Morphology and ultrastructure of *Sphaeromyxa noblei* sp. n. (Myxozoa), parasite of *Heteroclinus whiteleggii* (Pisces) from Australian New South Wales coast

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Abstract. A new species, *Sphaeromyxa noblei* sp. n., is described from *Heteroclinus whiteleggii* (Perciformes: Clinidae), a marine fish from the coast of New South Wales in Australia. This raises the number of *Sphaeromyxa* species to 38; their list is presented. The species is characterised by a layer of branched glycostyles, which is about 2.4 µm thick and is a feature rather unique in Myxosporea. Pansporoblasts form one or two spores. The study of ultrastructure of this species and of those described to date result in recognition of a combination of patterns characterising the genus: plasmodia have marked surface projections, the endoplasm is full of vacuoles larger than in any other myxosporean genus, and contains a special kind of cells, the lobocytes. Sections through polar capsule reveal different appearance of subsequent stretches of the polar filament unlike in other Myxosporea.

The genus *Sphaeromyxa* Thélohan, 1892 has included thus far 37 species, which have a rather peculiar morphology in that their polar filament is a flat ribbon folded several times back and forth instead of being tube-like and coiled in several turns like in all other myxosporeans. *Sphaeromyxa* species are coelozoic parasites of gallbladders of marine fish and form typically a large, flat plasmodium. *Sphaeromyxa sabrazesi* Laveran et Mesnil, 1900 was actually the first myxozoan to be investigated by the electron microscope (Grassé 1960). Since then, three species only of this genus were studied ultrastructurally (Lom 1969, Uspenskaya 1982, Gracia et al. 1997) although this genus offers interesting cytological features.

MATERIALS AND METHODS

The parasite was found in the gallbladder of *Heteroclinus whiteleggii* (Ogilby, 1894) (Perciformes: Clinidae) collected in September 1990 in the tide pools at the shore near the Arrawara biological station of the University of New England in Armidale, Australia. The station is situated 24 km north of Coff’s Harbour at the New South Wales coast. The spores were observed, measured and photographed while fresh. The plasmodia were fixed for 60 min in cold 2% osmic acid in 0.1 M cacodylate buffer and embedded in Epon-Araldite. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined under a Philips 420 B electron microscope at 80 kV accelerating voltage.

RESULTS

*Sphaeromyxa noblei* sp. n. Figs. 1–10

Gallbladders of two fish specimens out of four collected were infected. The average size of the leaf-like plasmodium $1 \times 0.5$ mm. The spores 20 (18.5–21.5) µm long (the straight distance between the tips of the arched spore), the thickness 5 (4.8–5.2) µm, the width 5.6 (5.2–6) µm. The elongate oval polar capsules 5.9 (5–6.5) µm long and 2.6 (2.5–2.7) µm wide; the ribbon-like polar filament folded twice longitudinally inside the capsule (Figs. 1, 2).

The surface of the shell valves with longitudinal ridges up to 1 µm deep (as calculated from the transverse section in the TEM). As counted in the TEM from sectioned spores, the number of ridges at about mid-length of the spore was 7 on one valve and 6 to 9 on the other.

Ultrastructure

The thickness of the plasmodium, as calculated from the TEM section, was about 70 to 80 µm. The plasmodium was covered with fine projections (Fig. 3), and from their tips extended ramified strands of mucus, forming together a network about 2.4 µm thick (Fig. 4). The surface membrane, curiously enough, did not reveal any marked pinocytotic vesicles or canals.

