
Myxozoan Affinities and Route to Endoparasitism

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Abstract

There is now strong evidence that myxozoans have evolved from free-living cnidarians but until recently their higher level relationships have been the subject of considerable controversy. This chapter reviews the morphological and molecular evidence that has contributed to problems in placement and how further collective support has finally resolved their cnidarian affinity. We then consider the inherently difficult but fascinating topic of how myxozoans may have evolved as endoparasitic cnidarians. We first explore how a close association of free-living precursors could have led to the evolution of myxozoans with simple life cycles and the nature of the first myxozoan hosts. We propose that either freshwater bryozoans or fish (or their precursors) were ancestral hosts (in view of the more derived nature of myxozoans that infect annelids and the fact that fish are hosts for most members of all major myxozoan clades) and suggest that the morphological complexity of myxozoans in freshwater bryozoans renders a scenario of fish as first hosts less likely. We then discuss how new hosts may have been adopted subsequently, resulting in the complex life cycles involving invertebrate and vertebrate hosts that now characterise all myxozoans. Cnidarian traits, including life cycle plasticity and a capacity to evolve novel propagative stages, ultimately support many different scenarios regarding the route to endoparasitism.

Keywords

Long branch attraction · Morphological simplification · Phylogeny · Polar capsules · SSU rDNA · Minicollagen · Mitochondrial genes · Cnidaria · *Buddenbrockia* · *Polypodium hydriforme* · Host acquisition · Life cycle evolution · Undiscovered diversity

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2.1 Introduction

The affinities of some metazoans have been exceedingly difficult to ascertain (Conway Morris 1991). For extant taxa this is largely because comparisons of species may have to bridge enormous time spans of independent evolution (Jenner and Littlewood 2008). Signs of ancestry may therefore have vanished because of extensive modification or loss of characters. In addition, convergent evolution may obscure phylogenetic signal. Thus, in practice, extensive morphological modifications may preclude straightforward comparisons with potential relatives for some problematic metazoan taxa while high levels of molecular divergence and associated long branch attraction may artificially infer close relationships. Prominent examples include certain sessile taxa (e.g. bryozoans and brachiopods), tiny and possibly miniaturised taxa (e.g. tardigrades and acoels) and parasitic taxa (e.g. pentastomids and strepsipterans) (Jenner and Littlewood 2008; McKenna and Farrell 2010).

A combination of features has rendered placement of the Myxozoa especially challenging. Apparently rapid molecular evolution has resulted in accumulation of homoplastic characters causing long branch attraction that has proven to be highly problematic for placement within the Metazoa (e.g. Hanelt et al. 1996; Zrzavý et al. 1998; Kim et al. 1999; Evans et al. 2010). In addition, myxozoans present the most extreme case of morphological simplification associated with parasitism. Thus, not only do they lack features associated with organs, such as a digestive tract and a nervous system, but cilia and centrioles are also absent as are recognisable gametes, embryonic and larval stages (Lom 1990; Canning et al. 2000; Canning and Okamura 2004). As a counter example, the development of cypris larvae enabled the parasite, *Sacculina*, to be recognised as a highly modified barnacle in the nineteenth century, although the specific affinities with barnacles and other cirripede crustaceans are not fully resolved (Høeg 1992). In this chapter we review the history of and difficulties in determining the higher level

relationships of the enigmatic Myxozoa and how their status as cnidarians has finally been confirmed on the basis of morphological and molecular data. We then explore the potential origins of parasitism including the nature of the first myxozoan hosts and how subsequent hosts may have been incorporated.

2.2 History of Higher Level Relationships

2.2.1 Discovery of Myxozoans and Placement with Protists

Myxozoans were discovered in the first half of the nineteenth century by Jurine (1825) and were assigned to the Sporozoa by Bütschli (1882). For a long time the Sporozoa comprised a diverse group of organisms regarded as unicellular, spore-forming parasites of animals including coccidians, gregarines, haemosporidians, *Plasmodium* spp. and piroplasms [today classified as Apicomplexa (Cavalier-Smith 1998)] along with the Microsporidia [now associated with Cryptomycota in the Kingdom Fungi (Hirt et al. 1999; James et al. 2013)] and the Myxosporida (now the Myxozoa). Myxozoan characters such as absence of centrioles and cryptomitosis and the presence of tubular (rather than plate-like) mitochondrial cristae in some taxa (Marquès 1987; Lom and Dyková 1997) were suggestive of a protistan nature. However, it is increasingly apparent that some of these features are variable (e.g. mitochondrial cristae, closed vs. open mitosis; Canning et al. 2000; Redondo et al. 2003).

2.2.2 Recognition as Multicellular Animals

Recognition of the multicellular nature of myxozoan spores led Štolc (1899) to propose that myxozoans should be included with Metazoa. This conclusion was echoed by others (e.g. Emery 1909; Ikeda 1912) and gained additional

support when Weill (1938) noted the similarity between the eversible, intracellular organelles present in both groups—polar capsules in myxozoans and nematocysts in cnidarians. Weill (1938) suggested that myxozoans are indeed cnidarians and further alluded to their potential similarity to the parasitic larval stages of *Polypodium hydriforme*, a cnidarian affiliated with the Narcomedusae and now placed by some in its own group, the Polypodiozoa (Raikova 1988; Bouillon et al. 2004). These similarities were re-emphasised by Lom (1990) and Siddall et al. (1995) (see below).

Myxozoa were accorded the status of a phylum within the Metazoa by Grassé (1970) as proposed previously by Grell (1956) and Lom (1969). This status was subsequently independently confirmed by molecular sequence data when Smothers et al. (1994) demonstrated that myxozoans grouped with bilateral animals on the basis of SSU rDNA and were possibly a sister group to the nematodes. This conclusion was similarly reached by Schlegel et al. (1996) who also analysed SSU rDNA. However, Siddall et al. (1995) concluded that myxozoans grouped within the Cnidaria as sister to *Polypodium hydriforme* on the basis of combined analyses of SSU rDNA and morphological data. At the time, morphological features that supported a metazoan nature included septate and adherens-type cell junctions, structural and functional differentiation of cells and separation of somatic and germ cells (Siddall et al. 1995; Lom and Dyková 1997). However, the separation of somatic and germ cells is not a clear-cut metazoan character as it has evolved several times in different lineages of multicellular organisms (and bacteria: Oliveiro and Katz 2014), and is also implemented in varying degrees in metazoans (Grosberg and Strathmann 2007). Further purported metazoan features used in phylogenetic analyses (the presence of collagen and acetyl-choline/cholinesterase activity; Siddall et al. 1995) were pointed out to be questionable (Lom and Dyková 1997; Canning and Okamura 2004). The putative protistan-like features of myxozoans (lack of centrioles, cryptomitosis and tubular mitochondrial cristae) have also been demonstrated more

broadly. Centrioles have been shown to be absent in planarians apart from in terminally differentiating ciliated cells (Azimzadeh et al. 2012) while structures resembling microtubule organising centres have been observed in *Enteromyxum scophthalmi* (Redondo et al. 2003). Furthermore, it is now evident that mitochondrial cristae can assume many different shapes (Griparic and van der Bliek 2001) often reflecting differences in biochemistry even within the same tissues (Riva et al. 2005) and tubular cristae are widely distributed across the eukaryotes (Cavalier-Smith 1993; Seravin 1993) including in free-living cnidarians (e.g. Gray et al. 2009).

