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Sphaerospora scardinii n. sp. (Myxosporea: Sphaerosporidae) observed in the kidney of rudd *Scardinius erythrophthalmus*

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ABSTRACT: *Sphaerospora scardinii* n. sp. is described from the kidney of rudd *Scardinius erythrophthalmus* obtained from the river Main. Three stages of development were observed: intracellular trophozoites consisting of one uninucleate cell in epithelial cells of the ureter, round to oval extrasporogonic stages (17.49 ± 2.29 µm in size) in the lumen of the ureter and pseudoplasmodial sporogonic stages (elongated to irregular in shape, 20.27 × 12.32 µm) in the lumen of kidney tubules. One or two subspherical spores (6.01 µm length, 5.79 µm width) developed within the surrounding pseudoplasmodium. Thin valves surrounded 2 spherical polar capsules (2.26 × 2.14 µm) which contained a polar filament with 4 to 5 coils.

INTRODUCTION

Many species of the genus *Sphaerospora* Thelohan, 1892 are known to have pathogenic potential in various fish hosts. These species have recently attracted special attention as agents of sphaerosporosis affecting kidney, swimbladder and blood, such as *Sphaerospora renicola* (Dykova & Lom 1982), or gills, such as *Sphaerospora molnari* (Lom et al. 1983), in common carp. The first *Sphaerospora* found to be pathogenic was *S. tinca* Plehn, 1925 infecting the head kidney of tench *Tinca tinca*.

To our knowledge 43 species of the myxosporean genus *Sphaerospora* are known. Arthur & Lom (1985) listed 36 species, of which most have been described from the lumina of the kidney tubules and urinary bladder of freshwater fishes. Since then, 7 other renal species have been described: *S. diminuta*, from the renal tubules of pumpkinseed *Lepomis gibbosus* (Li & Desser 1985); *S. truttae* in brown trout *Salmo trutta* (Fischer-Scherl et al. 1986); *S. paulini* and *S. hankai* from the renal tubules of creek chub *Semotilus atromaculatus* and brown bullhead *Ictalurus nebulosus* respectively (Lom et al. 1989); *S. ictaluri* in channel catfish *Ictalurus punctatus* (Hedrick et al. 1990); *S. colomani* in sterlet *Acipenser ruthenus* (Baska 1990); and *S. epinepheli* in grouper *Epinephelus malabaricus* (Supamattaya et al. 1991). Lom et al. (1985) reported *Sphaerospora* spp. from renal tubules of roach *Rutilus rutilus* in a fish pond near Bohemia. This infection was so light that the material was insufficient for the authors to describe the species adequately.

Most species of *Sphaerospora* are known only from a single host. The species appear to be strictly host-specific, e.g. *S. molnari* and *S. renicola* are parasitic in *Cyprinus carpio*, and *S. tincae* is known only from *Tinca tinca*. Some *Sphaerospora* species are characterized by vegetative reproduction in the blood and tissues of other organs. In this paper we describe *Sphaerospora scardinii* n. sp. from the kidney of rudd *Scardinius erythrophthalmus*.

MATERIALS AND METHODS

In a parasitological survey during February and March 1992 rudd *Scardinius erythrophthalmus* and roach *Rutilus rutilus* were collected from the Main River near Würzburg, Germany. In this study 10 rudd and 15 roach (50 to 150 g, 18 to 23 cm) were examined. For histological studies, tissue samples from gills, kidney, liver, spleen, heart, muscle, gut and brain were fixed in 5% buffered formaldehyde and embedded in Paraplast or Historesin. Sections were stained with

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H&E, Giemsa's solution, and Toluidine Blue and using the Periodic Acid-Schiff (PAS) reaction.

For examination of fresh material, portions 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 of these stages 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.

DESCRIPTION

Sphaerospora scardinii n. sp. (Fig. 1)

Host: Rudd *Scardinius erythrophthalmus*.

Locality: The river Main, near Würzburg, Germany.

Location: Lumen of the kidney tubules, distal and proximal tubules, collecting ducts and ureter.

Prevalence: 7 of 10 host specimens examined.

Developmental stages: Extrasporogonic stages were round to oval (n = 20, mean diameter = 17.49 ± 2.29 µm). They contained numerous highly refractile granules and were found only in the ureter of the kidney.

Sporogonic stages: These were composed of 6 or 12 cells within a pseudoplasmodium in the lumen in the kidney tubules. One or two spores developed within each pseudoplasmodium.

