

SESSION 8

3rd MYXOZOAN WORKSHOP

Convenors:

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(Spain)

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Morphological, molecular and pathological comparison of two *Unicapsula* species from the Mediterranean

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Histozoic myxozoa of fishes have been reported to cause problems in aquaculture and fisheries. Important examples are species of the genus *Kudoa* (Meglitsch 1947) which, by proteolytic enzymes, can cause post-mortem myoliquefaction in commercial fishes. In general, the life cycle of myxozoans is indirect, which could be a problem when the intermediate host is present in the environment of the sea cages.

The striped sea bream, *Lithognathus mormyrus* (L.) (Sparidae), is appreciated for his excellent flesh in the Mediterranean fish markets. This species is a new candidate for aquaculture in the attempt to diversify the sea bass and sea bream monocultures in the Mediterranean. Thus, the parasitological study of *L. mormyrus* provides information on future culture problems. Myxozoan cysts were found in the muscle of the striped sea bream from the Spanish Mediterranean (Murcia and Valencia). The myxospores inside these cysts were identified as representatives of the genus *Unicapsula* Davis, 1924 characterised by three valves and one polar capsule. Due to the close relatedness of *Unicapsula* and *Kudoa* and the identical host tissue localisation, the two genera might cause similar pathological effects in their hosts.

In the Mediterranean, only one species of *Unicapsula*, i.e. *Unicapsula pflugfelderi* Schubert, 1975 has been described in the muscle of the picarel *Spicara smaris* (L.) (Centracanthidae). The *Unicapsula* species from *L. mormyrus* was compared morphologically and molecularly with *U. pflugfelderi* collected from *S. smaris* using optic and scanning electron microscopy as well as 18S rDNA sequencing. The potential damage that these species might cause in the muscle of the fish is being investigated on paraffin sections and by TEM.

Morphological and developmental adaptations of some novel North American myxozoans

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Myxozoan infections in annelids result in production of actinospores – the life stage infectious to fish and other vertebrates. The tremendous range of spore shapes, sizes and surface augmentations allows these spores to infect vertebrate hosts with vastly different life histories. In the course of a survey of myxozoan parasites in North America, we discovered several novel spore types that possess interesting morphological or developmental features.

Most actinospores have smooth, feature-less valve surfaces. We found spores in three collective groups - Triactinomyxon, Echinactinomyxon and Raabeia - with augmentations to their valve cells. Hair-like, barb-like, or finger-like extensions were present at the distal ends of their caudal processes. Presumably these modifications affect the hydrodynamic properties of the spores in the water column, or serve to anchor the spores to substrate, facilitating encounter with the alternate host.

Myxozoans are regarded as microscopic parasites. However, we discovered the largest known members of two groups, Raabeia and Triactinomyxon, spores of which approach 1 mm across, almost double the size of known types. Myxozoans have now been shown to exist as spores whose sizes range across three orders of magnitude.

Whereas the majority of myxozoans develop asynchronously in the intestinal epithelium of their oligochaete hosts with mature spores shed into the lumen and expelled from the host with faeces, we encountered several infections of the circulatory system of *Ilyodrilus templetoni*. Spores underwent synchronous development in typical pansporocysts, which circulated freely in the dorsal and ventral blood vessels. When nearly mature, the pansporocysts clustered in the posterior third of the oligochaete giving the worm a distinctive 'white tail'. The oligochaetes then underwent autotomy and lost the infected portion. Oligochaetes which had shed their parasite load were maintained in culture for several weeks to demonstrate that they remained healthy after clearing the infection. This mode of release of spores which would otherwise be trapped within the host until its predation or death represents a highly evolved host-parasite relationship.

These findings broaden the definition of myxozoan spores and indicate there likely remains a diverse range of forms and *modi vivendi* of Myxozoa to be discovered.

***Parvicapsula minibicornis*: a Myxozoan species complex?**

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For many myxozoans a paucity of morphological characters impedes taxonomic classification and the number of true species is likely underestimated. The presence of such cryptic species is especially probable for myxozoans described from a wide range of hosts. DNA sequencing is now firmly established as a tool for augmenting traditional taxonomy and is well suited for resolving clades of closely related species.

Parvicapsula minibicornis is a pathogenic myxozoan parasite described from a range of salmonids in North America. During the elucidation of its life cycle, we observed differences in 18S rRNA gene sequences of parasite isolates from different hosts and geographic locations. We designed a primer pair to amplify 934 bp of the most variable region of *P. minibicornis* 18S, then sequenced isolates from multiple salmonid species of the genus *Oncorhynchus*: coho (*O. kisutchi* - 13 isolates), Chinook (*O. tshawytscha* - 14), sockeye/kokanee (*O. nerka* - 17/3) and pink salmon (*O. gorbuscha* - 3), and rainbow and steelhead trout (*O. mykiss* - 17/1). Both juvenile and adult fish were sampled from California, Idaho, Oregon, Washington and British Columbia.

Given that the parasite has a two-host life cycle, which involves the freshwater polychaete worm *Manayunkia speciosa* and two water-borne spore stages, we also sequenced 11 isolates from polychaetes and 25 water samples, primarily from the Klamath River basin in northern California/southern Oregon. One polychaete was found infected with a freshwater *Parvicapsula* that had a significantly different genotype (< 93% similar to *P. minibicornis* #AF41147) which we have designated *P. manayunkia*, in recognition of its invertebrate host.

Phylogenetic analysis of 12 informative sites in the sequences revealed that *P. minibicornis* clustered into 7 distinct genotypes which were largely consistent across fish species and the extensive geographic range of the study. Four genotypes were found in Chinook, sockeye, rainbow and coho, and one genotype was found in rainbow, Chinook and sockeye/kokanee. One genotype exclusively parasitized coho salmon, and in turn, coho were only parasitized by this genotype. Multiple genotypes were found in most water samples and in some individual fish whose sequence chromatograms had multiple superimposed peaks at the polymorphic loci. Although the sequence similarity between the majority of *P. minibicornis* genotypes was high (99.1 - 99.8 %) compared with typical variation observed (< 98%) for morphologically distinct species of myxozoans, the distinct preferences of some *P. minibicornis* genotypes for specific salmonids is indicative of a complex of cryptic freshwater *Parvicapsula* species.

Myxozoans in waterfowl: an example of parasite host range expansion?

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Here, we characterize the first myxozoan infection in birds, documenting developmental stages and mature spores in the bile ducts of a Pekin Duck (domesticated *Anas platyrhynchos*) from Georgia as well as eight additional cases of infection in both captive exotic and free-flying native ducks (Anseriformes, Family Anatidae), from locations across the United States (Lowenstine LJ *et al.* 2002, 2002 Proc Am Ass Zoo Vet 86-87). Cases occurred both from the wild and from zoological parks where birds were in enclosures with open water. Inflammation associated with these infections varied from mild to severe. In severe cases, bile ducts were ruptured and parasite spores were dispersed in the hepatic parenchyma. Often there were other significant health problems and there was no clear link between myxozoan infection and morbidity.

Based on the morphological and molecular data, the novel parasite belongs to the myxozoan genus *Myxidium*. Phylogenetic analysis of the small subunit 18S rRNA gene revealed closest affinity with freshwater *Myxidium* species described from 'higher' vertebrates - chelonids (tortoises) and anurids (frogs). The number of infection records and the large geographic range indicates this is not an incidental occurrence and expands the known host range of myxozoans from poikilotherms to homeotherms. Whether this points to an emerging pathogen or an overlooked parasite of waterfowl will remain unknown until archived samples and birds from more locations are examined. In either case, this finding will require a redescription of the group and has implications for the evolution of this phylum.

Phylogenetic relationships among myxosporeans based on ribosomal and protein-coding data

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The recent published study of myxosporean phylogeny based on the small subunit ribosomal RNA gene (SSU rDNA) sequences showed discrepancies between the existing myxosporean taxonomy and molecular phylogeny. Phylogenetic analysis revealed many paraphyletic and polyphyletic genera. The formation of myxosporean clades was not in accordance with the spore morphology but with the environment of host species or the site of infection (Fiala I, 2006, *Int J Parasitol*, 36: 1521-1534).

Our research was focused on phylogenetic analyses of other three genes to ascertain whether myxosporean relationships correspond to the SSU rDNA phylogeny or current taxonomy based on spore morphology. Concatenated alignment should also increase the resolution of the myxosporean phylogenetic tree. The large subunit ribosomal DNA (LSU rDNA) dataset consisted of 20 newly obtained sequences and 14 sequences retrieved from the GenBank. This dataset contained LSU rDNA sequences of 34 species from 11 myxosporean genera. Members of 7 genera were sequenced for the first time. Results of phylogenetic analysis based on LSU rDNA sequences were congruent with SSU rDNA phylogeny.

