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Recent Advances in Our Knowledge of the Myxozoa

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ABSTRACT. In the last few years two factors have helped to significantly advance our understanding of the Myxozoa. First, the phenomenal increase in fin fish aquaculture in the 1990s has lead to the increased importance of these parasites; in turn this has lead to intensified research efforts, which have increased knowledge of the development, diagnosis, and pathogenesis of myxozoans. The hallmark discovery in the 1980s that the life cycle of *Myxobolus cerebralis* requires development of an actinosporean stage in the oligochaete, *Tubifex tubifex*, led to the elucidation of the life cycles of several other myxozoans. Also, the life cycle and taxonomy of the enigmatic PKX myxozoan has been resolved: it is the alternate stage of the unusual myxozoan, *Tetracapsula bryosalmonae*, from bryozoans. The 18S rDNA gene of many species has been sequenced, and here we add 22 new sequences to the data set. Phylogenetic analyses using all these sequences indicate that:1) the Myxozoa are closely related to Cnidaria (also supported by morphological data); 2) marine taxa at the genus level branch separately from genera that usually infect freshwater fishes; 3) taxa cluster more by development and tissue location than by spore morphology; 4) the tetracapsulids branched off early in myxozoan evolution, perhaps reflected by their having bryozoan, rather than annelid hosts; 5) the morphology of actinosporeans offers little information for determining their myxosporean counterparts (assuming that they exist); and 6) the marine actinosporeans from Australia appear to form a clade within the platysporinid myxosporeans. Ribosomal DNA sequences have also enabled development of diagnostic tests for myxozoans. PCR and in situ hybridisation tests based on rDNA sequences have been developed for *Myxobolus cerebralis, Ceratomyxa shasta, Kudoa* spp., and *Tetracapsula bryosalmonae* (PKX). Lectin-based and antibody tests have also been developed for certain myxozoans, such as PKX and *C. shasta.* We also review important diseases caused by myxozoans, which are emerging or re-emerging. Epizootics of whirling disease in wild rainbow trout (*Oncorhynchus mykiss*) have recently been reported throughout the Rocky Mountain states of the USA. With a dramatic increase in aquaculture of fishes using marine netpens, several marine myxozoans have been recognized or elevated in status as pathological agents. *Kudoa thyrsites* infections have caused severe post-harvest myoliquefaction in pen-reared Atlantic salmon (*Salmo salar*), and *Ceratomyxa* spp., *Sphaerospora* spp., and *Myxidium leei* cause disease in pen-reared sea bass (*Dicentrarchus labrax*) and sea bream species (family Sparidae) in Mediterranean countries.

Key Words. Actinosporea, *Ceratomyxa,* disease, fin fish, *Kudoa, Myxidium,* Myxosporean, Myxozoa, nomenclature, phylogeny, *Sphaerospora, Tetracapsula,* whirling disease.

THE myxozoans are a speciose and economically important group of microscopic metazoan parasites (Fig. 1). Whereas they are best known for the diseases they cause in commercially important fish hosts, these species represent only a fraction of the some 1,350 described species assigned to about 52 genera of Myxozoa. They have also been reported rarely in platyhelminths, reptiles, and amphibians, and recently a myxozoan-like parasite was found in the brain of a mole *Talpa europrea* (Friedrich et al. 2000). The Myxozoa have been reviewed in a number of book chapters and scientific articles up to about 1995 (El-Matbouli et al. 1992a; Garden 1992; Lom and Dyková 1992; Lom and Dyková 1995; Moser and Kent 1994), and recently a monograph of the Myxozoa by Chen and Ma (1998) greatly increased the species richness of the group. Because of the noteworthy advances in recent years we review and up-date the knowledge of the Myxozoa, based largely on reports that have appeared since the mid-1990s.

Several events have precipitated these advances. Molecular systematics has become a mainstream approach in taxonomic and phylogenetic studies. Smothers et al. (1994) were the first to use ribosomal DNA (rDNA) sequence analysis to study the phylogenetics of the Myxozoa. Since then, small subunit rDNA sequences of myxozoans have been employed by several investigators to address systematics and life cycle questions, and for the development of highly sensitive and specific diagnostic tests. These studies have resulted in proposals for dramatic revisions regarding relationships of the Myxozoa, both within the group (Table 1) and in comparison with other taxa. An outgrowth of these studies has been the development of sensitive PCR tests based on rDNA sequences, which are becoming routine diagnostic tools in fish health laboratories.

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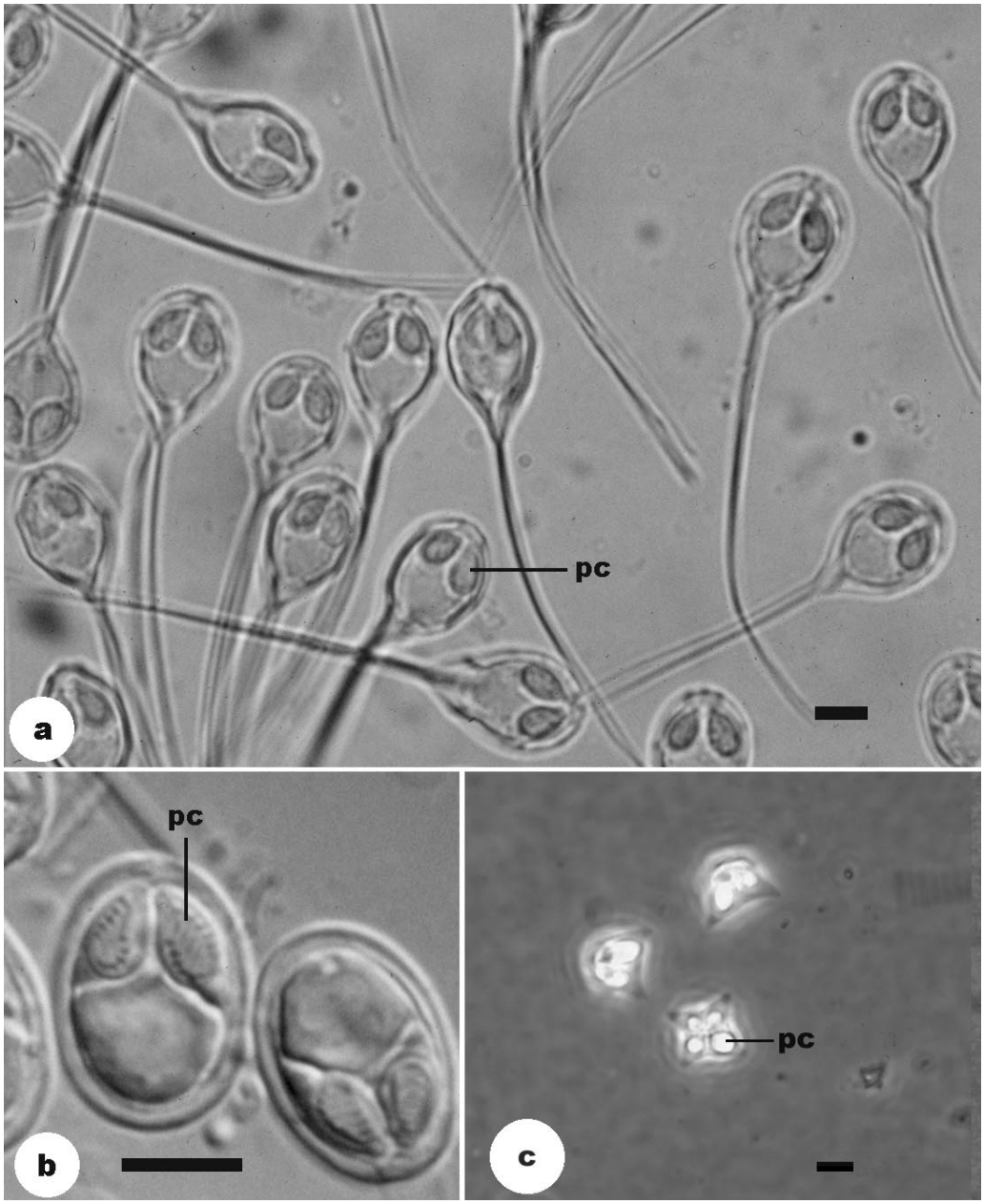

Fig. **1**. Myxosporean spores of Myxozoa infecting fish hosts. PC = polar capsules. **a.** *Henneguya salminicola.* Note bifurcate tails. **b.** *Myxobolus* sp. **c.** *Kudoa thyrsites* with 4 polar capsules. Bar = 5 μm.

After Wolf and Markiw's (1984) landmark discovery that the life cycle of *Myxobolus cerebralis* involves alternation with an actinosporean form in *Tubifex tubifex* (Markiw and Wolf 1983; Wolf and Markiw 1984), involvement of annelid worms in the life cycles of some 25 other freshwater myxozoans have been documented (Table 2). This has lead to nomenclatural discrepancies and problems in how we should deal with descriptions of new actinosporean forms (Kent et al. 1994b; Lester et al. 1998, 1999; Lom et al. 1997). Furthermore, these studies have permitted establishment of laboratory infections, leading to a more precise understanding of the early development of myxozoans (El-Matbouli et al. 1995, 1999a; El-Matbouli and Hoffmann 1998).

The realization that whirling disease, caused by *M. cerebralis*, is responsible for high mortality in wild rainbow trout (*Oncorhyncus mykiss*) populations in the western United States (Hedrick et al. 1998) has lead to increased research on this parasite. This research has advanced our knowledge of *M. cerebralis* and myxozoans in general, particularly in the area of myxozoan development and host interactions (El-Matbouli et al. 1995; Hedrick et al. 1998). With the huge expansion in marine fish aquaculture in the 1990s, particularly netpen culture of salmonids and sea bream species, several myxozoans have been recognized or elevated in status as important pathogens. Increased research efforts on these marine myxozoans have advanced our understanding of their development and pathogenesis.

LIFE CYCLES AND DEVELOPMENT

Wolf and Markiw (1984) discovered that an actinosporean (triactinomyxon) (Fig. 2) is a required alternate life cycle stage

Table **1**. Abbreviated classification of the class Myxosporea based primarily on Lom and Dyková (1992).

Phylum Myxozoa
- Class Myxosporea
  - Order Bivalvulida (marine and freshwater, with two valves to spore)
    - Suborder Variisporina (marine and freshwater, mostly coelozoic)
      - Includes *Ceratomyxa, Chloromyxum, Hoferellus, Myxidium, Myxobilatus, Ortholinea, Parvicapsula, Polysporoplasma, Sinuolinea, Sphaerospora, Zschokkella,*
    - Suborder Platysporina (marine and freshwater, mostly histozoic)
      - Includes *Myxobolus, Henneguya,* and *Thelohanellus*
    - Suborder Sphaeromyxina (marine, with ribbon-like polar filaments in polar capsules at opposing end of spore)
      - *Sphaeromyxa*
  - Order Multivalvulida (marine, with greater than 2 spore valves)
    - Includes *Hexacapsula, Kudoa, Trilospora,* and *Unicapsula*
- Class Malacosporea (freshwater, with soft valves, parasites of bryozoans; one order, family and genus)
  - Order Malacovalvulida
    - *Tetracapsula* (with 4 polar capsules)

of *M. cerebralis*. Similar life cycles have now been described for some 25 species assigned to the genera *Myxobolus, Henneguya, Sphaerospora, Ceratomyxa, Myxidium, Zschokkella, Thelohanellus, Hoferellus,* and *Tetracapsula* (Table 2). In addition to definitive laboratory transmission studies, confirmation of myxosporean-actinosporean relationships have used rDNA gene sequence comparisons, as first demonstrated by Andree et al. (1997) for *Myxobolus cerebralis.* This approach has now linked life stages of the PGD agent of catfish with *Henneguya ictaluri* (Pote et al. 2000) and PKX with *Tetracapsula bryosalmonae* (Longshaw et al. 1999).

Some general trends are apparent when correlating actinosporean morphotypes with myxosporeans: *Myxobolus* species (suborder Platysporina) usually develop into triactinomyxons, while members of the suborder Variisporina (e.g. *Ceratomyxa, Myxidium, Sphaerospora*) have been reported to develop into aurantiactinomyxon, neoactinomyxum or tetractinomyxon forms. Although actinosporeans forms may provide some suggestion of their myxosporean affinities, this is often not absolutely predictable. For example, the actinosporean of *Myxobolus pavlovskii* would be assigned to the former genus *Hexactinomyxon,* whereas members of *Thelohanellus* and *Henneguya* (suborder Platysporina) form aurantiactinomyxon spores. *Zschokkella nova* (closely related to the genus *Myxidium*) forms

Table **2**. A summary of life cycles of myxozoans

Myxosporean	Fish host	Actinosporean	Invertebrate host	References
Myxobolus cerebralis	*Oncorhynchus mykiss*	triactinomyxon	*Tubifex tubifex*	Wolf and Markiw 1984
Myxobolus cotti	*Cottus gobio*	triactinomyxon	mixed oligochaetes	El-Matbouli and Hoffmann 1989
Myxobolus pavlovskii	*Hypophthalmichthys molitrix*	hexactinomyxon	mixed oligochaetes	Ruidisch et al. 1991
Myxobolus cultus	*Carassius auratus*	raabeia	*Branchiura sowerbyi*	Yokoyama et al. 1995
Myxobolus carassii	*Leuciscus idus*	triactinomyxon	*Tubifex tubifex*	El-Matbouli and Hoffmann 1993
Myxobolus arcticus (Canada)	*Oncorhynchus nerka*	triactinomyxon	*Stylodrilus heringianus*	Kent et al. 1993b
Myxobolus arcticus (Japan)	*Oncorhynchus masu*	triactinomyxon	*Lumbriculus variegatus*	Urawa 1994*
Myxobolus drjagini	*Hypophthalmichthys molitrix*	triactinomyxon	*T. tubifex*	El-Mansy and Molnár 1997a
Myxobolus portucalensis	*Anguilla anguilla*	triactinomyxon	*T. tubifex*	El-Mansy et al. 1998a
Myxobolus hungaricus	*Abramis abramis*	triactinomyxon	*T. tubifex, L. hoffmeisteri*	El-Mansy and Molnár 1997b
Myxobolus dispar	*Cyprinus carpio*	raabeia	*T. tubifex*	Molnár et al. 1999a
Myxobolus pseudodispar	*Rutilus rutilus*	triactinomyxon	*T. tubifex, L. hoffmeisteri*	Székely et al. 1999, 2000
Myxobolus bramae	*Abramis brama*	triactinomyxon	*T. tubifex*	Eszterbauer et al. 2000
Henneguya exilis	*Ictalurus punctatus*	*Aurantiactinomyxon janiszewskai*	*Dero digitata*	Lin et al. 1999
Henneguya ictaluri	*Ictalurus punctatus*	aurantiactinomyxon	*D. digitata*	Burtle et al. 1991; Styer et al. 1991; Pote et al. 2000
Hoferellus carassii (Germany)	*Carassius auratus*	aurantiactinomyxon	mixed species	El-Matbouli et al. 1992b
Hoferellus carassii (Japan)	*Carassius auratus*	neoactinomyxum	*B. sowerbyi*	Yokoyama et al. 1993
Hoferellus cyprini	*Cyprinus carpio*	aurantiactinomyxon	*Nais* sp.	Grossheider and Körting 1992
Thelohanellus nikolskii	*Cyprinus carpio*	aurantiactinomyxon	*B. sowerbyi*	Székely et al. 1998
Thelohanellus hovorkai	*Cyprinus carpio*	aurantiactinomyxon	*B. sowerbyi*	Yokoyama 1997; Székely et al. 1998; Anderson et al. 2000
Sphaerospora renicola	*Cyprinus carpio*	undetermined neoactinomyxum	*unknown B. sowerbyi*	Grossheider and Körting 1993; Molnár et al. 1996b
Sphaerospora truttae	*Salmo truta*	echinactinomyxon	*L. variegatus*	Özer and Wootten 1999
Ceratomyxa shasta	*Oncorhynchus mykiss*	tetractinomyxon	*Manayunkia speciosa*	Bartholomew et al. 1997
Zschokkella sp.	*Carassius auratus*	echinactinomyxon	*B. sowerbyi*	Yokoyama et al. 1993
Zschokkella nova	*Carassius carassius*	siedleckiella	*T. tubifex*	Uspenkaya 1995
Myxidium giardi	*Anguilla anguilla*	aurantiactinomyxon	*T. tubifex*	Benajiba and Marques 1993
PKX	*Oncorhynchus mykiss*	*Tetracapsula bryosalmonae*	*Plumatella* sp. and *Fredericella sultana*	Longshaw et al. 1999

* Urawa, S. 1994. Life cycle of *Myxobolus arcticus,* a myxosporean parasite of salmonid fishes. In: Program and Abstracts Intl. Symposium, Aquat. Animal Health Seattle, WA. 4–8 Sept. 1994. University of California, Davis. p. W-10.3.

