

MYXOZOAN BIOLOGY AND ECOLOGY

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Summary

The phylum Myxozoa is a group of microscopic metazoans with an obligate endoparasitic lifestyle. Traditionally regarded as protists, research findings during the last decades have dramatically changed our knowledge of these organisms, nowadays understood as examples of early metazoan evolution and extreme adaptation to parasitic lifestyles. Two distinct classes of myxozoans, Myxosporea and Malacosporea, are characterized by profound differences in rDNA evolution and well supported by differential biological and developmental features. This notwithstanding, most of the existing Myxosporea subtaxa require revision in the light of molecular phylogeny data. Most known myxozoans exhibit diheteroxenous cycles, alternating between a vertebrate host (mostly fish but also other poikilothermic vertebrates, and exceptionally birds and mammals) and an invertebrate (mainly annelids and bryozoans but possibly other

groups). Examples of horizontal (direct) transmission also exist, and the vast majority of the cycles remain unknown. In their hosts, myxozoans develop different kinds of spores presenting polar capsules, structures containing a coiled extrudable filament, which are reminiscent of –and are likely to be homologous to– cnidarians nematocysts. Their development includes a characteristic cell-within-cell pattern in trophic and proliferative stages, and a differentiation of cells into valvogenic, capsulogenic and sporoplasmic cells, which build the spores during sporogenesis. The repertory of developmental strategies and morphologies is extremely diverse and reflect the plasticity of these organisms and their adaptation to their unique lifestyles.

In the vertebrate hosts, most myxozoans develop in the cavities and ducts or in the organs parenchyma. Many infections are asymptomatic but often result in effects such as variable degrees of tissue hyperplasia, unsightly cysts, erosive and necrotic lesions, myoliquefaction, deformities, or even epizootics and severe losses in wild and cultured fish. The pathogenicity of myxozoans depends on the host and parasite species involved and the intensity of infection and the organs affected. An immunopathological component often plays a part in severe syndromes.

1. Introduction

Myxozoans are microscopic multicellular organisms with an obligate parasitic lifestyle. Discovered in the first half of the 19th century, they were regarded and studied for long as a minor lineage of protists parasitizing fish, collectively known as “myxosporidia” or “myxosporeans” (Greek etymology: slime animals harboring spores). The most fascinating and unique feature of these organisms in these early studies was their development into spores harboring complex structures (polar capsules) of striking similarity with cnidarian nematocytes and bearing, like those, a coiled, eversible and stinging filament (the polar filament). By the turn of the century, another group of parasites named “actinomyxidians” or “actinosporidians” was discovered in annelid worms. Although their biology and morphology seemed rather different, the presence of structures similar to the polar capsules of myxosporeans suggested some kinship with them.

For most of the 20th century, these two groups were studied in parallel as lineages of protozoans related with other spore-forming microscopic organisms, using different taxonomic labels such as Sporozoa, Neosporidia, Cnidosporidia or Amoebosporidia, nowadays obsolete. As the diversity of morphotypes and taxa widened steeply, research findings have changed our understanding of these organisms dramatically. Nowadays myxozoans are recognized as a species-rich and diverse Phylum (Myxozoa) of metazoan parasites of vertebrates (mostly aquatic poikilotherms) and invertebrates (mainly annelids and bryozoans). The typical myxozoan parasitizes a vertebrate host, usually a fish, in which the myxospores develop. These myxospores are infective for the invertebrate host, culminating with the generation of actinospores, which can infect new fish closing the model, heteroxenous cycle.

Myxozoans are fascinating organisms. Although marginally studied due to their minute size, cryptic biology and the relatively minor economical and zoological relevance of their hosts, they present many intriguing biological aspects. They also illustrate extreme

adaptations to parasitic lifestyles and living examples of early metazoan evolution. In addition, dramatic worldwide expansion of fish aquaculture and increasing study of aquatic habitats, have evidenced substantial ecological and economical impact of many myxozoan species due to their pathogenic action.