Despite the relatively early recognition of their multicellularity, textbooks largely continued to classify myxozoans as protists (e.g. Hyman 1940; Kudo 1966; Margulis and Schwartz 1998; Lom 1990) until very recently (e.g. Ruppert et al. 2004; Brusca and Brusca 2003; Pechenik 2009). This may, in part, reflect a propensity to adhere to the prior, entrenched classification, particularly in view of the conflicting evidence over the specific metazoan affinities of myxozoans. The extent that misclassification can muddy the taxonomic waters is exemplified by the ‘honorary’ inclusion of myxozoan papers in, for instance, the annual conferences of the British Section of the Society of Protozoologists (now the British Society for Protist Biology).

2.2.3 Clarification of Myxozoan Life Cycles and Diversity

The complex parasitic life cycles of myxozoans were not appreciated until the causative agent of salmonid whirling disease, *Myxobolus cerebralis*, was shown to incorporate tubificid worm hosts in a common life cycle (Markiw and Wolf 1983; Wolf and Markiw 1984). Only then was it recognised that actinospores and myxospores represented two different spore types produced within a common life cycle, thus uniting Actinosporea and Myxosporea within a single class (the Myxosporea). Previous to this work, actinospore- and myxospore-producing taxa were classified separately. For instance, an early

classification placed the Myxozoa (myxozoans producing myxospores) with Microsporidia and Actinosporea (myxozoans producing actinospores) in the class Cnidosporidia Doflein, 1901. The discovery of life cycle complexity simultaneously reduced the diversity of myxozoan species and opened the stage for exploring which actinospores and myxospores are involved in a common life cycle.

2.2.4 Recognition of the Malacosporea and Inclusion of *Buddenbrockia*

The most significant developments in understanding the higher level relationships of the Myxozoa have arisen from the discovery of myxozoans parasitic in freshwater bryozoans followed by the inclusion of the bizarre, worm-like endoparasite of freshwater bryozoans, *Buddenbrockia plumatellae*, as a myxozoan. This evidence was based on both molecular and morphological features (see Chap. 4 for further discussion). Thus, SSU rDNA analyses demonstrated a close relationship of *Buddenbrockia plumatellae* to *Tetracapsuloides bryosalmonae* (the causative agent of Proliferative Kidney Disease) (Monteiro et al. 2002) and hence an association with the Malacosporea, an early diverging clade of myxozoans so far associated with freshwater environments, utilising freshwater bryozoans and fish as hosts (Anderson et al. 1999; Canning et al. 2000). Meanwhile, an ultrastructural study simultaneously revealed that diagnostic polar capsules were present in the body wall of *Buddenbrockia* (Okamura et al. 2002). This study confirmed the early light-microscopy observations by Schröder (1910) that *Buddenbrockia* possesses a vermiform body plan with four sets of longitudinal muscles but lacks a digestive tract and anterior/posterior differentiation. Okamura et al. (2002) suggested these muscles were congruent to the four longitudinal muscles of nematodes and noted the presence of a basal lamina. These two complementary studies based on molecular and morphological characters thus provided the first insights into the body plan

of a myxozoan that may have retained more in the way of ancestral features than observed in any other taxon.

2.3 Evaluation of Current Phylogenetic Evidence

Myxozoa are generally viewed as a monophyletic group in almost all recent studies. However, due to the paucity of phylogenetically informative morphological characters, apparently high rates of sequence evolution, and problems in resolving basal metazoan relationships (see e.g. Dohrmann and Wörheide 2013) support for the position of Myxozoa within the animal kingdom in most studies has remained moderate. Furthermore, the conflicting hypotheses of Myxozoa as sister-group to the Bilateria and as ingroup of the Cnidaria receive support from different sets of phylogenetic data and analytical approaches. Other related phylogenetic questions include the exact position of the Myxozoa within the Cnidaria and relationships of myxozoans to the endoparasite *Polypodium*. In the following sections we review the hypotheses and problems for interpreting the phylogenetic relationships of the Myxozoa that result from various data sets and show how the collective evidence now strongly supports their cnidarian nature (Fig. 2.1).

2.3.1 Morphological Evidence

The general body architecture of myxozoans offers few clues regarding their phylogenetic affinities. As mentioned earlier the presence of cell junctions clearly identifies myxozoans as metazoans. Epithelial characteristics in the stages of malacosporeans infecting invertebrates further support inclusion in the Eumetazoa (Ctenophora, Cnidaria, Bilateria). Other crucial apomorphic features (e.g. nerve cells) are lacking in myxozoans. The recognition of an independent, mesodermal-like muscle layer in *Buddenbrockia* first appeared to link myxozoans to bilaterians. However, three dimensional reconstruction of the

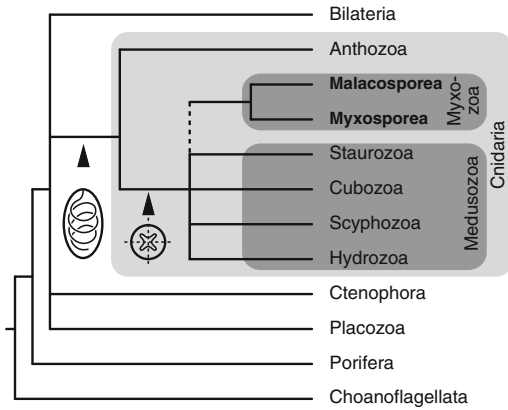


Fig. 2.1 Consensus tree of basal metazoan relationships and proposed position of Myxozoa as supported by phylogenomic studies (see text). Further support is gained from morphological characters: nematocysts (included in figure), with the inclusion of polar capsules constitute an apomorphic character of cnidarians; tetradial body symmetry (included in figure) is a shared apomorphic feature of medusozoans and myxozoans

muscle architecture demonstrates a tetradial symmetry (Gruhl and Okamura 2012), a unique trait of Medusozoa, the cnidarian taxon comprising Hydrozoa, Cubozoa and Scyphozoa. Also, contrary to traditional views, mesodermal-like musculature may occur in cnidarians as well (Seipel and Schmid 2006; Technau and Scholz 2003; but see Burton 2008), and may therefore be equally consistent with a cnidarian affinity. Characters of embryonic development such as cleavage patterns, modes of gastrulation and cell lineages, have proven useful to resolve some metazoan relationships (e.g. Valentine 1997; Nielsen 2012). However, with no regular pattern of cleavage, gastrulation or germ layer specification, myxozoan development currently does not offer any clear links with other metazoan phyla.