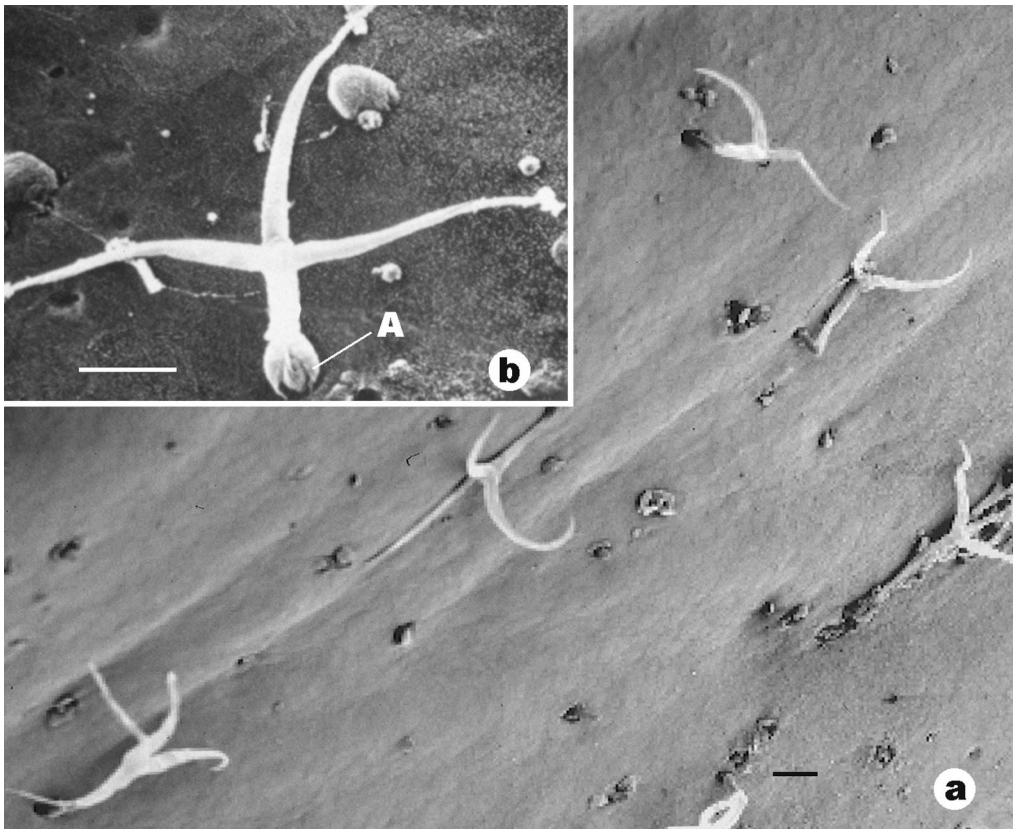

Fig. **2**. Triactinomyxons of the myxozoan, *Myxobolus cerebralis,* associated with fish skin. **a.** Five triactinomyxon spores. **b.** Detail of one spore. A = anterior end with polar capsules. Bar = 100 μm.

actinosporean spores similar to those of *Siedleckiella.* Xiao and Desser (2000a) conducted phylogenetic analysis of two partitioned data sets of life cycle stages and showed a lack of taxonomic congruence between the two life stages of myxozoans.

Several actinosporeans from marine oligochaetes collected in Queensland, Australia have recently been described (Hallett et al. 1998; Hallett and Lester 1999; Hallett et al. 1999). Whereas some forms were consistent with those previously described from the marine environment (e.g. sphaeractinomyxons), they assigned others to new genera. The genus *Endocapsa* was erected because its spore appendages differ from those of other actinosporeans and its polar capsules are submerged within the spore. The genus *Tetraspora* is distinguished by its spores, which develop in groups of four pansporoblasts, rather than eight as in all other actinosporeans. Lester et al. (1998) suggested that this difference may warrant assigning these actinosporeans to a separate family within the Myxosporea.

Many actinosporeans have been described from oligochaetes, including species in the major families Naiidae, Tubificidae, and Lumbriculidae, while only two actinosporeans have been described from polychaetes. Bartholomew et al. (1997) elucidated the life cycle of *Ceratomyxa shasta,* and showed that it forms tetractinomyxons in the freshwater polychaete, *Manayunkia speciosa.* Køie (2000) found actinosporeans in the marine polychaete, *Nereis diversicolor,* for which the myxosporean stages are still unknown.

Myxosporean development in the fish host. To date, the complete details of development have been resolved only with *M. cerebralis.* Salmonid fish are exposed to *M. cerebralis* through contact with waterborne triactinomyxon spores or through ingestion of infected *Tubifex tubifex* (El-Matbouli and Hoffmann 1989a; Wolf and Markiw 1984). As early as one-min post-exposure, the waterborne triactinomyxon spores accumulate at the openings of the mucous cells over the entire epidermis (Fig. 2), the buccal cavity, and the respiratory epithelial cells of the gills. The triactinomyxon spores extrude their polar filaments and inject them directly into the mucous cell openings or into the surrounding epidermis cells to anchor the spores and allow the sporoplasm to penetrate into the epidermis (Fig. 2, 3: 1, 2).

Presporogonic/Extrasporogonic phase (Fig. 3: 3–13). During the first 60 min following penetration, the sporoplasm migrates intercellularly in the epidermis and gill epithelium. Then, the cell enveloping the sporoplasm internal cells disintegrates and each cell penetrates a host epidermal or gill epithelial cell. These cells then undergo an endogenous cleavage producing an inner secondary cell within an enveloping primary cell. Secondary cells then proliferate through rapid, synchronous mitosis, and the host cell nucleus is compressed between the large parasitic aggregate and the host cell plasmalemma (Daniels et al. 1976; El-Matbouli et al. 1995). The secondary cells then undergo endogenous divisions to produce new cell-doublets with an enveloping cell and inner cell. These cell-doublets rupture the membrane of the original primary cell and enter the host cell cytoplasm. At this point, some cell-doublets seem to be destroyed within the cytoplasm of the host cell. When cell-doublets are free within the host cell cytoplasm, they pierce the host cell plasmalemma and enter the extracellular space. These now extracellularly situated cell-doublets either penetrate neighbouring epithelial cells or migrate deeper into the dermis and subcutis layers and penetrate new host cells, where they start the cycle again.

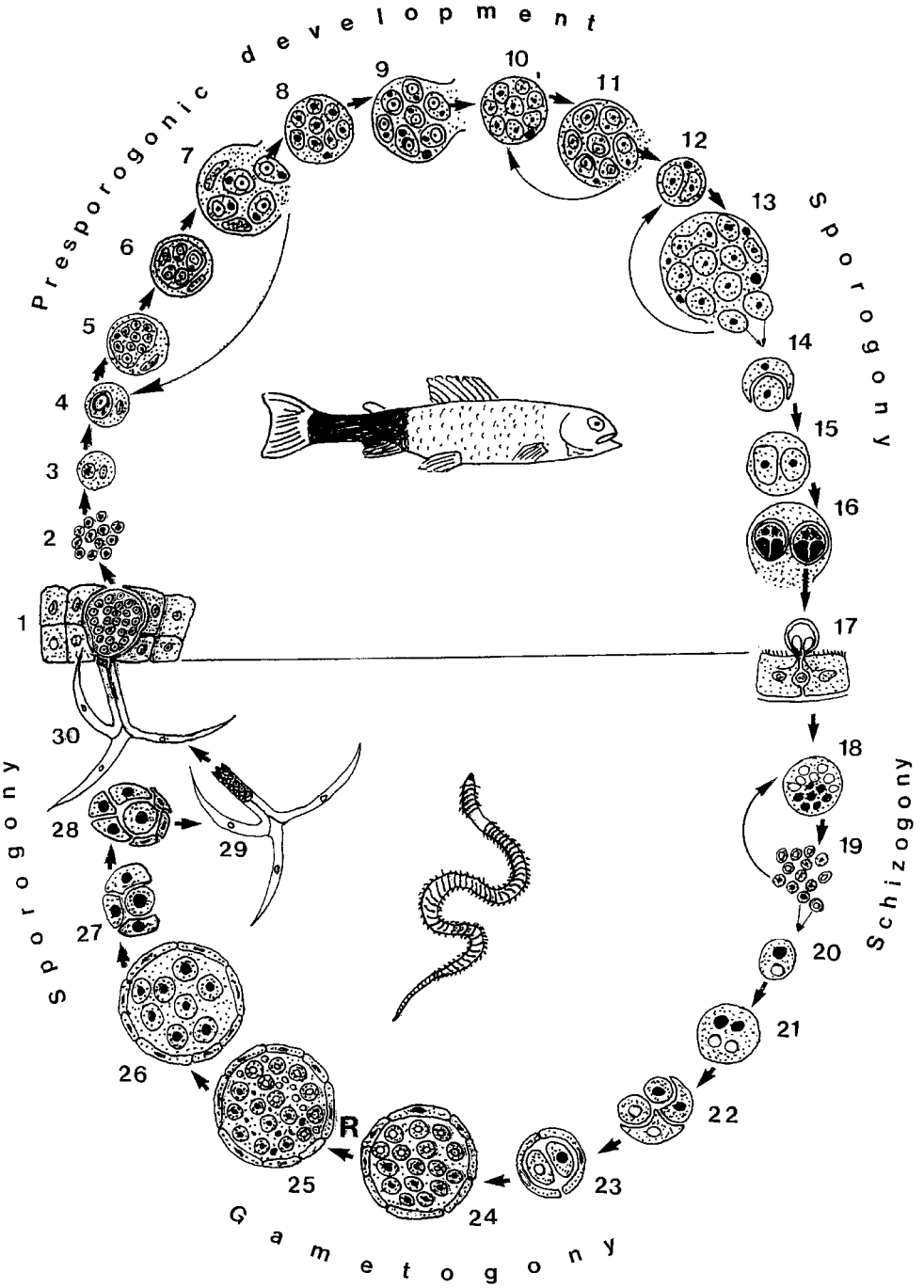

Fig. **3**. The life cycle and development of the Myxozoa, based largely on the life cycle of *Myxobolus cerebralis*, described in the text. **1–16.** Myxosporean development in the fish host. **17–30.** Actinosporean development in the annelid host. **1.** Actinospore attaches to surface of the fish, and releases sporoplasm into the fish. **2.** Sporoplasm internal cells divide by endogeny. **3–13.** Presporogonic or extrasporogonic vegetative replication. **14–16.** Sporulation with formation of multicelluar spores within plasmodia. **17.** Fully-developed myxospores released from fish host and ingested by annelids. **18–20.** Schizogony in gut epithelium of the worm. The resulting binucleate cells have an α and a β nucleus, which develop into complementary gametes by the end of gamogony. **21–26.** Gamogony. Internal cells in pansporocysts undergo 3 mitotic and 1 meiotic divisions. **24–25.** Resulting gametes fuse to form a pansporocyst with 8 zygotes. **27–29.** Sporogony. Multicellular spores are formed with 3 valves, 3 polar capsules, and a sporoplasm. Inflated spores (**29**) are released with the worm's faeces, float in the water, and contact the fish host to complete the life cycle.

Shortly after exposure, aggregates of cell-doublets can be found intercellularly in the subcutis. These stages continue the proliferative cycle of secondary cell mitosis to form cell-doublets. Around four days post-exposure cells of *M. cerebralis* migrate intercellularly in nervous tissue, where proliferation of cell-doublets continues as the parasite migrates through the central nervous system. From day 6–14 most parasitic stages can be found in the spinal cord; from day 16–24 most are found in

the brain. Some myxozoans (e.g. PKX, *Sphaerospora* spp.) exhibit massive presporogonic replication in tissues other than those in which sporulation occurs, and this has also been referred to as ''extrasporogonic development''. This occurs in several pathogenic *Sphaerospora* species and the PKX myxosporean, where massive proliferation of these stages may cause severe tissue damage (Lom and Dyková 1992).

Sporogonic phase (Fig. 3: 13–16). At the site of sporulation (e.g. cartilage for *M. cerebralis*), a plasmodium develops. The primary cell grows and its nucleus divides to produce numerous internal vegetative nuclei. The enveloped cell divides to produce many cells termed generative cells. According to the observations of El-Matbouli et al. (1995), after disintegration of the enveloping cell of the plasmodium, each enveloped cell either repeats the cycle producing numerous plasmodia, or unites with another cell, one becoming the pericyte envelope and the inner one forming the sporogonic cell; this unit generates pansporoblasts with internal spores (two spores for *M. cerebralis*). For each spore, valvogenic cells (which become the spore valves) enclose capsulogenic cells (which become the polar capsules) and a binucleate sporoplasm or two uninucleate sporoplasms. Myxospores are eventually released from the fish host and are infective to annelids.

Actinosporean development within annelids. The following description is based largely on that of El-Matbouli and Hoffmann (1998).

Schizogony (Fig. 3: 17–20). The actinosporean stage of development occurs when spores released from the fish host are ingested by the annelid worms (e.g. *Tubifex tubifex*). In the gut lumen of the worm, spores extrude their polar filaments by which they attach to the gut epithelium. The valves of the spore then open along the suture line, and the binucleated sporoplasm penetrates between the gut epithelial cells. The factors that induce myxospores to extrude their polar filaments remain unknown. Afterwards, both nuclei of the sporoplasm undergo multiple division to produce multinucleate cells. These stages then divide by plasmotomy to produce numerous uninucleate cells, which wander intercellularly through the gut epithelia of the worm. Some of these stages undergo further nuclear and cellular divisions, forming additional multinucleate and uninucleate cells. Others fuse to form binucleate stages.

Gametogony (Fig. 3: 20–25). The nuclei in the binucleate stage divide to form a stage with four nuclei, which then divides to form an early pansporocyst with four cells, two enveloping somatic cells and two generative cells termed α and β. Three mitotic divisions of the two generative cells yield 16 diploid gametocytes (8a and 8b) which then undergo one meiotic division to produce 16 haploid gametocytes and 16 polar bodies. Each gametocyte from the α line unites with one from the β line to produce eight zygotes. Based on the life cycle of *M. cerebralis,* this is the only phase of the life cycle in which sexual stages occur. Meanwhile, the somatic cells divide twice to produce eight enveloping cells (El-Matbouli and Hoffmann 1998).

Sporogony (Fig. 3: 27–29). At the end of gametogamy, the eight zygotes in each pansporocyst are surrounded by eight somatic cells. Each zygote then undergoes two mitotic divisions to produce a four-cell stage. Three cells are located peripherally and divide to form three capsulogenic and three valvogenic cells, while the fourth cell, centrally located, undergoes numerous mitotic divisions to form the sporoplasm of the actinosporean spore with its numerous internal cells. Subsequently, the capsulogenic cells and the sporoplasm are enclosed within a shell composed of three valves. Behind the sporoplasm, the valvogenic cells extend infolded membranes that ultimately turn into the shell valves of the style and the three projections of the triactinomyxon spore. This final stage with pansporocysts containing 8 (or 4 with *Tetraspora*) folded actinosporean spores begins to appear 90 days post-exposure in *M. cerebralis* (El-Matbouli and Hoffmann 1998). Actinosporean spores released from worms may remain viable for up to 2 wk (Xiao and Desser 2000b).

***Tetracapsula* in bryozoans and PKX.** Korotneff (1892) observed a myxozoan in the bryozoan, *Plumatella fungosa,* which he described as *Myxosporidium bryozoides.* Myxozoans in bryozoans were not reported again until 1996. Canning et al. (1996) and Okamura (1996) described *Tetracapsula byrozoides* from the phylactolaemate bryozoan *Cristatella mucedo.* This myxozoan was rather unique in that it formed soft spores with 4 polar capsules within large sac-like structures (Canning et al. 1996, 2000). Feist (1997) and Kent et al. (1998) noted the remarkable similarity between the ''haplosporosomes'' of the sporoplasm of *T. bryozoides* and those of the mother cell (primary cell) of PKX, the cause of proliferative kidney disease (PKD) of salmonid fishes (see review by Hedrick et al. 1993). Thus, they suggested that the two myxozoans may be related.

The identity of the PKX myxozoan had long been an enigma because its myxospores in fish were presumed to be immature or incomplete (Clifton-Hadley and Feist 1989; Kent and Hedrick 1986; Kent et al. 1998; Marin de Mateo et al. 1993). Many researchers, including some of the present authors, suggested that PKX may be a *Sphaerospora* or *Parvicapsula* species, but 18S rDNA comparisons proved otherwise (Kent et al. 1998). Then Anderson et al. (1999a,b) compared the 18S rDNA of PKX to undescribed species of tetracapsulids from the USA, and found that they were very similar ($>$ 99%). Longshaw et al. (1999) found similar results with tetracapsulids from *Fredericella sultana* and *Plumatella* sp. from PKX enzootic waters in England. This led to almost simultaneous descriptions of PKX as a *Tetracapsula* species. Canning et al. (1999) described PKX as *T. bryosalmonae* based on its development in Bryozoa, while Kent et al. (2000) assigned it the name *T. renicola* based on its myxosporean stages. Due to rules of priority, *T. renicola* becomes a junior synonym of *T. bryosalmonae.*

The potential for other fish to act as hosts for this group of parasites is apparent as PKX-like cells have been found in carp (*Cyprinus carpio*) (Voronin and Chernysheva 1993). It is expected that more species of *Tetracapsula* will be discovered although the features used to identify species will need careful consideration, as at present the best method to differentiate tetracapsulids is by rDNA comparisons. In an early report, Schröder (1910) described a species of Mesozoa in *Plumatella repens* and *P. rugosa* that was named as *Buddenbrockia plumatellae.* Examination of the drawings and mode of development strongly support the possibility that it is in fact a species of *Tetracapsula* with a vermiform spore sac and similar spore morphology. Recently, a species with a similar sac structure to *B. plumatellae* has been found in a *Plumatella* sp. in a small loch in Scotland (D. Morris, pers commun.). It seems likely that other myxozoans of Bryozoa will be detected as more attention is given to these organisms in both the freshwater and marine environments.

