STUDIES ON SPHAEROMYXA SPP. (THÉLOHAN, 1892) (MYXOZOA) FROM SELECTED FISH SPECIES

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Introduction

Sphaeromyxa species (Myxozoa: Bivalvulida) are coelozoic parasites of marine fish infecting the gall bladder. Characteristics are based on Lom and Noble's (1984) description of the family *Sphaeromyxidae* with spores being elongate or curved with tapering and truncate ends, opening in the level of the suture line. Spores possess one binucleate sporoplasm and two polar capsules at opposite ends of the spore. Valves may be smooth or ridged. Flat, leaf-like plasmodia form polysporic pansporoblasts and float freely in the gall bladder. Plasmodium may be over 2cm in diameter (e.g. *S. maiyai*, Morrison and Pratt, 1973) and are in fact amongst the largest protists known.

Myxosporean coelozoic infections are usually less harmful to the fish than histozoic infections and it has therefore been postulated that coelozoic myxosporeans are the more primitive form of the two (Shulman, 1966). *Sphaeromyxa* spp. infections are fairly innocuous and cause little pathological effect to the host. However, pathological changes have been observed including hypertrophied gall bladders and discoloration (*S. ganapatii*, Kalavati & Vaidehi, 1991).

This poster provides a description on the structure and development of several *Sphaeromyxa* species from various marine fish and data on the 33 *Sphaeromyxa* spp. discovered to date.

Materials and Methods

Fish were caught from various localities off the coast of United Kingdom (see Table 1 for details). Each were dissected and examined under a stereomicroscope for evidence of parasitism. Plasmodia found in the gall bladders were removed and were initially observed on a Nikon Eclipse E800 light microscope. If spores were detected, measurements were taken. Material was then processed for electron microscopy. Tissues were prefixed with 2.5% glutaraldehyde in cacodylate buffer (0.1M, pH 7.4, 2h, 4°C) and post-fixed with 2% OsO_4 in the same buffer (30 min), dehydrated with increasing concentrations of ethanol/acetone. For TEM, samples were embedded in epoxy resin, semi-thin (1µm) sections were obtained and stained with toluidine blue for preliminary observation. Ultra-thin (75-80nm) sections were floated onto copper grids and double stained with uranyl acetate and Fahmy's lead citrate and were observed using JEOL JEM-1210 Electron Microscope. For SEM, samples were CO_2 critical point dried and coated in gold (10nm thick) and observed using JEOL JSM-5200 Scanning Microscope.

Results

Two-spot goby (*Gobiusculus flavescens* Fabr. 1779): Plasmodia discoid and found floating freely within the gall bladder. Up to 4mm in diameter. Spores straight in frontal view, fusiform in sutural view and with truncated blunt ends. Polar capsules ovoid in shape.
Dimensions based on fresh spores (n=10) in micrometers with the mean followed by the range in parentheses: spore L=19.7 (18.2-21.0); spore W=4.3 (3.8-4.7); polar capsule L=5.8 (5.3-6.4) polar capsule W=2.9 (2.5-3.2).

Haddock (*Melanogammus aeglefinus* L. 1758): Plasmodia discoid and very large, more than 2cm in diameter in some cases, with some gall bladders containing multiple plasmodia. Spores arcuate with rounded ends in frontal view and with pyriform polar capsules.
Dimensions based on fixed spores (n=15) in micrometers with the mean followed by the range in parentheses: spore L=19.4 (17.7-21.9); spore W=4.8 (4.0-5.9); polar capsule L=6.2 (5.0-7.2); polar capsule W=2.7 (2.2-3.2).