This chapter will provide basic information on myxozoan biology and ecology, with emphasis on those aspects related to their pathogenic action and examples of different lifestyles. This notwithstanding, the diversity of myxozoans and the plasticity of their developments make it difficult to provide a simple, common model. Exceptions exist to almost any known myxozoan characteristic and many are still matter of active scientific debates. Since the number of known myxozoan species probably represents an insignificant fraction of their true diversity, many discoveries related to myxozoan biology are still waiting ahead.

2. Phylogeny

As outlined above, the myxozoans (myxosporeans and actinosporeans) have been classically studied as protists and much of the early scientific discussion on their phylogeny was focused on their relationships and position in the -currently deprecated- phylum Protozoa. Phylogenetic relationships with Amoeba, Foraminifera, Microsporidia, and Haplosporidia were argued at the time, but sustained on little more than speculative grounds. Nevertheless, their complex organization and cell specialization – particularly that of the spores- suggested a position somehow intermediate between unicellular and multicellular organisms, or even a closer relationship to mesozoan and metazoan lineages.

Although higher-rank phylogeny was unclear, myxosporeans and actinosporeans were considered subgroups of a unique lineage of organisms granted different taxonomic categories until 1970, when the Phylum Myxozoa was proposed to accommodate both. General acceptance of the rooting of the phylum within a higher-rank supertaxon Protozoa prevailed for most of the 20th century. A dramatic paradigm shift was faced in 1994 with the results of the first molecular phylogenetic studies of myxozoans based on small subunit ribosomal RNA genes (SSUrDNA), which clearly demonstrated, even with very limited datasets, a clear rooting within the Metazoa.

The metazoan nature of the Phylum Myxozoa has been further verified in numerous studies expanding the number of available rDNA sequences. However, inference of the closest metazoan lineage(s) has proved controversial. Most analyses of ribosomal sequences tend to resolve myxozoans as a sister clade of bilateral animals. In 1995, the incorporation of SSUrDNA data from the aberrant parasitic cnidarian *Polypodium hydriforme* and a re-interpretation of myxozoan polar capsules as homologous to cnidarian nematocysts lead to postulating an alternative rooting with the cnidarians (Medusozoa).

The astounding identification of a vermiform myxozoan stage, the long-known but unclassified worm *Buddenbrockia plumatellae*, added some arguments to the debate favoring Bilateria. Besides putative characterization of Bilateria-like *Hox* genes from this and other myxozoans (it was later proved to be from host's origin), a wormlike

organism with four apparent muscle blocks counterweighed for Bilateria the morphological evidence posed by polar capsules favoring Cnidarians. The resolution of these two alternative hypotheses on molecular grounds is very much complicated by the extraordinary divergence of myxozoan SSU rDNA genes, which makes these datasets vulnerable to phylogenetic inference artifacts and limited phylogenetic signal. Although the controversy is not yet settled, the compelling morphological, developmental and functional similarities between myxozoan polar capsules and cnidarian nematocysts have recently received further support from two independent molecular studies with malacosporeans: the analysis of protein-coding datasets including sequences from *B. plumatellae*, and the sequencing of a putative homologue of a minicollagen gene (a cnidarian-specific gene coding nematocysts proteins) from *T. bryosalmonae* EST gene libraries. If these findings are confirmed, any phylogenetic hypothesis on the origin of Myxozoa needs to reconcile the fact that these organisms, indeed, have nematocysts.