Spore characteristics: Spores were almost spherical with a somewhat projecting anterior pole and enveloped by a shell composed of 2 valves connected by a clearly visible suture. At the posterior pole of the spore 3 to 4 fine ridges were seen. Mean length of the spore (n = 20) was 6.01 ± 0.58 µm, mean width 5.79 ± 0.26 µm. At the apical end of the spore were 2 almost spherical polar capsules with a polar filament showing 4 to 5 coils. Mean length of polar capsules was 2.26 ± 0.28 µm, mean width 2.14 ± 0.18 µm.

RESULTS

From a total of 10 rudd, 7 were parasitized by *Sphaerospora scardinii* n. sp. Developmental stages and mature spores were found in fresh preparations, in Giemsa-stained imprints from all parts of the kidney and in tissue sections. Kidneys of infected rudd appeared to be slightly swollen. Histologically a hyperplasia of lower urinary ducts could be seen. In PAS-stained sections, mature spores and pseudoplasmodia exhibited PAS-positive granules in the cytoplasm and in the shell valves. In infected rudd, *S. scardinii* was found exclusively in the kidney. No other organs were infected. Infections with *S. scardinii* or with any other *Sphaerospora* species were absent from all roach examined.

Extrasporogonic stages

In infected rudd the lumen of the ureter and collecting ducts of the kidney were filled with trophozoite developmental stages (Figs. 2 & 3). Trophozoites were round in shape, up to 17 µm in size, and contained numerous refractile granules in the cytoplasm. Rounded primary cells included up to 8 secondary cells. In infected fish the ureter epithelial cells had proliferated and intracellular trophozoites were located in the apical region of the cells. In histological sections, trophozoites were seen singly in the epithelial cells (Fig. 4).

Sporogonic stages

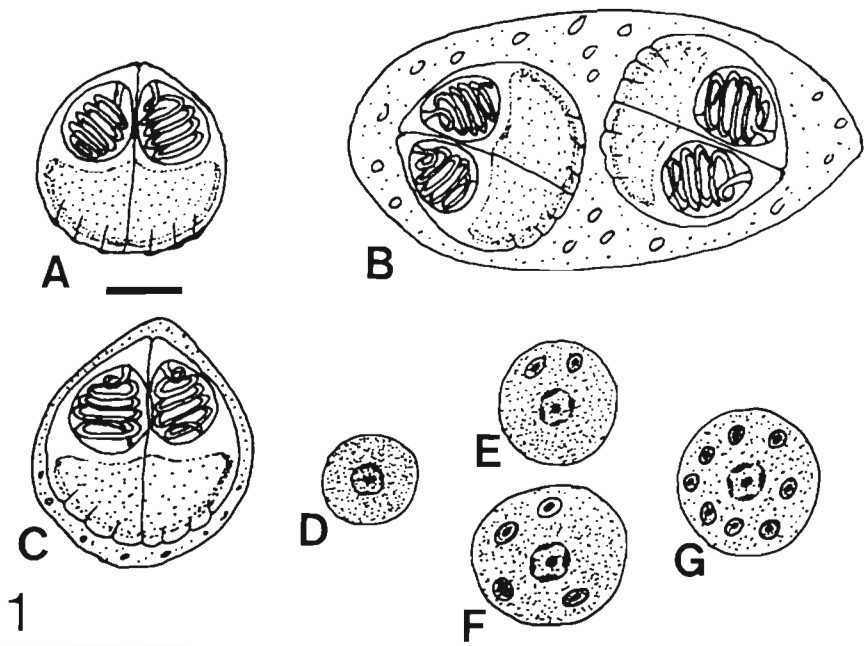
Pseudoplasmodia in various stages of development were observed (Fig. 5) within the lumina of the proximal tubule. They were elongated or irregular in shape, up to 23 µm in length and 15 µm in width, with numerous refractile granules in the cytoplasm. These pseudoplasmodia ranged from simple unicellular stages to stages with up to 12 cells within the surrounding pseudoplasmodium. In the lumina of the kidney tubules both mono- and disporic pseudoplasmodia were observed in fresh preparations (Figs. 6 & 7) and stained sections (Fig. 8).