To avoid problems connected with the use of ribosomal genes (long branch attraction) we have analyzed protein-coding genes. The phylogenetic utility of two genes, the elongation factor 2 (EF 2) and the heat shock protein 70 (HSP 70), has been assessed. Analysis of 10 sequences of the EF 2 gene confirmed the results of ribosomal phylogeny and the studied gene has appeared as a useful phylogenetic marker at this taxonomic level. The only discrepancy in the ribosomal and the EF 2 gene tree topologies was the different position of *Chloromyxum cyprini*.

Four sequences of HSP 70 have been obtained so far which does not allow us to construct a reliable phylogenetic tree of myxosporean relationships. Nevertheless, sequence comparison of myxosporean HSP 70 gene sequences showed that this gene could be a useful molecular marker for resolving myxosporean relationships. Our analyses of ribosomal (SSU rDNA and LSU rDNA) and protein-coding data (EF 2) and also combined analyses of these datasets confirmed previous results based only on SSU rDNA data. This phylogenetic reconstruction also helped to resolve some internal nodes unresolved in SSU rDNA tree topology. We suppose that the presented molecular phylogeny could reflect the organismal phylogeny.

Genetic diversity of kudoid parasites: including new examples from pomacentrid fishes

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Small subunit ribosomal DNA has been the standard for genetic species indicators of Myxosporea since the mid 1990's. Since then, there have been radical changes in the taxonomy of multivalvulids, reflecting ribosomal DNA relationships, for example synonymy of three families into Kudoidae (see Whipps *et al.*, 2004, *J Parasitol*, 90: 618-622). However, the problem still remains that even with an increasing genetic dataset, no biological correlates have yet been found that reflect ribosomal genetic relationships within this order of parasites. During the course of the present project, new species of kudoid parasites were collected from pomacentrid fishes from the Great Barrier Reef and their partial small and large subunit ribosomal DNA was obtained. Phylogenetic analyses have revealed two conflicting patterns of morphological and molecular relationships. Significant genetic diversity was discovered within a single morphotype from several different host species; while a single morphotype from a pomacentrid fish had almost identical (99%) similarity in ribosomal sequence (over > 2300 bp) with that morphotype from a carangid fish. These results and comparison with data available from GenBank, brings forth the question: Is SSU ribosomal DNA data becoming misleading in multivalvulidan taxonomy, and should we be searching for alternative or combined genetic regions that reflect biological data?

Phylogeography of *Tetracapsuloides bryosalmonae* in Italy

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Tetracapsuloides bryosalmonae is a myxozoan parasite causing Proliferative Kidney Disease (PKD) of salmonid fish in Europe and North America. In Italy the historical endemic area of the disease is the north side of the Po river where the parasite is largely diffused in *Oncorhynchus mykiss* (Gustinelli A *et al*, 2005, *Ittiopatologia*, 2:43-51). In order to study the genetic composition of the population of *T. bryosalmonae* in relation to the catchment's basin and the geographical region, a phylogenetic analysis has been carried out. Samples collected from 12 catchment's basins located in 5 regions (Lombardia, Piemonte, Veneto and Trentino/Friuli computed together) of northern Italy, were subjected to PCR amplifying the internal transcribed spacer 1 (ITS1), with the primers described by Henderson and Okamura (2004, *Proc. R. Soc. Lond.*, 271: 1729-1736). All PCR products were cloned and 10 clones for each sample were sequenced and aligned. The percentage of divergence was calculated in MEGA3 (Pairwise Distance option with Kimura2P and Pairwise Deletion) as the percentage of polymorphic sites among sequence within clonal groups, while the variation within clonal groups of interest was measured by calculating the mean. Phylogenetic analysis was performed in MEGA3 using Minimum Evolution strategy (Kimura2P, Gamma1, Pairwise Deletion, 100 bootstrap replicates).

The overall mean of variation observed within the clonal groups was about 1.5%. Considering the region, the percentages of polymorphic sites were 1.7% for Lombardia, 1.5% for Piemonte, 1.4% for Veneto, and 0.9% for Friuli/Trentino and within the clones of each catchment's basin ranged from 0.8% to 2%. Phylogenetic analysis by minimum evolution gave results which identified 3 distinct clades, with more than 81% bootstrap value. Inside each clade we observed the presence of *T. bryosalmonae* clones from different catchment's basins and regions, and in particular one of these clades contained clones from all the sites considered. Furthermore 8 clones were distributed in at least 2 catchment's basins and one clone (A6) in 11 out of 12 basins.

The percentage of divergence observed among the samples was very similar to those described for the EU group by Henderson & Okamura (2004), confirming the low genetic divergence in the ITS1 of *T. bryosalmonae* in Italy. Our results indicate that probably only one lineage is present in all the sites considered (i.e. clone A6 is present in 11 out of 12 sites) even if one cluster containing clones from Lombardia seems to begin diverging from the others.

Use of molecular biological methods for determining the host range of parasites: molecular, biological and histological studies on fish-parasitic *Myxobolus* species

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Regarding the parasitic infections of fish, one of the most important questions is whether a given parasitosis can be transmitted to other species or age groups. Until quite recently, the host range of parasites could be determined only empirically or using complex experiments. Molecular biological methods can give an almost perfect answer to the above question.

During our investigations on the host specificity of myxosporeans, which are among the commonest causes of parasitic infection in fish, we studied 1250 base pairs of the 18S rDNA of some relatively common *Myxobolus* species, and obtained new data on *Myxobolus dogieli* parasitising the heart of bream and *M. macrocapsularis* forming cysts on the gills of bream and white bream. These results are presented together with the findings of histological examinations carried out simultaneously.

The species *M. dogieli* is a common carp parasite occurring in Russia and Poland, which sometimes gives rise to rather severe infection. Developing in the epicardium and endocardium it produces numerous fairly large plasmodia. Most intensive infection occurred in the bulbus arteriosus but could be detected in the ventricles of the heart as well. We often found this parasite in bream from Lake Balaton in the summer months. This infection was not detected on common carp and in other fish species collected from Lake Balaton together with bream. The 18S rDNA gene sequences of the spores were well distinguishable from those of the hitherto known species. They showed the closest relatedness (94%) to the species *M. ellipsoides*. As we do not have samples from common carp, despite the morphological identity we cannot identify the species found with that recorded from the type host with absolute certainty.

At the same time, we have more conclusive data on the species *M. macrocapsularis*. We detected this species on multiple occasions from the gills of bream, where it formed plasmodia well visible also with the unaided eye in the gill arteries, typically at the tip of the gill filaments. Last year we observed similar signs in white bream as well, and studied the morphologically similar spores removed from the cyst by molecular methods. The 18S rDNA sequences of spores obtained from white bream showed 99.9% identity with those of spores obtained from bream. On that basis, we can state that the species *M. macrocapsularis* is a joint parasite of the above two bream species. Setting out from the above examples, we plan to analyse the DNA of *Myxobolus* species parasitising the same organs of different cyprinids and having morphologically similar spores in order to get data on the host range of different *Myxobolus* species by comparing the sequences obtained.

***Kudoa* sp. (Myxozoa, Multivalvulida) infection in *Sardina pilchardus* (Walb., 1792) from a Portuguese fish market**

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In order to identify the prevalence of the infection with *Kudoa* spp. in *Sardina pilchardus*, 40 specimens obtained from a fish market of Oporto were examined for the presence of the parasites in muscle. Three samples of skeletal muscle were collected behind the head from the median region and from the caudal region were screened separately. Each sample of muscle (1g) were mixed with 1 ml of saline and minced through pressed between two glass plates, the fluid was collected and allowed to stand 30 min in an eppendorf tub. A drop (approximately 20 µl) was collected from the bottom of the tube onto a slide and the spores were counted under 400x magnification.

Kudoa spores observed were stellate in apical view (length = 9.6 µm, width = 13.8 µm, thickness = 10.8 µm) with 4 unequal sized polar capsules (large PC = 4.6 µm x 3.2 µm, medium PC = 3.3 µm x 2.6 µm, small PC = 2.5 µm x 2.2 µm).

The abundance of spores from the 3 regions screened was significantly different (Friedman test = 8.9, $p < 0.05$). The higher values were observed in the muscle collected behind the head.

The spores were found in 77.5 % of the specimens. The intensity of infection were generally low: 42 % of the infected fish presented light infections (1 – 9 spores/ 100 fields), 29 % showed moderate infections (10 – 29 spores/ 100 fields), 26 % were heavy infected (30 – 99 spores/100 fields) and only 3 % showed very heavy infections (> 100 spores / 100 fields). All the specimens with heavy or very heavy infections showed softening of the muscle.

