Myxozoa

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Secondary article

Article Contents

Myxosporidians, members of the phylum Myxozoa, are common multicellular parasites of cold-blooded vertebrates, primarily fishes. They have a complex life cycle and many species are highly pathogenic in commercially important fishes, particularly in aquaculture.

Introduction

Myxozoa are common multicellular parasites of cold-blooded vertebrates, particularly fishes. A few unusual members of the Myxozoa have been reported from other hosts, e.g. *Tetracapsula bryozoides* from bryozoans, and *Fabespora vermicola* in a digenean trematode. They undergo complicated development within the hosts, and many (perhaps almost all) utilize alternate development within an annelid worm in their life cycle. Many species are highly pathogenic in commercially important fishes, particularly in aquaculture. The following article presents an overview of the development, nomenclature, taxonomy and phylogeny of the Myxozoa. In addition, some of the most important members in the group are reviewed.

Myxozoa infect a wide variety of tissues (histozoic species) or the lumina of organs such as the gall bladder, urinary bladder or kidney (coelozoic species). Histozoic species usually form small, confined white cysts with little associated tissue damage. However, when these cysts are numerous in vital organs, such as the gills or heart, they can cause disease. Furthermore, heavy infections of certain histozoic myxozoa in the order Multivalvulida infect the flesh and may lower the market value of fish. Pathogenic coelozoic species generally cause more diffuse infections without macroscopically visible cysts.

Life Cycle and Development

The development of myxozoa within their two hosts is complicated (**Figure 1**). They contain several vegetative stages (trophozoites) and development in the fish culminates in the formation of multicellular spores (the myxospore). Attempts to infect fish with 'myxospores' collected from infected fish have been unsuccessful, unless they were 'aged' in organic mud for several months. In 1983, it was first demonstrated that the spores of *Myxobolus cerebralis*, an important parasite of salmonid fishes, are in fact infectious for an aquatic oligochaete, *Tubifex tubifex*. In the worm, the parasite continues to undergo complicated development, culminating in a completely different spore (**Figure 1**). These stages in the worm host were formerly considered to be completely different parasites from those occurring in fish, and were assigned to a different class – the Actinosporea. Similar heteroxenous life cycles involving oligochaete worms have now been demonstrated or strongly suggested for about 15 freshwater myxozoan species, belonging to six genera in four families. In addition, polychaetes (e.g. *Manyunkia speciosa*) may also serve as alternate hosts for myxozoa. Although yet to be demonstrated, it is possible that this alternate development also occurs in myxozoa that infect strictly marine fishes. Marine oligochaetes and polychaetes

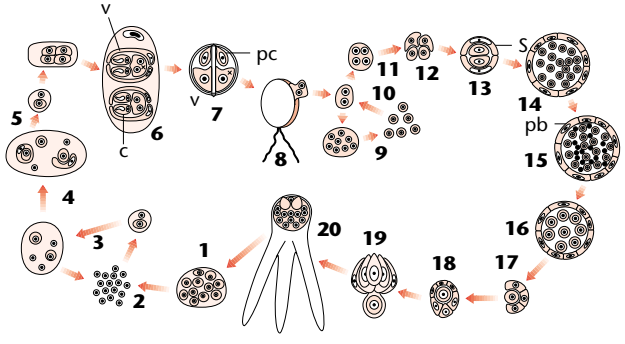

Figure 1 Generalized life cycle of myxozoa. 1–7; Development in the fish host (myxosporean phase). 1, Actinosporean contacts surface of fish and discharges sporoplasm; 2, free unicellular sporoplasms in fish skin shortly after infection; 3, extrasporogonic multiplication; 4, trophozoites develop into sporogonic plasmodia, vegetative nuceli (v) and internal generative cells (g) formed by endogeny (internal budding); 5, sporogenesis: internal daughter cells form sporoblasts; s, sporogonic cell; 6, sporoblasts differentiate into the cells comprising the spore; c, capsulogenic cell; x, sporoplasm; v, valvulogenic cells; 7, fully-formed myxospore (*Sphaerospora* sp.), v, valve; pc, polar capsule; x, sporoplasm.