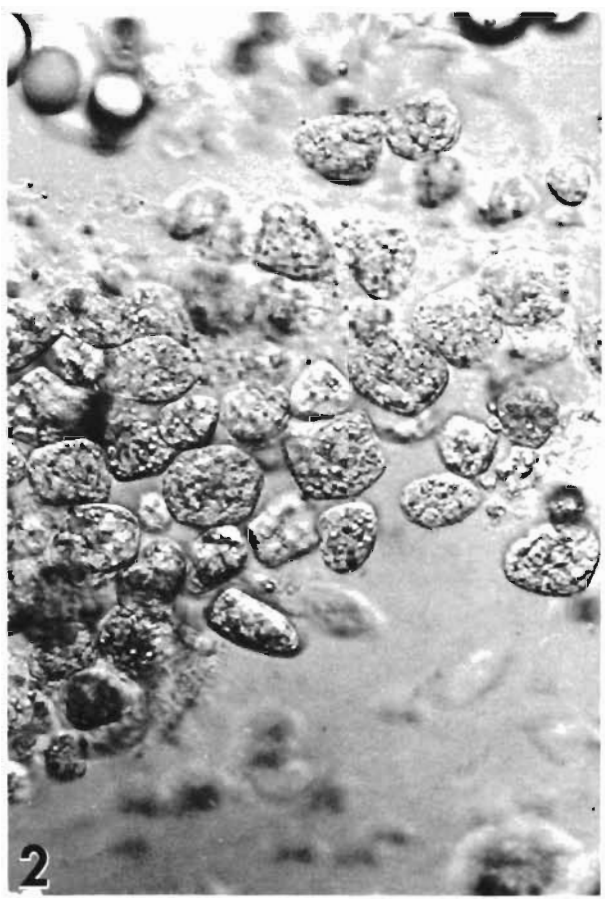
Mature spores (Figs. 1 & 7) were subspherical, had a pointed anterior end and contained 2 spherical polar capsules that opened towards the anterior end of the spore near the sutural ridge. Spore length was 6.01 ± 0.58 µm, width 5.79 ± 0.26 µm (n = 20). The suture of the 2 valves protruded only slightly anteriorly Both polar capsules were of equal size with a length of 2.26 ± 0.28 µm and a width of 2.14 ± 0.18 µm. The polar filament was coiled 4 to 5 times, wound almost perpendicularly to the longitudinal axis of the capsule.

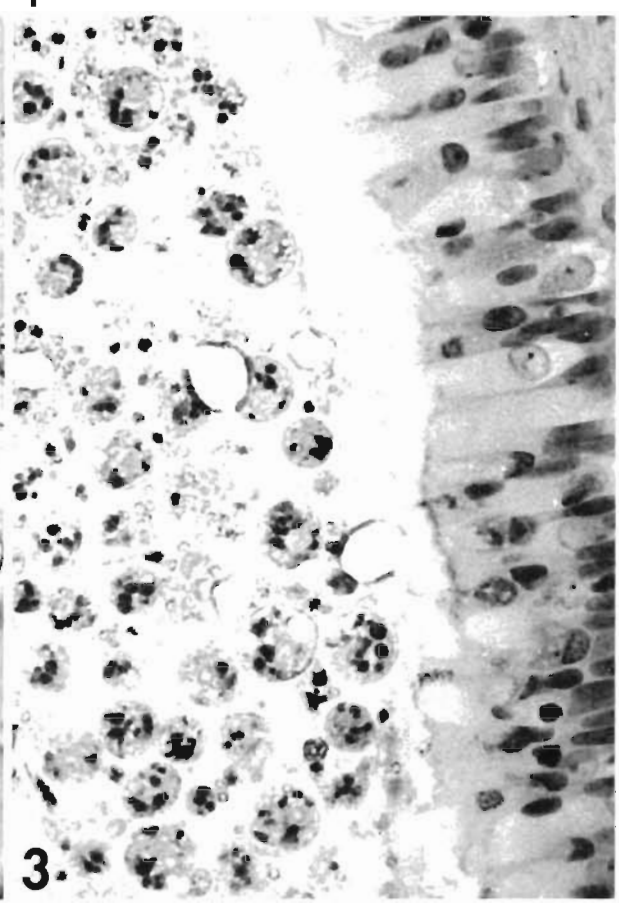
In blood smears no myxosporean developmental stages were observed.