Comments on the recent knowledge and some of the topics to be solved in the research on Myxozoa

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The research of Myxozoa has resulted thus far in a vast assemblage of described species - about 2.200 in 65 genera. This number itself should prompt a requirement to verify the validity of descriptions. To match the actinospore phases – thus far detected 18 forms in oligochaetes, polychaetes and sipunculids – to their myxosporean counterparts is a standing task of research.

Presently, research interest in Myxozoa centres primarily on Myxozoa as pathogens in fish cultures. Emerging pathogens in freshwater and marine aquaculture have been registered and new species are continuously added on the list. Myxozoa as pathogens in fish farming offer an array of aspects: source of infection (can it increase by the *in vivo* fragmentation of definitive hosts known in some species?); transmission (does the direct transmission also occur beyond the genus *Enteromyxum*?); scope of hosts (very broad in the most pathogenic species like *Ceratomyxa shasta* or *E. leei*); diagnostics (four different techniques are available for *Myxobolus cerebralis*); histopathology (it has not yet been fully exploited in explaining the mechanisms of pathogenic action); tissue and host specificity (arguments pro and contra are being supplied); control (could *Tubifex* strains resistant to myxosporean infection contribute to the attempts to control the infection?).

Thus far, complete life cycles involving both the intermediate vertebrate host and the oligochaete definitive host are known in 36 myxosporeans, only one taking place in the marine environment. As for the amphibian and reptile myxosporeans, and rare findings in anatid ducks and terrestrial mammals, their life cycles open a big challenge. Although one enigma has been solved (the life cycle of *Tetracapsuloides bryosalmonae*), the cycle of other malacosporans is still in limbo. Do the actinosporean stages reflect strictly the taxonomy and/or phylogeny of the myxosporean stage they belong to or is their morphology also defined by some other factors? Some types of actinosporean stages may occur at random throughout the existing taxonomic spectrum: aurantiactinomyxon in *Myxidium*, *Chloromyxum*, *Henneguya*, *Thelohanellus*; neoactinomyxon in *Sphaerospora* and *Chloromyxum*; tetractinomyxon in *Ceratomyxa*, *Ellipsomyxa* and *Parvicapsula*. Moreover, species of the genus *Myxobolus* exhibit three different actinospore collective groups. Will all this have an impact on the taxonomy? And is there any actinosporean propagating itself without involving a myxospore stage in an intermediate (fish) host?

Phylogeny based on SSU rDNA sequences is often at variance with the existing taxonomic schemes. A proposal has already been made in Multivalvulida to abandon most of the existing genera in favour of *Kudoa*. The schism found between “marine” and “freshwater” species of many genera predicts other possible changes in taxonomy. An important task is to confront the small and large subunit phylogenies with that of other markers. The views on bilaterian nature of Myxozoa, although contravened by structural identity of polar capsules and nematocysts, still prevail. In addition, there are indications that some species might not belong to either of these large groupings of organisms.

Relevance of different fish species as vertebrate host of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea)

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Proliferative Kidney Disease (PKD) of salmonids is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. Thus far, several PKD-susceptible salmonid species are known, but laboratory infection of bryozoa has been achieved only by exposure of bryozoan colonies to water from a tank with PKD-infected brown trout (*Salmo trutta*) (Morris DJ *et al.* 2006, Parasitology 133: 701-709). Spores have been demonstrated in the urine of an American rainbow trout strain (Hedrick RP *et al.* 2004, Parasitol Res 92: 81-88). In the present study the role of three salmonids as vertebrate hosts of *T. bryosalmonae* were investigated by experimental infections.

Infected *Fredericella sultana* (Bryozoa: Phylactolaemata) colonies were collected from the field and kept in laboratory culture according to Morris DJ *et al.* (2002, Folia Parasitol 49: 25-34). Statoblasts were harvested from the colonies and raised on plastic Petri dishes in a separate container to obtain parasite free colonies. Four rainbow trout (*Oncorhynchus mykiss*), four brown trout and four brook trout (*Salvelinus alpinus*) were infected by cohabitation with infected bryozoan colonies for two weeks and then removed in three separate aquaria. Three weeks after the start of the cohabitation, one fish from each group was killed and a sample of the kidney was taken for analysis by *T. bryosalmonae*-specific PCR (Kent M *et al.* 1998, J Aquat An Health 10: 12-21). Approximately eight weeks after cohabitation, a *F. sultana* colony obtained from statoblasts was kept in each fish aquarium for 8 h per day. For the remaining time they were transferred to separate culture containers for feeding. Several statoblast-raised colonies were kept without contact with fish, as a negative control.

The three fish species exposed to infected bryozoans were PCR-positive, and thus the transmission of the infection can be considered successful. Four weeks after exposure of the parasite-free bryozoan colonies to infected brook trout, typical *T. bryosalmonae* stages were observed in a small part of the colony. One week later, the first developmental stages were observed in the brown trout-exposed bryozoa, while the bryozoa cohabitated with the rainbow trout showed no visible *T. bryosalmonae* stages until six weeks post exposure. None of the control bryozoan colonies showed malacosporean developmental stages.

These results might indicate the existence of two strains of *T. bryosalmonae*, one American and one European being adapted to rainbow trout and brown trout respectively.

Studies on differentially expressed genes involved in the host invasion of *Myxobolus cerebralis*

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The economic and ecological importance of fish-parasitic myxozoans is considerable worldwide. Species, such as *Myxobolus cerebralis* and *Tetracapsuloides bryosalmonae* cause high mortality among salmonids in aquaculture, and also significant declines in wild salmonid populations. For the development of an effective treatment, it is essential to discover the genetic background of the invasion mechanisms of myxosporeans. The objective of the present study was to identify parasitic genes regulating host invasion, which may be potential targets for further development of preventive methods. In the case of *Myxobolus cerebralis* and other myxosporeans, observations suggest that a combination of mechanical and chemical stimuli is required for the attachment of actinospores (triacinomyxon, TAM) and thus for initiation of host infection by active penetration. The transcriptomes of TAMs of *M. cerebralis* and actinospores activated *in vitro* were compared using suppression subtractive hybridisation (SSH).

For TAM activation, an *in vitro* experimental system was developed. The activation efficiency data were statistically analysed, and a significant difference in sporoplasm release was detected between activated and non-activated samples. For the molecular genetic study, 8×10^5 TAMs/SSH sample were collected. After RNA extraction and cDNA synthesis, forward and reverse SSH were performed on the cDNA of the activated and non-activated TAMs. The hybridized DNA products were PCR amplified using adaptor-specific primers and the PCR products were cloned into a plasmid vector. The created SSH cDNA library was differentially screened using an adaptor-specific PCR, non-radioactive dot blot, DNA sequencing and quantitative real-time PCR.

Upon DNA sequencing, 15 candidate genes were identified, from which seven were examined using quantitative real-time PCR. The transcription level of the candidate genes was determined in different developmental stages of the infection in fish and also in the non-activated and activated TAMs. The expression levels of some genes were rather variable showing no trend in the infection process, whereas in the case of two genes, definite tendencies were recognizable in the changes of their expression at different stages of infection. For three genes coding an actin-related protein, a Ca²⁺-binding protein of the EF-hand superfamily, and a pleckstrin homology domain respectively, an increased activity was detected during host invasion. Their expression level was highest in the activated TAMs, while relatively low in non-activated TAMs and in infected fish samples. The finding of the present study suggests that the latter genes are involved in the host invasion of the TAM of *M. cerebralis*, and further study is required to investigate their function in the complicated process of host recognition and invasion.

Myxozoan relationships among Metazoa: conflicting phylogenetic signals of ribosomal RNA genes and protein coding genes

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Although the discovery that *Buddenbrockia plumatellae*, an enigmatic worm-like organism, is a myxozoan with distinct bilaterian features probably resolved the origin of Myxozoa, the evidence for bilaterian or non-bilaterian relationships from molecular taxonomy is still lacking. The analyses of 18S rDNA have resulted into the controversial tree topologies for the relation of Myxozoa to Bilateria or Cnidaria (or *Polypodium hydriforme*). It provokes the debate about Long Branch Attraction (LBA) artefacts in phylogenetic analyses and about appropriateness of 18S rDNA for phylogenetic reconstruction at this phylogenetic level. In the present study, we enlarge the rRNA gene dataset with almost entire 28S rDNA and 16S mitochondrial rDNA sequences (more than 4500 unambiguous characters in concatenated alignment). Elongation factor 2 (EF2), heat shock protein 70 (HSP70) and myosin heavy chain type II (myosin-II) were chosen as potential protein coding gene markers for reconstructing phylogenies within the Myxozoa and the main metazoan clades. Independent phylogenetic analyses of the three rRNA genes as well as their concatenated analysis placed the Myxozoa as a sister taxon of the Bilateria (with possible influence of LBA). Myosin-II appeared to be very useful gene marker for reconstructing metazoan relationships in published studies, but we were unable to obtain any myxosporean myosin-II amplicon by PCR from both DNA and cDNA, except the one, which appeared as a different homologue of myosin gene superfamily. Phylogenetic analyses of myxosporean EF2 and HSP70 sequences showed Myxozoa as a sister group of all Metazoa. It disagrees with rRNA genes phylogenies, which placed Myxozoa inside the Metazoa group. These results will encourage the subsequent seeking for other genes appropriate for the Myxozoa/Metazoa phylogenies.

