

MYXIDIUM-LIKE MYXOSPOREAN HYPERPARASITES OF GILL MONOGENEANS ARE BASAL MULTIVALVULIDS

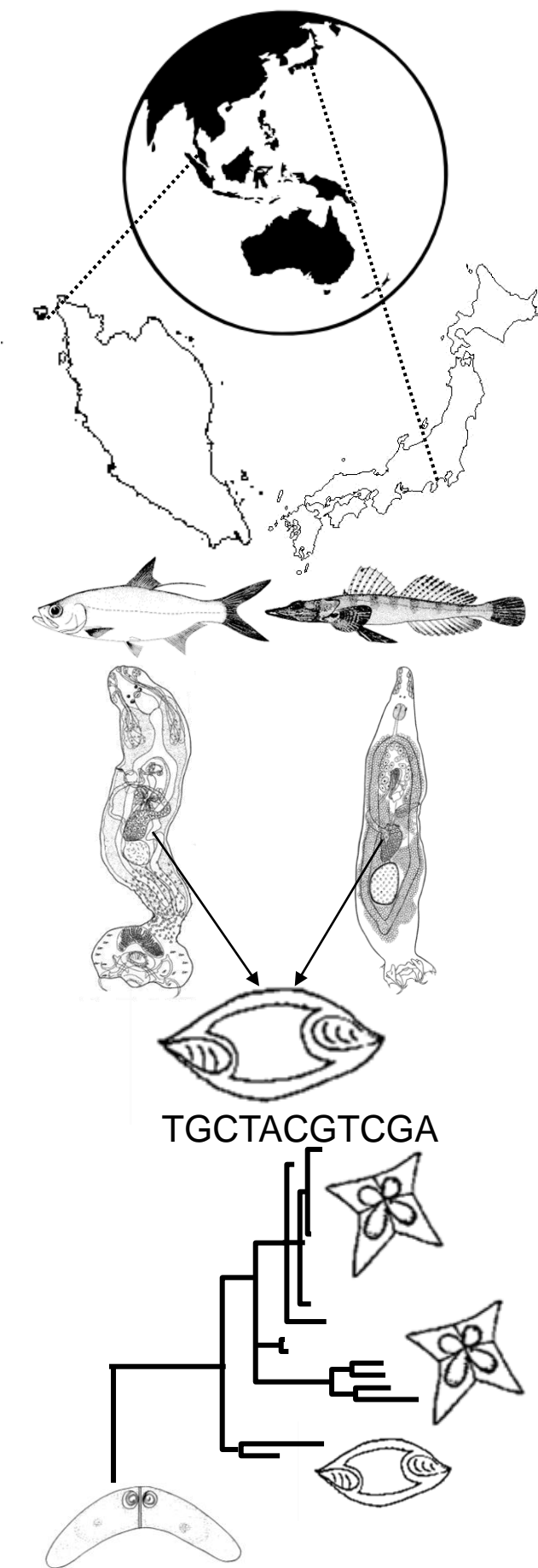
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Abstract

Gill monogeneans *Haliotrema* sp. infecting *Platycephalus* sp. from Lake Hamana, Japan and *Diplectanocotyla* sp. infecting *Megalops cyprinoides* from Langkawi, Malaysia were found to contain myxozoan myxospores. Infected monogeneans showed marked pathological changes having poor internal structure definition and parenchymal tissues packed with disporous plasmodia and free myxospores. Spore morphology was similar to that of *Myxidium* being basically fusiform with a slightly sigmoid curve to pointed ends where opposing pyriform polar capsules were located. Analysis of the small subunit rDNA (SSU) sequence data revealed a similar BLAST profile for both hyperparasites each having approximately 90% similarity to numerous multivalvulid myxosporeans. The two hyperparasites are 93% similar to each other over the entire SSU of approximately 1700 bases. Phylogenetic analyses showed them to group together at the base of the Kudoa / Multivalvulida clade, suggesting that they are ancestral to the group and the last known bivalvulid ancestors to the marine multivalvulid order. Simultaneous infections in the fish gills were not microscopically apparent, nor were they detectable using specific PCRs performed from DNA extracted from gill filaments, suggesting that the infection may be limited to the monogenean worms. The life cycles of marine myxosporeans are poorly understood compared to the freshwater group, and little data on invertebrate or non-fish hosts are currently available for the multivalvulid order. As the Myxozoa have been shown to infect a wide range of both vertebrate and invertebrate hosts, alternate hosts for the multivalvulids should not be assumed to be limited to annelid worms. Therefore, there remains the possibility that monogeneans could be involved in the life cycles of some marine myxozoans.