Early branching of the Myxozoa stem in two main lineages, Myxosporea and Malacosporea, can be inferred by profound differences in SSU rDNA sequences, morphology, and lifestyles. The malacosporeans are an ancient, rather aberrant myxozoan clade currently comprising only two known genera (*Tetracapsuloides* and *Buddenbrockia*) and a handful of species or SSU rDNA genotypes. The cryptic spores present soft, inconspicuous shells (valves). Their invertebrate hosts are freshwater bryozoans and species or genotypes of *Tetracapsuloides* can be transmitted to fish, causing a virulent syndrome known as proliferative kidney disease (PKD). The other myxozoan lineage, Myxosporea, includes over 50 genera and 2700 species for which all known life cycles (although from a small portion of the known species) use an annelid as invertebrate host. Using rDNA gene data, three main clades can be inferred within the Myxosporea. The earliest splitting gave rise to a basal lineage, the sphaerosporids. Known species in this group are characterized by long insertions in variable regions of their rDNA and development in the vertebrate genitourinary systems.

The myxospores present rather homogeneous morphology and can be assimilated in the genus *Sphaerospora* (although many species included in this genus do not actually belong to this evolutive lineage according to their rDNA data, see taxonomy below). The remaining myxosporeans are grouped in a very specious clade comprising diverse myxospore and actinospore morphotypes, life cycles, and tissue tropisms in the vertebrate host. Two main lineages can be distinguished in this clade according to rDNA-based phylogeny, grouping species from primarily freshwater or marine habitats respectively (although both include exceptions to this rule).

The number of species for which the entire life cycle has been elucidated is still quite limited, particularly in the marine environment, but an emerging pattern indicates good correspondence between the type of invertebrate host and the rDNA-inferred phylogeny in these two lineages. Thus, known life cycles in the “freshwater clade” use an oligochaete worm (including earthworms), whereas they involve polychaetes as hosts in species of the “marine” clade. Interestingly, apparent exceptions of freshwater species whose position in rDNA-based phylogenies fall in the “marine” clade have been found to involve a freshwater polychaete. Within both clades, tissue tropism in the vertebrate host seems to be the character that correlates best with molecular phylogeny. As a consequence, rDNA-based trees generally group species whose myxospores develop in

the vertebrate urinary system, biliary ducts and gallbladder, or histozoic species, but these niches seem to have been colonized independently in both freshwater and marine clades.

A modern interpretation of myxozoan evolution assumes that the main adaptations of ancestral myxozoans leading to radiation of the Myxosporea were the development of hardened spore shells (valves) and possibly the incorporation of annelids (and probably other invertebrates) as hosts. The branching of malacosporeans and myxosporeans appears as an ancient event in myxozoan evolution and it seems plausible that bryozoans were ancestral hosts for the entire phylum. However, modern malacosporeans are known only from freshwater environments where a single lineage of bryozoans (Class Phylactolaemata) evolved. A large diversity of malacosporeans in marine bryozoans and other invertebrates –besides annelids- would be expected if these are indeed the ancestral hosts. Similarly, the evolutionary link to the sphaerosporids is not clear. The life cycle of this clade outside the vertebrate hosts is currently unknown and in spite of extensive searches for their actinospores in freshwater annelids such phase has not been identified, even in environments with rather limited biodiversity. This might indicate that a different invertebrate type is representative of this lineage.

The evolutive radiation of the myxosporeans would be driven by an extraordinary plasticity of morphotypes and developments, which facilitated multiple events of host and environment switching. According to this view, an ancestral malacosporean-like myxozoan would spread with the asexual reproduction of its hosts, and with limited power to propagate by itself through wormlike or amoeboid stages, until spores with shell valves and some degree of protection for the infective cell (sporoplasm or germ cell) were evolved. Hardened spore shells would allow protection and space-temporal dispersion facilitating the colonization of new hosts and environments by means of small adaptations (differential floatability, sizes and shapes, chemical signals, etc.).

The capture of vertebrate hosts probably represented a key innovation, which drove extensive radiation adapted to the unique demands of efficient infection and asexual propagation in multiple vertebrate niches. At the same time, the radiation in vertebrates could have facilitated the colonization of new environments and hosts when compatible invertebrates could be utilized. Further knowledge on other myxozoan cycles will clarify if this hypothesis holds and whether host-parasite coevolution in the invertebrate phase has shaped the main evolutionary trends of the myxozoa. In this scenario, host switching or coevolution with vertebrate hosts would be of secondary importance although able to generate great diversity.

