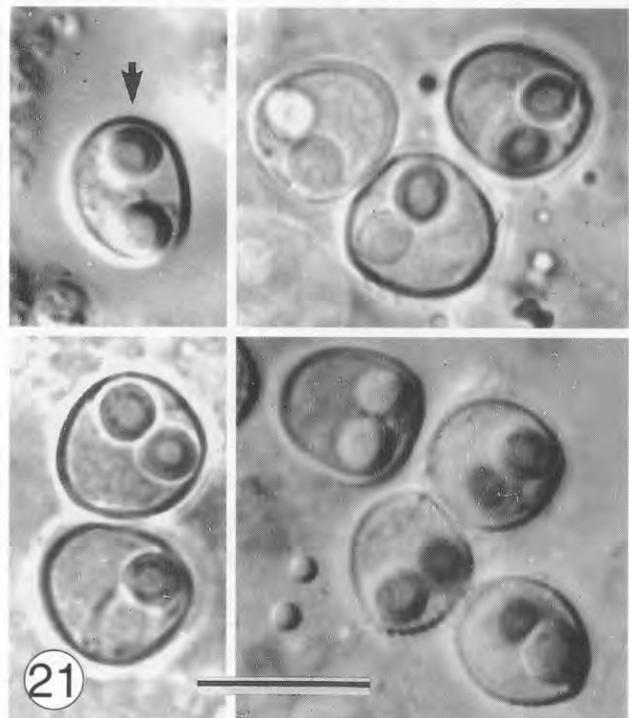


Fig. 21. Spores of *Ortholinea fluviatilis* in fresh mount. Arrow points at an abnormally short spore. Bar = 5 μm .

alata Kent et Moser, 1990 has spores with posteriorly directed lateral projections of valves, similar to some extent to projections of *Bipteria admiranda* Kovaleva, Zubchenko et Krasin, 1983.

Because of all these differences, we propose to establish the present species as a new one, *Ortholinea fluviatilis* sp. n., the specific name being derived from that of the host.

Kent and Moser (1990) mentioned that unless more ortholineas with projections similar to those in *O. alata* are found, their species should be confined to *Ortholinea*, the erection of a new genus being not desirable for a single species. In this point we disagree and propose a new genus, *Kentmoseria* gen. n., to be assigned into the family Ortholineidae Lom et Noble, 1984 with the following diagnosis:

Elongated spores, wider anteriorly than posteriorly are slightly flattened parallel to the straight suture. Pointed projections extend backwards from the posterior half of the shell valves. Pyriform polar capsules open laterally.

Trophozoite mono- to disporic, coelozoic in the urinary tract of marine fishes.

Type species *Kentmoseria alata* (Kent et Moser, 1990) comb. n. by monotypy.

Specimens of *Ortholinea fluviatilis* in semithin section of the kidney are deposited in the Institute of

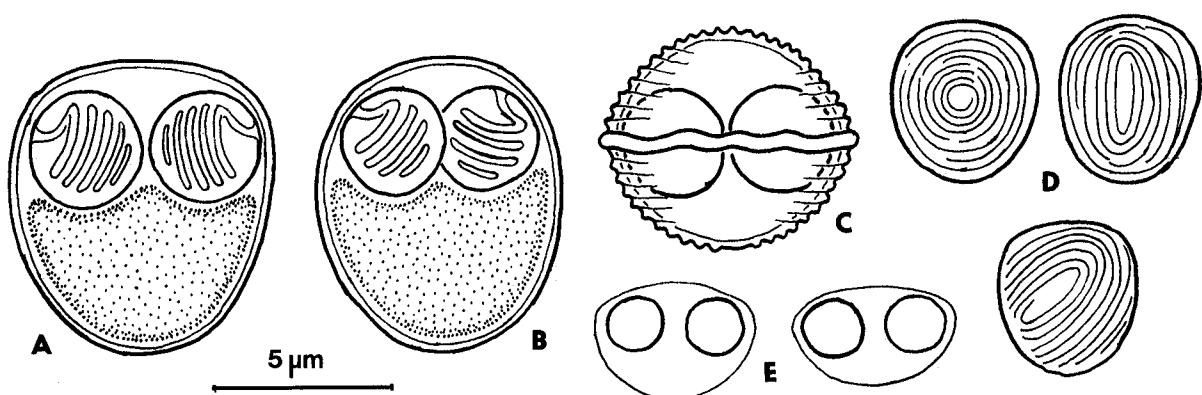


Fig. 22. A – a typical spore of *O. fluviatilis*, B – a rather rounded spore. (Bar = 5 μ m). C – apical view of the spore showing the ridges, D – three different arrangements of ridges on the shell valve, E – diagram of abnormally short spores.

Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Protozool. Coll. PM – 025.

Sinuolinea cf. *tetraodoni* El-Matbouli et Hoffmann, 1994

In the renal tubules of one specimen of the examined *T. fluviatilis* a small number of spores was found closely resembling *Sinuolinea tetraodoni*. The average size was 9.3 (length) \times 9.3 (width) μ m, length of polar capsules with seven turns of the polar filament coil averaged 3.6 μ m. Because of insufficient quantity of spores observed, the identity of the species remains uncertain.

DISCUSSION

Tetraodon fluviatilis is one of the members of its family which are largely adapted to freshwater habitat. Thus *Ortholinea fluviatilis* sp. n. is the first freshwater exception among the rest of the species – seven to our knowledge – which infect marine hosts. Migration of fish hosts from brackish waters to rivers makes for the existence of species of “marine genera” in freshwater habitats. Similar exceptions can be found in the marine genus *Ceratomyxa*. The notorious pathogen *C. shasta* Noble, 1950 came to being certainly through oceanic migrations of its salmonid hosts. *C. anguillae* described by Tuzet and Ormieres (1957) and occurring in the Mediterranean brackish lagoons has probably evolved through contacts with marine fish. *Sinuolinea tetraodoni* El-Matbouli et Hoffmann, 1994, the sole freshwater *Sinuolinea*, is a case similar to *O. fluviatilis*; its host, *Tetraodon palembangensis*, is also adapted to freshwater.

In this way, ascension of myxosporeans into freshwater from the supposedly original marine habitat seems to lend support to the theory of Shulman (1966) that myxosporeans originated in perciform marine fish as coelozoic parasites and that *Platysporina* are rather evolved, histozoic freshwater forms. While there are very few purely freshwater genera (e.g., *Thelohanellus*) there are several with species almost equally divided between marine and freshwater habitats (e.g., *Zschokkella*, *Myxidium*).

The changes of size and shape during final morphogenesis of *Zschokkella pleomorpha* spores are rather unique. The only comparable reduction in size in maturing spores was observed in *Sphaerospora molnari* (Lom et al. 1983) when the originally very thick, *Leptotheca*-like spore shrinks to almost subspherical shape and similar phenomenon was observed in final stages of spore development in some other *Sphaerospora* species (e.g., in *S. truttae* Fischer-Scherl, El-Matbouli et Hoffmann, 1986 – our unpublished observations). However, while in *Sphaerospora* the mature spore is subspherical, in *Zschokkella pleomorpha* the early almost spherical shape turns later into an elongated ellipsoid. The immature spore of *Z. pleomorpha* with its sinuous suture looks like *Sinuolinea* to an extent inviting speculations on possible kinship of the two genera.

In species with considerably sinuous suture line as in *Z. tetrafluvi* it is impossible to distinguish which view of the spore is frontal and which is valvular, and that is why in the preceding descriptions only “width” was given. The same problem exists sometimes in *Myxidium* and *Zschokkella*; the distinction between the two genera is more or less a matter of convention (Diamant et al. 1994). Actually, there is a gradual transition between some species described as *Myxidium* (e.g., *M. triangulum* Shulman, 1962) or *Zschokkella* (e.g., *Z. stettinensis* Wierzbicka, 1987) with spores having polar capsules set

close to each other and sporoplasm situated not between the capsules but set aside and species of *Neomyxobolus* and *Ortholinea*. These problems of myxosporean taxonomy can hardly be solved but by involving life cycle studies and molecular biology. Classification according

to the type of plasmodial stages will not solve much; in the genera *Myxidium*, *Zschokkella*, *Ortholinea* and *Sinuolinea* sporogonic stages of a given species may be represented by small mono- or disporic pseudoplasmodia or by large polysporic plasmodia.

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Received 5 June 1995

Accepted 9 August 1995