Long-spined sea scorpion (*Taurulus bubalis* Euph. 1786): Plasmodia discoid and up to 4mm in diameter. Spores straight or sometimes slightly curved in frontal view with truncated, blunt ends. Polar capsules ovoid with extruded polar filament measuring at 23µm in length.
Dimensions based on fresh spores (n=15) in micrometers with mean followed by the range in parentheses: spore L=18.7 (17.6-19.7); spore W=4.9 (4.2-6.1); polar capsule L=6.3 (5.3-7.4); polar capsule W=3.8 (3.3-4.5)

Four-bearded rockling (*Enchelyopus cimbrius* L. 1766): Plasmodia round and up to 8mm in diameter. Spores arcuate in frontal view with gently rounded ends. Polar capsules very large and pyriform in shape. Dimensions based on fixed spores (n=15) with the mean followed by the range in parentheses: spore L=20.6 (18.0-21.8); spore W=5.0 (4.2-5.9); polar capsule L=8.1 (6.7-9.0); polar capsule W=2.7 (2.2-3.3).

Fig 1. Sphaeromyxa balbianii from two-spot goby (Gobiusculus flavescens).

Fig 2. Sphaeromyxa cf. hellandi from haddock (Melanogrammus aeglefinus).

Fig 3. Sphaeromyxa balbianii from long-spined sea scorpion (Taurulus bubalis) showing extruded polar filament.

Fig 4. Sphaeromyxa cf. curvula from four-bearded rockling (Enchelyopus cimbrius.)

Host Species	Plasmodium	Spore Dimensions	Polar Capsule Dimensions	Sphaeromyxa sp.
Two-spot goby (Gobiusculus flavescens)	Up to 4mm in diameter	[illegible]	[illegible]	S. balbiani
Haddock (Melanogrammus aeglefinus)	Up to 20mm in diameter	[illegible]	[illegible]	S. cf. hellandi
Long-spined sea scorpion (Taurulus bubalis)	Up to 4mm in diameter	[illegible]	[illegible]	S. balbiani
Four-bearded rockling (Enchelyopus cimbrius)	Up to 8mm in diameter	[illegible]	[illegible]	S. cf. curvula (or previously undescribed species)

Table 1: Summary of results

Fig 5a. Plasmodium surface of Sphaeromyxa from M. aeglefinus. Bar = 500nm.

Fig 5b. Plasmodium surface of Sphaeromyxa from T. bubalis. Bar = 500nm.

Fig 5c. Plasmodium surface of Sphaeromyxa from E. cimbrius. Bar = 500nm.

Fig 6. Longitudinal section of sporoblast showing two capsulogenic cells (c), one valvulogenic cell (v) and a binucleate sporoplasm (s). Bar = 1µm.

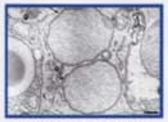

Fig 7. A binucleate sporoplasm, note the presence of nuclear pores (arrow) and Golgi (g). Bar = 500nm.

Fig 8. High power view showing various profiles of dome-shaped sporoplasmosomes. Bar = 200nm.

Fig 9. Longitudinal section of an almost mature polar capsule showing the coiled polar filament(s) with two exit points(arrows)? Bar = 500nm.

Table 2: Summary data on *Sphaeromyxa* species.