Important data on the life cycles and transmission of this interesting group of myxozoans is still needed. For example, does *T. bryozoides* also have a fish host, and is it similar to PKX? Unlike actinosporean stages from annelids, can *Tetracapsula* species complete their life cycles without fish hosts? And can PKX infect bryozoans, or are fish dead-end hosts for PKX as suggested by Canning et al. (2000).

Direct transmission. Direct fish-to-fish transmission without the requirement of alternate actinosporean development may occur in some species. Diamant (1997) demonstrated that *My-*

xidium leei, a parasite of the gut epithelium of various marine fishes, could be transmitted directly from fish to fish. As these transmissions studies were conducted by cohabitation, rather than with purified myxospores, it is still possible that the infection was transmitted by trophozoites. Therefore, myxospores of this species may also require development within an alternate host to complete the life cycle. Swearer and Roberston (1999) reported that *Kudoa ovivora,* which infects the eggs of the wrasse *Thalassoma bifasciatum,* could be directly transmitted from fish to fish. However, this study utilized fish collected from the field, and a more defined, laboratory transmission study is needed to confirm this observation.

NOMENCLATURE

At first, the phylum Myxozoa was divided into 2 classes: members of the Class Myxosporea infect fishes (and rarely reptiles and amphibians) while members of the Class Actinosporea infect annelid worms. The final developmental stage in both hosts is a spore, identified by distinct polar capsules. This initially appeared to be a reasonable taxonomic division: the smaller, simpler and mostly bilaterally symmetrical myxozoans, or Myxosporea, were parasites of vertebrate hosts while the larger, ornate Actinosporea, often exhibiting 6- to 12-fold geometric symmetry were found exclusively in annelid hosts. This broad taxonomic subdivision remained until it was discovered that these were merely alternating stages of the same species. This life cycle has now been confirmed in 25 myxozoan species from several genera (Table 2). Consequently, Kent et al. (1994b) proposed that the class Actinosporea be suppressed. They also proposed, based on their interpretation of the International Code of Zoological Nomenclature (1985), that all actinosporean generic names should be treated as collective groups, and thus would not compete in priority with myxosporean generic names. In addition, they proposed that new taxa of Myxozoa should be described based on the myxosporean stages: new myxozoans should **not** be described based only on actinosporean forms. Lom et al. (1997) proposed that new actinosporeans be described in the vernacular (e.g. 'triactinomyxon actinosporean stage'), a concept not new in parasitology, and that, similar to what has been used for other collective groups, the authority in each instance should be quoted (e.g. triactinomyxon actinosporean stage of Marques 1984). Many researchers have followed this suggestion for describing actinosporeans in which their myxosporean stages are unknown (El-Mansy et al. 1998b,c; McGeorge et al. 1997; Xiao and Desser 1998a,b). However, some have considered this proposal premature and have thus continued to assign binomial names to actinosporeans (Hallett et al. 1998; Hallett and Lester 1999; Hallett et al. 1999; Lester et al. 1998; Lin et al. 1999). Furthermore, the PKX myxozoan was named *Tetracapsula bryosalmonae* based on its bryozoan stage (Canning et al. 1999), but this may be equivalent to an unusual actinosporean stage, with its myxosporean stage occurring in salmonid fishes. The controversy on naming actinosporeans has been played out in letters and rebuttals to *Parasitology Today* (Kent and Lom 1999; Lester et al. 1998, 1999).

In our view, the major discrepancies in these divergent practices in nomenclature depend on interpretations of the significance of new and unusual actinosporean forms, and not on interpretations of the International Code of Zoological Nomenclature. While Kent, Lom, and colleagues (Kent and Lom 1999) contend that actinosporeans should be treated as life stages of known or unknown myxosporeans, Lester and colleagues (Lester et al. 1998, 1999) proposed that new forms discovered in the marine environment may not possess myxosporean forms. Eventually a consensus should be reached, as it is certain that many new actinosporean forms with unknown myxosporean stages will continue to be discovered and resolution of life cycles cannot keep pace with the descriptions of new actinosporean forms.

PHYLOGENETIC PLACEMENT AND ORIGINS OF THE MYXOZOA

The Myxozoa were usually grouped with protistan taxa until the early 1990s. Largely unnoticed, or perhaps merely dismissed, a century ago Štolc (1899) claimed that myxozoans are not protists. Rather, he asserted that their spores are multicellular and that they should be included with Metazoa. Later, Weill (1938) reaffirmed this hypothesis and was more specific. Because the polar capsules of myxozoans showed identical discharge properties to nematocysts, Weill (1938) suggested that they are cnidarians. Moreover, the coelozoic myxozoans showed such remarkable pathological similarities to some parasitic cnidarians that Weill (1938) suggested an affinity with the narcomedusan, *Polypodium hydrifome.* Although these positions were noted from time to time (Dogiel, 1965; Grassé and Lavette, 1978), it took molecular sequence data to confirm them.

When sequences of the nuclear small 18S rDNA became available, Smothers et al. (1994) showed that myxozoans grouped with the Metazoa as a sister group to nematodes, an unusual hypothesis, and not with the three cnidarian sequences that they used. Shortly thereafter, using a combination of molecular and morphological data and an additional dozen available cnidarian sequences, Siddall et al. (1995) showed that the myxozoans nested within the Cnidaria, as sister to the narcomedusan fish parasite, *Polypodium hydriforme,* precisely as predicted by Weill (1938). Siddall et al. (1995) employed both MALIGN (Wheeler and Gladstein, 1996a) and optimization alignment (Wheeler and Gladstein, 1996b), a then novel approach to simultaneously solving the problems of sequence alignment and phylogeny reconstruction. Specifically, in terms of metazoan affinities, Siddall et al. (1995) were the first to note desmosomes, tight junctions, and collagen production. With regard to their cnidarian origins, Siddall et al. (1995) provided ultrastructural characterization of the development of myxozoan ''polar capsules'' from capsulogenic cells, finding this to be indistinguishable from that seen in narcomedusan nematocysts. Lom and Dyková (1997) also concluded that the Myxozoa are very similar to the Cnidaria based on ultrastructural comparisons, particularly comparing polar capsules and nematocysts.

Since those studies were published, the issue of myxozoan origins has been revisited on three occasions. Though not a principal component of their study, Hanelt et al. (1996) reported that the Myxozoa were sister to the triploplasts, rather than cnidarians using a maximum likelihood approach. Actually, even using the maximum likelihood approach, Hanelt et al. (1996) reported that myxozoans were sister to the narcomedusan, *P. hydriforme,* but they argued that this was due to long branch attraction and then excluded the narcomedusan from further analyses. Siddall and Whiting (1999) re-evaluated the phylogenetic position of the Myxozoa with a much broader array of taxa than was employed by Siddall et al. (1995), and once again found a cnidarian origin for the Myxozoa. Moreover, placing the Myxozoa squarely at the forefront of the likelihood-vs-parsimony debate, Siddall and Whiting (1999) argued that nematocysts, developmental stages, and other morphological characters could not be explained away as the result of long-branch attraction in the same way as nucleotide data could be.

These latter findings are by no means considered to be the last word on the matter of myxozoan origins and affinities (An-

derson 1998; Anderson et al. 1998; Hanelt et al. 1996; Schlegel et al. 1996). A factor confounding final resolution of this matter is the reliance on a single data set: the 18S rDNA gene. Anderson et al. (1998) and Anderson (1998) have suggested a triploblast affinity for myxozoans as well, but this was based on an 82-bp homeobox (*Hox*) gene that looked like an antennapedial sequence. Poriferan *Spox* genes also resemble *Hox* genes in molluscs and polychaetes (Degnan et al. 1995), and no one seriously argues that sponges should be placed among the Eutrochozoa or even with triploblasts. So far *Cnox* (cnidarian-like homeobox) homologous genes in the Myxozoa have not been reported.

RELATIONSHIPS AMONG THE MYXOZOA

Shulman (1966) suggested that the first myxozoans were coelozoic, inhabiting the gall bladder and later the urinary bladder of marine teleost fishes in the late Cretaceous period. Myxozoans later evolved to infect other tissues, with some forms becoming histozoic. Shulman (1966) also suggested that ancestral myxozoans were bipolarids (e.g. *Myxidium, Sphaeromyxa*), and that in fresh water they gave rise to the platysporinids (*Henneguya, Myxobolus*). The class Myxosporea, until recently, contained two orders; the Bivalvulida (spores with 2 valves and generally 2 polar capsules) and the Multivalvulida (with spores containing greater than 2 valves and usually more than 2 polar capsules) (Table 1). Shulman (1966) proposed that the order Multivalvulida (all marine histozoic forms) was derived from ancestors similar to *Ceratomyxa* (a coelozoic, marine genus). Canning et al. (2000) proposed the class Malacosporea and the order Malacovalvulida for *Tetracapsula* spp. (family Saccosporidae), unusual myxozoans that infect bryozoans. Some special features of these unusual myxozoans include a sac-like proliferative body and valve cells that do not cover the apex of the spore where the polar filament exits.

Aside from the new sequences that we report here, 18S rDNA sequences for about 35 species of Myxozoa, belonging to about 9 genera are available from GenBank and published reports (Table 3). Phylogenetic trees using 18S rDNA sequences from species available at the time largely agreed with Shulman's phylogenetic hypotheses (Kent et al. 2000; Kent and Palenzeula 2001). For example, *Ceratomyxa* clusters with *Kudoa* species, and the histozoic characteristic appears to have arisen at least twice; with the freshwater myxozoans as represented by the suborder Platysporina (e.g. *Myxobolus* and *Henneguya* spp.), and with the marine order Multivalvulida (represented by *Kudoa* species). Based on 18S rDNA analyses most marine and freshwater taxa examined at that time were separated on two major branches, with the single exception of *C. shasta,* a freshwater species that infects salmonid fishes (Kent et al. 2000; Kent and Palenzeula 2001). This organism falls between the marine genera *Sinuolinea* and *Parvicapsula* (Fig 4). All other members of this genus are marine species, suggesting the possibility of later or secondary colonization of the freshwater environment by this myxozoan. Interestingly, the alternate host of *C. shasta* is a freshwater polychaete, which as a group are typically marine organisms. Analysis of 18S rDNA sequences indicated that the enigmatic PKX myxosporean is distinct from other myxosporeans and that its roots lie within the Myxozoa before divergence of the other major groups (Kent et al. 1998). However, when *T. bryozoides,* an unusual myxozoan of bryozoans (Okamura et al. 1996), is included in these analyses a distinct clade is formed with PKX and *T. bryozoides* (cf. Anderson et al. 1999a; Kent et al. 2000). This provided Canning et al. (2000) further support to erect a separate class and order for these organisms.

Although at the genus and family level 18S rDNA results are fairly consistent with traditional taxonomic schemes, some interesting inconsistencies arise. The taxonomic classifications within the Myxozoa are largely based on spore morphology (Kent et al. 2001; Lom and Arthur 1989; Lom and Noble 1984). However, developmental stages (Lom and Arthur 1989) and non-morphological attributes (Meglitsch 1957) should also be included in taxa descriptions. Examinations of five species of *Kudoa* suggested that members of this genus are related more by hosts and geography than by spore morphology (Hervio et al. 1997): *Kudoa* species from the eastern Pacific temperate areas (*Kudoa minibicornis, K. paniformis,* and *K. thyrsites*) form a distinct clade from *Kudoa amamiensis* from yellowtail (*Seriola quinqueradiata*), a warm-water pelagic fish from the western Pacific. In contrast, based on spore morphology, *K. thyrsites* would be considered an outlier as its spores are stellate, whereas the others all have round or quadrate spores. Furthermore, these phylogenetic trees of *Kudoa* based on 18S rDNA sequence disagreed with those constructed by Swearer and Roberston (1999) based on morphologic characters. In the latter study, the taxonomic affinities of 45 *Kudoa* species were analysed using 12 phenotypic variables including various spore measurements, site of infection, and number of spores per trophozoite. Clearly more molecular systematics research is warranted to resolve this apparent discrepancy among *Kudoa* species.

While *Kudoa* appears to be a monophyletic group, many genera are not monophyletic, based on 18S rDNA analyses. *Myxidium* and *Myxobolus* are paraphyletic, while *Henneguya* and *Sphaerospora* are polyphyletic. The discrepancies within the Platysporina were reported by both Smothers et al. (1994) and latter expanded upon by Andree et al.(1999b), for example, 18S rDNA sequence data do not support a phylogenetic separation of the two major genera *Henneguya* and *Myxobolus. Henneguya salminicola,* which was used as an outgroup in the analysis along with *K. thyrsites* and *C. shasta,* grouped within the clade formed by the *Myxobolus* spp. This suggests that caudal appendages on spore valves, which distinguishes *Henneguya* from *Myxobolus,* arose on multiple occasions, and is not a valid character to separate these speciose groups of platysporinids into two separate genera. Using 18S rDNA sequences of ten *Myxobolus* species, Andree et al. (1999b) suggested that members of this genus tend to group by tissue locations. In contrast, Salim and Desser (2000), using partial 18S rDNA sequences of seven different *Myxobolus* species from cyprinid fishes, found that they group by spore morphology. Xiao and Desser (2000c) used riboprinting analysis of the 18S rDNA of some 18 myxozoans from Lake Sasajewun, Ontario, including both myxosporean and actinosporean forms. Consistent with our view on alternate development between these two stages in fish and worms, they found that neither the myxosporeans nor actinosporeans formed monophyletic groups. Also consistent with sequence analysis based on 18S rDNA studies, *Myxidium* sp. was an outlier taxon from the clade containing *Myxobolus* species (i.e. the platysporinids). The ITS sequence of only one myxozoan has been examined thus far (Andree et al. 1999a). They found no differences in the ITS-1 of *M. cerebralis* from Europe and the United States, supporting the hypothesis that the parasite was transferred to North America in recent years.

NEW rDNA SEQUENCES AND ANALYSIS

In addition to 18S rDNA sequences available in GenBank, we present here new sequence data from 22 myxosporeans and actinosporeans published for the first time (Table 3; Hallet 1997; Xiao 1999). New sequences were obtained using standard PCR approaches as previously described (Andree et al. 1999b; Hervio et al.1997; Kent et al. 2000).