3. Morphology and Taxonomy

A characteristic feature of myxozoans is the presence of a cell-within-cell pattern in most of their life cycle. However, the morphology of trophic and developmental stages is quite variable and plastic, and will be described in more detail under the “Cell Biology and Development” entry. Spores are the most conspicuous morphological stage of myxozoans. The variability of sizes, shapes and number of constitutive elements is significant and thus it has been used traditionally as the main character for species descriptions and the key for taxonomical classification.

3.1. Spore Morphology

Myxozoans develop into multicellular spores consisting of an assemblage of different, specialized cell types: capsulogenic cells that develop into the polar capsules; valvogenic cells that form the shell valves; and the amoeboid, naked sporoplasms, which are the infective stages protected by this cluster of cells. The number of these elements is very variable in different taxa. Polar capsules are the most prominent structures and contain a coiled, stinging polar filament that can be extruded and is interpreted as an anchor for the spore that allows the infective agent to emerge from the open spore valves, entering the next suitable host.

Polar capsules are very much like cnidarian nematocytes and they are interpreted as homologous to these (see phylogeny above). Myxozoan spores differ fundamentally from resistant protistan stages (cysts or spores). In protists, the wall is a non-living protective coat either secreted by or surrounding the living cell, which may or may not divide within it before emerging to infect a new host. In myxozoan spores, the infective stage (sporoplasm) is surrounded by several living cells that play a protective role and are discarded when infection is achieved by the emergent sporoplasm. A typical myxozoan can develop into two types of spores: the myxospore present in vertebrate hosts and the actinospore in invertebrates. Malacosporean spores (malacospores) present unique features and are described separately.

Myxospores (Figure. 1A & Figure 2: 8-25, 27, 28, 30) are typically 10-20 μm in size and can be spherical, subspherical, ellipsoid, pyramidal, stellate, crescent or sigmoid shaped. Valves can be smooth, striated or ridged, with or without lateral or caudal appendages or projections, sometimes asymmetrical. The joint of the valves closing the spore or sutural line can be very delicate or prominent, straight or sinuous. Polar capsules are spherical or pyriform, sometimes unequal in size, and can be located at various positions relative to the valves and their sutures, together at the same spore pole or set in opposite poles and oriented at variable angles.

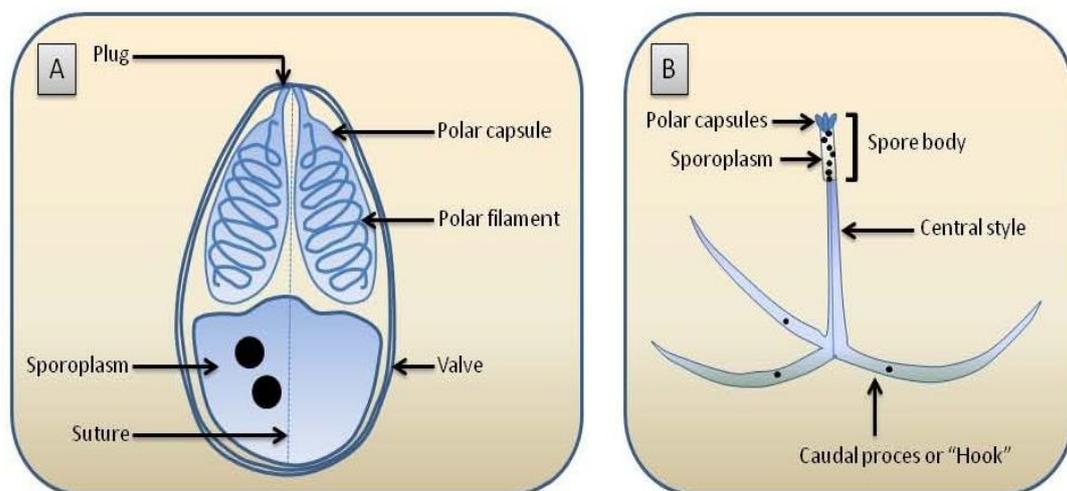


Figure 1. Diagrammatic scheme of a myxospore (A) and an actinospore (B). Drawing by A. Sitjà-Bobadilla.