8–20: Actinosporean phase in the annelid worm host (actinosporean phase). 8, Myxospore is ingested by worm and released sporoplasm penetrates epithelium; 9, sporoplasms form multiple schizogonic stages; 10, plasmotomy (fusion) of two uninucleate cells to produce one binucleate cell; 11, division to form cell with four nuclei; 12, division of '11' into four unicellular cells; 13, formation of early pansporocysts with two somatic cells (S) and two internal generative cells; 14, gametocytes form within pansporocysts; 15, meiosis of gametocytes results in transformation of diploid to haploid cells and polar bodies (pb); 16, fusion of gametocytes to form diploid 'zygotes'. 17–20: Sporoblasts develops into actinospores, which are then released from the worm. Adapted from Moser and Kent (1994), El-Matbouli and Hoffmann (1998), El-Matbouli *et al.* (1995).

are common and actinosporeans have been described from the former. Myxozoa are also found in pelagic fishes, and the possibility that planktonic invertebrates serve as the alternate hosts for some of these species should be considered.

Direct fish-to-fish transmission without the requirement of alternate actinosporean development is also possible, at least for some species. Diamant (1997) demonstrated that *Myxidium leei*, a parasite of various marine fishes, could be transmitted directly from fish to fish. These studies were not done with purified myxospores, and it is possible that myxospores of this species also require development within an alternate host to complete the life cycle.

In the myxozoan heteroxenous life cycles known to date, development occurs within the intestinal epithelium or body cavity of aquatic annelids (the actinosporean phase), culminating in an 'actinospore' (= actinosporean spore) (**Figures 2** and **3**). The stages infective to fish are the sporoplasms, which are released from the multicellular actinospore. The following description of early development in the fish host is based largely on experimental infections of trout with *Myxobolus cerebralis* (El-Matbouli *et al.*, 1995). The actinospore is released from the oligochaete and sporoplasms penetrate (or are injected) through the surface epithelia after contact of the actinospore with the skin of the fish host. Shortly after infection, clusters of dividing cells are found in epithelia cells. The parasite then migrates to the site of infection where it continues to develop. With *M. cerebralis*, the extrasporogonic forms migrate to the cartilage via peripheral nerves. These extrasporogonic forms continue to divide during migration to the target tissue. Some species (e.g. *Sphaerospora* spp.) exhibit prominent extrasporogonic multiplication in the circulatory system or other sites where sporulation does not occur. At the site of sporulation, a multinucleate plasmodium develops which contains free vegetative nuclei and generative cells formed by endogenous budding (internal budding). In many genera, sporogenesis is initiated by fusion of generative cells; one cell envelops the other to form an internal sporoblast. In other genera, the sporoblast is formed by division of a single generative cell. The sporoblast divides and differentiates into valvogenic, capsulogenic and sporoplasmic cells, which form the valves, polar capsules and sporoplasm(s), respectively in the fully developed myxospore (**Figure 3**).