The most informative ubiquitous morphological character is the polar capsule, an intracellular organelle found in all myxozoan spores (Weill 1938; Lom 1990; Canning and Okamura 2004). The concordance to cnidarian nematocysts is striking and includes ultrastructural features (e.g. capsule wall and inverted tubule), formation (Golgi-secretory pathway, tubule invagination), and molecular architecture (proteins involved in

capsule walls; see below and Chap. 3 for further discussion). Homology of the two structures appears highly likely, but of course this inference assumes that the nematocyst is a bona fide apomorphy of Cnidaria. For instance, nematocyst-like elements in a small number of unicellular eukaryotes have led to the hypothesis that nematocysts are a general eukaryote feature and have been lost repeatedly in various lineages (Özbek et al. 2009). Although this cannot be completely discounted it seems unlikely given that, in addition to crucial differences in the fine structure of these various organelles, there is not a single known case of nematocyst loss in the entire Cnidaria. It has also been hypothesised that nematocysts originated as endosymbionts derived from free-living unicellular organisms either once or multiple times during eukaryote evolution (Shostak 1993). This theory is now mostly refuted by: (a) the fact that, unlike other endosymbiotic organelles like mitochondria or plastids, nematocysts and polar capsules lack genetic material, and; (b) proteins involved in nematocyst formation appear to be unique, bearing little relationship with other eukaryote proteins (Balasubramanian et al. 2012). Horizontal transfer of nematocysts, as seen in the cleptocnidae of e.g. nudibranch molluscs (Edmunds 1966) and some ctenophores (Carré et al. 1989), is also highly unlikely as in all known cases these have to be acquired anew in each individual since, due to lack of genetic material, they do not reproduce.

In summary, both morphological and molecular data on polar capsules now provide convincing support for Myxozoa belonging to Cnidaria (Fig. 2.1). Chapter 3 describes how polar capsules may also be phylogenetically informative with regard to their position within Cnidaria by pointing out similarities of the lid-like apical plugs that seal polar capsules and medusozoan nematocysts. The absence of apical plugs in nematocysts of anthozoans adds further support for a myxozoan-medusozoan affinity. However, the evolution of nematocysts has not been fully resolved (David et al. 2008; Fautin 2009) and convergence cannot be entirely discounted. The relative simplicity of polar capsules

may reflect their modified function of host attachment (see Chap. 13) and whether this is primary or due to secondary reduction remains obscure.

2.3.2 Molecular Evidence

2.3.2.1 rDNA Data Sets

The SSU ribosomal RNA gene was the first molecular marker widely applied to resolve metazoan relationships (Field et al. 1988). Myxozoans were firstly included in a metazoan-wide data set in a study by Smothers et al. (1994) who found good support for a position within the Bilateria, as sister-group to nematodes. However, exclusion of fast-evolving nematode sequences removed Nematoda from its basal bilaterian position and demonstrated major changes among bilaterian relationships (e.g. Aguinaldo et al. 1997). In addition, studies on lower metazoan groups (e.g. Hanelt et al. 1996; Pawlowski et al. 1996; Winnepenninckx et al. 1998) demonstrated the importance of long branch attraction in impeding phylogenetic resolution of the Myxozoa (see Canning and Okamura 2004, for review). Analyses of SSU rDNA sequences were also demonstrating variable phylogenetic placement for myxozoans depending, for instance, on the incorporation of *Polypodium hydriforme* and the analyses of partial versus full length sequences (see Canning and Okamura 2004, for review). Kim et al. (1999) attempted to control for long branch attraction by analysing only full (or near full) length SSU rDNA sequences obtained for the species with the shortest branch to the ancestral node in each monophyletic lineage but their results were relatively inconclusive. Thus, using distance analyses weak support was obtained for a sister taxon relationship between myxozoans and *Polypodium hydriforme* and they did not fall within the Cnidaria. However, maximum likelihood analyses identified Myxozoa as sister to the triploblasts while *Polypodium hydriforme* was unresolved within the diploblasts.

More recently, Evans et al. (2010) examined the effects of missing data, model choice and

inference methods in placing highly divergent taxa and confirmed the two relatively stable placements that previous researchers had found for myxozoans, with Cnidaria or Bilateria, based on various types of analyses of SSU rDNA and LSU rDNA sequences. The analyses by Evans et al. (2010) thus exemplified the importance of careful model selection, taxon and data sampling, and in-depth data exploration when investigating the phylogenetic placement of highly divergent taxa such as the Myxozoa.

2.3.2.2 Protein Coding Genes, Expressed Sequence Tags, and Genomic and Transcriptomic Data Sets

The conflicting conclusions and generally weak support gained by phylogenetic analyses based on nuclear rDNA data suggested that information of another type was required to gain meaningful insights into myxozoan origins. The identification of central class Hox genes in myxozoans was therefore of great interest since the absence of these genes in cnidarians implied a bilaterian affinity for the Myxozoa (Anderson et al. 1998). However, these genes were later shown to be host contaminants (Jiménez-Guri et al. 2007). Success in developing suitable markers for phylogenetic studies has clearly been highly problematic due to the extreme divergence of myxozoan genes which precludes the use of universal primers for obtaining sequence data.

More recent technological innovations that generate data on multiple gene loci, such as large scale sequencing of expressed sequence tags (ESTs), transcriptomes or genomes, have overcome the limitations of earlier phylogenetic analyses based on only a single or a few genes. The breakthrough in understanding myxozoan higher level phylogeny came when Jiménez-Guri et al. (2007) were able to construct an EST library for *Buddenbrockia*. This enabled a phylogenomic investigation based on 50 protein coding genes which provided evidence that *Buddenbrockia* groups within the Cnidaria and, with strongest support, as sister to the Medusozoa (Jiménez-Guri et al. 2007). Subsequent

genomic and transcriptomic studies have consistently provided further confirmation of the cnidarian nature of myxozoans. Thus, Nesnidal et al. (2013) came to similar conclusions based on phylogenomic analysis of 128 protein-coding genes identified by whole genome shotgun sequencing of the myxosporean, *Myxobolus cerebralis* (the causative agent of whirling disease). In addition to confirming the cnidarian status of the Myxozoa and the sister group relationship between the Myxozoa and Medusozoa, they also explicitly tested the effects of missing data and showed that these cannot explain the placement of Myxozoa within the Cnidaria as posited by Evans et al. (2010). Most recently, Feng et al. (2014) obtained genomic and transcriptomic data from the myxosporean *Thelohanellus kitauei* and gained strong support for Myxozoa as sister to the Medusozoa by analysing a subset (86 genes) of the 128 genes analysed by Nesnidal et al. (2013).

2.3.2.3 Taxonomically Restricted Genes (Minicollagens)

Taxonomically restricted genes (TRGs) represent another useful and independent source of data relevant for evaluating myxozoan affinities. TRGs may be identified in the genomes and transcriptomes of a wide range of organisms and will contribute to the high percentage (~20–50 %) of genes with no detectable homologies to proteins in public databases and which are thus referred to as “orphan” or “novel” genes (Khalturin et al. 2009). In cnidarians certain genes involved in nematocyst formation are regarded as TRGs (Milde et al. 2009) since nematocysts are unique to cnidarians and genes coding for proteins specifically involved in nematocysts (e.g. minicollagens, NOWA) have not been found in bilaterian, sponge or protist genomes sequenced to date (David et al. 2008; Khalturin et al. 2009) (although sequence data are lacking for protists with similar organelles).