DISCUSSION

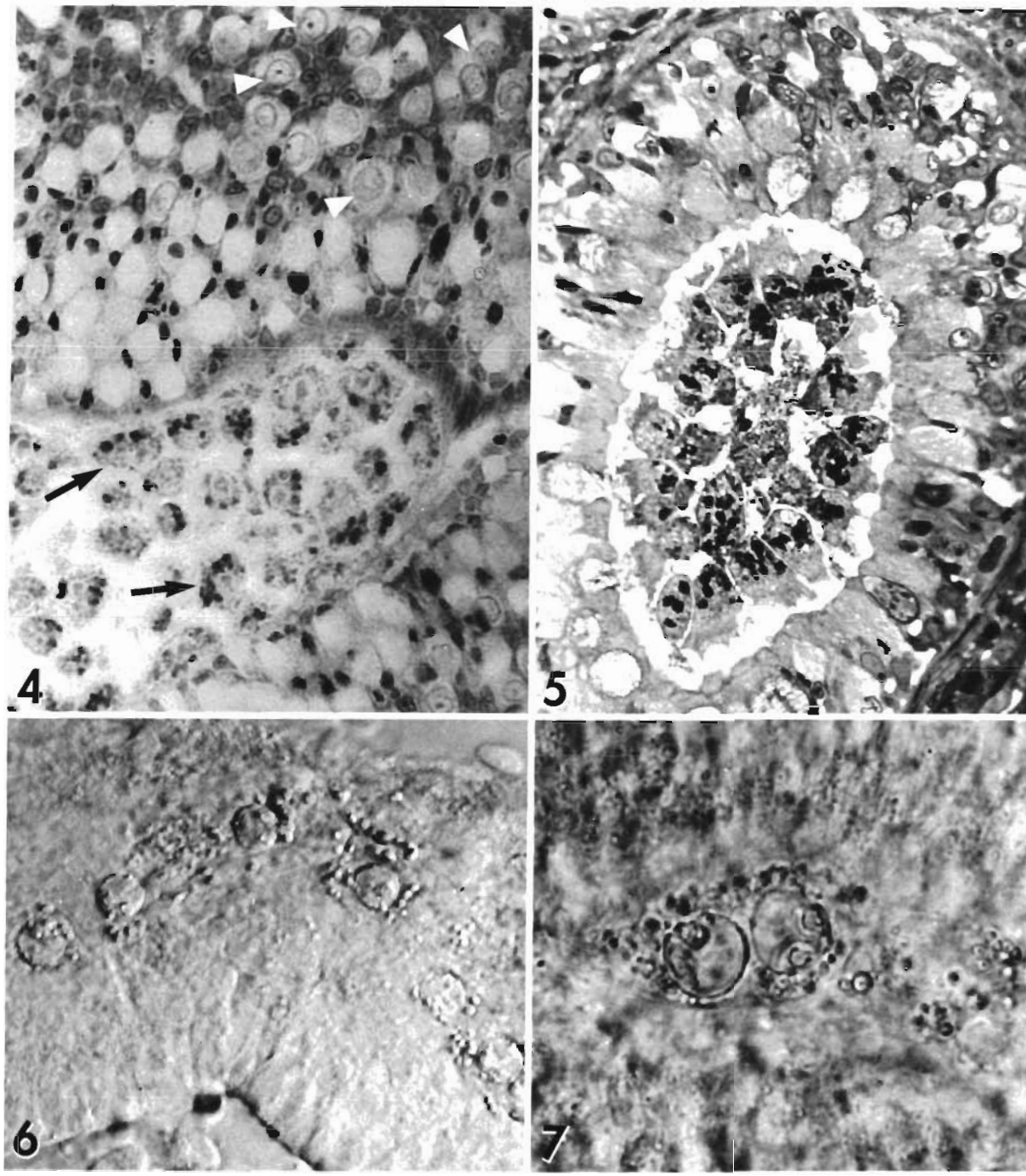
The *Sphaerospora scardinii* detected in the kidney of rudd in this study are the first to be reported in detail from rudd. A similar *Sphaerospora* sp. in rudd was







Figs. 1 to 3. *Sphaerospora scardinii* infecting *Scardinius erythrophthalmus*. Fig. 1. Line drawings of various stages. (A) Mature spore; (B) disporic pseudoplasmodium; (C) monosporic pseudoplasmodium; (D to J) extrasporogonic stages found in the lumen of the ureter. Scale bar = 2.5 µm. Fig. 2. Extrasporogonic stages detected in the lumen of the ureter. Squash preparation, unstained, × 600. Fig. 3. Histological section with extrasporogonic stages in the lumen of ureter H&E, × 600