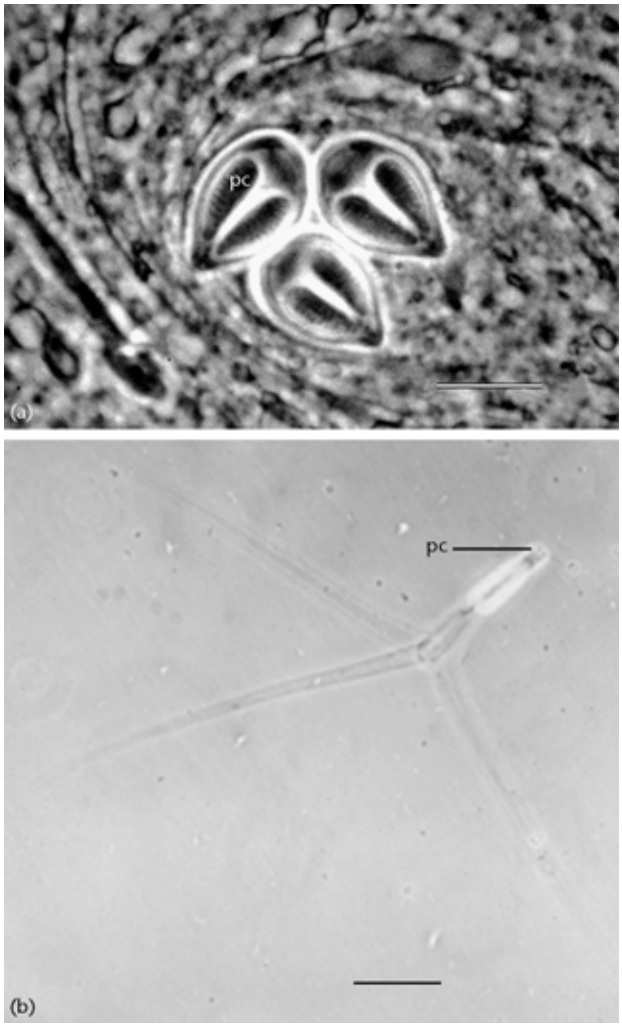

Figure 2 Wet mounts of *Myxobolus arcticus*, a myxozoon infecting the brain of salmonid fishes. (a) Myxospore from brain. Bar, 10 μm. (b) Triactinomyxon actinospore from oligochaete worm host. Bar, 50 μm; pc, polar capsule.

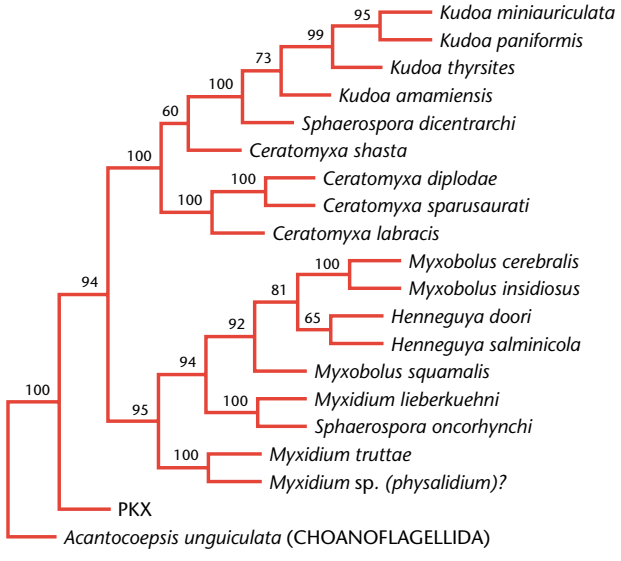

Figure 3 Phylogeny of the Myxozoa based on analysis of small subunit ribosomal DNA (SSU rDNA) sequences. Maximum parsimony analysis of the Myxozoa inferred from complete SSU rDNA sequences with a bootstrap resampling of the data set. The numbers at the forks represent the percentage of times the group occurred out of 100 trees. Branch lengths are proportional to the scale given in substitutions per sequence position.

The myxosporean spore contains one or two sporoplasms (except for *Polysporoplasma* which contains many sporoplasms) and 1–7 (usually two) polar capsules, each of which contains a coiled, extrusible polar filament. The spore shell is composed of 2–7 valves that are adhered together along one or more sutures.

The stage infective to the annelid alternate host is the 'myxospore' released from the fish following death or discharge with body fluids. Early actinosporean development in the oligochaete was described by El-Matbouli and Hoffman (1998) for *Myxobolus cerebralis*. The myxosporean spore is ingested, the sporoplasm is released from the spore, penetrates the gut epithelium and multiplies by schizogony. The earliest recognizable stages are amoeboid, uninucleate or multinucleate stages located intercellularly in the intestine. Uninucleate cells fuse to form binucleate oval cells. Each nucleus divides to form a four-cell stage with two inner cells (generative cells) and two enveloping cells (pericytes or somatic cells). Inner cells divide by meiosis to produce gametes. Gametes fuse to form zygotes, which then divide into sporoblasts. Sporoblast cells differentiate into the multicellular actinospore. The actinospore has three valves, three polar capsules and multiple sporoplasms. These are released from the digestive tract of the worm, inflate and float about in the water until they come in contact with a suitable fish host.