The opening in the polar capsule wall, named capsular foramina, is sealed with a plug that may protrude over the anterior face of the capsule. The valves enclose the apices of the polar capsules and, when discharged, the filament must break through both the plug and the valve. The polar filament is coiled in a variable number of turns and with a distinct angle from the main axis of the polar capsule. Sporoplasms are naked cells filling the space between or beneath the polar capsules. Most myxospores contain a single binucleate sporoplasm but in some species there can be two uninucleate sporoplasms, multiple sporoplasms (up to 12 nuclei in the genus *Polysporoplasma*), or one sporoplasmic cell enveloping another (a cell doublet).

By contrast, actinospores (Figure. 1B & Figure 2: 1-7) typically present a tri-radiate symmetry with three polar capsules (exceptionally one) and three valves, which often have elongated or bulbous extensions and a multinucleate sporoplasm. In most actinospores there is an anterior spore body containing the polar capsules and three shell valves, which in many actinosporean morphotypes extend in very long, hollow and divergent caudal processes.

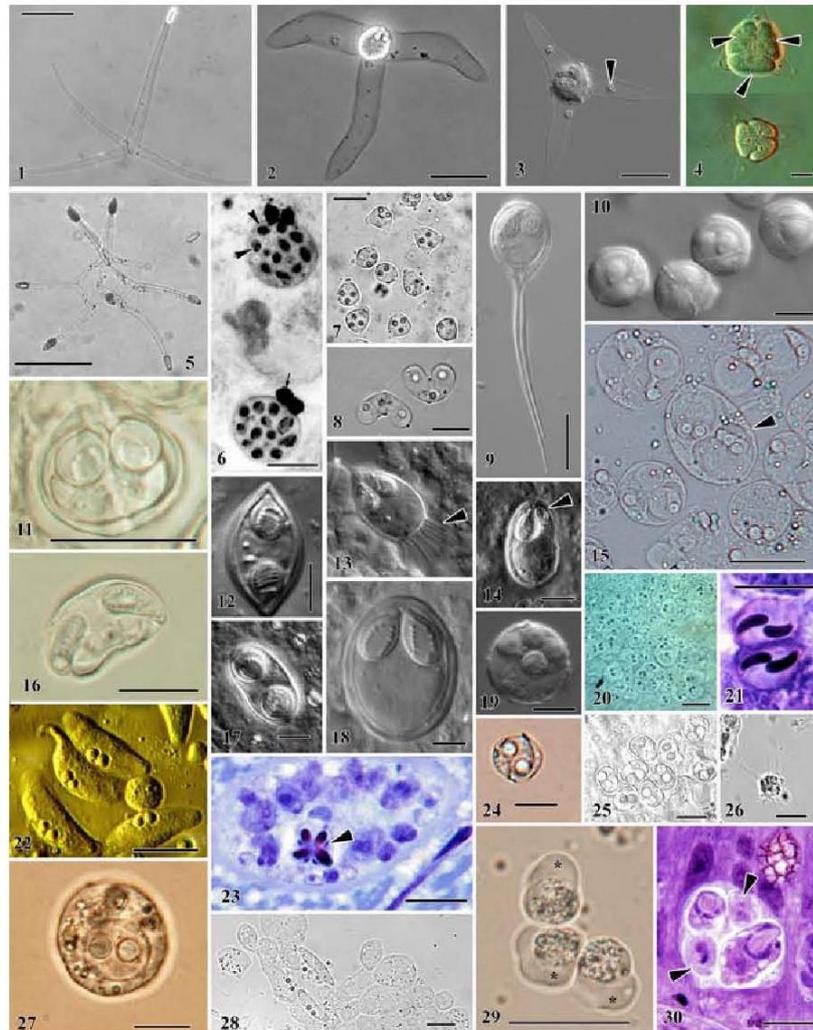


Figure 2. Light microscopy images of different types of actinospores (1-7) and myxospores (8-25) and other developmental stages of myxosporeans (26-30).