Hyperparasitic Myxozoan parasites of Monogenea

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Myxozoan parasites are commonly found infecting numerous fish species in many different target organs and tissues. In the current study, ancyrocephalid gill monogeneans infecting a *Platycephalus* sp. from lake Hamana in Japan was found to be infected with myxospores. Certain individual worms were observed with the body cavity filled with spores, mostly seen as mature disporous trophozoites. Spore morphology was similar to that of *Myxidium* spp., being basically fusiform with a slightly sigmoid curve to pointed ends where the opposing pyriform polar capsules were located. However, analysis of the 18s rRNA gene showed a closer identity to members of the genus *Kudoa*, albeit only with a maximum identity of 90% over 1627 bases of sequence data.

Simultaneous infections in the fish gills were not microscopically apparent, nor were detectable using PCR's performed from DNA extracted from gill filaments, suggesting that the infection may be limited to the monogenean worms. However, potential infection sites other than gill tissue were not analysed during the current study, so it remains possible that the infection seen in the monogenean worms was simply opportunistic.

A similar type of infection was observed in another ancyrocephalid monogenean infecting a *Megalop* sp. from marine waters off Pulau Langkawi, Kedah, Malaysia.

Actinospores from an Icelandic lake

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Numerous myxozoan parasites infecting brown trout (*Salmo truttae*) and arctic char (*Salvelinus alpinus*) in Lake Hafravatn in Iceland have been recently sampled (Richter SH and Kristmundsson Á, 2007, *Læknablaðið* [Icelandic Med J], 93 (53): 110-111). Lake Hafravatn is 1.06 square kilometres in area with a maximum depth of 28m and is attached to the sea by a small river. The only other fish species found in the lake are three-spined stickleback (*Gasterosteus aculeatus*), the European eel (*Anguilla anguilla*) and, during the summer months, breeding Atlantic salmon (*Salmo salar*).

Chloromyxum sp. (*truttae*) and *Myxobolus neurobius* were common in brown trout, *M. arcticus* and *M. cerebralis* were common in arctic char, and *Sphaerospora truttae* was occasionally sampled from brown trout. Furthermore, *Myxobolus* sp. was found in the lateral line nerve of both fish species.

In an attempt to understand the life cycle of these myxozoan parasites, oligochaete worms were collected from sediment samples and incubated at 5°C in 24 well cell culture plates containing lake water. The worms were observed after 48 hours to look for signs of actinospore release. Only tubificid oligochaetes were collected, of which 20% (6/30) released actinosporean spores. Raabeia-like actinospores with three long caudal processes (80-90µm) were released from a single worm. A second type of actinospore was observed with only two caudal processes that opposed each other in direction and terminated to a fine point. Fixed or terminal polar capsules were not immediately apparent. However, electron lucent inclusions were visible inside the spore-like form. A third form, without any obvious caudal processes was also observed. This tubular-like form narrowed to one end and terminated with a single polar capsule, often observed with the polar filament extruded and the sporoplasm released.

The morphology and taxonomic relationship of these actinospore-forms will be discussed.

Monitoring *Henneguya ictaluri* infection in channel catfish, blue catfish and channel x blue backcross hybrids using histopathology and real-time PCR

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Proliferative gill disease (PGD) caused by the myxozoan parasite *Henneguya ictaluri* is one of the most economically damaging parasitic infections in channel catfish (*Ictalurus punctatus*) aquaculture. Currently, there is no effective treatment for *H. ictaluri* and outbreaks can result in >50% mortality in commercial channel catfish ponds. Challenge studies have shown that the closely related blue catfish (*Ictalurus furcatus*) does not exhibit as severe an inflammatory response to *H. ictaluri* and mortalities are significantly lower than in channel catfish. Comparisons of PGD severity and *H. ictaluri* infection in channel catfish, blue catfish and channel x blue catfish backcross hybrids were carried out by gross examination of gill clip wet-mounts, histopathology and real-time PCR (QPCR). This study was conducted in an attempt to elucidate factors attributing to variation in host response in susceptible and unsusceptible species of catfish. Fish were held in floating net pens and placed in a three separate commercial catfish ponds experiencing clinical outbreaks of PGD. Fish from each species were sampled at days 1, 3, 5, and 7 and gill clip (40-80 filaments) wet mount preparations were examined for chondrocytic lysis and gross manifestation of the disease. Molecular analysis was performed on gill clips and blood samples. Whole gill arches were fixed in formalin and processed for routine histological examination to confirm the presence of the developing organism. Preliminary data showed significant gill damage and *H. ictaluri* development in channel catfish and channel x blue catfish hybrids but not in blue catfish. No significant gill damage was observed grossly in blue catfish and parasitic organisms were absent from histological preparations of blue catfish tissues. *H. ictaluri* DNA was detected in blue catfish gills, however this could be attributed to the presence of the organism in the water and not signify infection. Presently, it is unclear whether *H. ictaluri* actinospores are infecting blue catfish and are rapidly cleared by the host's defenses, or if *H. ictaluri* spores are actually unable to penetrate blue catfish tissue. Future research will focus on identifying the mechanisms utilized by blue catfish to prevent infection by this enigmatic organism.

Relationships within the *Ceratomyxa* Thélohan, 1892 (Myxosporea: Bivalvulida) in marine fishes from Queensland, Australia

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The genus *Ceratomyxa* contains over 160 formally described species, most of which are coelozoic and cause apparently little pathology in their host teleost fishes. *C. shasta* is a notable exception occurring systemically in salmonid fish and causing morbidity and mortality. Relationships within the genus are unclear due to a paucity of data, with only 10 species for which genetic sequence data are currently available. This study will provide a dataset from Australian fishes to allow more robust exploration of relationships within this genus of parasites. *Ceratomyxa* spp. were recorded in the gall bladder of 49 host species representing 19 families of marine teleost from Lizard Island, Heron Island and Moreton Bay. *Ceratomyxa* spp. from three host families, Serranidae, Labridae and Pomacentridae were characterised through a combination of morphometrics, host occurrence and rDNA sequence data. Phylogenetic analyses of SSU ribosomal DNA provide evidence that the genus as currently recognised is not monophyletic, with *C. shasta* more closely related to members of other genera within the Bivalvulida.

Effects of water flow on the infection dynamics of *Myxobolus cerebralis*

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Myxobolus cerebralis, the myxozoan parasite responsible for whirling disease in salmonid fishes, has a complex life cycle involving an invertebrate host and two spore stages. Water flow rate is an environmental variable thought to affect the establishment and propagation of *M. cerebralis*; however, experimental data that separates flow effects from those of other variables are scarce. To compare how this parameter affected parasite infection dynamics and the invertebrate and vertebrate hosts, dead, infected fish were introduced into a naïve habitat with susceptible hosts under two different flow regimes: slow 0.02 cm/s and fast 2.0 cm/s. Throughout the one year study, uninfected fry were held in both systems, the outflows were screened weekly for spores and the annelid populations were monitored. Clear differences in prevalence of infection in the worms, prevalence and severity of infection in the fish, and host survival were found. Both flows provided environments in which *M. cerebralis* could complete its life cycle, however, both the parasite and its invertebrate host proliferated to a greater extent in the slow flow environment over the one year study period. This finding is of significance for aquatic systems where the flow rate can be manipulated, and should be incorporated into risk analysis assessments.

Acknowledgements: We appreciate the contribution of the following people to the success of this large project: Donald Stevens; Lindsey Osborn; Jenny Dubanoski; Harriet Lorz; Kyle Thames; Stephen Atkinson; Sarah Bjork; Lan Xue; and Ted Ernst. This project was funded by the National Partnership for the Management of Wild and Coldwater Fishes, Whirling Disease Initiative.

