**Introduction**

Multivalvid myxospores represent the marine order Multivalvulida that are predominantly histozoic and found infecting the musculature of marine fishes. The order currently contains only two families, Trilosporidae and Kudoidae. The Trilosporidae have three polar capsules and three shell valves and are considered to be the basal group of the order. The Kudoidae now accommodate all marine myxospores with four or more shell valves and polar capsules and contains over 60 described species. The multivalvids are important parasites that can have a considerable negative impact to the condition of farmed marine fish in and commercial fisheries. Currently, they are the only large group of myxozoans that no life cycle or alternate host data is available for.

Hyperparasitism of platyhelminths by myxozoa is not common. However, myxospores from the genus Fabespora have been reported, on two separate occasions, infecting the tegument and parenchyma of different digenene trematodes and the assumed hyperparasitism by Myxidium giardi has also been reported in the gill monogenean Pseudodactylogyrus bini from the European eel Anguilla anguilla.

**Materials and methods**

Gill monogeneans were removed from host fish and mounted on glass slides for microscopy. Some specimens were fixed, stained with Gomori’s trichrome stain and mounted in Canada balsam, others were pooled and used in the DNA study. Small subunit ribosomal DNA sequences were compared to others in the data bases and phylogenetic analyses performed using representatives of all major myxozoan taxa.

**Results**

Myxosporean hyperparasites with Myxidium-like myxospores were observed infecting the gill monogeneans from both Platycopheus sp. from Japan (Figs 1-4) and Megalops cynocephalus from Malaysia (Figs 5 & 6). In both cases infected monogeneans showed marked pathological changes, when compared to uninfected individuals from the same fish host (Figs 1 & 2). Infected monogeneans had poor internal structure definition and parenchymal tissues packed with free spores and disporous plasmodia (Figs 3-6). Spore morphology was similar to that of Myxidium, being fusiform with opposing pyriform polar capsules. Average size measurements for fixed spores of the Japanese hyperparasite were 9 x 4.5 µm and unfixed spores of the Malaysian hyperparasite measured 11.5 x 4.3 µm (n=8).

The two hyperparasites are 93% similar to each other over the entire SSU of approximately 1700 bases. Phylogenetic analyses (Figs 7 & 8) showed that both hyperparasite DNA sequences formed a discrete, well-supported clade immediately below Unicapsula spp. in the phylogenetic tree and were basal to the entire multivalvid clade.

**Discussion**

Hyperparasitism by myxozoans has been reported rarely, and the discovery of two geographically distinct cases with morphological and phylogenetic similarities, occurring within different gill monogeneans, is significant. Infections in both monogeneans were often advanced causing significant tissue damage to the worms. The presence of disporous plasmodia and the sheer scale of the infection strongly suggest that myxosporean development took place inside the worms and that the presence of myxospores in the internal tissues was not a result of accidental consumption by grazing epithelium from myxosporan-infected fish tissues, and indeed, no such infection was evident on the fish gill tissues or was detectable with the specific PCRs.

Spor morphology was difficult to assess during the study and features such as the location and orientation of the sartorial line and hence definitive evidence of a bivalvid formation was not clear. However, no evidence of a third reduced valve, as seen in the Trilosporidae, was observed only inside the gill monogeneans, in some cases after fixation and staining. Both hyperparasites had similar shaped spores; the Malaysian spores were longer and had a more slender appearance, however, non-fixed spore dimensions are not available the Japanese samples. The spores of Fabespora, hyperparasitic myxospores of digeneans, are morphologically distinct from those in the present study having blunt ends with elongated valves perpendicular to a prominent central transverse sartorial line. Myxidium-shaped spores have a polyphyletic distribution throughout the Myxozoan, and hence classification on spore form alone is not possible.

The length of the SSU was relatively short, approximately 1700 bases, but consistent with other multivalvid taxa. In the phylogenetic analyses the hyperparasite sequences are consistently and robustly located together at the base of the multivalvid clade irrespective of the tree-building methodology used, strongly suggesting they are basal multivalvids and ancestral to the group. In both Bayesian analysis (not shown) of all multivalvid taxa and the maximum likelihood analysis of truncated sequence data, the previously unexplained Sphaerospora clade that has been shown to be weakly supported inside the Kudoidae now appears to be basal to the main Kudoidae group, which makes its presence easier to explain than when previously embedded within the Kudoidae clade.

The Myxozoan are known to infect an extremely wide range of both vertebrate and invertebrate groups. Therefore, it is not surprising that monogeneans and other platyhelminths can also act as myxospore hosts. The life cycles of marine myxospores are poorly understood compared to the freshwater group, and little data on invertebrate or non-fish hosts are currently available for the entire multivalvid clade. Thus, it is imperative to keep an open mind as to what organisms could potentially be involved and how host transmission events could take place in multivalvid myxosporean life cycles.

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**Figures**

- **Fig. 1** - Hyperparasitic Myxidium-like myxozoan infecting Halotrematidae sp. from Platycopheus sp. in Japan. Fig 1-2 Infected and uninfected Halotrematidae sp. stained with Gomori’s trichrome. Fig 3. Higher power magnification of the boxed section in Fig 1. revealing a lack of internal structures in the underlying tissues surrounding the male copulatory organ (m) and the scerotised parts of the vagina (v). Fig 4. Higher power magnification of Myxidium-like myxosporean spores, some of which appear to be contained within a plasmoidal membrane (black arrows).

- **Fig. 2** - Myxidium-like spores in Megalops cyanasps from Malaysia. Fig 5 litter visible internal organisation is present and numerous spores replace tissues between the testes (Te) and the tegumental spines to the posterior (black arrows). Fig 6 ammonium picrate / glycerine cleared unstained temporary mount with four disporous plasmodia visible (black arrows).

- **Fig. 3** - Unrooted maximum likelihood phylogram of approximately 630 bases of SSU data including the V4 region for selected multivalvid taxa. Based on the evolutionary model TIN-G numbers at the nodes indicate bootstrap support that was assessed using 1000 replications.

- **Fig. 4** - Maximum parsimony tree of marine myxozoans. Bootstrap support values are calculated from 1000 replications, a solid circle at a node represents a support value of >95% (scale = 50 changes).

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**References**

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