Introduction

Multivalvulid myxosporeans 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 accommodates all marine myxosporeans with four or more shell valves and polar capsules and contains over 60 described species. The multivalvulids are important parasites that can have a considerable negative impact to the condition of farmed marine finfish 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 platyhelminthes by myxozoans is not common. However, myxosporeans from the genus *Fabespora* have been reported, on two separate occasions, infecting the tegument and parenchyma of different digenean 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 *Platycephalus* sp. from Japan (Figs 1-4) and *Megalops cyprinoides* 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 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 multivalvulid order.

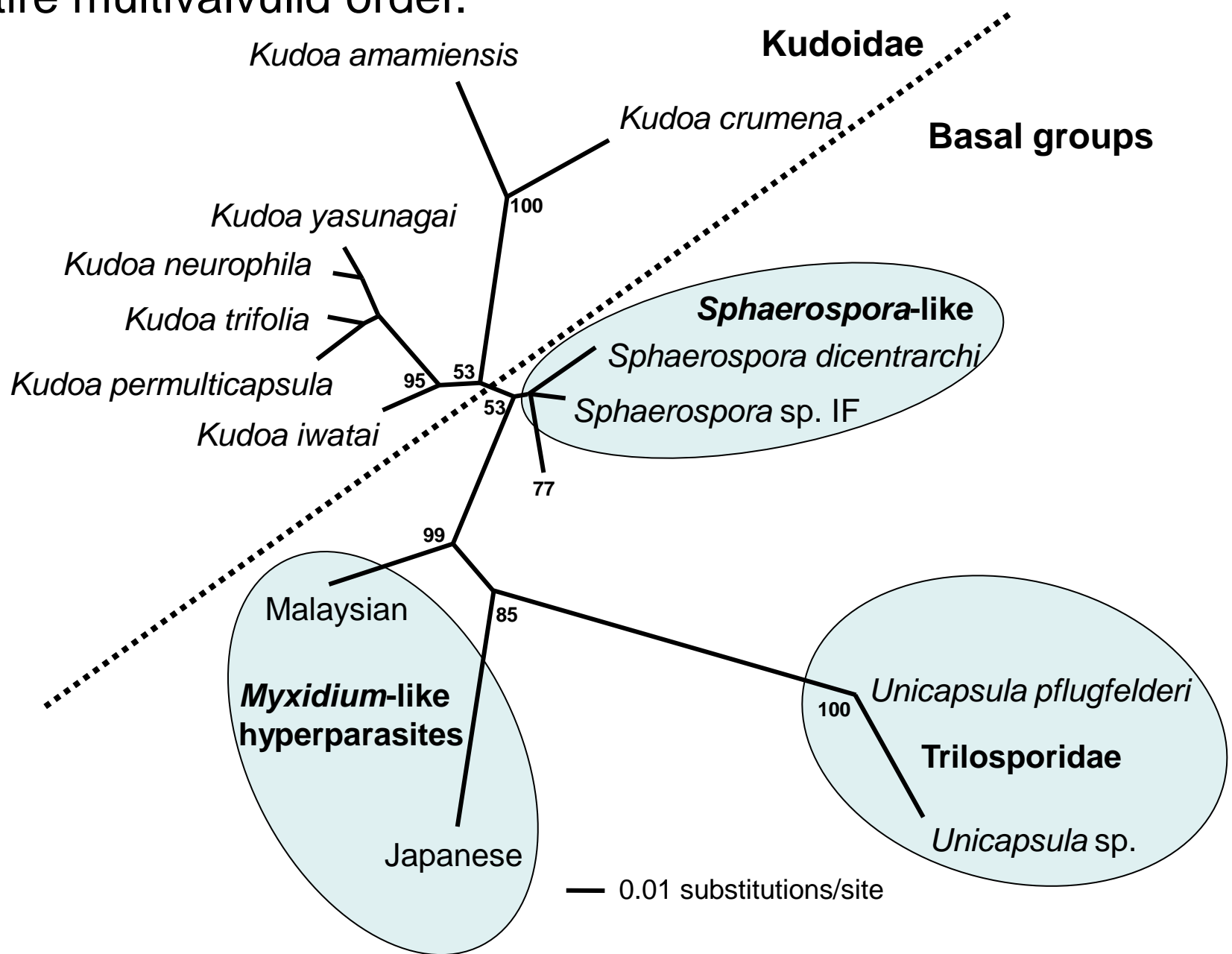
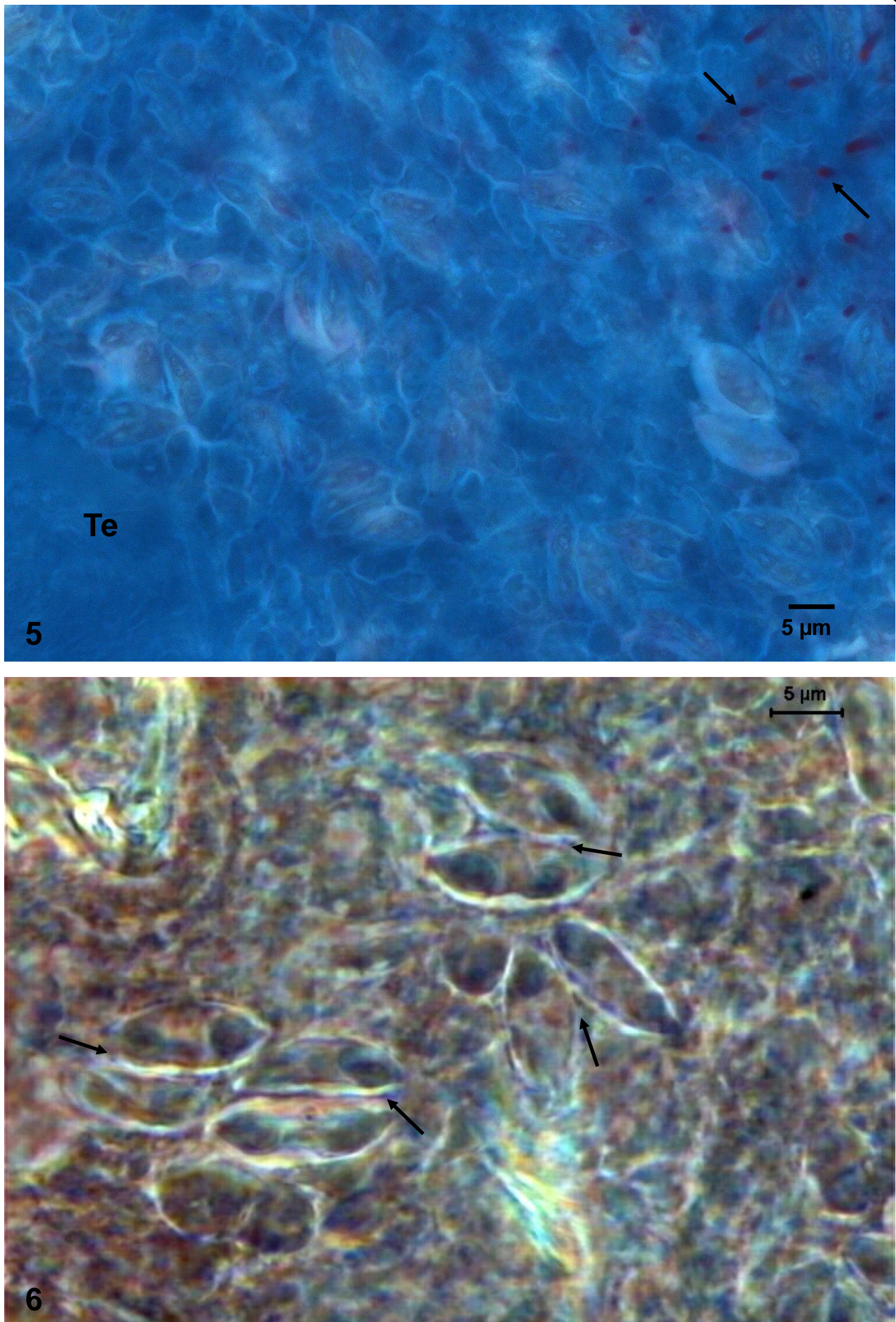