Biology, morphology and molecules of some extraordinary myxozoans and implications from the synopsis of this information

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Two findings have dramatically changed our knowledge and understanding of the Myxozoa in recent years: 1. The discovery of the myxozoan life cycle in 1986 (Wolf K and Markiw ME, Science 225: 1449-1452) with more than 30 life cycles since elucidated, and 2. Phylogenetic relationships based on a rapidly growing database of ribosomal sequences. The molecular data show that most of the morphologically defined genera form paraphyletic groups (Fiala I, 2006, Int J Parasitol 36: 1521-1534). A striking example is the "genus" *Sphaerospora* Thélohan, 1892 with 9 representatives sequenced, 3 of which form a monophyletic clade in a basal position of the phylogenetic tree of myxozoans whereas the remaining four are scattered throughout the tree, despite similar spore morphology of all representatives. Two *Sphaerospora* species, i.e. *Sphaerospora dicentrarchi* Sitjà-Bobadilla and Alvarez-Pellitero, 1992 and *Sphaerospora* sp. cluster within the marine, multivalvulid genus *Kudoa* (Meglitsch, 1947). Based on molecular data, the diagnosis of the genus *Kudoa* has been widened several times in recent years to include different morphologies, all of which are multivalvulid. It is demonstrated here that, despite the bivalvulid character of the spores, *S. dicentrarchi* also shows biological similarities with *Kudoa*, explaining their close molecular relatedness. The current study also presents, for the first time, secondary structure analysis of myxozoan 18S rRNAs, which shows that the basal clade of *Sphaerospora* species has very distinct and unique features with long inserts in the variable regions V4 and V7 of the rRNA, differentiating this clade from all other *Sphaerospora* species and other myxozoans sequenced to date. Thus, molecular data, in context with the life cycle and biology, demonstrates that the *Sphaerospora* group is a multi-genus complex, characterised by a spore shape which possibly represents a very basic myxozoan structural plan and, as a consequence, explains its presence in more than one phylogenetic lineage. In general, the myxozoan spore morphology seems to be of limited value for taxonomy, but various features suggest its importance for spore distribution and the infection process.

Sphaerosporosis in the Indian carp, *Labeo rohita*, in Chilka Lake, a brackish water lagoon on the east coast of India

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During a study (1989-1990 and 1995-1996) of myxozoan parasites of fish from of Chilka Lake, a brackish water lagoon in the east coast of India, a new myxosporean species belonging to the genus *Sphaerospora* was found parasitizing various organs of the carp, *Labeo rohita*. Prevalence of infection was up to 6 % in the fish caught in the open lagoon, whereas it reached 56 % in fish reared under captivity in an enclosure in the same area. Seasonal changes were registered, being the highest prevalence during March-January. Sub-spherical to spherical spores measuring 8.0-11.2 (9.6) x 10.8-12.6 (11.8) x 10.0-11.2 (10.0) μm , and developing pseudoplasmodia ranging in size 14.6-19.2 x 8.0-11.2 μm were observed in the lumen of renal tubules. Different developing stages were found in different organs. Within the gills, *Sphaerospora* sp. formed oval cysts measuring 48-64 x 64-96 μm which deformed the gill lamellae and caused necrotic changes in the epithelium. Histopathological changes in the kidneys consisted of varying degrees of renal tubular dilation and necrosis, and mild to severe non-suppurative nephritis. Several changes were noticed in the blood, which included decreased serum proteins, serum globulins, haemoglobin and RBC count, and increased leucocytes. Distinct nuclear changes, such as karyolysis and pycnosis, were evident in erythrocytes from infected fish. Anaemia evidently is induced by the extrasporogonic stages.

Myxosporeans infecting Atlantic cod (*Gadus morhua*) in Norway: pests in cod culture?

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Cod culture is a modest but fast growing industry in Norway, and is already plagued by viral, bacterial and parasitic diseases. During a project establishing base-level prevalences of important viral and bacterial disease agents in wild cod stocks, we also examined some of the fish for myxosporeans. Adult cod was collected along the coast of W and N Norway. In addition, broodstock and juveniles from several cod farms were examined for myxosporean infections. Bile and urine were examined at 400-1000x magnification, while the eye-balls (i.e. sclera) was systematically examined for *Myxobolus aeglefini* infection.

A total of 210 fish were examined, representing both local and migratory stocks, and 6 species of myxosporeans were discerned. *Myxobolus aeglefini* infected the cartilage of the eyes and cranium of 6% of the fish. In the gall bladder, spores of two species were encountered, *Myxidium oviforme* Parisi, 1912 *sensu* Auerbach (1912) part (ex cod) and *Myxidium bergense* Auerbach, 1910, infecting the liver bile ducts and the gall bladder, respectively. The overall prevalence of *M. oviforme* was 53%, but was higher in local stocks (56-76%) than in migratory fish (31%, Borgundfjorden, northern W Norway, N=52). *Myxidium bergense* was absent in local cod from southern Norway, but occurred in local fish from northern Norway and in migratory fish caught in Borgundfjorden (37%). A novel parvicapsulid-type infected the kidney, ureters and urinary bladder, found in the urine of 69% of the fish, but with a higher prevalence in migratory (88%) than local stocks (35-77%). However, in this case season was likely important, lowest prevalence occurred in fish caught during summer (35% July), while high prevalences occurred in winter (69-88%). *Zschokkella hildae* Auerbach, 1910 infections are common throughout Norway (44% with spores) but always occurred in low intensities. Of the migratory cod, 53% were infected. *Zschokkella* sp. was only detected in a few cod of Barents Sea origin.

Myxidium oviforme infections were observed in wild-caught broodstock cod kept for up to 4 years in tanks on land, with water treatment that should exclude myxosporean re-infections. Hence these infections must be long-lasting, likely due to an observed budding of plasmodia in the liver. *Zschokkella hildae* was the most common myxosporean in cage reared cod, while infections in the biliary system were absent. Parvicapsulidae gen. sp. infects juvenile cod reared in shallow closed bays, and hence likely has a life cycle involving annelid hosts living in shallow waters. Among Myxozoa, so far only *Myxobolus aeglefini* have been observed to cause significant pathology in Norwegian farmed cod.

Redescription of a long lost *Myxobilatus* sp. (Myxozoa) from the ovaries of the three-spined stickleback (*Gasterosteus aculeatus*)

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Sticklebacks are a ubiquitous species throughout the northern hemisphere, occurring in a number of habitats ranging from still waters and rivers in the freshwater environment through to estuarine and full strength seawater. Included in the list of parasites of sticklebacks are a number of myxozoan species such as members of the genera *Henneguya*, *Myxobilatus*, *Ceratomyxa*, *Myxidium* and *Sphaerospora*. However, as with many early descriptions of Myxozoa, these have not always been properly figured or adequately described, making comparisons between species difficult.

Sticklebacks from a number of sites in England were examined for the presence of pathologies potentially associated with contaminants. Whole fish were processed using standard histological techniques and sections stained with haematoxylin and eosin. In two sites, female sticklebacks were infected with myxozoan spores in the ovaries. Using morphological criteria, the parasites were identified as a *Myxobilatus* sp. Measurements from ethanol fixed material were as follows: Spore body length 10-11µm; spore width 4-6µm; caudal appendages 2-5µm; total spore length (including caudal appendages) 14-16µm; polar capsules 4-5µm x 2-3µm, with 9-10 turns of the polar filament. Polysporous plasmodia formed within ovaries. Only two ovarian myxozoan species have been reported in sticklebacks, namely *Myxobilatus* (= *Henneguya*) *medius* (= *media*) (Thélohan, 1892) and *Henneguya brevis* (Thélohan, 1892). The measurements and site of infection of the parasite found in the current study most closely correlates to *H. brevis*. However, the presence of striations on the body and polar capsules perpendicular to the sutural plane clearly place the parasite within the genus *Myxobilatus*. We propose that *H. brevis* is transferred to *Myxobilatus* to reflect this. Further information is provided on the pathological responses to the parasite and consideration is given to the impact of the parasite on host population dynamics.

Occurrence of actinosporean stages (Myxozoa) in the Nera River system (Umbria: Central Italy)

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Diseases caused by myxozoans both in marine and freshwater fish, have received increasing attention over the last years in Italy, nevertheless data about the actinosporean stages are still poor. In order to study the occurrence of these alternate stages in oligochaetes from Nera River basin (Central Italy), where wild populations of brown trout (*Salmo trutta*) and farmed rainbow trout (*Oncorhynchus mykiss*) are present, a survey was carried out from April 2005 to April 2007. Mud was collected from 24 sampling stations in April 2005; oligochaetes were isolated, sorted by presence/absence of hair-chetae and then individually placed into cell-well plates according to Yokoyama *et al.* (1991, J. Parasitol 81: 446-451).