Taxonomy, Phylogeny and Nomenclature

The life cycle stages found in oligochaetes were originally considered to be completely different parasites, which were assigned to the class Actinosporea. Because these stages are not separate taxa from myxosporeans, Kent *et al.* (1994) proposed changes in the taxonomy and nomenclature for the phylum Myxozoa. In brief, the class Actinosporea, the order Actinomyxidia, and all families in the Actinosporea (except Tetractinomyxidae) should be suppressed. They proposed that actinosporean generic names be treated as collective group names, and thus they do not compete in priority with myxosporean generic names. We opted to refer to the actinosporean stage as the 'alternate stage' (Kent *et al.*, 1994). However, El-Matbouli and Hoffmann (1998) reported that sexual stages occur in the actinosporean phase of *M. cerebralis* (see **Figure 1**). Nevertheless, even if the actinosporean stage is determined to be the definitive stage (i.e. the stage where meiosis and fusion occurs), this does not require that this stage replace the myxosporean stage for taxonomic and nomenclature purposes. For example, malaria (*Plasmodium* spp.) taxonomy is based on stages occurring in vertebrates, which are technically the intermediate host.

With the proposal to suppress the class Actinosporea, the phylum Myxozoa contains one class, Myxosporea Bütschli, 1881, two orders, several families, about 50 genera, and about 1200 species. Taxonomy is based primarily on morphological differences in shell valves, polar capsules, and associated structures of the spores. The major taxa of the Myxozoa as adapted from Lom and Noble (1984) and Moser and Kent (1994) are listed in **Table 1**.

Several independent phylogenetic analyses based on small subunit ribosomal DNA (SSU rDNA) sequences, and recently on *Hox* genes, have demonstrated that Myxozoa roots within the kingdom Animalia (Smothers *et al.*, 1994; Siddall *et al.*, 1995; Anderson *et al.*, 1998). Although protozoan affinities are no longer defended, the precise relationship of the Myxozoa with other metazoan groups is still controversial. Pioneer studies based on SSU rDNA reported myxozoa to be related to Nematoda

Table 1 The major taxa of the Myxozoa

Phylum Myxozoa	
Class Myxosporea	
Order Bivalvulida	
Suborder Variisporina	
	Includes *Myxidium, Zschokkella, Parvicapsula, Ortholinea, Ceratomyxa, Sphaerospora, Chloromyxum, Hoferellus* and *Myxobilatus*
Suborder Platysporina	
	Includes *Myxobolus* and *Henneguya*
Suborder Sphaeromyxina	
	Sphaeromyxa
Order Multivalvulida	
	Includes *Kudoa, Hexacapsula* and *Unicapsula*

(Bilateria) (Smothers *et al*., 1994), and this has been supported by other authors (Anderson *et al*., 1998). However, Siddall *et al*. (1995), including the rDNA sequence of the cnidarian fish parasite *Polypodium hydriforme*, and a combination of morphological and molecular data, concluded that myxozoa are cnidarians and that the phylum Myxozoa should be suppressed. Affinities of the Myxozoa with the Cnidaria have been suggested for longer than a century by several authors. The striking similarity of the myxozoan polar capsules and the cnidarian nematocysts, which display essentially identical morphology, ultrastructure and development, has been used as the main argument favouring this relationship. Nevertheless, the suppression of the phylum Myxozoa seems premature with the available evidence. Because a taxon may have evolved from another existing taxon does not necessitate that the former be suppressed. Indeed, the Myxozoa are quite distant from cnidarians based on the length of branches in phylogenetic trees and on several developmental and morphological differences between the two groups.

Shulman (1966) suggested that the first myxozoa were coelozoic, i.e. inhabitated the gall bladder and later the urinary bladder of marine teleost fishes in the late Cretaceous period. From these organs they later evolved to infect other tissues, with some forms becoming histozoic. Shulman (1966) also suggested that the ancestral myxozoa were bipolarids (e.g. *Sphaeromyxa*, *Myxidium*), and that in freshwater they gave rise to the platysporinids (*Myxobolus* and *Henneguya*). He also proposed that the order Multivalvulida (marine histozoic forms) was derived from ancestors similar to *Ceratomyxa* (a coelozoic, marine genus).