Fig. 7 Unrooted maximum likelihood phylogram of approximately 630 bases of SSU data including the V4 region for selected multivalvulid taxa. Based on the evolutionary model TrN+G numbers at the nodes indicate bootstrap support that was assessed using 1000 replicates.



Figs. 5 & 6 *Myxidium*-like spores in *Diplectanocotyla* sp. from *Megalops cyprinoides* from Malaysia. Fig 5 little 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)

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 myxosporean-infected fish tissues, and indeed, no such infection was evident on the fish gill tissues or was detectable with the specific PCRs.

Spore morphology was difficult to assess during the study and features such as the location and orientation of the sutural line and hence definitive evidence of a bivalvulid formation was not clear. However, no evidence of a third reduced valve, as seen in the Trilosporidae, was present. Spores were only observed 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 myxosporeans of digeneans, are morphologically distinct from those in the present study having blunt ends with elongated valves perpendicular to a prominent central transverse sutural line. *Myxidium*-shaped spores have a polyphyletic distribution throughout the Myxozoa, 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 multivalvulid taxa. In the phylogenetic analyses the hyperparasite sequences are consistently and robustly located together at the base of the multivalvulid clade irrespective of the tree-building methodology used, strongly suggesting they are basal multivalvulids and ancestral to the group. In both Bayesian analysis (not shown) of all multivalvulid 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 *Kudoa* clade.