In some actinospores, the three processes are fused to some extent forming a cylindrical style separating the projections from the spore body. Actinospores can be quite large compared to most myxospores (up to 1 mm) and some types even develop as a web-like structure containing multiple tri-radial elements. The caudal processes and style, in the actinospores that have them, are inflatable osmotically into full length when the spore is released from the invertebrate host. Just beneath the polar capsules the spore body is filled by a plamodium-like sporoplasm containing multiple germ cells. As in myxospores, the valvogenic cells become progressively thinner during spore maturation until they are practically membrane doublets. However, unlike most myxospores, there is not a conspicuous build-up of material hardening the structure. The three valves fuse, enclosing the capsulogenic cells except for apertures over the polar capsule apices. The mature polar capsules develop like those of myxospores and are also sealed with a prominent plug. Malacosporae from fish have four soft valve cells (which retain their cytoplasmic integrity in mature spores), two polar capsules and one sporoplasm, which contains sporoplasmosomes but no secondary cells. Bryozoan malacosporae are composed of eight soft shell valves, four polar capsules and two sporoplasms with secondary cells and sporoplasmosomes (Figure. 3). The valve cells envelop the sporoplasms and extend over the four capsulogenic cells except at the polar filaments egression points. Polar capsules are spherical and their foramina lie at the spore surface sealed by an umbrella-shaped plug, which is overlaid by a pad. Even in mature polar capsules, the capsulogenic cells retain their nuclei and functional mitochondria.

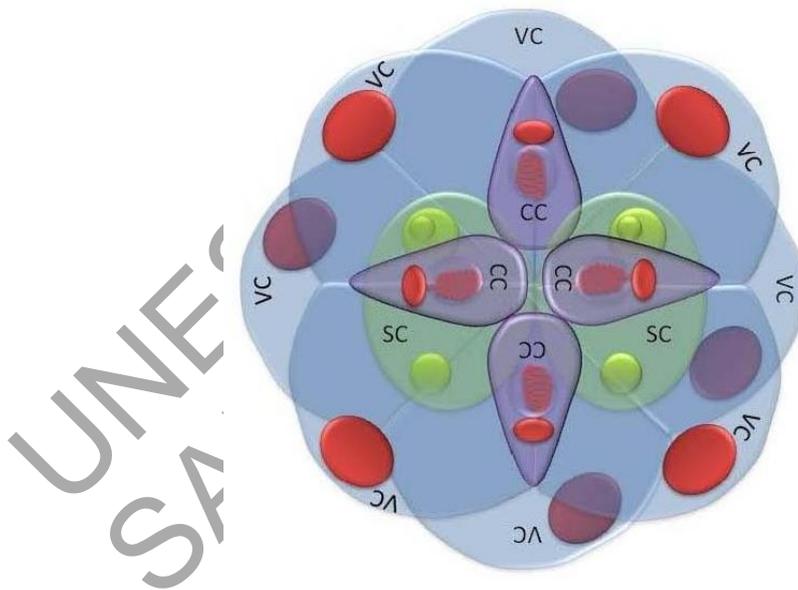


Figure 3. Interpretative image showing all layers of the different cell types of *Tetracapsuloides bryosalmonae* malacospore.