To date, SSU rDNA sequence is available for over 20 species of Myxozoa, belonging to about seven genera. We constructed phylogenetic trees using species with the most complete representative SSU sequences (**Figure 3**), and this analysis largely agrees with Shulman's phylogenetic hypotheses. For example, *Ceratomyxa* clusters with *Kudoa* species, and the histozoic characteristic appears to have arisen at least twice; with the freshwater myxozoa as represented by the suborder Platysporina (e.g. *Myxobolus* and *Henneguya* spp.) and with the marine order Multivalvulida (represented by *Kudoa* sp.).

Marine and freshwater taxa examined to date are separated in two major branches of the tree, with the single exception of *Ceratomyxa shasta*. Almost all other members of this genus are marine species, and the life cycle of *C. shasta* involves a freshwater polychaete. This suggests the possibility of later or secondary colonization of the freshwater environment by this myxozoon. Most members of the genus *Sphaerospora* are coelozoic parasites of freshwater fishes. One marine *Sphaerospora* species, *S. dicentrarchi*, clusters with the Multivalvulida, whereas others (e.g. *S. oncorhynchi*) fall, as expected, with *Myxidium*, forming a clade of the coelozoic suborder Variisporina. However, in support of molecular systematics, phenotypic characters of *S. dicentrachi* suggest that it is somewhat atypical for the genus *Sphaerospora*, e.g. it is marine, it is histozoic (rather than coelozoic), and its spores are unusually small and are subtriangular (rather than spherical in lateral view). Indeed, *S. dicentrarchi* appears to be a link between the marine coelozoic myxozoans with two spore valves (e.g. *Ceratomyxa*) and the Multivalvulida (which are marine, have more than two valves, and are histozoic).

Within the Platysporina, as reported by Andree *et al*. (1998), a phylogenetic separation of the two major genera *Henneguya* and *Myxobolus* based on SSU rDNA sequence is not clear. For example, *M. squamalis* represents an outgroup from *Henneguya* spp. and other *Myxobolus* spp. In other words, *Henneguya salminicola* appears more closely related to certain *Myxobolus* species than does *M. squamalis*.

Analysis of SSU rDNA sequences indicate that the enigmatic PKX myxosporean is very distinct from other myxosporeans examined thus far, and its roots lie within the Myxozoa before divergence of the other major groups.

Examples of Some Important Myxozoa

Myxobolus (suborder Platysporina)

Myxobolus is the most specious genus in the phylum Myxozoa, with some 450 described species. Spores of this genus are characterized by having two polar capsules, and two thick spore valves divided by a suture in the plane of the polar capsules (**Figure 2**). Probably the best known species is *Myxobolus cerebralis*, which causes whirling disease in salmonid fishes (particularly rainbow trout *Oncorhynchus mykiss*) in hatcheries in Europe and the USA. In recent years, high mortality in wild rainbow trout in the western USA has been attributed to this myxozoon. *Myxobolus cerebralis* infects the cartilage and bone of juvenile salmonids, causing neuropathology due in most part to pressure on the central nervous system related to malformed bone.

A few other *Myxobolus* species are also important pathogens, e.g. *M. cyprini* causes systemic disease in various cyprinid fishes (family Cyprinidae). Other members of the genus *Myxobolus* are less pathogenic, but cause unsightly cysts in the skin or muscle (e.g. *M. squamalis* and *M. insidiosis* in salmonid fishes).

Henneguya (suborder Platysporina)

This genus is the other major genus in the suborder Platysporina and also has members that are pathogenic or cause unsightly lesions. *Henneguya* differs from *Myxobolus* in that it has two posterior tail-like projections on the

spore. *Henneguya salminicola* has an impact on salmon fisheries because it forms large cysts in the somatic muscle and thus reduces the market value of affected fish. Some investigators (e.g. Shulman, 1966) believe that *H. salminicola* is synonymous with *H. zschokkei*. This myxozoon was originally reported from whitefish (*Coregonus* spp.) in Europe, and is known from various salmonid and nonsalmonid fishes from Eurasia. *Henneguya* species that infect the gill may be highly pathogenic. For example, *H. exilis* infects the gills of pond-reared channel catfish (*Ictalurus punctactus*) in the southeastern USA, and has caused high mortality due to obstructive lesions in the gills.