The Myxozoa are known to infect an extremely wide range of both vertebrate and invertebrate groups. Therefore, it is not surprising that monogeneans and other platyhelminthes can also act as myxozoan hosts. The life cycles of marine myxosporeans are poorly understood compared to the freshwater group, and little data on invertebrate or non-fish hosts are currently available for the entire multivalvulid order. 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 multivalvulid myxosporean life cycles.

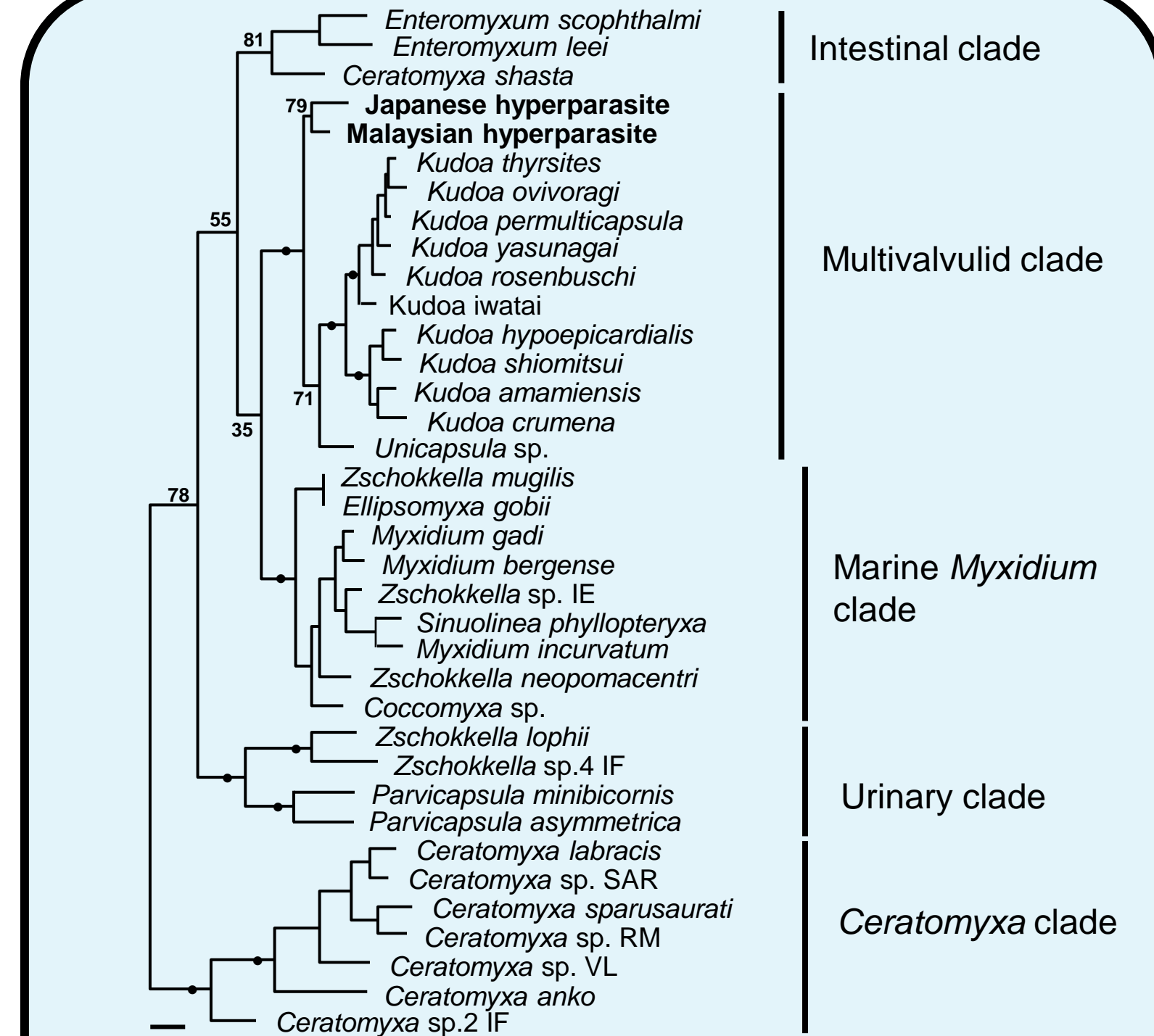


Fig. 8 Maximum parsimony tree of marine myxozoans. Bootstrap support values are calculated from 1000 replicates, a solid circle at the node represents a support value of >90% (scale = 50 changes).