The layer of the ectoplasm was homogeneous with some dense granules, was about 15 µm thick, being rather sharply delimited from the endoplasm (Fig. 3). The ectoplasmic margin was full of various vesicles, mostly dense, and of mitochondria. At its peripheral layer, the endoplasm was full of dense vesicles and of vacuoles with granular content, in addition to a few developing spores. At the plasmodium centre, where it was thicker, the endoplasm was replete with large lucent vacuoles with rather straight boundaries with many interspersed vesicles, dark granules, mitochondria, vegetative nuclei of the plasmodium, generative cells and pansporoblasts (Figs. 4–6, 8) with developing and mature spores.
As in other myxosporeans, the sequence of sporogenic cell divisions in the sporoblast could not be safely revealed; it was mostly the final stages of sporogenesis which could be observed. Curiously enough, while some pansporoblasts contained two spores (Fig. 5), the majority of pansporoblasts produced a single spore (Figs. 6, 8). In some pansporoblasts, next to a maturing spore there was a group of isolated cells, probably the result of an aborted sporogenesis.

Membrane of the pericyte cell persisted around the maturing spore, harbouring for some time still cell components like pericyte mitochondria and nucleus. The sporoplasm, occupying the space between the two capsules, had two large nuclei close to each other. The shell valves in a maturing spore had a condensed, dark-appearing cytoplasm. In the grooves between the elevated ridges, there were approximately ten microtubules beneath the cell membrane (Fig. 8, arrow), which were no more visible in the completely dense, homogeneous shell valve of the mature spore. In the not yet mature spore, there was a thin, dense cap (“stopper” – Fig. 5, arrowhead) covering the flat discharge canal of the polar filament; this cap was not distinct in the mature spore.

At the sutures of the two shell valves, the borders of the valves did not face each other but overlapped each other. Thus one valve (the one which bore more ridges at mid-spore length) was fixed in the other one like in a cradle, which was especially evident near the tips of the spore (Fig. 6, arrowhead).

The nascent polar capsule, from which the external tube had already disappeared, contained dense matrix of a more or less homogeneous structure. On a transverse section it revealed plate-like sections (rarely undulated in transverse section) of the future polar filament, with a dark inside, bearing on the surface fine ridges appearing as sectioned microtubules set 12.5 nm apart (Figs. 9, 10). In longitudinal section the filament appeared as an irregularly wound structure reaching outside through the filament discharge canal. The narrow layer of the remnant of capsulogenic cell was squeezed between the rather thin dense layer of the capsule wall, while the lucent layer, known to be chitinous (Lukeš et al. 1993), was about 0.23 µm thick.

**Type and only host:** *Heteroclinus whiteleggii* (Ogilby, 1894) (Perciformes: Clinidae).

**Type locality:** Arrawara, New South Wales, Australia.

**Site of infection:** Gallbladder.

**Prevalence:** 50% (2 fish infected / 4 fish examined).

**Type material:** Phototypes deposited in the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, no. PT 7001.

**Etymology:** The new species is named in honour of Elmer R. Noble, the eminent American parasitologist known also for his studies on Myxosporea.

**DISCUSSION**

**Identity of the species**

Out of the 37 *Sphaeromyxa* species recorded to date, only about 6 species have an arched spore with the two valves equally long. None of these species has a host that lives in the Pacific Ocean at the Australian coast—all live either near Japan or in the Atlantic or Indian Ocean. Their hosts also belong to different orders of fish except for *Pholis pictus*, host of *S. parva* Dogiel, 1948 from the Japan Sea. *S. parva*, however, is smaller in its dimensions (only 15 to 17 µm in length), and the spore is essentially less arched than that of the *Sphaeromyxa noblei*. *S. cottidarum* Dogiel, 1948, except for different hosts (scorpaenid fishes) and their different area of distribution (Japan Sea, Bering Sea and Atlantic Ocean), has considerably longer spores (33–35 µm). *S. elegini* Dogiel, 1948, except for a different host (*Eleginus gracilis*) with a different area of distribution (Japan Sea, Bering Sea), has a spindle-shaped spore only slightly arched, unlike *S. noblei*. *S. exneri* Awerinzew, 1913 also...
Lom: *Sphaeromyxa noblei* sp. n.