Species Reference	Plasmodium	Spore Shape	Spore Dimensions	Polar capsule Shape	Polar capsule Dimensions	Type Host	Geographical Distribution
1. S. balbiani Thélohan, 1892	3-4mm	Straight, fusiform. Ends truncate.	[illegible]	Ovoid	[illegible]	[illegible]	France, USA, Canada, Brazil
2. S. incurvata Doflein, 1898	5-7mm	Arcuate. Ends truncate.	[illegible]	Pyriform	[illegible]	Blennius ocellaris	Napoli, Black Sea
3. S. sabrazesi Laveran & Mesnil, 1900	2mm	Arcuate. Ends truncate.	[illegible]	Pyriform	[illegible]	Hippocampus sp.	France, Hungary, Norway, Monaco
4. S. hellandi Auerbach, 1909	-	Arcuate. Ends truncate.	[illegible]	Pyriform	[illegible]	Molva molva (=Molva vulgaris)	Norway, Barents Sea
5. [illegible] Awerinzew, 1913	-	Arcuate. Ends slightly tapering.	[illegible]	Pyriform	[illegible]	[illegible]	Africa
6. [illegible] Georgévitch, 1916	Large	Large, elongated, fusiform.	2-3 times larger than S. balbiani	Ovoid	-	Spinachia spinachia (=Gasterosteus spinachia)	France
7. [illegible] Dunkerly, 1921	-	Slightly curved, fusiform.	[illegible]	Ovoid	-	[illegible]	England
8. [illegible] Jameson, 1929	Very small	Straight or slightly curved. Fusiform.	[illegible]	Ovoid	-	[illegible]	California
9. [illegible] Fantham, 1930	-	Arcuate, ends bluntly rounded.	[illegible]	Pyriform	[illegible]	[illegible]	S.Africa
10. S. curvula Fantham, 1930	-	Arcuate, ends bluntly rounded.	[illegible]	Pyriform	[illegible]	[illegible]	S.Africa
11. [illegible] Noble, 1939	[illegible]	Slightly curved, elongate.	[illegible]	Ovoid	[illegible]	[illegible]	California
12. [illegible] Noble, 1939	1mm	Oval, rounded ends.	[illegible]	Ovoid	[illegible]	[illegible]	California
13. [illegible] Noble, 1941	[illegible]	Slightly curved, oval	[illegible]	Ovoid	[illegible]	[illegible]	California
14. [illegible] Dogiel, 1948	Small	Large, arched with blunt ends.	[illegible]	Pyriform	[illegible]	[illegible]	Sea of Japan, Bering Sea
15. [illegible] Dogiel, 1948	[illegible]	[illegible]	[illegible]	Ovoid	[illegible]	[illegible]	Sea of Japan
16. [illegible] Dogiel, 1948	-	Arched, blunt ends.	[illegible]	Pyriform	[illegible]	[illegible]	Sea of Japan
17. [illegible] Dogiel, 1948	-	Small, slightly curved.	[illegible]	Pyriform	[illegible]	[illegible]	Sea of Japan
18. S. pultai Tripathi, 1952	[illegible]	Long, straight with truncated ends.	[illegible]	Pyriform	-	[illegible]	India
19. S. theraponi Tripathi, 1952	-	Arched in front view.	[illegible]	Pyriform	[illegible]	Therapon jarbua	India
20. [illegible] Laird, 1953	5-7mm	Arcuate, truncated ends.	[illegible]	Pyriform	[illegible]	[illegible]	New Zealand
21. [illegible] Polyanskii, 1955	-	[illegible], straight, with blunt ends.	[illegible]	Ovoid	[illegible]	[illegible]	Barents Sea
22. [illegible]	[illegible]	Large, straight, with blunt ends.	[illegible]	Ovoid	[illegible]	[illegible]	Barents Sea
23. [illegible] 1970	-	Short ends, straight	[illegible]	Ovoid	[illegible]	Gobius niger	Black Sea, USA
24. S. maiyai Morrison & Pratt, 1973	[illegible]	Elongate, arcuate in front view.	[illegible]	Pyriform	[illegible]	[illegible]	USA
25. [illegible] 1982	-	Straight, blunt ends.	[illegible]	Ovoid	[illegible]	[illegible]	SW Atlantic
26. [illegible] Sarkar & Majumder 1983	-	Large, broad, serrate.	[illegible]	Pyriform	[illegible]	[illegible]	India
27. [illegible] 1984	-	Straight, arched on one side.	[illegible]	Ovoid	[illegible]	[illegible]	-
28. [illegible] Sarkar, 1984	-	Fusiform, slightly curved.	[illegible]	Ovoid	[illegible]	[illegible]	India
29. S. ganapatii Kalavati & Vaidehi, 1991	-	Crescent shaped, rounded ends.	[illegible]	Pyriform	[illegible]	[illegible]	India
30. [illegible] Xie & Gong, 1993	-	-	-	-	-	-	China
31. [illegible] Su & White, 1994	-	Elongate, fusiform, serrate.	[illegible]	Pyriform	[illegible]	[illegible] sp.	Australia
32. [illegible] Timi & Sardella, 1998	[illegible]	Elongate, fusiform, serrate.	[illegible]	Pyriform	[illegible]	[illegible]	Argentine Sea
33. [illegible] Timi & Sardella, 1998	[illegible]	Fusiform, slightly serrate.	[illegible]	Ovoid	[illegible]	[illegible]	Argentine Sea

Note: [illegible]

Fig 10. SEM of plasmodium from M. aeglefinus showing rugose folds.