Table **3**. Small subunit ribosomal DNA sequences of Myxozoa used in phylogenetic analysis

Species	Host	Location	GenBank number	Reference
Myxobolus sp. 1	*Cottus bairdii*	Idaho	U13828	Smothers et al. 1994
Myxobolus sp. 2	*C. bairdii*	Idaho	U13830	Smothers et al. 1994
Myxobolus cerebralis	*Oncorhynchus mykiss*	West Virginia	U96493	Andree et al. 1997
Myxobolus arcticus	*O. nerka*	British Columbia	AF085176	Andree et al. 1999
Myxobolus bramae	*Abramis brama*	Hungary	AF085177	Andree et al. 1999
Myxobolus drjagini	*Hypophthalmichthys molitrix*	Hungary	AF085179	Andree et al. 1999
Myxobolus insidiosus	*O. tshawytscha*	Oregon	U96494	Andree et al. 1997
Myxobolus ellipsoides	*Rutilus rutilus*	Hungary	AF085178	Andree et al. 1999
Myxobolus neurobius	*O. mykiss*	California	AF085180	Andree et al. 1999
Myxobolus portucalensis	*Anguilla anguilla*	Hungary	AF085182	Andree et al. 1999
Myxobolus sandrae	*Stizostedion lucioperca*	Hungary	AF085181	Andree et al. 1999
Myxobolus squamalis	*O. tshawytscha*	California	U96495	Andree et al. 1997
Myxobolus algonquinensis	*Notropis cornutus*	Ontario	AF378335	Present study
Myxobolus bibullatus	*Catostomus commersoni*	Ontario	AF378336	Present study
Myxobolus ichkeulensis	*Mugil* sp.	Tunisia	AF378337	Present study
Myxobolus osburni	*Lepomis gibbosus*	Ontario	AF378338	Present study
Myxobolus pellicides	*Semotilus atromaculatus*	Ontario	AF378339	Present study
Myxobolus pendula	*Semotilus atromaculatus*	Ontario	AF378340	Present study
Myxobolus spinicurvatura	*Mugil cephalus*	Tunsia	AF378341	Present study
Myxobolus sp. (rainbow trout)	*Oncorhynchus mykiss*	California	AF378342	Present study
Myxobolus sp. (white sucker)	*Catostomus commersoni*	California	AF378343	Present study
Henneguya sp. 1	*Cottus bairdii*	Idaho	U13826	Smothers et al. 1994
Henneguya zschokkei	*P. williamsonii*	British Columbia	AF378344	Present study
Henneguya salminicola	*O. nerka*	British Columbia	AF031411	Hervio et al. 1997
Henneguya lesteri	*Sillago analis*	Queensland	AF306794	Hallett and Diamant 2001
Henneguya exilis	*Ictalurus punctatus*	Mississippi	AF021881	Lin et al. 1999
Henneguya ictaluri	*I. punctatus*	Mississippi	AF195510	Pote et al. 2000
Henneguya doori	*Perca flavescens*	Nova Scotia	U37549	Siddall et al. 1995
Thelohanellus hovorkai	*Cyprinus carpio*	Japan	AJ133419	Anderson et al. 2000
Myxidium sp.	*Cottus bairdii*	Idaho	U13829	Smothers et al. 1994
Myxidium lieberkuehni	*Esox lucius*	Czech Republic	ML16SLR1	Schlegel et al. 1996
Myxidium truttae	*Oncorhynchus kisutch*	British Columbia	AF201374	Kent et al. 2000
Sphaerospora oncorhynchi	*O. nerka*	British Columbia	AF201373	Kent et al. 2000
Sphaerospora molnari	*Carassius auratus*	Japan	AF378345	Present study
Parvicapsula minibicornis	*O. nerka*	British Columbia	AF201375	Kent et al. 2000
Ceratomyxa shasta	*O. mykiss*	Oregon	AF031579	Bartholomew et al. 1997
Sinuolinea sp.	*Scophthalmus maximus*	Spain	AF378346	Present study
Kudoa thyrsites	*Salmo salar*	British Columbia	AF031412	Hervio et al. (1997)
Kudoa amamiensis	*Seriola quiqueradiata*	Japan	AF034638	Hervio et al. (1997)
Kudoa miniauriculata	*Sebastes paucispinis*	California	AF034639	Hervio et al. (1997)
Kudoa paniformis	*Merluccius productus*	British Columbia	AF034640	Hervio et al. (1997)
Kudoa crumena	*Thunnus albacares*	North Carolina	AF378347	Present study
Kudoa ciliatae	*Sillago ciliata*	Queensland	AF378348	Present study
Tetracapsula bryosalmonae	*O. mykiss*	France	U70623	Saulnier et al. 1997
Tetracapsula bryozoides	*Cristatella mucedo*	England	AJ133564	Anderson et al. (1999)
Tetraspora discoidea	Tubificidae	Queensland	AF306793	Present study
Sphaeractinomyxon ersei	Tubificidae	Queensland	AF306790	Present study
Endocapsa rosulata	Tubificidae	Queensland	AF306791	Present study
Triactinomyxon sp.–marine	Tubificidae	Queensland	AF306792	Present study
Triactinomyxon ignotum	*Lumbriculus hoffmeisteri*	Ontario	AF378349	Present study
Triactinomyxon C	*L. hoffmeisteri*	Ontario	AF378350	Present study
Triactinomyxon F	*L. hoffmeisteri*	Ontario	AF378351	Present study
Raabeia B	*L. hoffmeisteri*	Ontario	AF378352	Present study
Neoactinomyxum	*L. hoffmeisteri*	Ontario	AF378353	Present study
Synactinomyxon	*L. hoffmeisteri*	Ontario	AF378354	Present study
Antonactinomyxon	*L. hoffmeisteri*	Ontario	AF378355	Present study
Aurantiactinomyxon	*L. hoffmeisteri*	Ontario	AF378356	Present study
Aurantiactinomyxon mississippiensis	*Dero digitata*	Mississippi	AF021878	Pote et al. 2000
Polypodium hydriforme	*Acipenser ruthenus*	Russia	U37526	Siddall et al. (1995)

Sequences were aligned with CLUSTAL and with MALIGN (Wheeler and Gladstein 1996a) in order to assess how sensitive relationships may be to alignment regime. Moreover, due to extremely high rates of insertions and deletions in some regions, analyses were conducted both with and without hypervariable sites using parsimony and neighbor-joining with the Kimura 2-parameter distance model. In order to assess stability to character information we used parsimony jackknifing (Farris et al. 1996) with 1000 replicates and deletion of e^{-1} proportion of the aligned sites. Furthermore, only those groups found in more than 60% of the replicates were retained (Fig. 4).

Although our present study included some 60 species of myxozoans, there remains instability in this data set depending on whether one aligns with CLUSTAL or with MALIGN and

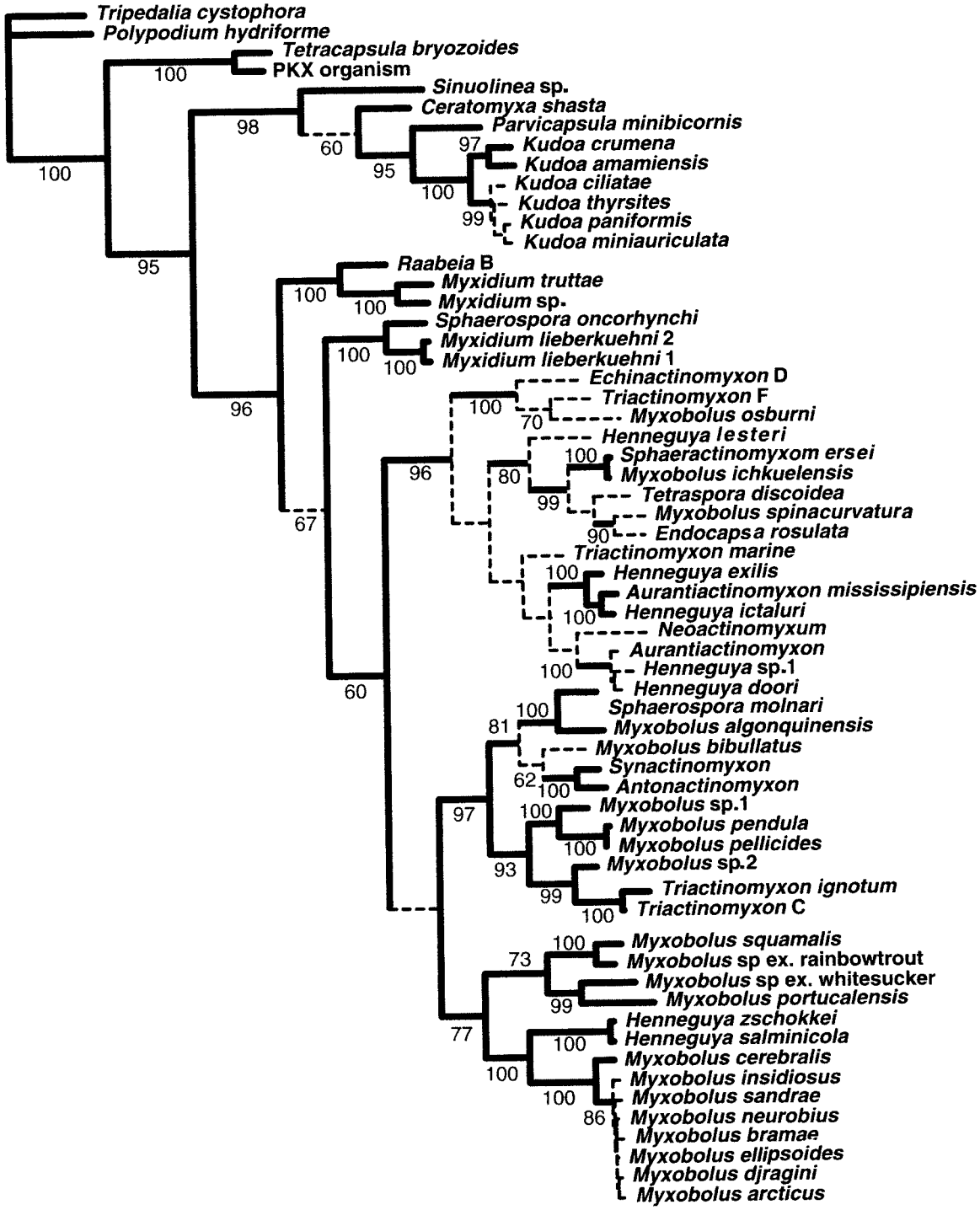

Fig. **4**. Single most parsimonious tree from phylogenetic analysis of all CLUSTAL aligned 18S rDNA data for myxozoans (length = 10,865, retention index = 64.5%). Solid lines indicate groupings that were also found in the most parsimonious tree found when hypervariable sites were excluded and that are also consistent with the trees found from distance-based neighbor joining methods. Branches with dotted lines indicate lack of stability in alignment, lack of stability where variable characters were included, or disagreement between parsimony and distance approaches. Numbers at nodes are parsimony jackknife support indices after 1,000 jackknife sampling replicates.

whether one assesses optimality in a parsimony or a distance framework. It is unusual among current phylogenetic studies to find such instability when afforded comprehensive taxonomic sampling of a group. Generally (Graybeal, 1998), but not always (Kim 1998), the confounding effects of multiple substitutions or unequal rates of change can be countered through judicious taxon sampling across the full range of taxa in a group of interest. For the CLUSTAL aligned data, where inclusion or exclusion of hypervariable regions has no effect and where the parsimony analyses agree with the distance-based methods,

branches are drawn solid. Branches with disrupted lines indicate lack of stability to alignment, lack of stability to inclusion of variable characters, or disagreement between parsimony and distance approaches. Still, some 38 out of 59 clades are stable to these parameters and there is considerable resolution of many of the substantive issues in myxozoan systematics.

Phylogenetic analysis of these data (Fig. 4) continues to suggest that the only genus that is clearly monophyletic is *Kudoa,* which falls out near the base of the tree with a basically marine clade containing *Sinuolinea, Ceratomyxa,* and *Parvicapsula.* Mirroring other l8S rDNA analyses (Anderson et al. 1999a,b; Kent et al. 1998, 2000) the earliest diverging myxozoan lineage appears to be the tetracapsulids. However, with the MALIGN alignment method this group was placed with *Sinuolinea* sp., irrespective of optimality criteria. *Sinuolinea* has rather unusual, spherical spores, and it is conceivable that, like the tetracapsulids, it may represent a myxozoan very distinct from multivalvulid and bivalvulid forms. The genus *Myxidium* is paraphyletic, successively diverging prior to the origins of the principally freshwater clade that contains (among others) *Myxobolus* and *Henneguya* species. *Henneguya* and *Myxobolus* (suborder Platysporina) group together within a large clade comprised of freshwater histozoic myxosporeans. Thus, these revised data still support the deep division of freshwater myxozoans from marine genera (e.g. *Ceratomyxa, Parvicapsula, Sinuolinea,* and *Kudoa*) and the affinity between *Myxobolus* and *Henneguya.* Although there are, of course, marine species of *Henneguya* and *Myxobolus* (e.g. *Henneguya lesteri, Myxobolus spinacurvatura,* and *Myxobolus ichkeulensis*), these optimize as recent reversals to a marine environment. Similarly, though *C. shasta* infects freshwater salmonids, all other members of the genus *Ceratomyxa* infect marine fishes and *C. shasta* thus groups within the marine clade (Fig. 4).

The two *Sphaerospora* species in our analysis, *Sphaerospora oncorhynchi* and *Sphaerospora molnari,* do not appear to be related (e.g. jackknife support for a sister group relationship between *S. molnari* and *Myxobolus algonquinensis* is 100%). Superficially this appears contrary to the phenotypic characters of spore morphology typical of the genus *Sphaerospora.* However, *S. oncorhynchi* is like most other *Sphaerospora* spp. in that its development is coelozoic in kidney tubules (Kent et al. 1993a), whereas *S. molnari* is histozoic in gills and skin (Lom et al. 1983). Notably, *S. oncorhynchi* clusters with the coelozoic genus *Myxidium* while *S. molnari* groups consistently with the histozoic platysporinid clade.

The basically freshwater clade (jackknife support of 60%, in Fig. 4) is comprised of three well-supported subclades (jackknife supports of 96%, 97% and 77%, Fig. 4) that are found irrespective of alignment method or of phylogenetic optimality criterion (parsimony or distance). Each of the three subclades separately includes several species of *Myxobolus.* Concerning first the myxosporean stages included, one of these well-supported subclades contains the histozoic *S. molnari* and two separately contain species of *Henneguya* rendering this genus polyphyletic and *Myxobolus* paraphyletic. With respect to the actinosporean stages included here (Fig. 4), like the broad separation of *Myxobolus* species, triactinomyxon morphotypes are seen in two of the three subclades but are rendered paraphyletic by the remaining representative actinosporean types in these clades. There are no included actinosporeans for the third major freshwater subclade, but several of these members have triactinomyxon actinosporean stages (Table 2). On the whole, these relationships suggest that the original freshwater myxozoan possessed a *Myxobolus* myxosporean stage in the fish host and a triactinomyxon actinosporean stage in the annelid host.

The aurantiactinomyxon actinosporean morphotype appears to be associated with one of the three origins of a *Henneguya* myxosporean type (Table 2, Fig. 4), but it also may appear in other myxosporean genera (Table 2). Beyond this, though, there is relatively little correlation (Fig. 4) with the various morphological forms of alternating spore stages. Xiao and Desser (1998a,b) examined the 18S rDNA of several actinosporeans from a lake in Canada in which their myxosporean counterpart stages were unknown. With few exceptions, the inclusion of sequences from these same actinosporeans did not clearly resolve their taxonomic placements. However, triactinomyxon C of Xiao (1999) may be conspecific with *Triactinomyxon ignotum,* which agrees with the riboprinting data of Xiao and Desser (2000c). Raabeia B of Xiao (1999) may well represent an expectation of the actinosporean morphotype for some *Myxidium* species, but in the riboprinting study (Xiao and Desser 2000c), raabeia B grouped with platysporinids and other actinosporeans, rather than with a *Myxidium* species (not included in our present analysis). Similarly, inclusion of the marine actinosporeans (such as *Endocapsa rosulata* and *Tetraspora discoidea*) places them near *Myxobolus spinicurvata* and not in a clade distinct from other myxozoans. Interestingly, though these two actinosporean types were collected from marine oligochaetes, they fall within one of the well-supported clades comprised mostly of freshwater platysporinids (e.g. *Henneguya, Myxobolus*). However, these actinosporeans are in a region of the tree that appears to be secondarily marine (i.e. they include marine *Henneguya* and *Myxobolus* species).

In addition to the higher-level questions addressed above there are some species-level phenomena worth noting. For example, there is very little genetic distinction among *M. cerebralis* and allies, or for that matter among species related to *Kudoa ciliatae.* Similarly, *Myxobolus pendula* and *Myxobolus pellicides* are separated by only eight substitutions, and Xiao and Desser (2000c) reported identical riboprints for these species. *Henneguya zschokkei* differs from *Henneguya salminicola* in about thirty nucleotide positions. Two possible explanations for these patterns are that there are over-split species or that there are several recent rapid radiations of various myxozoan groups. Indeed, many researchers consider that *H. salminicola* of salmonids is a junior synonym of *H. zschokkei,* which infects closely related whitefishes (Lom and Dyková 1992).

Molecular systematics has helped to elucidate relationships within the Myxozoa, but the support for the branches in phylogenetic trees made from this large assemblage of taxa is weak. However, some general trends are now apparent: 1) marine taxa at the genus level branch earlier than genera that are generally freshwater; 2) taxa cluster more by development and tissue location than by spore morphology; 3) the tetracapsulids branched off early in myxozoan evolution, perhaps reflecting their bryozoan as opposed to annelid hosts; 4) actinosporean morphology so far offers little information for determining myxosporean counterpart morphology (assuming that they exist); 5) the unusual marine actinosporeans from Australia appear to form a clade with the marine platysporinids and; 6) the overall lack of generic monophyly may eventually require the more complete accommodation of *Myxobolus, Henneguya,* some *Sphaerospora* species and probably others yet to be sequenced, collectively with the genus *Myxobolus,* which has taxonomic priority (Bütschli 1881). So far we have yet to include representative taxa from the families Sphaeromyxidae, Ortholineidae, Fabesporidae, Chloromyxidae, Auerbachiidae, Alatosporidae, and Trilosporidae. Additional myxozoan taxa, as well as the putatively related narcomedusans, combined with more and different character sets should continue to aid in unravelling of the historical patterns of infection and global biogeography of myxozoans. Furthermore, the only large data set for molecular sys-

tematics for this group is the 18S rDNA, and it is imperative that other genes be analysed as well to verify or refute these observations. Therefore, at present, we are reluctant to revise the higher taxonomy of the Myxozoa based on the molecular data available at this time.

We do, however, encourage researchers to provide rDNA sequences when describing new species in the group, particularly when describing those that are morphologically similar or indistinguishable from exisiting species (e.g. many *Myxobolus* species). However, the 18S rDNA sequences of myxozoans are remarkably variable between even closely related taxa, and it is often very difficult to purify these organisms from host tissue. Both of these factors make it often very difficult to obtain rDNA sequences from new taxa when genus-specific primers are not available. Therefore, while recommended, we do not propose that rDNA sequences must be provided before new taxa can be described.