Holland et al. (2011) sequenced and characterised a minicollagen in the malacosporean, *Tetracapsuloides bryosalmonae*, demonstrating the presence of a gene homologous to those

encoding for nematocyst proteins in the Myxozoa. This minicollagen protein has now been localised to polar capsules (Gruhl et al. in prep.). Feng et al. (2014) have identified two further minicollagens in the myxosporean, *Thelohanellus kitauei*, which were distinct from that identified in *Tetracapsuloides bryosalmonae*. The three myxozoan minicollagens identified so far cluster with minicollagens of medusozoans but because taxon sampling is very poor these results should be viewed with caution. Further minicollagens have been detected in original EST and in new transcriptomic libraries for both malacosporeans and myxosporeans (Holland et al. unpub. data; Gruhl et al. unpub. data) and Shpirer and Chang (2014) report three minicollagens and three nematogalactins in genomic and transcriptomic libraries of *Kudoa iwatai*, *Enteromyxum leei* and *Sphaeromyxa zaharoni*. Nematogalactins represent a further family of cnidarian-specific genes (Hwang et al. 2010).

TRGs offer a promising alternative to support relationships where conflicting evidence or low support obscures resolution of problematic taxa. The power of TRGs is limited by the comprehensiveness of the reference data used for identification, but as the number of sequences deposited in public databases increases, precision is likely to increase as well.

2.3.2.4 Mitochondrial Genes and Genomes

Mitochondrial gene sequences and especially the arrangement of genes in the mitochondrial DNA molecule are important data that are largely independent of nuclear DNA evolution. In addition, the aberrant evolution of mitochondrial genomes within the Cnidaria is now coming to light. The Medusozoa in particular do not retain the archetypal circular mitochondrial chromosome typical of animals, but possess linear chromosomes and in some groups these are fragmented into more than one chromosome (see Kayal et al. 2012 for review). Further unique features of at least some medusozoan mitogenomes include the telomeres forming inverted repeats and duplicate genes and pseudogenes in

the subtelomeric regions as revealed in some species of *Hydra*. Comparative mitogenome architecture, gene arrangements and telomere sequences may therefore be relevant for understanding the cnidarian affinities of the Myxozoa. However, until recently difficulties imposed by extreme molecular divergence precluded the development of mitochondrial markers by a number of groups including our own. Indeed, consistent lack of success in amplifying mitochondrial sequence data from myxozoans despite persistent efforts led authors of a conference abstract to claim that myxozoans are amitochondriate (Wood et al. 2002)! This claim is of course countered by the numerous mitochondria evident in ultrastructural studies.

Data on myxozoan mitochondrial sequences have now been obtained by several groups. In a recent poster abstract Fiala et al. (2013) reported sequences of the 12S, NADH and COX1 genes from six myxosporean species and additionally retrieved a partial mitochondrial genome sequence for *Polypodium*. Preliminary analyses support a medusozoan affinity for Myxozoa and suggest that *Polypodium* is distinct and closely related to the Narcomedusae. In another recent poster abstract Yahalomi et al. (2013) present mitochondrial sequences from genomic data for three myxosporeans and found that myxozoans, like some medusozoans, are characterised by fragmented mitogenomes that have unusually high rates of sequence evolution and are comprised of several linear chromosomes. Preliminary data suggest that malacosporean mitogenomes may be similarly comprised of fragmented, linear molecules (Hartikainen and Okamura, unpub. data).

2.4 Myxozoans and *Polypodium*: Close Relatives or Independently-Evolved Lineages?

It is of interest to further explore the possibility that *Polypodium* and myxozoans are indeed sister taxa with a common origin supporting assignment to the clade Endocnidozoa (Zrzavý and

Hypša 2003). Molecular phylogenetic analyses based on SSU data have often concluded that both *Polypodium* and myxozoans are sister to bilaterians (e.g. Cavalier-Smith et al. 1996; Pawlowski et al. 1996; Winnipenninckx et al. 1998; Kim et al. 1999). However, as discussed above, myxozoans are now confirmed as cnidarians. For *Polypodium* there is now also tentative support based on broad SSU sampling of the Cnidaria that *Polypodium* is a cnidarian (Evans et al. 2009). However, if myxozoans are included in these comprehensive analyses, cnidarian affinities of both *Polypodium hydriforme* and myxozoans disappear (Evans et al. 2008, 2009), highlighting the generic problem of long branch attraction based on SSU data. New evidence based on analyses of 128 genes presented by Rubinstein et al. (2013) indicates *Polypodium* and myxozoans cluster together and form the sister clade to the Medusozoa. However, because the study is ongoing and results may be altered with further cnidarian sampling it is relevant to undertake comparison of other features.

Polypodium is an intracellular parasite infecting the eggs of primitive freshwater bony fish (sturgeon and paddlefish). It undergoes an extraordinary although simple life cycle with a larval stage infecting fish eggs as intracellular parasites and an adult, free-living stage that produces gametophores which infect fish upon contact (Raikova 1994). The life cycle entails development as a so-called planuliform larva with the gastrodermis located externally within a fish egg. The larva is enclosed by a cell called the trophamnion which is likely to have a protective and nutritive role (Raikova 1994). The larval form inverts prior to host spawning to produce a free-living stolon comprised of a chain of tentaculate units with the gastrodermis situated internally. At spawning the fish egg membranes are disrupted to release the stolon which subsequently fragments into tentaculate individuals that actively feed, walk on their tentacles and produce infectious gametophores as a result of sexual reproduction. Gametophores containing binucleate cells have been observed to infect larval fish. Nothing is known about how fish eggs eventually become infected. Infection of

larval fish implies that prolonged periods of arrested development may be required since the time of first spawning may be up to 16 years (see Raikova 1994 for review).

Some basic features present in *Polypodium* that are absent in myxozoans include centrioles, flagellated gastrodermal cells, a cnidocil (a cilium-derived structure associated with nematocysts), gonads and a network of nerve fibres underlying the epidermis (see Raikova 2008 for review). Features shared by *Polypodium* and myxozoans include parasitism of fish, infection via nematocysts, a similar type of nematocyst (putatively atrichous isorhiza), longitudinally arranged and mesodermal-like muscle cells, cell-within-cell stages (see Chap. 8 for review of endogeny processes in myxozoans) and mitochondria with tubular cristae (Raikova 2008). Many of these shared features are, however, also found in other cnidarians. For instance, atrichous isorhizas are broadly distributed and mesodermal-like muscle arrangements characterise many cnidarians (reviewed in Seipel and Schmidt 2006). Furthermore, cell-within-cell stages have been observed during development of the trachylinid *Pegantha smaragdina* which Bigelow (1909) described as parasitizing the parent (which lacks gonads) by developing within the gelatinous matrix close to the gastric cavity. During this development a nurse cell surrounds an embryonic cell that then subsequently divides while continuing to be enclosed within the nurse cell (Bigelow 1909). We have already reviewed how the shape of mitochondrial cristae is highly variable and should no longer be considered a character of phylogenetic significance.