Each well was examined for the presence of released actinospores every 2-3 d for about two months. The oligochaetes releasing actinospores were fixed in buffered 10% formalin for histology while the actinospores were identified both by morphology and PCR. During the first sampling (April 2005), a single type of echinactinomyxon actinosporean stage was released by immature lumbriculids, in 4 out of 24 stations (1 in Campiano R., 3 in Sordo R.). Subsequently the 4 positive stations were further monitored (December 2005, March, July, October 2006 and April 2007). During the survey a total of 1068 lumbriculids were collected from these sites (38% of the total worms) with an overall prevalence infection of 6.8% with the highest values (8.5%) in the Sordo River just upstream a trout hatchery. In the same site a single lumbriculid releasing aurantiactinomyxon spores was collected in April 2007. The actinospores isolated from lumbriculids were identified on the basis of morphological characters. The echinactinomyxon type shows some similarity with raabeia type 1 from *Lumbriculus variegatus* and with echinactinomyxon type from *Rhyacodrilus komarovi*. The morphology of the aurantiactinomyxon type fit well with several forms already described and, among them, with the bigger form of *Aurantiactinomyxon pavinsis*. Both the two spore types were submitted to nested PCR of the SSU rRNA. Nucleotide sequences of the PCR products were determined and analyzed by BLAST. The echinactinomyxon showed a 97% of identity with *Myxobolus* sp. isolated from *O. mykiss*, while the aurantiactinomyxon showed 100% identity with *Chloromyxum truttae/A pavinsis* from *Salmo trutta/Stylodrilus heringianus*.

It was impossible to identify the genus of the lumbriculids collected because all the worms were immature. The other oligochaetes collected and identified as *Tubifex tubifex*, *Psammoryctides barbatus*, *Limnodrilus hoffmeisteri*, *Spirosperma velutinus* didn't release any actinospores during the observation period.

Molecular analyses confirm that the vertebrate hosts of the myxozoan, whose actinosporean stages have been found in this study, are rainbow/brown trout which represent the most abundant species in the positive sites.

Further analyses on fish population are required in order to detect myxosporean stage in the vertebrate hosts and confirm the molecular data.

Comparative morphological and phylogenetic studies on *Myxobolus* spp. infecting chub

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The European chub, *Leuciscus cephalus* is one of the most common fish in Europe and in the Middle East. We studied the myxozoan fauna of chub collected from River Danube, Hungary.

The collected fish were subjected to a complete parasitological dissection, and when mature plasmodia were found, morphological and molecular examination was performed on the myxospores.

During the survey, 8 *Myxobolus* spp. were observed, and two of them were described as new species. Every parasite species found had a specific location within the fish host. *M. cycloides* formed plasmodia in the swim bladder wall, the plasmodia of *M. ellipsoides* infected fins between two fin rays, *M. pseudodispar* was found in muscle cells, the cysts of *M. sp. 1* were frequently found in the intestinal wall, *M. dujardini* were located in the epithelium of the non-lamellar part of gill filaments, *M. muelleri*, and *M. sp. 2* formed large elongated plasmodia in the afferent gill artery of filaments, while *M. muellericus* filled the capillary network of the gill lamellae. The plasmodia of the latter four species very often occurred on the gills in the form of mixed infection. Despite similarities of some species in spore morphology, 18S rDNA sequences showed clear differences between the species examined.

According to our findings, chub specimens collected from the same habitat could be infected by different *Myxobolus* species at the same time. The *Myxobolus* spp. examined were characterized by a relatively strict site-specificity, and besides their morphological appearance and 18S rDNA sequences, the majority of these species differed from one another in the intrapiscine location as well.

Although slight intraspecific variabilities were detected in the 18S rDNA sequences, the samples of the examined species grouped together on the phylogenetic tree, supported by high bootstrap values. Within groups containing morphologically similar species, differences in tissue location were reflected in the phylogenetic positions. The result of the phylogenetic analyses demonstrated that both, tissue tropism and spore morphology, play an important role in the genetic relationships among *Myxobolus* species, whereas host-specificity correlates slightly with the phylogenetic results.

Acknowledgements: The study was supported by the Hungarian Scientific Research Found (OTKA, Projects No. T042464 and F045908).

Histoxic developmental stages and pathological effects of *Myxobolus karuni* and *Myxobolus persicus*

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A survey of developmental stages and pathological effects of *Myxobolus karuni* and *M. persicus* in the gills of Mesopotamian Barboid fishes of Iran was carried out from spring 2004 to autumn 2006. Altogether, 296 specimens of *Barbus pectoralis*, *B. barbolus*, *B. esocinus*, *B. grypus* and *B. sharpeyi* were examined. As a result, 98 fishes (33%) were infected by *M. karuni* and *M. persicus*. Histological investigation revealed that *M. karuni* developed within primary filaments but *M. persicus* were developed in the secondary filaments. Developing plasmodia of *M. karuni* were found inside the blood vessel of the primary filaments, *M. persicus* developed in epithelia and endothelia of secondary filaments. The pathologic effects of the parasites on the gills of Barboids were that the developmental stages of *M. karuni* caused hyperplasia around the secondary filaments and finally destroyed the connective tissue of the primary lamella, while *M. persicus* caused not only hyperplasia but also cellular infellamination, atrophy and respirational dysfunction. According the results of this study, *B. pectoralis*, *B. barbolus* and *B. esocinus* are new hosts for *M. karuni* and *M. persicus* from Iranian freshwater fishes.

How many *Myxobolus* species exist?

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At the present time, about 800 *Myxobolus* spp. are known to exist. However, several species have been inadequately described and identified, and due to the high number of possible synonyms, this number will probably decrease after thorough revisions. On the other hand, incorrect identification of some species from genetically different hosts suggests that some of the best known *Myxobolus* species represent several non-separated, morphologically similar ones, and in this way the number of valid species may increase. The above controversies regarding the genus *Myxobolus* can be avoided if in the future we pay closer attention to host specificity, tissue affinity and molecular evidences.

As regards host specificity, it should be accepted as a general rule that a given *Myxobolus* species will infect only the typical host and some closely related fish species. When a species is described by the original author from systematically far standing hosts, only one of the hosts can be accepted as type host. Generally the first mentioned is suggested as type-host and the last mentioned should be omitted from the range of hosts. In most cases, the original authors correctly designated a single, well-defined typical host but subsequent studies incorrectly inflated the number of additional hosts. In such cases most of the recorded hosts should be disregarded and only the original host can be accepted as type-host.

Studying the tissue affinity of a given species greatly facilitates correct identification. *Myxobolus* spp. developing in muscle cells, nerve cells, within the capillaries or in epithelial, cartilaginous and connective tissues can easily be differentiated from each other despite their similar spore morphology. However, the best method enabling correct species identification is a molecular study that firmly supports morphological evidences.

The basic requirements for describing a new species should be as follow:

(a) acceptance of the rules of description suggested by Lom and Arthur (1989, J Fish Dis, 12: 151-156); (b) presentation of suitable schematic drawings; (c) description of the site of development and tissue affinity of plasmodia; (d) designation of a type host and additional hosts; (e) identification with a proper name according to the rules of the Latin grammar and following the International Code of Zoological Nomenclature. Additional suggested tasks are: (a) histological study of plasmodia and of the infected organs, (b) collection of spores for molecular studies, and (c) sending sequences from the typical hosts to the GenBank.

Endoparasitism in colonial hosts and implications for salmonid disease

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Research on the evolutionary ecology of host-parasite interactions has made great progress since the fundamental theory was developed concerning the population dynamical consequences of host and parasite interactions. For parasites, this research has focused on life history trade-offs associated with patterns of virulence and transmission. For hosts, the research has investigated trade-offs that maximise future reproductive success by minimizing the effects of parasitism on fitness components such as survival, growth and reproduction. The majority of studies on host-parasite interactions have involved unitary, exclusively sexually-reproducing animal hosts (such as *Drosophila*, other non-social insects, snails and vertebrates) and hosts that incorporate parthenogenesis in their life cycles (*Daphnia* and bumble bees). However, to achieve a general overview of the evolutionary ecology of hosts and parasites, it is important to study a range of systems. While parasitism in colonial animals has received some investigation at the population level (e.g. emerging diseases of corals), there is a notable lack of studies on life history strategies and trade-offs. Our work on myxozoan endoparasites of freshwater bryozoans begins to redress the lack of knowledge of the interactions between colonial hosts and their parasites and demonstrates a tractable model system for such investigations. Field- and laboratory-based studies provide evidence for high levels of vertical transmission achieved through colony fission and the infection of dormant stages. Increased fission rates may be a strategy for hosts to escape from parasites but the parasite can also exploit the fragmentation of bryozoans to gain vertical transmission and dispersal. The research provides evidence that opportunities and constraints for host-parasite coevolution can be highly dependent on organismal body plans and that low virulence may be associated with exploitation of colonial hosts. The latter is highly relevant to the persistent outbreaks of salmonid proliferative kidney disease which are caused by myxozoans that develop in bryozoans.