Sexual reproduction in the Myxozoa apparently occurs only in the actinosporean phase found in annelids. Because annelids evolved before fishes, annelids may indeed be the original hosts for myxozoans, which subsequently may have developed a two-host life cycle to include fishes. In contrast, there is evidence that fish may be the original hosts: 1) fish are hosts for most members of three clades of Myxozoa (i.e. the tetracapsulids, the freshwater clade, and the marine clade), while different invertebrates (bryozoans, oligochaetes, and a polychaete) are hosts for their respective actinosporean stage or its equivalent; and 2) the closest ancestor to the Myxozoa, *Polypodium*, is a parasite of freshwater fishes.

EMERGING AND RE-EMERGING MYXOZOAN DISEASES

***Kudoa thyrsites* and post-harvest soft flesh.** During the 1990s marine aquaculture expanded at a phenomenal rate, particularly with netpen culture of salmonids (family Salmonidae) and seabream species (family Sparidae). Atlantic salmon (*Salmo salar*) is the primary salmonid species reared in netpen aquaculture, and *K. thyrsites* has emerged as a serious problem in this fish reared in Ireland (Palmer 1994) and British Columbia, Canada (Kent et al. 1994a).

In British Columbia, Atlantic salmon account for approximately 70% of the farmed salmon production. One of the most important concerns to this industry is infection by *K. thyrsites.* Myxozoans of the genus *Kudoa* and related genera infect the muscle of many marine fishes, and heavy infections can cause unsightly white cysts or soft texture in fillets (Moran et al. 1999b). Although these changes can lower the market value of the infected fish, they seldom cause morbidity. *Kudoa thyrsites* is a cosmopolitan parasite that infects many species of marine fish (Moran and Kent 1999; Moran et al. 1999b). Heavy infections are associated with soft flesh and the unsightly white patches in pen-reared Atlantic salmon that are either held on ice for 3–6 days or smoked (Whitaker and Kent 1991; St.-Hilaire et al. 1997). Unfortunately, such infections are not detected in fresh fish during processing, and thus fish destined to develop the condition are usually sold in the market place. *Kudoa thrsites* has also been detected in farmed coho salmon (*Oncorhynchus kisutch*) (cf. Whitaker and Kent 1992), but never in farmed chinook salmon (*Oncorhynchus tshawytscha*). Interestingly, Kabata and Whitaker (1989) found infections in the heart muscle of wild Pacific salmon (*Oncorhynchus* spp.), but not in the skeletal muscle. Given that *K. thyrsites* infects a wide variety of fish hosts in many families, it is an enigma why chinook salmon are resistant.

Little is known about early development and transmission of *K. thyrsites* in fish. By experimentally exposing fish at a netpen site where the infection is indigenous, Moran et al. (1999a) found that it takes about 5–6 mo (i.e. about 2,000 degree-days) after infection before spores are detected in the flesh. A high prevalence of infection (often 50–70%) occurs in post-smolts after the first 5 mo in sea water, and then the fish recover. Atlantic salmon that undergo sexual maturation (referred to as grilse) in netpens will eventually absorb their gonads and their external characteristics will revert back to those of sexually-immature fish. At this stage they are referred to as ''reconditioned grilse'' and are often sold as normal fish in the market place. St.-Hilaire et al. (1998) found that the infection was much more prevalent in Atlantic salmon grilse or reconditioned grilse than in market-size fish that had not undergone sexual maturation. Infection prevalence in grilse may be as high as 70%, whereas less than 10% of immature fish from the same stocks were infected. It is not known if the high prevalence of the infection in grilse is due to reinfection or proliferation of a cryptic infection that originally occurred when the fish were first transferred to sea water. An infectious stage of the parasite occurs in the blood: fish injected with blood from infected fish and then held in fresh water developed the infection (Moran et al. 1999c). This experiment also demonstrated that once a fish is exposed, the parasite could complete its development even if fish were transferred to fresh water. Moran et al. (1999c) also found that direct per os exposure of Atlantic salmon with heavily-infected tissue did not cause infections.

Myxozoans in sea bass and sea bream. Mariculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) has dramatically expanded in the last decade in Mediterranean countries, such as Greece, Israel, Italy, Turkey, and Spain. Sea bream production leads this expansion, with a 412% increase from 1991 to 1996 (Basurco and Abellán 1999). Diversification of production through the culture of other sparid fishes with high local market value, such as *Dentex dentex, Puntazzo puntazzo, Diplodus* spp., and *Pagrus* spp., is a recent strategy that, together with the adoption of netpen-based growing facilities, characterize the rapidly growing industry of Mediterranean mariculture. Under these circumstances, outbreaks of diseases due to myxozoans are increasingly being reported.

Sphaerospora dicentrarchi and *Sphaerospora testicularis* are common parasites of sea bass (Álvarez-Pellitero and Sitjà-Bobadilla 1990, 1993a). *Sphaerospora testicularis* is a coelozoic species infecting the seminiferous tubules; the infection can eventually result in parasitic castration of valuable broodstock males. *Sphaerospora dicentrarchi* is a histozoic, systemic species infecting connective tissue at a very high prevalence (70-100%) in wild and cultured animals. Massive infections with this species have been found associated with extensive mortalities of juvenile fish. Recent analyses of molecular data indicate that this species is closely related to *Kudoa* spp. and to other marine histozoic myxozoans rather than to typical *Sphaerospora* species (Kent and Palenzeula 2001; Palenzeula and Bartholomew 1999).

Ceratomyxa labracis and *Ceratomyxa diplodae* are also highly prevalent in sea bass. They are not usually associated with clinical disease but they can induce histological lesions in the gall bladder, including epithelial necrosis and thickening and inflammation of the underlying connective tissue, as well as damage to the neighboring pancreatic tissue (Álvarez-Pellitero and Sitjà-Bobadilla 1993b).

Several myxozoan species have been reported from gilthead sea bream *Sparus aurata,* three of which are associated with histopathological damage or episodes of mortality or morbidity. *Polysporoplasma sparis* infects the trunk kidney causing glomerulonephritis (Palenzeula et al. 1999a). *Ceratomyxa sparu-*

saurati is very common in the gall bladder, generally causing limited histopathological damage but sometimes in heavy infections it is associated with clinical signs and mortality (Palenzeula et al. 1997). *Myxidium leei* is the most significant myxozoan in cultured sea bream in the Mediterranean. Since its description as a significant contributor to mortality of cultured *S. aurata* in eastern Mediterranean waters (Diamant 1992; Diamant et al. 1994) it has been found to cause important losses in other sparid fishes. The parasite invades the intestinal tract causing severe chronic enteritis that frequently causes emaciation and death. Losses reach 80% of some stocks, especially in *P. puntazzo,* which seems to be the most susceptible sparid (Athanassopoulou et al. 1999). Although assigned to the genus *Myxidium,* this parasite presents rather unique characteristics, such as direct transmission between fish and an extremely wide host specificity. Susceptible animals can become infected by cohabitation with infected fish, by ingestion of developmental stages, and by receiving water from infected sources (Diamant 1997). This range of susceptible fish species is astonishing for a myxozoan. Infections have been recorded in most cultured sparid species, in wild mullets in the vicinity of sea bream growing systems, and in *Sciaenops ocellatus* (an Atlantic, North American species introduced in the Mediterranean). Recently, *M. leei* has been detected in marine aquarium fishes belonging to 25 species in four separate orders (Padrós et al. 2001). Furthermore, related enteric histozoic species have been reported recently in other marine fish, such as a *M. leei*-like myxozoan in anemone fish held in the Pacific coast of the U.S. (Kent 1999) and a species responsible for significant mortalities in cultured turbot on the Atlantic coast of Spain (Branson et al.1999). Preliminary studies of molecular data showed that *M. leei* and the species from turbot cluster with other marine histozoic species and are very distant from *Myxidium* spp. (Palenzeula and Bartholomew 1999). These studies and the biological characteristics of these organisms challenge their classification in the genus *Myxidium.*

Whirling disease. Concerns about the whirling disease of freshwater salmonid fishes, caused by *M. cerebralis,* have dramatically increased in recent years. Whirling disease was first described as the cause of significant losses of farm-reared rainbow trout (*Oncorhynchus mykiss*) in Germany by Dr. Bruno Hofer of Munich University in 1898 (Hofer 1903). Disease symptoms are caused by destruction of cartilage and associated tissues in juvenile salmonids. The cartilage associated with the spinal column and cranium (skull, jaws, and gills) can be massively infected leading to death of the fish or life-long skeletal deformities. It is during the course of this cartilage destruction (e.g. at 6-8 wk at water temperatures of 15 °C) that the characteristic erratic tail chasing behavior and blackened caudal area becomes apparent in infected fish (Hofer 1903; Schaperclaus 1931). The severity of the disease depends in part on the age of the fish and on the intensity of the infection (Hoffman and Byrne 1974; Markiw 1991; E. Ryce, pers. commun.). These impairments, if not initially fatal, are thought to severely compromise survival and, in part, explain losses of up to 90% in certain year classes of rainbow trout (Nehring and Walker 1996; Vincent 1996).

Rainbow trout are considered the most susceptible of the 11 host species of salmonid fish (El-Matbouli et al. 1999a; Halliday 1976; Hedrick et al. 1999a; Hofer 1903). The parasite has been spread by the movements of live or frozen fish from its presumed origins in Eurasia to many countries in central and western Europe, Australia/New Zealand, and the U.S. (Hoffman 1970, 1990). Following its introduction into the USA, an intensive research program largely led by the U.S. Fish and Wildlife Service, was initiated to develop means to detect and control the disease (Hoffman 1975, 1977; Hoffman and Hoffman 1972; Hoffman and Putz 1969).

These investigations provided many insights into minimizing the effects of the infection in hatchery-reared rainbow trout but the focus on whirling disease was surpassed by more pressing problems due to other pathogens. This complacency ended in 1989 when U.S. fisheries biologists in the states of Montana and Colorado began to associate whirling disease with catastrophic declines among populations of wild rainbow trout in premier angling waters (Hedrick et al. 1999a; Nehring and Walker 1996; Vincent 1996). The significant negative ecological and economic consequences of these population declines has prompted the most intensive and coordinated research efforts ever mounted to understand and attempt to control a pathogen among wild fish.

The ability to maintain *M. cerebralis* alternately in trout and oligochaetes was key to subsequent experimental studies that provided details on how the parasite recognized and attached to its fish host (El-Matbouli et al. 1999a; Markiw 1989), on the complex development of the parasite following penetration of the trout or following ingestion of the spores by the oligochaete hosts (El-Matbouli et al. 1995, El-Matbouli et al. 1998), and on how this development differed between different host fish species (Hedrick et al. 1999 a,b). Thus, our section on the life cycle and development of myxozoans (see above) is based largely on the *M. cerebralis* model.

Variations in susceptibility among salmonid fish species have been observed in field studies and now among controlled laboratory trials (Hedrick 2000 a,b; Hedrick et al. 1999 a,b; Thompson et al. 1999). Laboratory studies have demonstrated that resistance to the disease in certain species (e.g. brown trout) can be overwhelmed by exposure to high concentrations of the infectious (triactinomyxon) stages. This may in part explain the recent occurrences of whirling disease among wild and hatchery-reared brown trout in the USA (S. Opitz and B. Nehring, pers. commun.) and Europe, respectively. Evidence for rainbow trout acquiring resistance to whirling disease has not yet been found. Several strains of rainbow trout show a genetic resistance to *C. shasta,* a parasite believed to have evolved with its salmonid hosts in the Pacific Northwest of North America (Bartholomew 1998). The mechanisms, which enable certain strains of rainbow trout to resist *C. shasta* however, do not extend to infections of *M. cerebralis:* two strains of rainbow trout and one strain of steelhead trout with resistance to *C. shasta* are highly susceptible to *M. cerebralis* (Hedrick et al. 2000b). Ryce et al. (2000) have examined whether natural resistance to *M. cerebralis* has arisen among wild rainbow from a river enzootic for whirling disease. In controlled laboratory exposures, progeny from adult wild trout, before and after whirling disease was known in the river system, were found equally susceptible to the disease. Hedrick et al. (2000a) also found no evidence for resistance among wild steelhead trout (anadromous rainbow trout) from one river where whirling disease has been known for 35 yr compared to a second steelhead trout population from a river system with no history of whirling disease. These observations are consistent with the hypothesis of Hoffman (1970) and supported by Andree et al. (1999b): 18S rDNA sequences from *M. cerebralis* isolates from the USA and Germany suggest that the parasite has only recently been introduced to North America. Thus, there has been insufficient time for resistant strains of rainbow trout to emerge. Nevertheless, one of us (El-Matbouli) has recently identified a population of rainbow trout in Germany, which has an apparent resistance to whirling disease, and which is now undergoing intensive laboratory analyses. If strains of rainbow trout genetically resistant to whirling disease are identified, they may, de-

pending on decisions of resource managers, be exploited for restocking in areas where current trout populations have undergone serious declines.

Understanding the role of the invertebrate host and how it may influence the severity of infection among wild trout has stimulated new investigations into the biology and ecology of aquatic oligochaetes and *T. tubifex* in particular. Determining factors that control the distribution and abundance of the oligochaete host are now appreciated as essential to explain the declines observed in some, but not in other, wild trout populations infected with *M. cerebralis.*

Water temperature influences not only the development and release (a range with 16 °C as optimal), but also the subsequent longevity of the triactinomyxon stages (El Matbouli et al. 1999b; Markiw 1986, 1992). When temperatures cycle seasonally, as occurs in most natural waters, there may be cyclical shedding of actinosporeans from a single worm. Our laboratory studies with infected worms that experience higher water temperatures (> 25 °C) show they may be cured of infection. Recovered worms, however, can be reinfected if new spores are available when water temperatures drop below 20 °C. In streams and rivers where whirling disease has caused major trout declines, there are most likely high releases of triactinomyxon spores in the spring and early summer as water temperatures approach 15–16 °C. Unfortunately, this is the same period when newly hatched rainbow trout are at their most susceptible age for infection and subsequent development of whirling disease.

Early studies demonstrated the specificity of the parasite for *T. tubifex* as experimental exposures of other oligochaetes, including *Limnodrilus hoffmeisteri, Ilyodrilus templetoni, Quistadrilus multisetosus, Tubifex ignotus, Lumbriculus variegatus,* and *Aelosoma* sp. have failed to result in production of triactinomyxon stages (El-Matbouli et al. 1989; Wolf et al. 1986; unpubl. data). However, the morphological criteria once used to identify *T. tubifex* have failed to separate clades that are now separable by sequences of 16S mitochondrial rDNA (Beauchamp et al. 2001). Some of these clades/species contain previously identified *T. tubifex* that are resistant to infections with *M. cerebralis.* Resistant *T. tubifex* ingest the spores, which hatch and invade, but fail to develop (El-Matbouli et al. 1999b). This process effectively inactivates the spore, preventing its contact with susceptible strains of *T. tubifex* in the same sediments. Environmental or other factors that would increase the abundance of worms resistant to *M. cerebralis* are now one focus of research aimed at controlling whirling disease in wild trout.

Other approaches to controlling whirling disease in wild trout populations have focused on preventing further spread of the parasite both by restricting movement of live fish for recreational sport fisheries, and identifying strains of trout that might possess genetic resistance to the parasite or whose life histories reduce the contact between young fish and large numbers of the parasite. Public education, particularly of those involved in the sport fishery, aims to explain how to prevent the spread of the parasite, either by restricting movement of live fish or fish parts and by removing contaminants on fishing gear (e.g. boots, waders or boats). Programs by federal and state resource agencies have emphasized eliminating or reducing the stocking of infected trout raised in hatcheries, which was identified earlier as one known method by which the parasite has been spread (Hoffman et al. 1990). The ability to ensure that fish from hatcheries are not infected with the parasite has been greatly improved by the use of new and sensitive diagnostic approaches, such as the PCR (Andree et al. 1998) (see below). In addition, ultraviolet irradiation of hatchery water supplies has been demonstrated as one practical approach to destroying the waterborne (triactinomyxon) stages infectious to trout (Hedrick et al. 2000c). Anti-myxozoan drugs, such as fumagillin, are also being investiged as a treatment to eliminate the parasite from infected fish in hatcheries (El-Matbouli and Hoffmann 1989b). These control measures offer immediate assistance, but the longer and more difficult task of working with wild trout populations is still a challenge.