At present, both molecules and other traits provide conflicting evidence. Myxozoans and *Polypodium* may represent independently-derived endoparasitic lineages or they may represent sister cnidarian taxa that have undergone extensive divergence following their separation. If they are sister taxa this could imply that primitive bony fish (Order Acipenseriformes) were ancestral hosts to myxozoans in freshwater environments since both *Polypodium* and malacosporeans (which retain primitive morphologies) both exploit freshwater hosts. Ancestral marine fish

hosts are less likely in view of the combination of primitive and simple features demonstrated in malacosporeans (see later discussion), the apparent restriction of malacosporeans to freshwater environments (see later discussion) and phylogenetic analyses of character evolution that identify freshwater fish as primitive hosts (Fiala and Bartošová 2010). In turn, myxozoans may have diverged to exploit a diversity of fish and to subsequently evolve a complex parasitic life cycle with adult stages exploiting invertebrate hosts. The alternative possibility, that a complex parasitic life cycle was ancestral to both myxozoans and *Polypodium*, would imply the unlikely event of *Polypodium* regaining free-living adult stages. The possibility that myxozoans and *Polypodium* may represent two independent transitions to endoparasitism within the Cnidaria is supported by the independent evolution of anthozoan and narcomedusan species with larval stages that develop parasitically in the gastrovascular cavities of other cnidarians (Spaulding 1972; Pagès et al. 2007) or in the stomach of a ctenophore (Bumann and Puls 1996).

2.5 New Markers, New Methods

As outlined above for myxozoans and as is also the case for several other problematic taxa in the tree of life, there are still numerous sources of error that prevent us from deciphering true phylogenetic relationships. General strategies to improve support include firstly an increase of both the number of taxa and the number of characters used (Philippe and Telford 2006). However, this is not always easily achievable, for example when the organisms are rare, difficult to sample, or highly divergent. Also, an uncritical use of more characters does not necessarily lead to higher support values, but can instead introduce further new sources of error (e.g. Nosenko et al. 2013). Systemic errors can only be reduced by optimising the algorithms and models used for phylogenetic analysis (e.g. Philippe and Roure 2011; Struck 2013).

A different approach is to use more complex characters as these are less likely to be

homoplastic. Such characters can be morphological as well as non-sequence genomic characters. Examples of the latter that have been used to resolve phylogenies include rare genomic changes (Rokas and Holland 2000), near-intron-pairs (Lehmann et al. 2013; Hill et al. 2013), microRNAs (Wheeler et al. 2009), retroposons (Suh et al. 2011), and protein indels (Gupta 2001). Mitochondrial genome structure data such as linearity versus circularity are also very informative in cnidarians (Bridge et al. 1992) and are now promising to add further resolution to the position of myxozoans (see earlier section).

Finally, in myxozoans as well as in many other understudied taxa, morphological and developmental studies are extremely valuable, because many traits may be unrecognised or have been characterised for only a few representatives. Such poor cover may potentially bias inferences of body plan evolution and development.

2.6 Evolution of Parasitism: From Free-Living Cnidarians to Endoparasites

After a long period of controversy, the cnidarian affinity of myxozoans appears at last to be clear. We are therefore now able to interpret the Myxozoa within the context of their cnidarian nature and the evolution of endoparasitism from free-living ancestors. In this section we explore the evolution of their complex parasitic life cycles, the nature of the first myxozoan hosts and the incorporation of new hosts. Chapter 3 considers the extent that ancestral cnidarian features may be reflected in their subsequent evolution and life histories as well as unique traits that may have enabled their radiation as endoparasites, the latter is also considered further in Chap. 4.

2.6.1 Evolution of a Complex Parasitic Life Cycle

Present knowledge indicates that all myxozoans incorporate invertebrate and vertebrate hosts in a

complex life cycle. A marine origin was inferred by Shul'man (1990) who suggested that the common ancestor was a coelozoic parasite of the gall bladder and urinary bladder of actinopterygian teleosts (ray fins) but this was proposed before invertebrate hosts and the malacosporeans were recognised. On the basis of current evidence a freshwater origin is also conceivable. This is implied by phylogenetic analyses of character evolution based on stages in fish (Fiala and Bartošová 2010) and the primitive characters of malacosporeans along with their simple spores (see below). It would also be supported if *Polypodium* is confirmed as sister to the Myxozoa. The evolution of the complex myxozoan life cycle would have first involved a transition from a free-living lifestyle to a parasitic form that exploited a single host. Such a transition would be preceded by the two organisms coming into contact for some time. Pre-adaptations of parasite precursors will then have enabled initial stages of host exploitation when greater fitness was attained by maintaining the association (Poulin 2007). Routes to parasitism may involve parasite precursors feeding on hosts, utilising hosts for dispersal (via phoresy) or to reduce environmental variability, and survival following predation (see Poulin 2007; Schmid-Hempel 2011 for further review and examples).

We note that the number of species observed in the present day is not informative about how old lineages may be and thus cannot provide potential insights about original hosts or habitats. For instance, relatively depauperate clades may once have been more speciose. In addition, speciose clades may have arisen by adaptive radiation enabled by the evolution of a key trait. Chapter 4 explores how such adaptive radiation may have resulted in the highly speciose myxosporeans. Other parasitic taxa are similarly characterised by depauperate clades sister to highly speciose clades with derived characters. For instance, the Aspidogastrea comprises four families and some 80 species and is sister to the Digenea which is composed of 100 families and >10,000 species (Cribb et al. 2003). Another example is the Cyclophyllidae, which is the most highly derived