Fig 11. Spore of S. cf. hellandi showing longitudinal striations.

Fig 12. Spore of S. cf. hellandi split along the sutural line showing polar capsule.

Fig 13. Spore of S. cf. hellandi emerging from surface of the plasmodium. Note the sinuous suture line (arrow).

Discussion

Laird (1953) unofficially split the genus into two distinct groups and this has aided in the identification and description of *Sphaeromyxa* species:

- *Balbianii* group: Straight or slightly curved fusiform or ovoid spores having ovoid polar capsules.
- *Incurvata* group: Arcuate spores having pyriform capsules.

Although some species such as *S. pultai*, Tripathi, 1952 and *S. maiyai*, Morrison & Pratt, 1973 can exhibit intermediate characteristics. In both groups, ends may be blunt or rounded.

In the current study, two species fall into the *balbianii* group (from sea scorpion and two-spot goby) and the other two fall into the *incurvata* group (from haddock and four-bearded rockling). *Sphaeromyxa* spp. have already been described in one of the hosts above. Kabata (1963) observed *S. hellandi* in *M. aeglefinus* (=*Gadus aeglefinus*). However, as Kabata did not include any spore measurements or diagrams of the parasite, the identification of the parasite is questionable. Auerbach's (1909) original description of *S. hellandi* had the following spore dimensions: length=21-26µm, width=5µm; polar capsule length=10-11µm. As can be seen in Table 1, the dimensions we have from *M. aeglefinus* are very much smaller than those of *S. hellandi*. It is possible that one host can harbour different species of the same protistan genus as in the case of fish species *Therapon jarbua* which has been noted to have two *Sphaeromyxa* species; *S. theraponi*, Tripathi, 1952 and *S. ganapatii*, Kalavati & Vaidehi, 1991. It may be that haddock also harbours more than one *Sphaeromyxa* species or that the spore dimensions were smaller due to the process of fixation. Until further research can be done, the *Sphaeromyxa* species from *M. aeglefinus* is recorded as *S.* cf. *hellandi*.

By comparing with other *Sphaeromyxa* species (see Table 2), those from sea scorpion and two-spot goby are identified as *S. balbianii* (Thélohan, 1892). *S. balbianii* was originally found in shore rockling (*G. mediterraneus*) and as both species of fish in the present study occupy the same habitat (rocky shores), it is reasonable to assume that they would harbour the same species of parasite.

The parasite from four-bearded rockling most closely resembles *S. curvula*, Fantham, 1930 in shape and dimensions, but as *S. curvula* was found in South African waters and in a different species of fish, the rockling *Sphaeromyxa* is likely to be a previously undescribed species.

Ultrastructure of *Sphaeromyxa* species

Sphaeromyxa spp. in the present study appear to follow the normal developmental patterns as other *Sphaeromyxa* spp. described (Lom, 1969, Gracia et *al.*, 1997). Mature spores were typical of the genus with fusiform or curved spores with truncated ends. Each possessed deep, longitudinal surface ridges, clearly visible by electron microscopy.

Although some features of sporgonic stages differed between the species examined, there were relatively few major differences recorded. Sporoplasmosomes were a prominent feature of the sporoplasm of the species from haddock but were not clearly seen in any other *Sphaeromyxa* species examined. Differences in the microvillosities on the plasmodial surface were apparent between species. However, the effect of maturation of the plasmodia in each species may have a significant effect on the ultrastructural appearance. Likewise, the appearance of sporoplasmosomes seem to occur later in sporogenesis.

Conclusions

A thorough review of the taxonomy of the genus *Sphaeromyxa* and other marine myxozoa is recommended especially due to recent revelations about the life-cycle of myxosporeans. It is clear that new research is necessary before a robust classification of this group can be achieved.

Due to the uncertainty and difficulty of identification of *Sphaeromyxa* spp. encountered in this study based on classical morphology, molecular biology will be one way to assist in the classification of *Sphaeromyxa* and *Myxozoa* in general.

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