NEW DIAGNOSTIC METHODS

Diagnosis of myxozoan infections has always been complicated by the complex developmental cycle of these parasites. In the fish, the parasites often migrate from sites of invasion to target tissues, and during this period dramatic morphological changes occur, which cause difficulties with identification using traditional microscopic methods. Antibodies, both monoclonal and polyclonal, have been produced against several myxozoans (i.e. *M. cerebralis, Ceratomyxa* spp., *Sphaerospora* spp., *Kudoa* spp., and PKX) for use in both diagnostic and functional studies (Adams et al. 1992; Bartholomew et al. 1989; Marin de Mateo et al. 1993; Markiw 1989; Morris et al. 1997; Muñoz et al 1998, 1999a; Pearson et al. 2001; Saulnier and de Kinkelin 1996). Lectin-based assays have also been exploited successfully, particularly for the detection of the PKX parasite (Castagnaro et al. 1991; Hedrick et al. 1992; Marin de Mateo et al. 1993, 1997). Lectins used in histochemical studies of PKX and other myxozoan species have also demonstrated significant differences in binding patterns that may be useful in diagnosis (Marin de Mateo et al. 1996, 1997; Muñoz et al. 1999b). However, for both lectin and antibodies, there are inherent limitations in their use for clinical diagnosis as they may exhibit stage-specific affinities (Bartholomew et al. 1989; Marin de Mateo et al. 1996; Morris et al. 1997; Saulnier and de Kinkelin 1996) and may show cross-reactivity with host tissues and with other parasites (Marin de Mateo et al. 1996, Muñoz et al. 1999b). Thus there is clearly a need for diagnostic tools that can specifically detect myxozoans in all life stages.

The 18S rDNA sequences generated in phylogenetic studies provide the means for detecting all life stages of an organism. This has resulted in development of DNA-based assays for detection of several economically important myxozoans. Because the specificity of these techniques is, in theory, limited only by the uniqueness of the genetic sequences targeted, molecular techniques offer the potential for highly specific diagnostic assays. The first DNA-based assay described for *C. shasta* (Bartholomew et al. 1995) actually used arbitrary primers to amplify unique DNA fragments. Although a unique approach, it is preferable to target known sequences to insure specificity of the assay. Subsequent development of molecular techniques has focused on using primers from variable regions of the 18S rRNA gene. Assays based on PCR amplification of this gene sequence have been developed for *C. shasta* (Bartholomew et al. 1997; Palenzeula et al. 1999b), *M. cerebralis* (Andree et al. 1998), *K. thyrisites* (Hervio et al. 1997), *K. amamiensis* (Yokoyama et al. 2000), and PKX (Kent et al. 1998; Morris et al. 2000; Saulnier and de Kinkelin 1997). Additionally, the procedures for PCR diagnosis of *C. shasta* have been adapted for non-lethal testing utilizing intestinal samples collected with a swab (Fox et al. 2000). This approach is simple and useful in obtaining information on populations that are too valuable to be sampled lethally.

In situ hybridisation procedures have also been developed that combine the specificity and sensitivity of DNA detection techniques with the ability to examine the parasite in its biological context (Antonio et al. 1998, 1999; Morris et al. 1999, 2000). At this time, these procedures are still rather labor-in-

tensive and therefore not applicable for routine diagnostic needs. However, they have been extremely useful in studies of parasite invasion and life cycles and in examining mechanisms of host resistance (Antonio et al. 1999).

CONCLUSIONS

Molecular systematics using rDNA sequences has resolved specific relationships within the Myxozoa, particularly with linking life cycle stages. While interesting phylogenetic relationships between the major groups have begun to be elucidated using these sequences, more representative taxa and other genes must be examined before proposals for revisions of the higher taxonomy of the Myxozoa should be put forward. Along with the increased importance of myxozoan diseases, our understanding of life cycles, host-pathogen relationships, and development of diagnostic tests has been greatly advanced in recent years. Unfortunately, advances in our understanding of life cycles strategies of freshwater myxozoans have not extended to marine myxozoans at the same rate. Another research need is better understanding of the specific intricacies of how fish respond to infections by these parasites (e.g. mechanisms of protection).

LITERATURE CITED

Adams, A., Richards, R. H. & Marin de Mateo, M. 1992. Development of monoclonal antibodies to 'PKX', the causative agent of proliferative kidney disease. *J. Fish Dis.,* **15**:515–521.

Alvarez-Pellitero, P. & Sitjà-Bobadilla, A. 1993a. Pathology of Myxosporea in marine fish culture. *Dis. Aquat. Org,*. **17**:229–238.

Alvarez-Pellitero, P. & Sitjà-Bobadilla, A. 1993b. *Ceratomyxa* spp. (Protozoa: Myxosporea) infections in wild and cultured sea bass, *Dicentrarchus labrax,* from the Spanish Mediterranean area. *J. Fish. Biol.,* **42**:889–901.

Anderson, C. L. 1998. Phylogenetic relationships of the Myxozoa. *In:* Coombs, G. H., Vickerman, K., Sleigh, M. A. & Warren, A. (ed.), Evolutionary Relationships among Protozoa. Chapman & Hall, London. p. 341–350.

Anderson, C. L., Canning, E. U. & Okamura, B. 1998. A triploblast origin for Myxozoa? *Nature,* **392**:346.

Anderson, C. L., Canning, E. U. & Okamura, B. 1999a. 18S rDNA sequences indicate that PKX organism parasitizes Bryozoa. *Bull. Eur. Ass. Fish Pathol.,* **19**:94–97.

Anderson, C. L. Canning, E. U. & Okamura, B. 1999b. Molecular data implicate bryozoans as hosts for PKX (Phylum Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. *Parasitology,* **119**:555–561.

Anderson, C. L., Canning, E. U., Schäfer, S. M., Yokoyama, H. & Okamura, B. 2000. Molecular confirmation of the life cycle of *Thelohanellus hovorkai* Achmerov, 1960 (Myxozoa: Myxosporea). *Bull. Eur. Ass. Fish Pathol.,* **20**:111–114.

Andree, K. B., Gresoviac, S. J. & Hedrick, R. P. 1997. Small subunit ribosomal RNA sequences unite alternate actinosporean and myxosporean stages of *Myxobolus cerebralis,* the causative agent of whirling disease in salmonid fish. *J. Eukaryot. Microbiol.,* **44**:208–215.

Andree, K. B., MacConnell, E. & Hedrick, R. P. 1998. A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss. Dis. Aquat. Org.,* **34**:145–154.

Andree, K. B., El-Matbouli, M., Hoffman, R. W. & Hedrick, R. P. 1999a. Comparison of 18S and ITS-1 sequences of selected geographic isolates of *Myxobolus cerebralis. Int. J. Parasitol.,* **29**:771–775.

Andree, K. B., Székely, C., Molnár, K., Gresoviac, S. J. & Hedrick, R. P. 1999b. Relationships among members of the genus *Myxobolus* (Myxozoa: Bivalvulida) based on small subunit ribosomal DNA sequences. *J. Parasitol.,* **85**:68–74.

Antonio, D. B., El-Matbouli, M. & Hedrick, R. P. 1999. Detection of early stages of *Myxobolus cerebralis* in fish and tubificid oligochaete hosts by in situ hybridization. *Parasitol. Res.,* **85**:942–944.

Antonio, D. B., Andree, K. B., McDowell, T. S. & Hedrick, R. P. 1998. Detection of *Myxobolus cerebralis* in rainbow trout and oligochaete tissues by using a non-radioactive in situ hybridization (ISH) protocol. *J. Aquat. Anim. Health,* **10**:338–347.

Athanassopoulou, F., Prapas, Th. & Rodger, H. 1999. Diseases of *Puntazzo puntazzo* Cuvier in marine aquaculture systems in Greece. *J. Fish Dis.,* **22**:215–218.

Bartholomew, J. L. 1998. Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: a review. *J. Aquat. Anim. Health,* **10**:112–120.

Bartholomew, J. L., Rodriguez, R. J. & Arakawa, C. K. 1995. Development of a DNA probe for the myxosporean parasite *Ceratomyxa shasta,* using the polymerase chain reaction with arbitrary primers. *Dis. Aquat. Org.,* **21**:215–220.

Bartholomew, J. L., Rohovec, J. S. & Fryer, J. L. 1989. Development, characterization, and use of monoclonal and polyclonal antibodies against the myxosporean, *Ceratomyxa shasta. J. Protozool.,* **36**:397–401.

Bartholomew, J. L., Whipple, M. J., Stevens, D. G. & Fryer, J. L. 1997. The life cycle of *Ceratomyxa shasta,* a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *J. Parasitol.,* **83**:859–868.

Basurco, B. & Abellán, E. 1999. Finfish species diversification in the context of Mediterranean marine fish farming development. *In:* Abellán, E. & Basurco, B. (ed.), Marine Finfish Species Diversification: Current Situation and Prospects in Mediterranean Aquaculture. Zaragoza, CIHEAM/FAO. Série B: Etudes et Recherches, No. 24, Options Méditerranéennes. p. 139.

Beauchamp, K. A., Kathman, R. D., McDowell, T. S. & Hedrick, R. P. 2001. Molecular phylogeny of tubificid oligochaetes with special emphasis on *Tubifex tubifex* (Tubificidae) as the host to the fish parasite *Myxobolus cerebralis. Mol. Phylogenet. Evol.,* **19**:216–224.

Benajiba, M. H. & Marques, A. 1993. The alternation of actinosporidian and myxosporidian sporal forms in the development of *Myxidium giardi* (parasite of *Anguilla anguilla)* through oligochaetes. *Bull. Eur. Ass. Fish Pathol.,* **13**:100–103.

Branson, E., Riaza, A. & Álvarez-Pellitero, P. 1999. Myxosporean infection causing intestinal disease in farmed turbot, *Scophthalmus maximus* (L.), (Teleostei: Scophthalmidae). *J. Fish. Dis.,* **22**:395–399.

Burtle, G. J., Harrison, L. R. & Styer, E. L. 1991. Detection of a triactinomyxid myxozoan in an oligochaete from ponds with proliferative gill disease in channel catfish. *J. Aquat. Anim. Health,* **3**:281–287.

Bütschli, O. 1881. Myxosporidien. *Zool. Jahrb. F.,* **1**:162–164.

Canning, E. U., Okamura, B. & Curry, A. 1996. Development of a myxozoan parasite *Tetracapsula bryozoides* gen. n. et sp. n. in *Cristatella mucedo* (Bryozoa: Phylactolaemata). *Folia Parasitol.,* **43**:249–261.

Canning, E. U., Curry, A., Feist, S. W., Longshaw, M. & Okamura, B. 1999. *Tetracapsula bryosalmonae* n. sp. for PKX organism, the cause of PKD in salmonid fish. *Bull. Eur. Assoc. Fish Pathol.,* **19**:203–206.

Canning, E. U., Curry, A., Feist, S. W., Longshaw, M. & Okamura, B. 2000. A new class and order of myxozoans to accommodate parasites of bryozoans with ultrastructural observations on *Tetracapsula bryosalmonae* (PKX organism). *J. Eukaryot. Microbiol.,* **47**:456–468.

Castagnaro, M., Marin, M., Ghittino, C. & Hedrick, R. P. 1991. Lectin histochemistry and ultrastructure of rainbow trout *Oncorhynchus mykiss* kidneys affected by proliferative kidney disease. *Dis. Aquat. Org.,* **10**:173–183.

Chase, J. C., Dawson-Coates, J. A., Haddow, J. D., Stewart, M. H., Haines, L. R., Whitaker, D. J., Kent, M. L., Olafson, R. W. & Pearson, T. W. 2001. Soft flesh disease in Atlantic salmon: analysis of *Kudoa thyrsites* (Myxozoa: Myxosporea) spore antigens using monoclonal antibodies. *Dis. Aquat. Org.,* (in press).

Chen, C. & Ma, C. 1998. Myxozoa, Myxosporea. Fauna Sinica. Ed. Committee of Fuanca Sinica. Science Press, Beijing.

Clifton-Hadley, R. S. & Feist, S. W. 1989. Proliferative kidney disease in brown trout *Salmo trutta*: further evidence of a myxosporean etiology. *Dis. Aquat. Org.,* **6**:99–103.

Daniels, S. B., Herman, R. L. & Burke, C. N. 1976. Fine structure of an unidentified protozoan in the epithelium of rainbow trout exposed to water with *Myxosoma cerebralis. J. Protozool.,* **23**:402–410.

Degnan, B. M., Degnan, S. M., Giusti, A. & Morse, D. E. 1995. A *hox/hom* homeobox gene in sponges. *Gene,* **155**:175–177.

Diamant, A. 1992. A new pathogenic histozoic *Myxidium* (Myxosporea)

in cultured gilt-head sea bream *Sparus aurata* L. Bull. Eur. Assoc. *Fish Pathol.*, **12**:64–66.
Diamant, A. 1997. Fish-to-fish transmission of a marine myxosporean. *Dis. Aquat. Org.*, **30**:99–105.
Diamant, A., Lom, J. & Dyková, I. 1994. *Myxidium leei* n. sp., a pathogenic myxosporean of cultured sea bream *Sparus aurata. Dis. Aquat. Org.*, **20**:137–141.
Dogeil, V. A. 1965. General Parasitology, 2nd ed. Oxford University Press, UK.
El-Mansy, A. & Molnár, K. 1997a. Extrapiscine development of *Myxobolus drjagini* Akhmerov, 1954 (Myxosporea: Myxobolidae) in oligochaete alternative hosts. *Acta Vet. Hung.*, **45**:427–438.
El-Mansy, A. & Molnár, K. 1997b. Development of *Myxobolus hungaricus* (Myxosporea: Myxobolidae) in oligochaete alternate hosts. *Dis. Aquat. Org.*, **31**:227–232.
El-Mansy, A., Molnár, K. & Székely, Cs. 1998a. Development of *Myxobolus portucalensis* (Myxosporea: Myxobolidae) in the oligochaete *Tubifex tubifex* (Müller). *Syst. Parasitol.*, **41**:95–103.
El-Mansy, A., Székely, Cs. & Molnár, K. 1998b. Studies on the occurrence of actinosporean stages of fish myxosporeans in a fish farm of Hungary, with the description of triactinomyxon, raabeia, aurantiactinomyxon, and neoactinomyxon types. *Acta Vet. Hung.*, **46**:259–284.
El-Mansy, A., Székely, Cs. & Molnár, K. 1998c. Studies on the occurrence of actinosporean stages of myxosporeans in Lake Balaton, Hungary, with the description of triactinomyxon, raabeia, and aurantiactinomyxon types. *Acta Vet. Hung.*, **46**:437–450.
El-Matbouli, M. & Hoffmann, R. 1989. Experimental transmission of two *Myxobolus* spp. developing bisporogeny via tubificid worms. *Parasitol. Res.*, **75**:461–464.
El-Matbouli, M. & Hoffmann, R. W. 1991. Prevention of experimentally induced whirling disease in rainbow trout *Oncorhynchus mykiss* by fumagillin. *Dis Aquat Org.*, **10**:109–113.
El-Matbouli, M. & Hoffmann, R. W. 1993. *Myxobolus carassii* Klokaceva, 1914 also requires an aquatic oligochaete, *Tubifex tubifex* as an intermediate host in its life cycle. *Bull Eur. Ass. Fish Pathol.*, **13**: 189–192.
El-Matbouli, M. & Hoffmann, R. W. 1998. Light and electron microscopic study on the chronological development of *Myxobolus cerebralis* in *Tubifex tubifex* to the actinosporean stage triactinomyxon. *Int. J. Parasitol.*, **28**:195–217.
El-Matbouli, M., Fischer-Scherl, T. & Hoffmann, R. W. 1992a. Transmission of *Hoferellus carassi* Achmerov, 1960 to goldfish *Carassius auratus* via an aquatic oligochaete. *Bull Eur. Assoc. Fish Pathol.*, **12**: 54–56.
El-Matbouli, M., Fischer-Scherl, Th. & Hoffmann, R. W. 1992b. Present knowledge on the life cycle, taxonomy, pathology, and therapy of some Myxosporea spp. important for freshwater fish. *Ann. Rev. Fish Dis.*, **3**:367–402.
El-Matbouli, M., Hoffmann, R. W. & Mandok, C. 1995. Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout cartilage. *J. Fish Biol.*, **46**:919–935.
El-Matbouli, M., Hoffmann, R. W., Schoel, H., McDowell, T. S. & Hedrick, R. P. 1999a. Whirling Disease: host specificity and interaction between the actinosporean stage of *Myxobolus cerebralis* and rainbow trout *Oncorhynchus mykiss. Dis. Aquat. Org.*, **35**:1–12.
El-Matbouli, M., McDowell, T. S., Antonio, D. B., Andree, K. B. & Hedrick, R. P. 1999b. Effect of water temperature on the development, release and survival of the triactinomyxon stage of *Myxobolus cerebralis*, the causative agent of whirling disease, in its oligochaete host. *Int. J. Parasitol.*, **29**:627–641.
Eszterbauer, E., Székely, Cs., Molnár, K. & Baska, F. 2000. Development of *Myxobolus bramae* (Myxosporea: Myxobolidae) in an oligochaete alternate host, *Tubifex tubifex. J. Fish Dis.*, **23**:19–25.
Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D. & Kluge, A. G. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, **12**:99–24.
Feist, S. W. 1997. Pathogenicity of renal myxosporeans of fish. *Bull. Eur. Assoc. Fish Pathol.*, **17**:209–214.
Feist, S. W., Longshaw, M., Canning, E. U. & Okamura, B. 2001. Induction of Proliferative Kidney Disease (PKD) in rainbow trout (*Oncorhynchus mykiss* Richardson) via the bryozoan *Fredericella sultana* (Blumenbach, 1779), infected with *Tetracapsula bryosalmonae* Canning, Curry, Feist, Longshaw &Okamura, 1999. *Dis Aquat. Org.*, (in press)
Felsenstein, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Syst. Zool.*, **22**:240–249.
Fox, M. D., Palenzeula, O. & Bartholomew, J. L. 2000. Strategies for diagnosis of *Ceratomyxa shasta* using the PCR: comparison of lethal and non-lethal sampling with microscopic examination. *J. Aquat. Anim. Health*, **12**:100–106.
Friedrich, C., Ingolic, E., Freitag, B., Kastberger, G., Hohmann, V., Skofitsch, G., Neumeister, U. & Kepka, O. 2000. A myxozoan-like parasite causing xenomas in the brain of the mole *Talpa europrea* L., 1758. *Parasitology*, **121**:483–492.
Garden, O. 1992. The Myxosporea of fish: a review. *Brit. Vet. J.*, **148**: 223–239.
Grassé, P.-P. & Lavette, A. 1978. La myxosporidie *Sphaeromyxa sabrazei* et le novel embranchement de Myxospoaire (Myxozoa). Recherches sur l'état pluricellulaire primitif et considerations phylogenetiques. *Ann. Sci. Nat., Zool. (Paris)*, **2**:193–285.
Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Syst. Biol.*, **47**:9–11.
Grossheider, G. & Körting, W. 1992. First evidence that *Hoferellus cyprini* (Doflein, 1898) is transmitted by *Nais* sp. *Bull Eur. Assoc. Fish Pathol.*, **12**:17–20.
Grossheider, G. & Körting, W. 1993. Experimental transmission of *Sphaerospora renicola* to common carp *Cyprinus carpio* fry. *Dis. Aquat. Org.*, **16**:91–95.
Hallet, S. L. 1997. Actinosporean and myxosporean parasites from marine oligochaetes and fish. Ph.D. thesis, University of Queensland, Brisbane Australia.
Hallett, S. L. & Diamant, A. 2001. Ultrastructure and small-subunit ribosomal DNA sequence of *Henneguya lesteri* n. sp. (Myxosporea), a parasite of sand whiting *Sillago analis* (Sillaginidae) from the coast of Queensland, Australia. *Dis. Aquat. Org.*, (in press)
Hallett, S. L. & Lester, R. J. G. 1999. Actinosporeans (Myxozoa) with four developing spores within a pansporocyst: *Tetraspora discoidea* n.g., n.sp. and *Tetraspora rotundum* n.sp. *Int. J. Parasitol.*, **29**:419–427.
Hallett, S. L., Erséus, C. & Lester, R. J. G. 1999. Actinosporeans (Myxozoa) from marine oligochaetes of the Great Barrier Reef. *Syst. Parasitol.*, **44**:51–59.
Hallett, S. L., O'Donoghue, P. J. & Lester, R. J. G. 1998. Structure and development of a marine actinosporean, *Sphaeractinomyxon ersei* n. sp. (Myxozoa). *J. Eukaryot. Microbiol.*, **45**:142–150.
Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*, the causative agent of whirling disease of salmonids. *J. Fish Biol.*, **9**:339–357.
Hanelt, B., Van Schyndel, D., Adema, C. M., Lewis, L. & Loker, E. S. 1996. The phylogenetic position of *Rhopalura ophiocomae* (Orthonectida) based on 18S ribosomal DNA sequence analysis. *Mol. Biol. Evol.*, **13**:1187–1191.
Hedrick, R. P., MacConnell, E. & de Kinkelin, P. 1993. Proliferative kidney disease of salmonid fish. *Ann. Rev. Fish Dis.*, **3**:277–290.
Hedrick, R. P., El-Matbouli, M., Adkison, M. A. & MacConnell, E. 1998. Whirling disease: re-emergence among wild trout. *Immunol. Rev.*, **166**:365–376.
Hedrick, R. P., Marin, M., Castagnaro, M., Monge, D. & de Kinkelin, P. 1992. Rapid lectin-based staining procedure for the detection of the myxosporean causing proliferative kidney disease in salmonid fish. *Dis. Aquat. Org.*, **13**:129–132.
Hedrick, R. P., McDowell, T. S., Mukkatira, K., Georgiadis, M. P. & MacConnell, E. 1999a. Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. *J. Aquat. Anim. Health*, **11**:330–339.
Hedrick, R. P., McDowell, T. S., Mukkatira, K., Georgiadis, M. P. & MacConnell. E. 2001a. Susceptibility of three species of anadromous salmonids to experimentally-induced infections with *Myxobolus cerebralis* the causative agent of whirling disease. *J. Aquat. Anim. Health*, **13**:43–50.
Hedrick, R. P., McDowell, T. S., Mukkatira, K., Georgiadis, M. P., MacConnell, E. 2001b. *Ceratomyxa shasta*-resistant salmonids are