Figs. 3–6. *Sphaeromyxa noblei* sp. n., transmission electron micrographs. **Fig. 3.** Edge of the plasmodium covered with small projections bearing mucous strands. E – ectoplasmic layer; En – endoplasm with various cell inclusions, generative cells (G) and somatic nuclei (N). **Fig. 4.** Part of the periphery of the plasmodium with a nascent spore with an immature polar capsule in the pansporoblast (P); arrows point at the surface mucous strands. **Fig. 5.** A pair of obliquely sectioned immature spores within a common pansporoblast (P); S – shell valve; Nr – dense mass representing the residual capsulogenic nucleus; arrow points at the point of exit of the polar filament through the capsular wall; arrowhead marks the dense cap on the filament discharge pore. **Fig. 6.** Section through two spore ends with almost mature polar capsules, both in monosporic pansporoblasts (P). Arrow points at the discharge canal of the polar filament; note the way in which one shell valve is embracing the other (at that point wider) valve (arrowhead).
lives in a different host (a scorpaeniform fish of the family Agonidae) with a different area of distribution (Japan Sea and Indian Ocean) and has only slightly arched spore shape. *S. hellandi* has considerably longer spores (20–26 µm) and lives in a gadid fish and though it was also reported from a perciform fish, it was from a different family (Pholidae). *S. hexagrammi* Dogiel, 1948 differs in living in different hosts (scorpaenid fishes in Japan Sea) and has slightly shorter spores (average 18 µm). *S. solomoni* Aseeva, 2002 from different hosts and Japan Sea has longer spores (25–27.5 µm). Although this account does not take into consideration the properties of the plasmodium (often disregarded), we can take for granted that the species under study is a new species.

We attach a list of *Sphaeromyxa* species at the end of the discussion, since according to our records the recent list of Aseeva (2002) is not complete. Browsing through the list, one can only wonder in how different hosts – even in different orders – some species of this genus live. This especially applies to *S. balbianii*; if all records really represent one species, which should be supported by molecular proof, it indicates an extremely low host specificity. The list also shows how little is the genus known – few of the species have been recorded more than once.

**Ultrastructural comparison**

The mucous “branches”, identifiable with glyco-styles, covering the surface of the plasmodium, are a

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**Figs. 7–10. Sphaeromyxa noblei** sp. n., transmission electron micrographs. **Fig. 7.** Section through an almost mature spore with coils of the polar filament still in formation. Arrow points at the narrow discharge canal for the flat filament; Nr – residual nucleus of the capsulogenic cell; S – shell valve. **Fig. 8.** Transverse section through the end of spore at the level of the polar capsule with coils of the almost mature polar filament in the dark capsular matrix. P – pansporoblast harbouring a single spore; arrowheads point at the junction of one shell valve covering the other; arrow points at the microtubules in the longitudinal furrow of the shell. **Figs. 9, 10.** Enlarged transverse sections of polar filament parts from Fig. 8; note the dark inner lining of the flat filament lumen and fine ridges of the surface of the filament. Scale bars: Figs. 9, 10 = 100 nm.
rather rare feature in myxosporean plasmodia. Grassé and Lavette (1978) did not observe any remarkable cell coat in *S. sabraezi*, while in *S. balbianii* Gracia et al. (1997) recorded a horizontal mucus layer, perhaps of plasmodial origin, touching the tips of the cell projections. In very few myxosporean species there exist similarly elaborate, glycostyle-like structures like in *S. noblei*. Thus at the surface of the histozoic plasmodium of *Myxobolus disparoides* there are pyramidal structures about 0.3 μm high composed of several layers (Uspenskaya 1984); tiny stratified tubercles are also on the surface of plasmodia of *Thelohanellus pyriformis* in blood vessels of tenches (Dyková and Lom 1987). In plasmodia of other species, the cell coat, if present at all, has the value of nanometres, not micrometres. Thus in the histozoic plasmodium of *Henneguya adipsa* the cell coat is about 80 nm thick (Current 1979); in *M. funduli* the granular cell coat is about 60 nm thick (Current et al. 1997), and coelozoic plasmodia of *Myxidium lieberkuehni* have a very thin layer of cell coat (Lom and de Puytorac 1965). Curiously, the surface of spores of *Henneguya pilosa* bears cell coat digitiform differentiation (Azvedo and Matos 2003); here these structures may serve for better buoyancy of spores in water. Factors that elicit the occurrence of different degree of cell coat development in plasmodia, however, have yet to be discovered.