Figs. 4 to 7 *Sphaerospora scardinii* infecting *Scardinius erythrophthalmus*. Fig. 4. Ureter with uninucleated trophozoite cells in epithelium (arrowheads) and coelozoic stages in the lumen (arrows). H&E, × 600. Fig. 5. Infected proximal tubule; the lumen is filled with pseudoplasmodia. Giemsa, × 600. Fig. 6. *S. scardinii* in the lumen of a kidney tubule; note monosporous pseudoplasmodia with mature spores. Fresh preparation, phase contrast, × 930. Fig. 7. Disporic pseudoplasmodium with many refractile granules in the lumen of a kidney tubule. Fresh preparation, × 1500

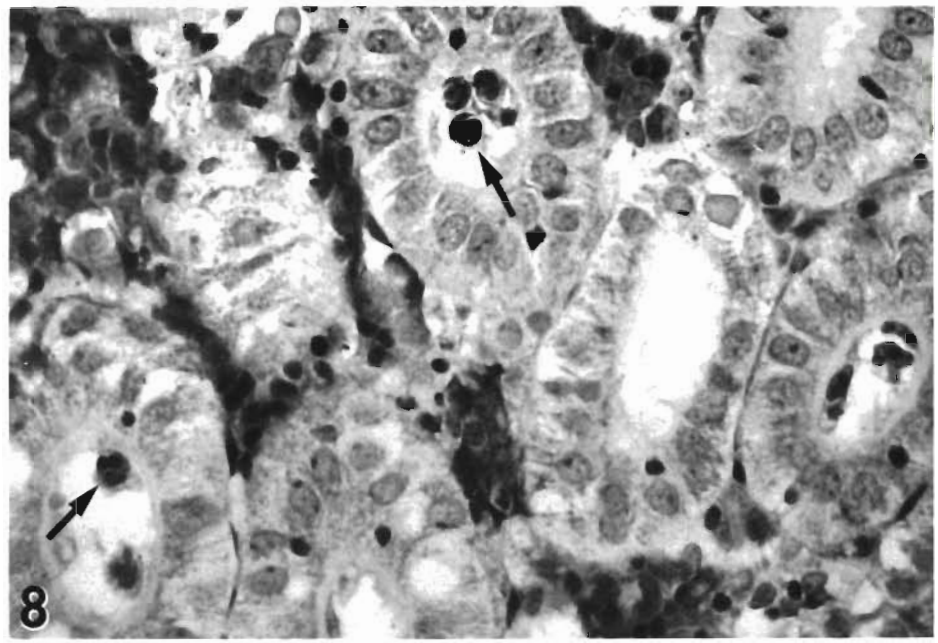


Fig. 8. *Sphaerospora scardinii* infecting *Scardinius erythrophthalmus*. Mature spores (arrows) in the lumen of renal tubules. Giemsa, × 930

mentioned by Hanjavanit (1991) from Ireland, but a detailed description which would have allowed an exact comparison to our material was not provided. The spores of *S. scardinii* are similar to those described from other species of fish, but their shape, size, host and mono- and disporic development distinguish them from other known sphaerospores. A comparison with other species of *Sphaerospora* (Table 1) from the kidneys of freshwater fish indicated that *S. scardinii* differs from them with respect to dimensions of spores and polar capsules, the number of coils of the polar filament and the occurrence of both mono- and disporous development. *Sphaerospora* species infecting kidney tubules of fishes of the subfamily Leuciscinae include *S. poljanski* Kulemina, 1969, *S. rota* Zaika, 1961 and *S. minima* Kashkowsky, 1974 (Shulman 1984). *S. poljanski* and *S. rota* differ from *S. scardinii* in having much larger polar capsules as well as spores (Table 1). Further, mature spores of both those species have no fine ridges at the posterior pole, which are clearly discernible in *S. scardinii*. The dimensions of *S. minima* (from *Rutilus rutilus*) are comparable to those of *S. scardinii*, but its shape differs clearly. Compared with spores of *S. scardinii*, which are almost spherical, those of *S. minima* are pyriformic with a narrow anterior end. According to our own unpublished observations on *S. minima*, the developmental stages of this species clearly diverge in size, shape and location in the kidney from those of *S. scardinii*. Furthermore roach living in the same river as the rudd infected with *S. scardinii* showed no sign of *Sphaerospora* infection. The presence of both mono- and disporic pseudoplasmodia in *S. scardinii* also distinguished them from *S. minima*, which have only disporic pseudoplasmodia. Lom et al. (1983, 1985) redefined the genus *Sphaerospora* and stated that the pseudoplasmodia generally are mono- or disporous according to the species. *S. scardinii* is the first species from a freshwater fish to have shown both mono- and disporic pseudoplasmodia. However, Supamattaya et al. (1991) recently described *S. epinepheli* from kidney tubules of a grouper from marine and brackish waters, which also developed both mono- and disporic pseudoplasmodia.