susceptible to experimentally-induced infections with *Myxobolus cerebralis*. *J. Aquat. Anim. Health*, **13**:35–42.
Hedrick, R. P., McDowell, T. S., Guy, M., Marty, G. D., Georgiadis, M. P. & MacConnell, E. 1999b. Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to *Myxobolus cerebralis*, the cause of salmonid whirling disease. *Dis. Aquat. Org.*, **37**:173–183.
Hedrick, R. P., McDowell, T. S., Marty, G. D., Mukkatira, K., Antonio, D. B., Andree, K. B., Bukhari, Z. & Clancy, T. 2000c. Ultraviolet irradiation inactivates the waterborne infective stages of *Myxobolus cerebralis*: a treatment for hatchery water supplies. *Dis. Aquat. Org.*, **42**:53–59.
Hervio, D. M. L., Kent, M. L., Khattra, J., Sakanari, J., Yokoyama, H. & Devlin, R. H. 1997. Taxonomy of *Kudoa* species (Myxosporea) using small subunit ribosomal DNA sequence. *Can. J. Zool.*, **75**: 2112–2119.
Hofer, B. 1903. Über die Drehkrankheit der Regenbogenforelle. *Allgemeinen Fischerei-Ztg., Jahrg.* **28**(1):7–8.
Hoffman, G. L. 1970. Intercontinental and transcontinental dissemination and transfaunation of fish parasites with emphasis on whirling disease (*Myxosoma cerebralis*). *In*: Snieszko, S. F., (ed.), Sympos. Diseases of Fish and Shellfishes. Washington, D.C. *Am. Fish. Soc.* p. 69–81.
Hoffman, G. L. 1975. Whirling disease (*Myxosoma cerebralis*): control with ultraviolet irradiation and effect on fish. *J. Wildl. Dis.*, **11**:505–507.
Hoffman, G. L. 1977. Control of whirling disease (*Myxosoma cerebralis*): use of methylene blue staining as a possible indicator of effect of heat on spores. *J. Fish Biol.*, **10**:181–183.
Hoffman, G. L. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. *J. Aquat. Anim. Health*, **2**:30–37.
Hoffmann, G. L. & Byrne, C. J. 1974. Fish age as related to susceptibility to *Myxosoma cerebralis*, cause of whirling disease. *Prog. Fish Cult.*, **36**:151.
Hoffman, Sr., G. L. & Hoffman, Jr., G. L. 1972. Studies on the control of whirling disease (*Myxosoma cerebralis*). I. The effects of chemicals on spores in vitro, and of calcium oxide as a disinfectant in simulated ponds. *J. Wildl. Dis.*, **8**:49–53.
Hoffman, G. L. & Putz, R. E. 1969. Host susceptibility and the effect of aging, freezing, heat, and chemicals on spores of *Myxosoma cerebralis*. *Prog. Fish Cult.*, **31**:35–37.
International Commission on Zoological Nomenclature. 1985. International Code of Zoological Nomenclature International Commission on Zoological Nomenclature. 1985. International Code of Zoological Nomenclature, 3rd ed. University of California Press, Berkeley.
Kabata, Z. & Whitaker, D. J. 1989. *Kudoa thyrsites* (Gilchrist, 1924) (Myxozoa) in the cardiac muscle of Pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*Salmo gairdneri*). *Can. J. Zool.*, **67**:341–342.
Kent, M. L. 1999. A myxozoan resembling *Myxidium leei* in the anemone fish *Amphiprion frenatus* from the Pacific Ocean. *Bull. Eur. Assoc. Fish Pathol.*, **19**:42–43.
Kent, M. L. & Hedrick, R. P. 1986. Development of the PKX myxosporean in rainbow trout *Salmo gairdneri*. *Dis. Aquat. Org.*, **1**:169–182.
Kent, M. L. & Lom, J. 1999. Can a new species of Myxozoa be described solely on their actinosporean stage? *Parasitol. Today*, **15**: 472–473.
Kent, M. L. & Palenzeula, O. 2001. Myxozoa. *In*: Encyclopedia of Life Sciences, MacMillan Reference Ltd. (Stockton Press), Basingstoke, Hampshire, England, (in press)
Kent, M. L., Margolis, L. & Corliss, J. O. 1994b. The demise of a class of protists: taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grassé, 1970. *Can. J. Zool.*, **72**:932–937.
Kent, M. L., Whitaker, D. J. & Margolis, L. 1993a. *Sphaerospora oncorhynchi* n. sp. (Myxosporea: Sphaerosporidae) from the kidney of sockeye salmon (*Oncorhynchus nerka*) in British Columbia and its possible relationship to the myxosporean causing proliferative kidney disease in salmonid fishes. *Can. J. Zool.*, **71**:2425–2430.
Kent, M. L., Whitaker, D. J. & Margolis, L. 1993b. Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, a myxosporean parasite of Pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (Lumbriculidae). *Can. J. Zool.*, **71**:1207–1211.
Kent, M. L., Khattra, J., Hedrick, R. P. & Devlin, R. H. 2000. *Tetracapsula renicola* n. sp. (Myxozoa: Saccosporidae); the PKX myxozoan–the cause of proliferative kidney disease of salmonid fishes. *J. Parasitol.*, **86**:103–111.
Kent, M. L., Khattra, J., Hervio, D. M. L. & Devlin, R. H. 1998. Ribosomal DNA sequence analysis of isolates of the PKX myxosporean and their relationship to members of the genus *Sphaerospora*. *J. Aquat. Anim. Health*, **10**:12–21.
Kent, M. L., Moser, M., Marques, A. & Lom, J. 2001. Phylum Myxozoa Grassé 1970. *In*: Lee, J. (ed.), An Illustrated Guide to the Protozoa. Soc. Protozool., Lawrence, Kansas, (in press)
Kent, M. L., Margolis, L., Whitaker, D. J., Hoskins, G. E. & McDonald, T. E. 1994a. Review of Myxosporea of importance to salmonid fisheries and aquaculture in British Columbia. *Fol. Parasitol.*, **41**:27–37.
Kim, J. 1998. Large-scale phylogenies and measuring the performance of phylogenetic estimators. *Syst. Biol.*, **47**:43–60.
Korotneff, A. 1892. *Myxosporidium bryozoides. Zeitsch. Wiss. Zool.*, **53**:591–596.
Køie, M. 2000. First record of an actinosporean (Myxozoa) in a marine polychaete annelid. *J. Parasitol.*, **86**:871–872.
Lester, R. J. G., Hallett, S. L., El-Matbouli, M. & Canning, E. U. 1998. The case for naming actinosporeans using the Zoological Code. *Parasitol. Today*, **14**:476–477.
Lester, B., Hallett, S., El-Matbouli, M. & Canning, E. 1999. Can a new species of Myxozoa be described based solely on their actinosporean stage. Reply. *Parasitol. Today*,**15**:508.
Lin, D., Hanson, L. A. & Pote, L. M. 1999. Small subunit ribosomal RNA sequence of *Henneguya exilis* (Class Myxosporea) identifies the actinosporean stage from an oligochaete host. *J. Eukaryot. Microbiol.*, **46**:66–68.
Lom, J. & Arthur, J. R. 1989. A guideline for the preparation of species descriptions in Myxosporea. *J. Fish Dis.*, **12**:151–156.
Lom, J. & Dyková, I. 1995. Myxosporea (Phylum Myxozoa). *In*: Woo, P. T. K. (ed.), Fish Diseases and Disorders, vol. 1. CAB Int'l., Wallingford. p. 97–148.
Lom, J. & Dyková, I. 1992. Protozoan Parasites of Fishes. Elsevier, New York. p. 315.
Lom, J. & Dyková, I. 1997. Ultrastructural features of the actinosporean phase of Myxosporea (phylum Myxozoa): a comparative study. *Acta Protozool.*, **36**:83–103.
Lom, J. & Noble, E. R. 1984. Revised classification of the Myxosporea Bütschli, 1881. *Folia Parasitol.*, **31**:93–205.
Lom, J., Dyková, I., Pavlásková, M. & Grupcheva, G. 1983. *Sphaerospora molnari* sp. nov. (Myxozoa: Myxosporea), an agent of gill, skin and blood sphaerosporosis of common carp in Europe. *Parasitology*, **86**:529–535.
Lom, J., McGeorge, J., Feist, S. W., Morris, D. & Adams, A. 1997. Guidelines for the uniform characterisation of the actinosporean stages of parasites of the phylum Myxozoa. *Dis. Aquat. Org.*, **30**:1–9.
Longshaw, M., Feist, S. W., Canning, E. U. & Okamura, B. 1999. First identification of PKX in Bryozoans from the United Kingdom—molecular evidence. *Bull. Eur. Assoc. Fish Pathol.*, **19**:146–148.
Marín de Mateo, M., Bovo, G., Comuzzi, M. & Adams, A. 1997. Lectin histochemical studies on *Sphaerospora* sp. (Myxosporea) from Italian brown trout, *Salmo trutta L. J. Fish Dis.*, **20**:51–58.
Marín de Mateo, M., McGeorge, J., Morris, D. & Kent, M. L. 1996. Comparative studies of PKX and *Sphaerospora* spp. from salmonids using lectin and monoclonal antibody staining techniques. *J. Fish Dis.*, **19**:55–63.
Marín de Mateo, M., Adams, A., Richards, R. H., Castagnaro, M. & Hedrick, R. P. 1993. Monoclonal antibody and lectin probes recognize developmental and sporogonic stages of PKX, the causative agent of proliferative kidney disease in European and North American salmonid fish. *Dis. Aquat. Org.*, **15**:23–29.
Markiw, M. E. 1986. Salmonid whirling disease: dynamics of experimental production of the infective stage—the triactinomyxon spore. *Can. J. Fish. Aquat. Sci.*, **43**:521–526.
Markiw, M. E. 1989. Portals of entry for salmonid whirling disease in rainbow trout. *Dis. Aquat. Org.*, **6**:7–10.
Markiw, M. E. 1989. Salmonid whirling disease: myxosporean and ac-