Sphaerospora (suborder Variisporina)

Several species of this mainly coelozoic genus are pathogenic, particularly in pond-reared cyprinid fishes. Spores of this genus are spherical, have two polar capsules, with a suture dividing the valves perpendicular to the plane of the polar capsules. *Sphaerospora renicola* infects the kidney tubules of common carp (*Cyprinus carpio*), where it is associated with impaired excretory and haematopoietic function. In addition, an extrasporogonic stage of the parasite develops in the swim bladder and is responsible for disease known as swim bladder inflammation. Other examples of pathogenic *Sphaerospora* species in cyprinid fishes include *S. molnari* that infects gills of carp and goldfish (*Carassius auratus*) and *S. tincae*, which infects the kidney of tench (*Tinca tinca*). The latter parasite may cause fatal epizootics due to massive replacement of the kidney tissue by parasites.

Proliferative gill disease or hamburger gill disease of pond-reared channel catfish is caused by an extrasporogonic stage of a myxozoon, probably *S. hanki* (syn. *S. ictaluri*). These stages infect the interstitium of the gill filament and cause massive inflammation. The spores occur in the lumen of kidney tubules, assuming that the parasites are the extrasporogonic stages of *S. hanki*. Extrasporogonic stages cause massive, chronic inflammation in the gill lamellae, and multifocal degeneration of the cartilaginous support filament of the gills. Infected gills also exhibit severe epithelial hyperplasia, resulting in a significant decrease in area of the respiratory surface. Massive mortalities may occur in affected fish following moderate declines in dissolved oxygen levels in ponds, which would normally not kill unaffected channel catfish.

Sphaerospora dicentriachi and *S. testicularis* species are important parasites in sea bass (*Dicentrarchus labrax*) in Mediterranean countries. The latter is a coelozoic species parasitizing the seminiferous tubules and it can cause parasitic castration. *Sphaerospora dicentrarchi* is a histozoic, systemic species infecting connective tissue, and has been associated with mortalities. This myxozoon was described as one of the rare examples of a marine and histozoic *Sphaerospora* species, but our taxonomic analysis demonstrated that it is not closely related to typical, freshwater *Sphaerospora* spp.

Hoferellus (suborder Variisporina)

The genus is related to *Sphaerospora* and also infects the kidneys of cyprinid fishes. *Hoferellus carassii* causes kidney bloater disease in goldfish. Histological examination of the kidney reveals bizarre lesions in which trophozoites infect the epithelium of tubules and elicit hypertrophy, hyperplasia and transformation of infected cells suggestive of neoplasia. This results in the formation of large cavernous lesions resembling polycystic kidney disease. *Hoferellus cyprini* of common carp also infects the kidney tubules and forms syncytia, but not the cavernous lesions as seen in goldfish.

Ceratomyxa (suborder Variisporina)

This is a very specious genus infecting many marine fishes. Most *Ceratomyxa* species infect the gall bladder, and normally cause only limited histopathological damage. Semi-intensive and marine netpen culture of European sea bass (*Dicentrarchus labrax*) and of fishes of the family Sparididae is a rapidly growing industry in the Mediterranean Sea, and some *Ceratomyxa* species can cause problems in these facilities. *Ceratomyxa labracis* and *C. diplodae* cause lesions in the gall bladder and pancreas of sea bass, and *Ceratomyxa sparusaurati* has been associated with mortalities in gilthead sea bream (*Sparus aurata*). Other pathogenic species include *Ceratomyxa drepanosettae* in several flatfish species.