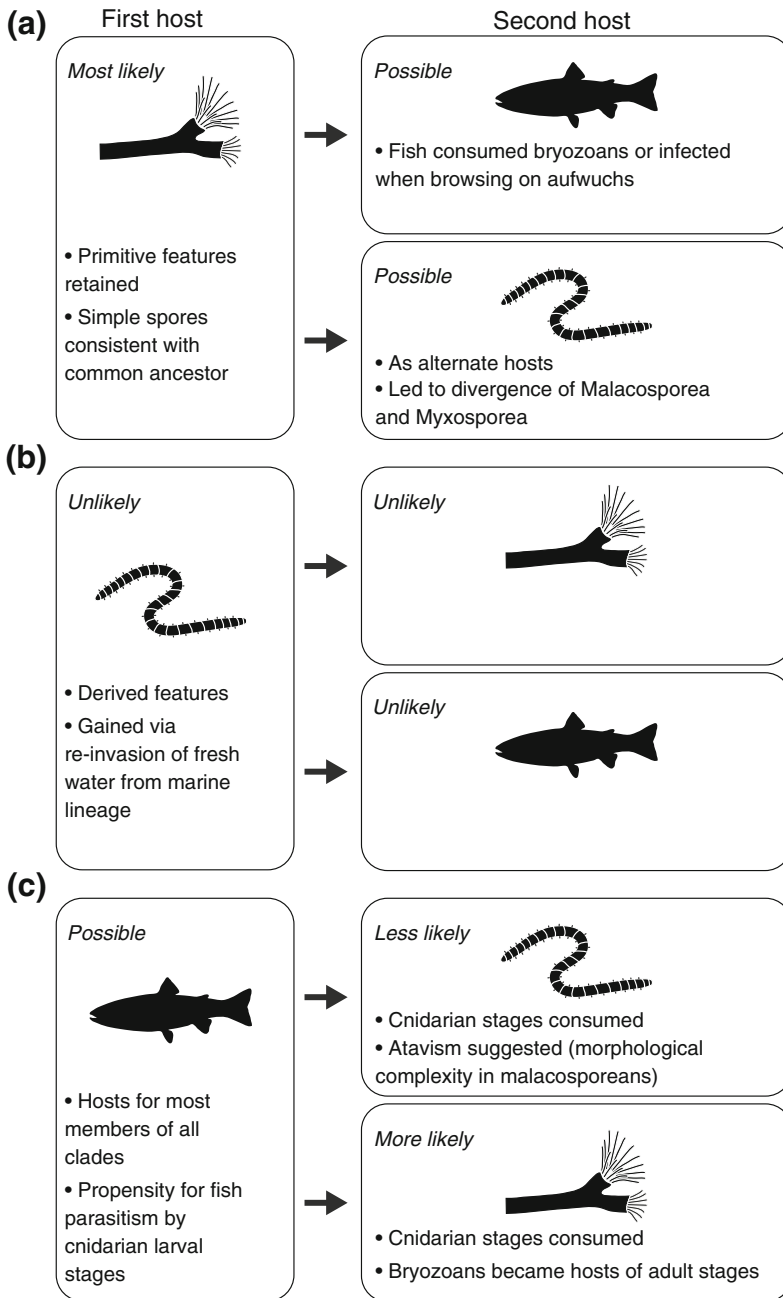


Fig. 2.2 The main hypothetical scenarios for the origin and evolution of myxozoan life cycles (see text for further details, including use of precursors to host groups, as well as consideration of further scenarios). **a** Freshwater bryozoans as first host with subsequent acquisition of fish as secondary host. Switch to annelids, initially as secondary and then as alternate hosts could have led to

divergence of Malacosporea and Myxosporea. **b** Annelids as first hosts. This scenario is unlikely given that myxozoans infecting annelids are derived. **c** Fish (or early fish-like vertebrates) as first hosts with later addition of bryozoans or annelids as second host. Subsequent switch of sexual phase to the invertebrate host



Fig. 2.3 *Hydra* sp. (double arrowhead) on the phylactolaemate bryozoan *Fredericella* sp. Several tentacular crowns (lophophores) of the bryozoan are extended for suspension feeding (arrowheads)

Eucestode order and contains 380–400 genera, while the other, less derived orders of eucestodes contain 1–66 genera (Brabec 2012).

The current topology of myxozoan phylogeny with a basal split into the subtaxa Malacosporea and Myxosporea enables us to identify characters that are shared by both groups as ancestral for Myxozoa. However, this is not possible for traits that show different character states between the two groups and are also absent in any potential myxozoan outgroup. Since the ancestral myxozoan was a free-living cnidarian we cannot determine whether the malacosporean condition (bryozoan host) or the myxosporean condition (annelid host) is plesiomorphic. One way to approach this dilemma is therefore to develop scenarios (Fig. 2.2) that can be examined for plausibility based on the evolution of other characters or on ecological or functional considerations. Below we use this approach to consider how endoparasitism and life cycle complexity may have arisen in the Myxozoa. The main scenarios for patterns of host acquisition are outlined in Fig. 2.2. The relationship between myxozoan life cycles and the complex life cycles of free-living cnidarians is explored in Chap. 3.

2.6.1.1 Invertebrates as First Hosts

The retention of primitive features (tissues, muscle blocks, tetradial symmetry) in freshwater

bryozoan hosts suggests that myxozoans may have evolved from a cnidarian ancestor in freshwater environments with bryozoans or their precursors acting as ancestral hosts of adult stages (since meiosis occurs in invertebrate hosts) (Fig. 2.2a). An alternate scenario is that myxozoans evolved to become parasites of marine bryozoan precursors that subsequently invaded freshwater environments to diversify as freshwater bryozoans. The relatively simple, soft-walled spores produced by malacosporeans in both bryozoan and fish hosts are similar to the simple spores identified as present in the common ancestor identified by phylogenetic analyses of character evolution based on stages in vertebrate hosts and which was inferred to have exploited freshwater hosts (Fiala and Bartošová 2010).

Relatively few cnidarians occur in freshwaters and current evidence suggests that all (except possibly *Polypodium*) are hydrozoans. Hydrozoans exhibit several instances of independent evolution to inhabit freshwaters (Jankowski et al. 2008), and *Hydra* is often found attached to bryozoan colony surfaces (Fig. 2.3) perhaps reflecting an association with future hosts by an ancestral hydrozoan form which preceded a transition to parasitism (Poulin 2007). Notably, the suspension feeding activity of bryozoans may predispose them to ingest a wide range of potential food items, including propagative stages of hydrozoans such as eggs, planulae, or small regressed stages (see Chap. 3 for review of the latter) that could potentially invade bryozoan tissues. Furthermore, the ability of cnidarians to absorb dissolved organic compounds through the integument (e.g. Ferguson 1982; Grover et al. 2008) and the incomplete digestion and even survival of organisms consumed by freshwater bryozoans (Hyman 1959; Raddum and Johnsen 1983; Okamura pers. obs.) may both be significant processes that enabled the invasion and development within the tissues of bryozoans (or their precursors) by ingested stages. Acanthocephalans provide evidence for such a postulated transition to parasitism with the stem species inferred to have lived epizootically on a marine arthropod ancestor prior to invading the host body cavity to establish an endoparasitic life

cycle. The present-day close association of the free-living *Seison nebaliae* (the sister taxon to acanthocephalans) with marine arthropods, may reflect such a situation (Herlyn et al. 2003).

It is also possible that myxozoans evolved from a cnidarian ancestor that became an endoparasite of annelid worms (Kent et al. 2001). Thus, myxozoans may have originated in the marine environment where annelids are a diverse and old group. Alternatively, freshwater oligochaetes could have been first hosts. However, we view either scenario of annelids as first hosts (Fig. 2.2b) as less likely than that of ancestral bryozoan hosts (Fig. 2.2a) since myxosporeans are highly derived. Furthermore, myxozoans infecting freshwater worms appear to have arisen via re-invasion of freshwater environments from marine myxosporean lineages (Kent et al. 2001; Fiala and Bartošová 2010). This suggests that if freshwater oligochaetes were first hosts these lineages have vanished without trace whilst malacosporeans have remained in freshwater environments exploiting a host group of low diversity (freshwater bryozoans) relative to the diversity of freshwater oligochaetes. Finally, it should be mentioned that myxozoans could have originated in other invertebrate hosts that remain undetected either because of extinction or lack of sampling.