tinosporean stages cross-react in a direct fluorescent antibody test. *J. Fish Dis.,* **12**:137–141.
Markiw, M. E. 1991. Whirling disease: earliest susceptible age of rainbow trout to the triactinomyxid of *Myxobolus cerebralis. Aquaculture,* **92**:1–6.
Markiw, M. E. 1992. Experimentally induced whirling disease. I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis. J. Aquat. Anim. Health,* **4**:40–43.
Markiw, M. E. 1992. Experimentally induced whirling disease. II. Determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. *J. Aquat. Anim. Health,* **4**:44–47.
Markiw, M. E. & Wolf, K. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of salmonid whirling disease requires tubificid worms (Annelida: Oligochaetea) in its life cycle. *J. Protozool.,* **30**:561–564.
McGeorge, J., Sommerville, C. & Wootten, R. 1997. Studies of actinosporean myxozoan stages parasitic in oligochaetes from the sediments of a hatchery where Atlantic salmon harbour *Sphaerospora truttae* infection. *Dis. Aquat. Org.,* **30**:107–119.
Mickevich, M. F. & Farris, J. S. 1981. The implications of congruence in *Menidia. Syst. Zool.,* **30**:351–369.
Molnár, K., El-Mansy, A., Székely, Cs. & Baska, F. 1999a. Development of *Myxobolus dispar* Thelohan, 1895 (Myxosporea: Myxobolidae) in an oligochaete alternate host *Tubifex tubifex* (Müller). *Folia Parasitol.,* **46**:15–21.
Molnár, K., El-Mansy, A., Székely, Cs. & Baska, F. 1999b. Experimental indentification of the actinosporean stage of *Sphaerospora renicola* Dykova and Lom 1982 (Myxosporea: Sphaerosporidae) in oligochaete alternate hosts. *J. Fish Dis.,* **22**:143–153.
Moran, J. D. W. & Kent, M. L. 1999. *Kudoa thyrsites* (Myxozoa: Myxosporea) infections in pen-reared Atlantic salmon in the Northeast Pacific Ocean with a survey of potential non-salmonid reservoir hosts. *J. Aquat. Anim. Health.,* **11**:101–109.
Moran, J. D. W., Whitaker, D. J. & Kent, M. L. 1999b. A review of myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture,* **172**:163–196.
Moran, J. D. W., Whitaker, D. J. & Kent, M. L. 1999c. Natural and laboratory transmission of the marine myxozoan parasite *Kudoa thyrsites* to Atlantic salmon. *J. Aquat. Anim. Health,* **11**:110–115.
Moran, J. D. W., Margolis, L., Webster, J. M. & Kent, M. L. 1999a. Development of *Kudoa thyrsites* (Myxozoa: Myxosporea) in netpen-reared Atlantic salmon determined by light microscopy and a polymerase chain reaction test. *Dis. Aquat. Org.,* **37**:185–193.
Morris, D. J., Adams, A. & Richards, R. H. 1997. Studies of the PKX parasite in rainbow trout via immunohistochemistry and immunogold electron microscopy. *J. Aquat. Anim. Health,* **9**:265–273.
Morris, D. J., Adams, A. & Richards, R. H. 1999. In situ hybridization of DNA probes to PKX, the causative organism of proliferative kidney disease (PKD). *J. Fish Dis.,* **22**:161–163.
Morris, D. J., Adams, A. & Richards, R. H. 2000. In situ hybridisation identifies the gill as a portal of entry for PKX (Phylum Myxozoa), the causative agent of proliferative kidney disease in salmonids. *Parasitol. Res.,* **86**:950–956.
Moser, M. & Kent, M. L. 1994. Myxosporea. *In*: Kreier, J. P. (ed.), Parasitic Protozoa. Academic Press, New York. **8**:265–318.
Muñoz, P., Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. 1998. Immunohistochemical characterization of a polyclonal antibody against *Sphaespora dicentrarchi* (Myxosporea: Bivalvulida), a parasite from sea bass (*Dicentrarchus labrax* L.)(Teleostei: Serranidae). *Parasitol. Res.,* **84**:733–740.
Muñoz, P., Palenzeula, O., Sitjá-Bobadilla, A. & Álvarez-Pellitero, P. 1999a. Immunohistochemical reactivity of polyclonal antibodies against *Sphaerospora testicularis* and *Ceratomyxa labracis* (Myxosporea: Bivalvulida), with other myxosporean parasites. *Int. J. Parasitol.,* **29**:521–525.
Muñoz, P., Palenzeula, O., Alvarez-Pellitero, P. & Sitjá-Bobadilla, A. 1999b. Comparative studies on carbohydrates of several myxosporean parasites of fish using lectin histochemical methods. *Folia Parasitol.,* **46**:241–247.
Nehring, R. B. & Walker, P. G. 1996. Whirling disease in the wild: the new reality in the intermountain west. *Fisheries* (Bethesda), **21**:28–32.
Okamura, B. 1996. Occurrence, prevalence, and effects of the myxozoan *Tetracapsula bryozoides* parasitic in the freshwater bryozoan *Cristatella mucedo* (Bryozoa: Phylactolaemata). *Folia Parasitol.,* **43**:262–266.
Özer, A. & Wootten, R. 2000. The life cycle of *Sphaerospora truttae* (Myxozoa: Myxosporea) and some features of the biology of both the actinosporean and myxosporean stages. *Dis. Aquat. Org.,* **40**:33–39.
Padrós, F., Palenzuela, O., Hispano, C., Tosas, O., Zarza, C., Crespo, S. & Alvarez-Pellitero, P. 2001. *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. *Dis Aquat. Org.,* (in press)
Palenzeula, O. & Bartholomew, J. L. 1999. Phylogeny of the genus *Ceratomyxa* and other marine myxozoans based on small subunit ribosomal RNA gene sequences. Abstract. *Proc. 5th Symp. Fish Parasitol.,* Ceské Budjovice. p. 106.
Palenueula, O. & Bartholomew, J. L. 1999. Phylogeny of the genus *Ceratomyxa* and other marine myxozoans based on small subunit ribosomal RNA gene sequences. Abstract. *Proc. 5th Int. Symp. Fish Parasitol.,* Ceské Budjovice. p. 106.
Palenzeula, O., Álvarez-Pellitero, P. & Sitjà-Bobadilla, A. 1997. *Ceratomyxa sparusaurati* (Protozoa: Myxosporea) infections in cultured gilthead sea bream *Sparus aurata* (Pisces: Teleostei) from Spain: aspects of the host-parasite relationship. *Parasitol. Res.,* **83**:539–548.
Palenzeula, O., Trobridge, G. & Bartholomew, J. L. 1999. Development of a polymerase chain reaction diagnostic assay for *Ceratomyxa shasta,* a myxosporean parasite of salmonid fish. *Dis. Aquat. Org.,* **36**:45–51.
Palmer, R. 1994. Kudoa–the Irish Experience. *In*: Conley, D. C. (ed.), *Kudoa* Workshop Proceedings, 17 and 18 February 1994, Nanaimo, B.C. *Aquaculture Industry Develop. Rep.,* **94**(01):21. Province of British Columbia, Ministry of Agriculture, Fisheries and Food, Victoria, B.C., Canada.
Posada, D. & Crandall, K. A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics,* **14**:817–818.
Pote, L. M., Hanson, L. A. & Shivaji, R. 2000. Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: Myxosporea). *J. Aquat. Animal Health,* **12**:230–240.
Rigos, G., Grigorakis, K., Christophilogannis, P., Nengas, I. & Alexis, M. 1997. *Ceratomyxa* spp. (Myxosporea) infection in cultured common dentex from Greece. *Bull. Eur. Ass. Fish Pathol.,* **17**:174–176.
Roubal, F. R., Hallett, S. L. & Lester, R. J. G. 1997. First record of triactinomyxon actinosporean in marine oligochaete. *Bull. Eur. Assoc. Fish Pathol.,* **17**:83–85.
Ruidisch, S., El-Matbouli, M. & Hoffmann, R. W. 1991. The role of tubificid worms as an intermediate host in the life cycle of *Myxobolus pavlovskii* (Achmerov, 1954). *Parasitol. Res.,* **77**:663–667.
Ryce, E. K. N., Zale, A. V. & Nehring, R. B. 2001. Lack of selection for resistance to whirling disease among progeny of Colorado River rainbow trout. *J. Aquat. Anim. Health,* **13**:63–68.
Salim, K. Y. & Desser, S. S. 2000. Descriptions and phylogenetic systematics of *Myxobolus* spp. from cyprinids in Algonquin Park, Ontario. *J. Eurkaryot. Microbiol.,* **47**:309–318.
Saulnier, D. & de Kinkelin, P. 1996. Antigenic and biochemical study of PKX, the myxosporean causative agent of proliferative kidney disease of salmonid fish. *Dis. Aquat. Org.,* **27**:103–114.
Saulnier, D. & de Kinkelin, P. 1997. Polymerase chain reaction primers for investigations on the causative agent of proliferative kidney disease of salmonids. *J. Fish Dis.,* **20**:467–470.
Schäperclaus, W. 1931. Die Dre-kankheit in der Forellenzucht und ihre Bekampfung. *Z. Fisherie,* **29**:521–567. (in German)
Schlegel, M., Lom, J., Stechmann, A., Bernhard, D., Leipe, D., Dyková, I. & Sogin, M. L. 1996. Phylogenetic analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: evidence that Myxozoa are Metazoa and related to the Bilateria. *Arch. Protist.,* **147**:1–9.
Schröder, O. 1910. *Buddenbrockia plumatellae,* eine neue Mesozoenart aus *Plumatella repens* L. und *Pl. fungosa* Pall. *Zeitschrift f. Wissensch. Zoologie,* **1056**:525–537.
Shulman, S. S. 1966. Myxosporidii fauny SSR. Izdatel'stvo Nauka.

Moscow-Leningrad. English translation 1988: Myxosporidia of the USSR. Amerind Publ. Co. Pvt. Ltd., New Dehli. p. 631.
Siddall, M. E. 1998. Stratigraphic fit to phylogenies: a proposed solution. *Cladistics,* **14**:201–208.
Siddall, M. E., Martin, D. S., Bridge, D., Desser, S. S. & Cone, D. K. 1995. The demise of a phylum of protists: phylogeny of the Myxozoa and other parasitic cnidaria. *J. Parasitol.,* **81**:961–967.
Siddall, M. E. & Whiting, M. F. 1999. Long-branch abstractions. *Cladistics,* **15**:9–24.
Sitjà-Bobadilla, A. & Alvarez-Pellitero, P. 1990. *Sphaerospora testicularis* sp. nov. (Myxosporea: Sphaerosporidae) in wild and cultured sea bass, *Dicentrarchus labrax* (L.), from the Spanish Mediterranean area. *J. Fish Dis.,* **13**:93–203.
Sitjà-Bobadilla, A. & Alvarez-Pellitero, P. 1993. Light and electron microscopical description of *Ceratomyxa labracis* n. sp. and a redescription of *C. diplodae* (Myxosporea: Bivalvulida) from wild and cultured Mediterranean sea bass *Dicentrarchus labrax* (L.) (Teleostei: Serranidae). *Syst. Parasitol.,* **26**:215–223.
Sitjà-Bobadilla, A., Palenzeula, O. & Álvarez-Pellitero, P. 1995. *Ceratomyxa sparusaurati* n. sp. (Myxosporea: Bivalvulida), a new parasite from cultured gilthead seabream (*Sparus aurata* L.) (Teleostei: Sparidae): light and electron microscopic description. *J. Eukaryot. Microbiol.,* **42**:529–539.
Smothers, J. F., von Dohlen, C. D., Smith, Jr., L. H. & Spall, R. D. 1994. Molecular evidence that the myxozoan protists are metazoans. *Science,* **265**:1719–1721.
St.-Hilaire, S., Hill, M., Ribble, C. S., Whitaker, D. J., & Kent, M. L. 1997. A comparative study of muscle texture and intensity of *Kudoa thyrsites* in pen-reared Atlantic salmon. *Dis. Aquat. Org.,* **31**:221–225.
St.-Hilaire, S., Ribble, C. & Kent, M. L. 1998. Prevalence of *Kudoa thyrsites* in sexually mature and immature pen-reared Atlantic salmon (*Salmo salar*) in British Columbia, Canada. *Aquaculture,* **162**:69–77.
Štolc, A. 1899. Actinomyxidies, nouveau groupe de Mesozoaires parent des Myxosporidies. *Bull. Intl. Acad. Sci. Boheme,* **22**:1–12.
Strimmer, K. & von Haeseler, A. 1996. Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.,* **13**:964–969.
Styer, E. L., Harrison, L. R. & Burtle, G. J. 1991. Experimental production of proliferative gill disease in channel catfish exposed to a myxozoan-infected oligochaete, *Dero digitata. J. Aquat. Anim. Health,* **3**:288–291.
Swearer, S. E. & Robertson, D. R. 1999. Life history, pathology, and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): an ovarian parasite of Caribbean labroid fishes. *J. Parasitol.,* **85**:337–353.
Székely, Cs., Molnár, K. & Eszterbauer, E. 2000. Infection of roach (*Rutilus rutilus*) by experimentally produced triactinospores of *Myxobolus pseudodispar*. [VIII Euro. Multicolloquium of Parasitology, Poznan, Poland, Sept. 10–14, 2000]. *Acta Parasitol.,* **45**:270.
Székely, Cs., El-Mansy, A., Molnár, K. & Baska, F. 1998. Development of *Thelohanellus hovorkai* and *Thelohanellus nikolskii* (Myxosporea: Myxozoa) in oligochaete alternate hosts. *Fish Pathol.,* **33**:107–114.
Székely, Cs., Molnár, K., Eszterbauer, W. & Baska, F. 1999. Experimental detection of the actinospores of *Myxobolus pseudodispar* (Myxosporea: Myobolidae) in oligochaete alternate hosts. *Dis. Aquat. Org.,* **38**:219–224.
Thompson, K. G., Nehring, R. B., Bowden, D. C. & Wygant, T. 1999. Field exposure of seven species or subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. *J. Aquat. Anim. Health,* **11**:312–329.
Trouillier, A., El-Matbouli, M. & Hoffmann, R. W. 1996. A new look at the life cycle of *Hoferellus carassii* in the goldfish *(Carassius auratus auratus)* and its relation to ''kidney enlargement disease'' (KED). *Folia Parasitol.,* **43**:173–187.
Uspenskaya, A. V. 1995. Alternation of actinosporean and myxosporean phases in the life cycle of *Zschokkella nova* (Myxozoa). *J. Eukaryot. Microbiol.,* **42**:665–668.
Van Oppen, M. J. H., Willis, B. L. & Miller, D. J. 1999. Atypically low rate of cytochrome b evolution in the scleractinian coral genus Acropora. *Proc. Royal Soc. B.,* **266**:179–183.
Vincent, E. R. 1996. Whirling disease and wild trout: the Montana experience. *Fisheries* (Bethesda), **21**:32–34.
Voronin, V. N. & Chernysheva, N. B. 1993. An intracellular gill parasite as the possible causative agent of mortality during swim-bladder inflammation in common carp, *Cyprinus carpio* L. *J. Fish Dis.,* **16**: 609–611.
Weill, R. 1938. L'interpretation des Cnidosporidies et la valeur taxonomique de leur cnidome. Leur cycle comparé à la phase larvaire des Narcomeduses Cuninides. *Travaux de la Station Zoologique de Wimereaux,* **13**:727–744.
Wheeler, W. 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics,* **12**:1–9.
Wheeler, W. & Gladstein, D. 1996a. MALIGN version 2.7, program and documentation. American Museum of Natural History, New York, New York. Software available from <ftp://ftp.amnh.org/>.
Wheeler, W. & Gladstein, D. 1996b. POY–Phylogeny Reconstruction via Direct Optimization of DNA data, version 2.0, program and documentation. American Museum of Natural History, New York, New York.
Whitaker, D. J. & Kent, M. L. 1991. Myxosporea *Kudoa thrysites*: a cause of soft flesh in farm-reared Atlantic salmon. *J. Aquat. Anim. Health,* **3**:291–294.
Whitaker, D. J. & Kent, M. L. 1992. *Kudoa thyrsites* (Myxosporea) and soft flesh in pen-reared coho salmon. *Fish Health Sec./Am. Fish Soc. Newslett.,* **20**:4–5.
Wolf, K. & Markiw, M. E. 1984. Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. *Science,* **225**:1449–1452.
Wolf, K., Markiw, M. E. & Hiltunen, J. K. 1986. Salmonid whirling disease: *Tubifex tubifex* (Müller) identified as the essential oligochaete in the protozoan life cycle. *J. Fish Dis.,* **9**:83–85.
Xiao, C. 1999. The biology and systematics of myxozoan parasites of fish and oligochaetes from Lake Sawajewun, Algonquin Park, Ontario. Dissertation, University of Toronto, Ontario.
Xiao, C. & Desser, S. S. 1998a. Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of triactinomyxon and raabeia. *J. Parasitol.,* **84**:998–1009.
Xiao, C. & Desser, S. S. 1998b. Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of echinactinomyxon, neoactinomyxon, aurantiactinomyxon, guyenotia, synactinomyxon, and antonactinomyxon. *J. Parasitol.,* **84**:1010–1019.
Xiao, C. & Desser, S. S. 2000a. Cladistic analysis of myxozoan species with known alternating life-cycles. *Sys. Parasitol.,* **46**:81–91.
Xiao, C. & Desser, S. S. 2000b. The longevity of actinosporean spores from oligochaetes of Lake Sasajewun, Algonquin Park, Ontario, and their reaction to fish mucus. *J. Parasitol.,* **86**:193–195.
Xiao, C. & Desser, S. S. 2000c. Molecular characterization of myxozoan parasites from Lake Sasajewun, Algonquin Park, Ontario, by riboprinting. *J. Eukaryot. Microbiol.,* **47**:85–89.
Yokoyama, H. 1997. Transmission of *Thelohanellus hovorkai* Achmerov, 1960 (Myxosporea: Myxozoa) to common carp, *Cyprinus carpio,* through the alternate oligochaete host. *Sys. Parasitol.,* **36**:79–84.
Yokoyama, H., Inoue, D., Sugiyama, A. & Wakabayashi, H. 2000. Polymerase chanin reaction and indirect fluorescent antibody technique for the detection of *Kudoa amamiensis* (Multivalvulida: Myxozoa) in yellowtail *Seriola quinqueradiata. Fish Pathol.,* **35**:157–162.
Yokoyama, H., Ogawa, K. & Wakabayashi, H. 1993. Some biological characteristics of actinosporeans from the oligochaete *Branchiura sowerbyi. Dis. Aquat. Org.,* **17**:223–228.
Yokoyama, H., Ogawa, K. & Wakabayashi, H. 1993. Involvement of *Branchiura sowerbyi* (Oligochaeta: Annelida) in the transmission of *Hoferellus carassii* (Myxosporea: Myxozoa), the causative agent of kidney enlargement disease (KED) of goldfish *Carassius auratus. Fish Pathol.,* **28**:135–139.
Yokoyama, H., Ogawa, K. & Wakabayashi, H. 1995. *Myxobolus cultus* n. sp. (Myxosporea: Myxobolidae) in the goldfish *Carassius auratus* transformed from the actinosporean stage in the oligochaete *Branchiura sowerbyi. J. Parasitol.,* **81**:446–451.