In addition our material showed that the development of *Sphaerospora scardinii* includes an early intracellular stage which occurs only in epithelial cells of the ureter. In each epithelial cell one mononucleated trophozoite develops (Fig. 4). Furthermore, the extrasporogonic stages of *S. scardinii* observed in the lumina of the ureter seems to be a characteristic feature in the development of this species. These stages, with diameters up to 17 µm, were very similar in shape and structure to those described from the blood of cyprinids infected by other sphaerosporeans (Csaba 1976, Molnar 1984, Lom et al. 1985). However, it cannot be determined by light microscopy whether secondary cells of *S. scardinii* also contain tertiary cells as observed in the stages from cyprinid blood.

Molnar (1988) described the development of *Myxobilatus legeri* in 10 cyprinid fishes. In rudd, the author found intracellular developmental stages and coelozoic plasmodia of this myxosporean in the lumen of renal tubules. Plasmodia and intracellular trophozoites of *M. legeri* were identical in morphology to those of

Table 1. Comparison of *Sphaerospora scardinii* n. sp. with some related *Sphaerospora* spp. L: length; W: width; all dimensions in µm

Parasite (Host)	Infection locus	Spore Range	Spore Mean	Polar capsule Range	Polar capsule Mean	No. of coils	Pseudoplasmodia Range	Pseudoplasmodia Mean
S. scardinii (*Scardinius erythrophthalmus*)	Kidney Tubules	L: 5.97–7.25 W: 5.31–6.30	6.01 5.79	L: 1.80–2.93 W: 1.82–2.41	2.26 2.14	4–5	Mono or disporic L: 10.27–23.64 W: 8.44–15.20	20.27 12.32
S. renicola (*Cyprinus carpio*)	Kidney Tubules	L: 6–8 W: 6.4–8.3	7.3 7.2	L: 1.7–2.3 W: 1.3–1.6	2 1.45	3–5	Disporic Up to 20 µm in size	
S. truttae (*Salmo trutta*)	Kidney Tubules	L: 6.58–8.68 W: 8.22–8.81	6.84 8.81	L: 1.8–2.5 –	2 –	4	Disporic – –	– –
Sphaerospora sp. (*Rutilus rutilus*)	Kidney Tubules	– –	L: 8.5 W: 8.2	– –	L: 3.5 W: 2.9	3	Disporic Up to 14 in size	
S. tincae (*Tinca tinca*)	Head Kidney	L: 7–8.9 W: 6–7.7	8 7	– –	L: 2.7 W: 2.5	3–4	Disporic Up to 18 µm in size	
S. minima (*Rutilus rutilus*)	Kidney Tubules	L: 6–7.7 W: 5.4–6	– –	L: 2.8–3 W: 2.3–2.8	– –	– –	– –	– –
S. poljanskii (*Rutilus rutilus*)	Kidney Tubules	L: 9.5–10 W: 9–10	– –	L: 3–4 W: 2.5–3	– –	– –	– –	– –
S. rota (*Brachymystax lenox, Leuciscus leuciscus, Cobitis taenia*)	Kidney	L: 8.4–11	–	W: 4.2–5.6	–	–	–	–

Sphaerospora scardinii; in addition, the extrasporagonic stages of both species displayed some similarity in shape, but there were differences in morphology.

During our observations no stages could be detected in the blood or in the glomerulus of infected rudd, but the low number of fish examined did not allow us to establish whether blood-stages occur or not. No effects on kidney tubules were apparent during the sporogonic stages of development (Figs. 5 & 8). However, the intracellular trophozoites in the ureter elicited a proliferation of the epithelial cells. A similar but more serious reaction was reported from the swimbladder of carp infected with *Sphaerospora renicola* (Molnar 1984).

The mode of transmission of all sphaerospores described to date is still unknown. However, several species of the family Myxobolidae are known to be transmitted by oligochaetes as intermediate hosts (Wolf & Markiw 1984, El-Matbouli & Hoffmann 1989, Kent et al. 1991, Ruidisch et al. 1991, El-Matbouli et al. 1992). Presently, studies are in preparation to examine the ultrastructure of *S. scardinii* as well as the mode of infection.

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