2.6.1.2 Fish as First Hosts

An alternative scenario (Fig. 2.2c) is that fish (or early fish-like vertebrates, see below) were original hosts for stages of myxozoans that are likely to have developed in renal tissues (Kent et al. 2001; Fiala and Bartošová 2010). The absence of meiosis in myxozoans in fish hosts suggests that fish may have supported the development of larval myxozoans. The adoption of fish as hosts may have occurred in freshwater or marine environments. Invertebrates would subsequently have been incorporated as hosts for adult stages in a two-host life cycle. Support for this scenario is that fish are hosts for most members of all major clades of myxozoans (malacosporeans and the freshwater and marine clades of myxosporeans) (Kent et al. 2001; see

also Chap. 4) and the propensity of other cnidarians to evolve parasitic larval stages (Spaulding 1972; Bumann and Puls 1996; Pagès et al. 2007). Additional support would be gained if *Polypodium* (which exploits fish hosts as larval stages) and myxozoans were determined to be sister taxa. The free-living adult stage may then, by association, have evolved endoparasitism using invertebrates as definitive hosts.

At present it is unclear whether myxozoans are sister to or were derived within the Medusozoa. This has implications for interpreting the nature of myxozoans under a scenario of utilising fish as first hosts. Analyses of mitochondrial protein-coding genes and the fossil record suggest that medusozoans diverged prior to the Cambrian (Park et al. 2012). The first cartilaginous and bony fish appear in the Devonian and Silurian, respectively. A sister-group relationship of myxozoans and medusozoans would therefore imply that myxozoans either must have been free-living for many millions of years prior to the utilisation of such fish as first hosts, or have used other hosts before switching to such fish. If, however, myxozoans were derived within Medusozoa then the period of time that myxozoan precursors were free-living prior to parasitising fish may have been greatly reduced, and the postulation of an additional host-switch becomes unnecessary. Similarly, the period of time may have been reduced if precursors to cartilaginous or bony fish served as first hosts. This would be supported if agnathans (hagfish and lampreys) turned out to be regular hosts of myxozoans, because the origin of vertebrates dates well back into the Ediacaran and agnathans diverged from gnathostomes in the Cambrian (Donoghue and Keating 2014). Thus far, only isolated findings of myxozoans in lampreys have been reported (Mori et al. 2000).

Larval stages of myxozoans may originally have been transmitted to fish by direct contact with the adult form, perhaps via a stage analogous or homologous to the gametophores of *Polypodium*. Alternatively, spores may have evolved as larval stages adapted to attach to fish hosts to enable phoresy (like glochidium larvae of some freshwater bivalves). This may have

been particularly advantageous in freshwater environments, providing a means of colonising habitats otherwise precluded or retention within favourable adult habitats rather than being swept downstream to unsuitable sites. It is difficult to envision how tiny larval stages released into a three-dimensional watery world would have achieved the close association with fish hosts required for spores to evolve in the first place unless release was somehow triggered by proximity of fish. The firing of polar filaments from polar capsules by exposure to fish mucous or mechanical contact of spores (see Chap. 13 for further discussion) may illustrate how such transmission may have been achieved.

The other possibility, that myxozoans first exploited fish hosts as adult stages, would entail a subsequent transition to using invertebrates as definitive hosts. The apparent flexibility of cnidarian life cycles (see Chap. 3) suggests a precedent for this, but as developed below, this scenario implies unlikely evolutionary events associated with the incorporation of secondary invertebrate hosts.

2.6.2 Incorporation of New Hosts

Complex life cycles have evolved independently in several groups of parasites with the drivers of host expansion likely to reflect historical events that affected parasite transmission or survival of the host (Poulin 2007). An increase in life cycle complexity may be explained, for instance, if parasites evolve to exploit predators or prey of the first host thereby enabling higher growth and fecundity or higher transmission rates (e.g. Choisy et al. 2003; Parker et al. 2003). This could be achieved by upward incorporation—when original hosts are frequently ingested and become intermediate hosts (Parker et al. 2003). Such upward incorporation could be driven by increased parasite fecundity in larger predator hosts with selection for delayed maturity and enhanced reproduction in this larger host. For example, upward incorporation appears to have occurred when the ancestor of acanthocephalans, an endoparasite of a marine arthropod,

incorporated a vertebrate predator as a second host (Near et al. 1998; Herlyn et al. 2003). Complex parasitic life cycles may also be achieved by downward incorporation—when prey of the original host frequently ingest parasite propagules and become intermediate hosts. This may enhance transmission to the original host which then becomes the definitive host (Parker et al. 2003). Platyhelminthes appear to present an example of downward incorporation with the lineage ancestral to digeneans and cestodes becoming parasitic in vertebrates (Littlewood et al. 1999) and the subsequent addition of invertebrate hosts in each group (see Poulin 2007; Schmid-Hempel 2011 for review).

Additional hosts may also be incorporated in parasite life cycles if there is an increased probability of finding a sexual partner (Brown et al. 2001). Note that this assumes there are selective benefits of cross-fertilization which may not be the case for myxosporeans (see Chap. 3) but could apply to malacosporans (see below). Finally, additional hosts may serve to transfer infectious stages from one host to the next. However, such paratenic hosts have no effect on completion of the parasite's life cycle. Below we consider how the complex myxozoan life cycles observed today may have evolved by expansion from various potential hosts. When this occurred is obscure—myxozoans may have remained endoparasites with a simple life cycle for millions of years. Our discussion focuses on how complex myxozoan life cycles may have arisen via expansion from precursors that exploited either invertebrate or vertebrate hosts. The subsequent adoption of a new host via host switching by myxozoans demonstrates a capacity for host substitution. For instance, myxosporeans have replaced fish with amphibian hosts on at least three times independently (see Chaps. 4 and 7 for further discussion).

2.6.2.1 Expansion from Bryozoans?

If freshwater bryozoans or their precursors were first hosts (Fig. 2.2a), fish may have been incorporated as secondary hosts by direct ingestion of infected bryozoans or of myxozoan

spores. So far there is no evidence for trophic transmission from invertebrate hosts to fish but the presence of bryozoan dormant stages (statoblasts) in fish guts in the present day (e.g. Dendy 1963; Applegate 1966) and demonstration that statoblasts carry myxozoan infections (Hill and Okamura 2007; Abd-Elfattah et al. 2014) suggests that trophic transmission may have been possible. On the other hand, ingestion of myxozoan spores might easily occur during predation and browsing of invertebrates commonly associated with and concentrated in dense stands of freshwater bryozoans (Bushnell and Rao 1979; Okamura pers. obs.). Utilisation of fish hosts may have enabled persistence during adverse conditions or amplifying transmission as a result of the greater biomass and longevity of fish. However, if the invertebrate hosts were highly clonal, as in present-day bryozoans, these advantages may not have pertained. For instance, malacosporeans infect dormant asexual bryozoan propagules (statoblasts) that enable survival during adverse conditions (Hill and Okamura 2007; Abd-Elfattah et al. 2014). In addition, extensive clonal growth in freshwater bryozoans combined with vertical transmission of infection in clonal fragments and propagules (see Chap. 11) could amplify parasite biomass and transmission to levels equal to if not greater than those achieved by exploiting fish. Alternatively or additionally, infection of fish may have enabled retention of larval stages within suitable habitats rather than being swept downstream or it may have enhanced outcrossing (Rauch et al. 2005) if infectious spores released by fish are more likely to be genetically distinct than those produced by parasites in highly clonal local bryozoan populations. For instance, fish movements may result in exposure to infection from multiple sources while extensive vertical transmission of parasites in bryozoans may amplify the biomass of only a single or a few parasite genotypes in local bryozoan populations.