Myxosporeans of the genera, *Myxobolus*, *Myxidium*, *Henneguya* and *Unicauda* from fish of Bay of Bengal, Visakhapatnam, South India

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A one year (1992-1993) study of the myxosporean parasites of fish from Bay of Bengal, East Coast of India revealed the presence of 6 species belonging to the genera *Myxidium*, *Myxobolus*, *Henneguya* and *Unicauda*. For this purpose, a total of 1511 fish, comprising 18 genera, obtained from the trawl catches at the Visakhapatnam Fishing Harbour, were examined at fortnightly intervals. Skin, gall bladder, intestine, gills, kidney, swim bladder, urinary bladder and gonads were examined following standard methodology. Host size, weight and sex were recorded. About 4 % of the sampled fish were infected by myxosporeans. A *Myxobolus* sp. was found in the skin of *Dussumeria acuta*; a *Unicauda* sp., and a *Myxidium* sp. in the gall bladder of *Caranx caranx*. *Sardinella gibbosa* harbored a *Henneguya* sp. in the gall bladder and a *Myxobolus* sp. in the intestine. The details on the morphology and morphometry of the different myxosporean species, following the guidelines of Lom and Arthur (1989, J Fish Dis, 12: 151-156), based on light microscopy observations, and their comparison with previously reported species are presented.

Description of a new species of *Myxobolus* Bütschli, 1882 (Myxozoa) from the gills of the common goby *Pomatoschistus microps* (Teleostei: Gobiidae) in Scottish waters

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Gobies are ubiquitous fish of estuarine and intertidal waters and although frequently studied, arguably little is known regarding their myxosporean fauna. A recent examination of a population of common gobies, *Pomatoschistus microps* Krøyer, in the Forth Estuary in Scotland, which set out specifically to investigate their myxozoan fauna, revealed the presence of numerous myxosporean cysts within the gill cartilage. The cysts were composed of large polysporoblastic plasmodia causing deformation of the surrounding cartilage. Cysts were found to contain myxobolid-like spores that were markedly different from the other known species of myxosporean recorded from this host *viz* the ceratomyxid *Ellipsomyxa gobii* Køie M *et al.*, 2004. Spores were spherical with two pyriform shaped polar capsules containing polar filaments coiled into 4-5 turns. Although myxobolid spores possess relatively few morphological characters they could, however, be differentiated on the basis of their spore dimensions from the other known myxobolids described from marine hosts. A phylogenetic analysis of the 18S rRNA gene of this myxosporean, based upon a consensus sequence of 1,558 base pairs, confirmed its identity as a member of the Myxobolidae. However, it was not well supported in any of the major clades containing other myxobolid species suggesting that this represents a new species.

In an attempt to elucidate the life-cycle of this new species and to identify the actinosporean stage, a total of 1,547 annelids, representing 1,355 oligochaetes and 192 polychaetes, were collected from the soft sediments of the Forth Estuary in the same vicinity in which the infected gobies were caught. Annelids were maintained singly in 5ml multi-well plates and screened daily, for a minimum of 7 days and a maximum of 21 days, for the presence of released actinosporean stages but none were found. At the same time, batches of uninfected oligochaetes (*Lumbriculus variegatus* Müller and *Pelosclex benedeni* Udekem) and polychaetes (*Nereis diversicolor* Müller, *Nereis pelagica* L. and *Nereis fucata* Savigny) were exposed to spores harvested from the gill cysts in attempt to infect potential intermediate hosts. After 7 days, each worm was examined using a compressor and screened for the presence of developing actinosporean stages within but no infection was found.

Hepatic myxosporosis in a terrestrial mammal

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A new myxosporean parasite was found in the liver of 19 out of 46 shrews (*Sorex araneus* L., 1758, Mammalia, Insectivora) captured from the Białowieża primeval forest (Poland). It was named *Soricimyxum fegati* Prunescu, Prunescu, Pucek, Lom 2007 (Myxozoa, Myxosporea) (Folia Parasitologica 54/3, in press). The parasite was detected in serial histological sections (5 µm thick), stained with haematoxylin-eosin and the Ziehl-Neelsen method used for polar capsule staining. Developmental stages and mature spores were observed and measured. Polysporic plasmodia were found either occupying intrahepatic bile ducts or in the hepatic parenchyma with an average size up to 30 µm and 80 µm, respectively. Most probably, parenchymal plasmodia reach this location after the disruption of the biliary ducts. In both locations, plasmodia elicited a strong inflammatory reaction with infiltration of lymphocytes and monocytes. A total of 21 spores were measured. Mature spores were ovoid, measuring 7.0 µm in length, 5.4 µm in width and 3.5 µm in thickness. Polar capsules, two, spherical, equal in size, 1.6 µm in diameter, located at opposite ends of the spore and with divergent orientation of the discharging filament. Polar filaments is very short (about 1¹/₂-1²/₃ turns). Spores had two valves with longitudinal surface ridges and a straight, longitudinal suture. The sporoplasm with two nuclei had a central position between the two polar capsules. *S. fegati* is the first myxozoan in which the development from plasmodia to spores has been observed in a terrestrial mammal. The whole developmental cycle (myxosporean and actinosporean stages) of this new myxosporean might be terrestrial, as *S. araneus*, the putative intermediate host, feeds exclusively on terrestrial animals, including lumbricids (Churchfield S and Rychlik L 2006 J Zool 269:381-390). The presence of *S. fegati* in the liver of *S. araneus* raises theoretical questions concerning the adaptation of the myxozoan life cycle to exclusively terrestrial hosts. The myxosporean-like developmental stages found in the mole brain (Friedrich C *et al.*, 2000 Parasitology 121: 483-492) might be another myxozoan adaptation to terrestrial life. The finding of more new myxozoan species in hosts of mammalian orders (Insectivora, Rodents) which feed especially on terrestrial oligochaetes is expected. Further studies on the life cycle of *S. fegati* in its natural definitive and intermediate hosts may contribute to the progress in the understanding of myxosporean biology and phylogeny.

Myxosporeans of two fish species in the Okavango Delta, Botswana

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In this study, myxosporean species invading internal organs of fish hosts in the Okavango River and Delta in Botswana were investigated.

Fieldwork involved the collection of fish from the river system using gill nets as well as rod and line. Fish were anaesthetized with clove oil and the internal organs were removed. These were compressed between two glass slides and the run-off liquid was examined for spores using light microscopy. Myxosporean cysts were also fixed in glutaraldehyde for scanning electron microscopy.

Results revealed the presence of 14 myxosporeans of two genera, *Myxobolus* Bütschli, 1882 and *Henneguya* Thélohan, 1892, from two fish hosts, namely *Hydrocynus vittatus* Castelnau, 1861 and *Hepsetus odoe* Bloch, 1794. Thirteen spores of the genus *Myxobolus* were recorded. Organs of *H. vittatus* revealed the following seven spores: Species A on the skin and gills, Sp. B on the skin and gills, in the urinary bladder and gonads, Sp. C in the kidneys (type 1) and urinary bladder, Sp. D in the gall bladder, Sp. E in the kidneys (type 2) and liver (type 1), Sp. F in the spleen, liver (type 2) and heart and Sp. G in the swimbladder. One new species of the genus *Henneguya* was also found to infect the gills. Six *Myxobolus* spores were found in the following organs of *H. odoe*: Sp. A on the gills, in the kidneys (type 1) and spleen, Sp. B in the kidneys (type 2) and urinary bladder (type 1), Sp. C in the gall bladder and testes, Sp. D in the urinary bladder (type 2), Sp. E in the kidneys (type 3) and Sp. F in the liver.

Previous studies in the Okavango Delta indicated the presence of myxosporean parasites on the gills and skin of various fish species. This is, however, the first results showing that histozoic myxosporeans also infect internal organ systems of fish species.

Coelozoic myxozoans from the south coast of Africa

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Very little is known about the diversity of fish-infecting marine myxozoans in Africa, with only 52 species recorded from the entire African coastline. Several surveys conducted along the Cape south coast of South Africa between 1998 and 2002 revealed the presence of numerous different coelozoic myxozoan species from a single locality near the southern most tip of Africa. Three species of the genus *Ceratomyxa* Thélohan, 1892 were recorded from the gall-bladders of *Amblyrhynchotes honckenii* (Bloch, 1795), *Clinus cottoides* Valenciennes, 1836 and *C. superciliosus* (Linnaeus, 1758). Two species from the genus *Myxidium* Bütschli, 1882 were recorded from the gall-bladders of *Chorisochismus dentex* (Bloch, 1795) and *Diplodus sargus capensis* (Smith, 1844), respectively. One species from the genus *Sphaeromyxa* Thélohan, 1892 was recorded from the gall-bladder of *Pavoclinus graminis* (Gilchrist and Thompson, 1908). Representatives of the genera *Chloromyxum* Mingazzini, 1890, and *Sphaerospora* Thélohan, 1892 were also recorded, respectively, from two individual surf zone fishes, *Torpedo fuscumaculata* (Peters, 1855) and *Lightognathus lithognathus* Cuvier, 1830.