The fine microtubule-like ridges on the surface of the nascent polar filament have a spacing (12.5 nm) similar to analogous structures on other filaments in myxosporean and actinosporean stages, where the spacing averages 11 to 12 nm (Lom and Dyková 1997) and also exemplifies the structural unity of these organisms.

Although only scarce data have been obtained on the fine structure of the genus *Sphaeromyxa* (Lom 1969, Grassé and Lavette 1978, Uspenskaya 1982, Gracia et al. 1997, the present paper), it appears that the structure of its species follows the same pattern: plasmodia have marked surface projections; the endoplasm is full of extremely large vacuoles; the lobocytes, absent in other genera, are of common appearance; and sections through polar capsule reveal different appearance of subsequent stretches of the polar filament unlike in other Myxosporea. The separateness of this genus should be confirmed by molecular analysis, which we—because of shortage of material—could not perform.

**List of species of the genus Sphaeromyxa**

(in alphabetical order, host species names follow mostly the original sources)

*S. arcuata* Fantham, 1930

*Macropius nasutus*, *Bathygobius sorporator*, *Argyrozoa argyrozona*; Atlantic Ocean off Namibia; Fantham 1930

*S. argentinensis* Timi and Sardella, 1998

*Engraulis anchoita*; South-west Atlantic, Argentine Sea; Timi and Sardella 1998

*S. atherinae* Karataev et Iskov, 1984

*Atherina mochon pontica*; Black Sea; Karataev and Iskov 1984

*S. balbianii* Thélohan, 1892 – type species


*S. bonaerensis* Timi et Sardella, 1998

*Anchoa marina*; South-west Atlantic, Mar del Plata Port; Timi and Sardella 1998

*S. cottidarum* Dogiel, 1948

*Hemitrupiterus villosus*, *Enophris diceraus*, *Enophris sp.*, *Blepsias sp.*; Atlantic Ocean, Bering Sea, Japan Sea; Dogiel 1948, Shulman 1966

*S. curvula* Fantham, 1930

*Helicolenus dactylopterus*, *Pachymetopon blochii*; Atlantic Ocean off Namibia; Fantham 1930

*S. digiae* Sarkar et Majumder, 1983

*Hilsa ilisha*; Bay of Bengal; Sarkar and Majumder 1983

*S. elegini* Dogiel, 1948

*Eleginus gracilis*; Japan Sea, Bering Sea; Dogiel 1948, Shulman 1966

*S. exneri* Awerinzew, 1913

*Thrysanophrys japonicus*, *Sarritor leptocephalus*; Indian Ocean (Mosambique Channel), Japan Sea; Awerinzew 1913, Kudo 1919

*S. ganapatii* Karataev et Iskov, 1984

*Terapon jarbua*; Chilka Lake, India; Kalavati and Vaidehi 1991

*S. gasterostei* Georgévitch, 1916

*Gasterosteus spinachia*; Mediterranean Sea; Georgévitch 1916, Kudo 1919

*S. gibbonsia* Noble, 1939

*Gibbonsia elegans*, *G. metzi*; Pacific Ocean; Noble 1939

*S. harenii* Sarkar, 1984

*Tachysurus platystomus*; Bay of Bengal; Sarkar 1984

*S. hellandi* Auerbach, 1909


*S. hexagrammi* Dogiel, 1948

*Hexagrammus octogrammus*, *H. stelleri*, *Pleurogrammus azonus*; Japan Sea; Dogiel 1948, Shulman 1966
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