3.2. Taxonomy

In the absence of significant differential characters and due to methodological difficulties in the study of vegetative stages, the current taxonomy of Myxozoa is strongly influenced by a myxospore-centric view of the organisms. Most taxa currently in use were defined using characters of these stages such as: number of valves, polar

capsules and sporoplasms; turns of the polar filament; shape and ornamentation of the valves; shape and relative orientation of the polar capsules; position of the plane of suture; or sizes and proportions of the elements. A representative scheme of normalized myxospore measurements can be found in Figure. 4. Similarly, morphometric analysis of actinospores was also used for the description of myxozoan taxa during most of the 20th century, including them in a different Myxozoa subclade (Class Actinosporea). However, the number of described species was modest compared to the myxosporean species described from fish spores, reflecting the marginal study of these parasites in annelids. By 1984, a remarkable study was published demonstrating that one of these actinospore taxa (i.e. *Triactinomyxon* from the worm *Tubifex tubifex*) was an alternating stage of the trout myxosporean *Myxobolus cerebralis*. This finding was later confirmed for other myxozoans (see life cycle entry), posing controversy on the status of many actinospore and myxospore taxa, which should be paired as distinct life stages of the same myxozoan species.

Generalization of these alternating actinospore-myxospore life cycles as a model for the phylum ultimately led to the demise of class Actinosporea and its integration in myxozoan life cycles, thus limiting the use of actinospore morphometrics for the description of new taxa. In addition, quite dissimilar actinospore morphology was found in genetically close species, and even in identical SSU rDNA genotypes. Thus, for practical purposes actinospores are currently classified in morphologically collective groups, some of which retain the Latin genus names originally used at the time of the description of the representative actinospore. There is no matching pattern between myxospore genera and actinospore morphotypes, except for the *Myxobolus* spp. assemblage and a cluster of actinospore morphotypes including *Tryactinomyxon* and the ERA types. Recent findings indicate that the number of germ cells in actinospores correlates with genetic data much better than the actinospores morphology.

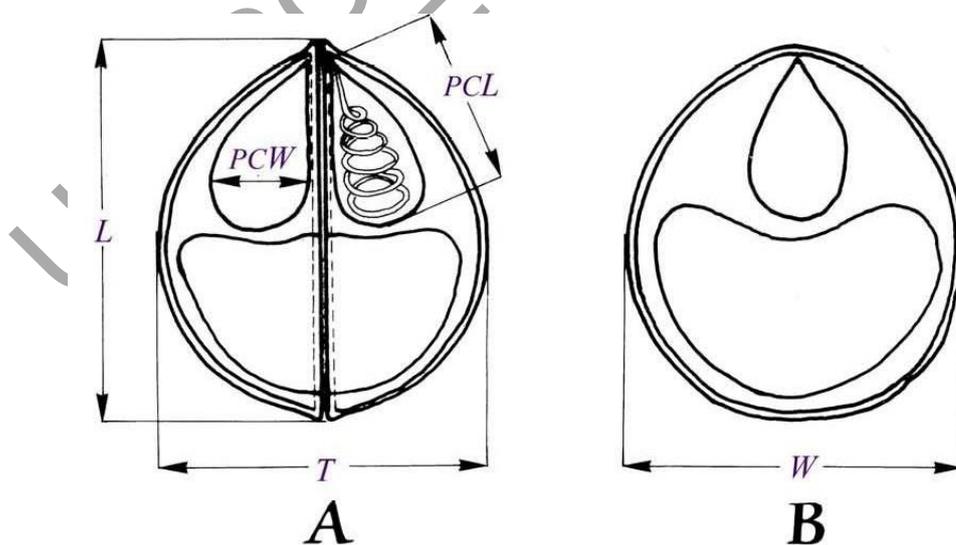


Figure 4. Diagrammatic drawings of the way of taking measurements of *Sphaerospora* spores (typical bivalvulid).

The analysis of DNA sequence data has shown that these morphological criteria do not reflect phylogenetic relationships of the main phylum subtaxa and has challenged the

cladistic congruence of most current families and genera due to paraphyly, polyphyly, or insufficient divergence to justify suprafamily ranks. Sequence data are available for roughly 500 genotypes of myxozoans (over 30 genera) and although it is still too unbalanced to justify a comprehensive revision of the phylum, the data is conclusive for some trends (see phylogeny section and comments below). The use of current taxonomic labels in Myxozoa must be understood as transitional.