Life cycle of *Myxobolus rotundus*, a myxosporean parasitizing the gills of bream (*Abramis brama*)

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The intraoligochaete development or entire life cycles of several fish parasitic myxosporeans have previously been reported. Among them, some experiments were aimed at elucidating the developmental cycle of freshwater *Myxobolus* species parasitizing the gills of bream (*Abramis brama*). At least 6 *Myxobolus* species, distinguishable by morphology and site of development, infect the gills of bream. Of these, the intraoligochaete development of *M. hungaricus* (El-Mansy A and Molnár K, 1997, Acta Vet Hun, 45: 427-438), *M. braelae* (Eszterbauer E et al., 2000, J Fish Dis, 23: 19-25) and *M. macrocapsularis* (Székely C et al., 2002, Dis of Aquat Org, 48: 117-123) were elucidated by our research team. Most recently, the complete life cycle of *M. parviformis* was described and validated by sequence data (Kallert DM et al., 2005, Dis Aquat Org, 66: 233-243). In this line of investigation, the complete life cycle of *M. rotundus*, another gill parasite of bream, was elucidated. This myxosporean species also alternates with a triactinomyxon actinospore stage in the oligochaete *Tubifex tubifex*. We conducted successful *in vivo* infection trials and confirmed the identity of the alternate spore stages with DNA sequence data.

A novel vital staining technique to determine the viability of *Enteromyxum leei* (Myxozoa)

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Enteromyxum leei is an enteric myxozoan parasite that causes serious damage to cultured marine fishes in Japan and Mediterranean countries. One of the most important characteristics of *E. leei* is the fish-to-fish transmission of the disease, and that the pre-sporogonic developmental stage is believed to be infectious to other fish. However, the biology of the infective stage is poorly understood. To date, a reliable method for determining the viability of myxosporeans has not been fully developed. Methylene blue staining, fluorescein diacetate (FDA) and propidium iodide (PI) staining, a polar filament extrusion method using KOH or urea were reported as possible indicators of spore viability. However, these have not been used commonly because of variable and inconsistent results. In case of *E. leei*, it is also necessary to distinguish early developmental stages from host cells. In this study, we aimed to develop a vital staining technique using Hoechst 33342 and PI to determine the viability of pre-sporogonic stages of *E. leei* through longevity and drug susceptibility tests. Hoechst yielded blue fluorescence in nuclei of both parasite and host cells. Because of the multinuclear characteristics of myxosporeans, developmental stages of *E. leei* were easily distinguishable from host cells. PI permeated only through the membrane of dead cells and emitted a red fluorescence. Thus, viability of *E. leei* was evaluated by PI staining. *In vitro* survivability tests showed that the longevity of *E. leei* in phosphate buffered saline was about 24 hours. Toltrazuril (symmetric triazinone) was found to be effective in killing the developmental stages of *E. leei*. The present study indicates that Hoechst-PI stain has the potential for screening chemotherapeutants against myxosporean infections.

Preliminary data on Myxosporea and Microsporidia of fish from the Charleston Harbour (Atlantic Ocean)

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For the first time, in October 2006 in the Charleston Harbour of Atlantic Ocean (USA), (South Carolina Department of Natural Resources) parasite fish microparasites has been studied. Totally, 96 specimens belonging to 12 fish species were studied. Representatives of 7 Myxosporea genera and 1 of Microsporidia have been found.

Kudoa spp. were doserved in 6 of 6 studied *Brevoortia tyrannus* of 23.6 – 26.1 cm length (TL) (looking like cysts); in 40 % of *Rhomboplites aurorubens* of 23.7 – 30.7 cm length, in 100 % of *Cynoscion nebulosus* of 34.7 – 54.3 cm length and in 1 of 5 *Mugil cephalus* of 31.7 cm length (like separate spores).

Ceratomyxa sp. was found in gall bladder of 1 of 4 *Pomatomus saltatrix* of 35.2 cm length.

Alataspora spp. were found in gall bladder of 33 % *Pagrus pagrus* of 29.2 – 36.1 cm length, and in 2 of 7 *Mycteroperca phenax* of 43.2 – 67.6 cm length.

Parvicapsula sp. was found in gall bladder of 27 % of *R. aurorubens* of 28.1 – 37.6 cm length.

Zschokkella sp. was found in gall bladder of 7 % *C. nebulosus* of 35.9 cm length.

Henneguya spp. was detected in muscles of 1 of 5 *M. cephalus* of 31.7 cm length (looking like separate spores) and in heart of 2 of 4 *P. saltatrix* of 35.4 – 38.2 cm (looking like circular yellowish cysts with 1,5 – 2,0 mm diameter).

Myxobolus spp. were found in heart (like separate spores) in 1 of 4 *P. saltatrix* of 35,2 cm length, and in liver (1 – 45 cysts), in kidney (1 – 10 cysts), heart (4 cysts), stomach (21 cysts) and in spleen (5 cysts) in 5 of 5 *M. cephalus* of 25.9 – 31.7 cm length.

Cysts of Microsporidia gen. sp. were found in muscles of 7 % *R. aurorubens* of 23,7 cm length, parasiting in common with *Kudoa* sp.

Paralichthys lethostigma, *Elops saurus*, *Menticirrhus americanus*, *Leiostomus xanthurus* and *Sciaenops asceletus* appeared to be free of the given parasites.

Acknowledgements: I am very grateful to Dr. Isaure De Buron – Connors and Bill Roumillat for the help in supply of material, as well as for creation of good conditions for work

About Validity of *Kudoa trifolia* Holzer *et al.*, 2006 (Myxosporea: Multivalvulida)

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The studies of glycerine-gelatinous preparations, photos and looking through the description of myxosporea, called *Kudoa trifolia* Holzer, Blasco-Costa, Sarabeev, Ovcharenko, Balbuena, 2006 (Holzer A *et al.*, 2006, J Fish Dis, 29: 743 – 755) convinced us of the fact, that the given species did not belong to the genus of *Kudoa*, but was a new genus. Its spores form differs considerably from all the representatives of the genus *Kudoa* (84 species, including 26 unnamed) and is more close to *Neoparvicapsula* (Gayevskaya A *et al.*, 1982) as it differs in tissue localization and multivalvulid structure. We propose to continue morphological (phylogenetic) row: *Ceratomyxa* – *Parvicapsula* – *Neoparvicapsula* (Schulman S *et al.*, 1997, Class of myxosporeans in the world fauna, Nauka, St.Petersburg), after including into it additional links – genus, described as *K. trifolia*, and *Kudoa* itself. This corresponds to Schulman's supposition about Multivalvulida originating from Eurysporina (*Ceratomyxa* and *Leptotheca*) and leads to the idea, that representatives of the new myxosporean genus, described as *K. trifolia*, are possibly direct ancestors of *Kudoa spp*. As for molecular biological analysis of the given myxosporean, we propose that its restudying is necessary. The description of the new species *Kudoa unicapsula* sp. n. Yurakhno, Ovcharenko, Holzer, Sarabeev, Balbuena (In press) from the same hosts – *Liza aurata* and *L. ramada* (Pisces: Mugilidae), partially from the same organs and the same region it appeared, that together with great differences in the given species spores morphology they had high similarity in 18s and 28s rDNA sequences, which contradicts any logic and can testify, possibly, only to high similarity of tissue tropism.

We present a more precise *K. trifolia* spores description. The spores have an elongated longitudinal shape and consist of 3 parts – “head”, “body” and “tail”. They feature a 4-valve head construction, connected with the body by a sutural line sharpened blade-form projections, each of which looks like a valve, bringing polar capsule and big valvagenous nucleus. That's why under the light microscope presence of big nuclei creates erroneous impression of great number of polar capsules (up to 8). One of the valves has diminished sizes, it is rounded and press the body, making impression that there are only 3 blades. The body of the spore is elongated – oval, narrowing in both sides, but more to the tail end. On the body they often observe asymmetrically situated longitudinal sutural line (or gutter), going from the tail to the head, more often by the side of the body. Big amoeboid germ with nucleus and big vacuole is located in the center of the body. On the opposite to the head end of the body there is a tail projection, most often it is wide and transparent, looking like a “beard” of different form and size, but in a number of cases it represents dense thin plait. Spore length without tail (head + body) 8.3 – 11.4 (10, 32 ± 0.77) µm, spore length with tail 9.5 – 13.7 (12.29 ± 0.81) µm, width (body) 4 – 5.7 (5,18 ± 0, 34) µm, tail length 1.1 – 5.4 (2.4 ± 1.15), tail width 0.5- 3.7 (2.30 ± 0.89) µm, polar capsule length 1.2 – 1.5 (1.3 ± 0.09), polar capsule diameter 0.5 – 1.1 (0.82 ± 0.15), head width 4.1 – 6.2 (5.50 ± 0.44), head height 3 – 5.1 (3.79 ± 0.48), filament length 3.1 – 3.3 (3.2).