Ceratomyxa shasta is a serious pathogen of salmonid fishes in western USA and Canada (Bartholomew *et al*., 1997), where it causes gut infections. As with *C. sparusaurati*, the parasite may breach the gastrointestinal tract and cause severe necrosis and inflammation in the viscera. In contrast other freshwater myxozoa, which use oligochaete annelids as alternate hosts, the freshwater polychaete *Manayunkia speciosa* is the alternate host for *C. shasta*. This fact might support a marine origin for this myxozoon. This also suggests that perhaps marine polychaetes may act as required alternate hosts for some marine myxozoa.

Myxidium (suborder Variisporina)

Myxidium, and the closely related genus *Zschokkella*, contain many species and are common coelozoic parasites of fishes, but only a few are pathogenic. Both are characterized by having two polar capsules at opposite ends of the spore. *Myxidium giardi* infects the gills, skin, kidney and intestine of eels (*Anguilla* spp.). Kidney lesions are the most severe, and the renal damage may cause mortality. *Myxidium lieberkuehni* infects the kidney of pike

(*Esox lucius*), in which extrasporogonic stages infect the glomeruli and cause large cyst-like nodules. The endothelial cells are infected and undergo severe hypertrophy.

A recently described species, *Myxidium leei*, infects the gut and causes mortality in sea bream in mariculture facilities in Israel and Greece. Furthermore, the red drum *Sciaenops ocellatus* (family Sciaenidae) and mullets (family Mugilidae) are also susceptible to the infection. This myxozoon is unusual in some important aspects; it has a wide host specificity and it is the only myxozoon that has been clearly demonstrated to be transmissible directly from fish to fish without the requirement of an alternate oligochaete host in the life cycle. Furthermore, *M. leei* is morphologically distinct from other *Myxidium* species described thus far, and may ultimately be assigned to another genus.

Chloromyxum (suborder Variisporina)

As with *Myxidium*, members of the mainly coelozoic genus are usually not pathogenic. This myxozoon has spherical spores, which contain four polar capsules and usually have very ornate valves. Heavy infections by some species of *Chloromyxum* in the biliary ducts (e.g. *C. truttae*) may cause liver damage and jaundice.

Kudoa species (order Multivalvulida)

The genus *Kudoa* is comprised of members with spores with four valves, each of which contains a polar capsule. Species within this genus are typically histozoic parasites of marine teleosts. However, since the establishment of the genus, a few coelozoic species have been described. This genus is of concern to both aquaculture and commercial fisheries because several of its species either produce unsightly macroscopic cysts in the musculature or are associated with post-mortem myoliquefaction, and thus reduce the market value of the infected fish products. This muscle degeneration, also referred to as 'soft flesh', is probably the result of proteolytic enzymes released by the parasite after the fish is harvested. Some of the most noted species are *K. thyrsites* in farmed Atlantic salmon and various wild marine fishes, *K. musculoliquefaciens* in swordfish (*Xiphias gladius*), *K. paniformis* in Pacific hake (*Merluccius productus*), and *K. clupeidae* in Atlantic herring (*Clupea harengus*). Other genera in the order Multivalvulida (e.g. *Unicapsuala* and *Hexacapsuala*) also infect the muscle of marine fishes and have been associated with 'soft flesh'.

PKX myxosporean

Proliferative kidney disease (PKD) of salmonid fishes is caused by the PKX organism, which is the extrasporogonic stage of an unidentified myxosporean. It occurs in most locations in North America and Europe where salmonid fishes are reared in freshwater at temperatures greater than 15°C. In Europe it is considered one of the most important diseases in rainbow trout culture.

The parasite infects primarily the kidney interstitium. Fish with PKD exhibit exophthalmos, lateral body swelling, distended abdomens and pale gills. Internally, gross signs are ascites and enlargement of the kidney and spleen. Affected fish are often anaemic, which may be the ultimate cause of death. The PKX myxosporean was originally thought to be related to the genus *Sphaerospora* or a related genus. However, based largely on SSU rDNA sequence data, it appears to be unrelated to *Sphaerospora* or other genera examined by this method thus far (Kent *et al*., 1998). Recently, PKX has been shown by both rDNA analysis (Anderson *et al*., 1999) and transmission studies (Longshaw *et al*., 1999) to be related to *Tetracapsula*.

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