Currently two classes: Myxosporea Bütschli 1881 and Malacosporea Canning, Curry, Feist, Longshaw and Okamura 2000 are recognized and they are both well supported by morphological, developmental and genetic data. The class Malacosporea contains only one order (Malacovalvulida) and one family (Saccosporidae). The diversity of known species in this class is very low (Table 1) and the current taxonomic scheme reflects well a cladistic classification. Nevertheless, the lack of morphological characters and the cryptic nature of malacosporians are handicaps for the assignment of newly discovered types.

Phylum: Myxozoa Grassé, 1970.

Class: Myxosporea Bütschli, 1881
Order: Bivalvulida Schulman, 1959
Suborders: Sphaeromyxina Lom and Noble, 1984
Family: Sphaeromyxidae
Variisporina Lom and Noble, 198
Families: Myxidiidae, Ortholineidae, Sinuolineidae, Fabesporidae, Ceratomyxidae, Sphaerosporidae, Chloromyxidae, Auerbachidae, Alatosporidae, Parvicapsulidae
Platysporina Kudo, 1919
Family: Myxobolidae
Order: Multivalvulida Schulman, 1959
Families: Trilosporidae, Spinavaculidae, Kudoidae
Class: Malacosporea Canning, Curry, Feist, Longshaw and Okamura, 2000
Order: Malacovalvulida Canning, Curry, Feist, Longshaw and Okamura, 2000.
Family: Saccosporidae Canning, Okamura and Curry, 1996
Genus: <i>Buddenbrockia</i> Schröder, 1910
Genus: <i>Tetracapsuloides</i> Canning, Tops, Curry, Wood and Okamura, 2002

Table 1. Classification of Myxozoa, according to Canning & Okamura, 2004. This classification is based on classical spore morphology and life cycles, and does not correspond with the phylogenetic clades thus far found.

The class Myxosporea is divided in orders Bivalvulida and Multivalvulida sorting myxospores built with two valves or with more than two, respectively. This division proves to be wrong on rDNA gene cladograms because: i) it does not recognize deeper branching of two-valve myxospore clades ii) Multivalvulida is indeed a monophyletic lineage but only as a subclade of the “marine group” (see phylogeny) and thus not deserving such a high taxon rank; and iii) multivalvulida lineage includes spores with two valves. Future subclass categories will need to accommodate at least the three main Myxosporea branches (see phylogeny) but, besides gene data, there is not a spore-based character that can support this sorting. Both Bivalvulida and Multivalvulida are formally

subdivided in suborders and families, which are equally flawed for classificatory purposes with their current definitional diagnoses.

An example of such classification is illustrated in Table 1. Comprehensive formal classification of Myxozoans is covered in the bibliography, though some of the families and genera have uncertain status. Future suprageneric taxa will need to address the apparent (from rDNA phylogenetic inference) existence of monophyletic clades grouping Myxosporaea species adapted to at least three different habitats in the vertebrate host: urogenital species, biliary/digestive species and systemic/histozoic species. Furthermore, adaptation to development in these niches seems to have evolved independently in the freshwater and in the marine clade.

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Dr. Ariadna Sitjà-Bobadilla is a Senior Research Scientist at the Institute of Aquaculture “Torre la Sal” (IATS), of the Spanish High Council for Scientific Research (CSIC) since 1996. She obtained her Ph.D. in Biological Sciences from the University of Barcelona (Spain) in 1991 and devoted her post-doc research to *Cryptobia salmositica* at the University of Guelph (Canada). She has been head of the Department of Marine Species, Biology and Pathology of IATS for more than ten years and she was appointed Assistant of the Committee of Stock-rearing and Fisheries Area of the National Evaluation and Foresight Agency (ANEP) in 2009. Her primary area of research is marine fish parasites, particularly myxozoans, monogeneans and coccidians, with special emphasis on fish immune response, host-parasite

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