An alternative or perhaps additional possibility is that annelid worms were incorporated as alternate hosts via ingestion of spores released

from bryozoans or direct consumption of infected bryozoans. This may have been facilitated by an association of oligochaetes with freshwater bryozoans—something commonly observed for a variety of species in the present-day (Okamura, pers. obs.). Indeed, oligochaetes are occasionally encountered that have ingested statoblasts (Okamura, pers. obs.) perhaps exemplifying the potential consumption of infected statoblasts by annelids that eventually were incorporated as hosts. This scenario would imply that life cycles subsequently evolved along different trajectories leading to the divergent Malacosporea and Myxosporea along with the capacity for adult development to be transferred from bryozoan to annelid hosts. The most parsimonious interpretation is that fish hosts were incorporated prior to the malacosporean/myxosporean split.

2.6.2.2 Expansion from Annelids?

Fish may have been incorporated as secondary hosts of myxozoans developing in annelid worms (Fig. 2.2b) by trophic transmission, with predation of infected annelids selecting for parasites with the ability to survive passage through fish and use of the gut as the primordial entry portal. This is supported by the common inclusion of annelids in fish diets. However, as outlined earlier, the derived nature of myxosporeans suggests this is unlikely to have involved expansion to fish hosts from basal myxozoans that infected annelids. A recent molecular phylogenetic analysis has identified *Bipteria* sp. to comprise the earliest diverging myxosporean branch of the derived marine lineage (Kodádková et al. 2014). Exploitation of the holocephalan fish, *Chimaera monstrosa*, as the vertebrate host by *Bipteria* sp. suggests that fish may have been incorporated as hosts as early as the Silurian when the oldest living group of jawed vertebrates (the cartilaginous fishes comprising the chimaeras, sharks, skates and rays) diverged from a common ancestor of bony vertebrates. It is possible that holocephalans were incorporated by myxozoans that infected annelids or even marine

precursors to freshwater bryozoans. It is, however, also possible that holocephalans were adopted as hosts more recently. In this respect it may be significant that the early branching malacosporeans in the present day do not infect particularly ancient fish.

Finally, if annelids were incorporated as alternate hosts to bryozoans (see above), it is possible that fish may already have acted as hosts in myxozoan life cycles prior to the addition of annelid hosts. Another scenario, in parallel with that outlined in the previous section, is that freshwater bryozoans were incorporated as alternate hosts via ingestion of spores released from annelid hosts. This would then entail subsequent evolution leading to the divergence of Malacosporea and Myxosporea and adult development transferred from annelid to bryozoan hosts. As argued previously, it is more likely that fish hosts were incorporated prior to the malacosporean/myxosporean split.

2.6.2.3 Expansion from Fish?

If fish were acquired as hosts of larval stages (Fig. 2.2c) trophic transmission of adult stages released from fish would seem difficult to achieve given the suspension-feeding and scavenging activities of bryozoans and annelids. However, the propensity of cnidarians to release small propagative stages by budding processes (see Chap. 3) may have enabled such transmission. Alternatively, if fish were acquired as hosts of adult stages (see above) trophic transmission via the ingestion (either of spores or of stages in which spores were present; see Chap. 3 for further discussion of myxozoan life cycle stages) by bryozoans and worms would be feasible. This scenario would require a switch to using invertebrates as definitive hosts. Since cnidarian life cycles demonstrate considerable plasticity, for instance with transition of sexual reproduction from medusa to polyp stages (e.g. in *Hydra*), the scenario is not entirely unfeasible (see Chap. 3 for further discussion). Nevertheless, the scenario does not readily explain why morphological complexity would subsequently characterise the adult malacosporean stages in bryozoans. Such

complexity is rather suggestive of atavism, with complex worm-like stages re-evolving in malacosporeans, unless of course early fish hosts harboured morphologically complex myxozoan parasites. Finally, cnidarians [e.g. *Polypodium*, anthozoans and narcomedusae (Bumann and Puls 1996; Spaulding 1972; Pagès et al. 2007)] demonstrate a proclivity to evolve parasitic larval rather than adult stages.

2.6.3 Undiscovered Diversity and Hosts

The diversity of myxosporeans described in fish and the discovery of new hosts for malacosporeans (see Chap. 4) provide evidence that myxozoan host ranges in these two invertebrate groups will continue to expand as further life cycles are resolved and new material encountered. Several other groups of invertebrates may have also been incorporated in myxozoan life cycles, such as octopus (Yokoyama and Masuda 2001) (see also Lom and Dyková 2006). However, because myxozoan infections are generally innocuous many are probably very often overlooked. The demonstration of extensive covert infections by malacosporeans in freshwater bryozoans (see Chap. 11) also suggests that many myxozoans may be unrecognised if they occur for prolonged periods of time as single cells associated with host tissues. The recent astonishing expansion in the diversity of Haplosporidia and Mikrocytida via both environmental DNA detection and sampling of invertebrate hosts (Hartikainen et al. 2014a, b) provides evidence that the diversities of innocuous, endoparasitic microbial taxa, such as Myxozoa, are likely to be greatly underestimated. Thus, it is possible that myxozoans have evolved to exploit a much broader range of invertebrate host groups than is currently evident.

Marine counterparts of freshwater bryozoans could be considered as likely candidate hosts for malacosporeans. However, we are unaware of any convincing observations in the literature of malacosporeans in marine bryozoans (Classes Gymnolaemata and Stenolaemata) nor have any

been found by specifically sampling marine bryozoans (Okamura, unpub. data on gymno-laemates). A number of early bryozoan researchers noted ‘vermiform’ and other apparently parasitic bodies in marine bryozoans. Some of these were likely to have been bryozoan organ systems (e.g. paired vestibular glands) including various specifically-located vermiform bodies described by Hastings (1943). Others apparently occurred collectively within a common matrix (e.g. Waters 1912). However, myxozoan stages are directly exposed to the host body cavity fluids. The description of cilia on one such stage is also inconsistent (Waters 1912). Some of the vermiform bodies observed by Hastings (1943) were examined by authorities who could not confirm their identity without fresh, properly fixed material although one authority suggested they could be Protozoa. The recent discovery of orthonectids endoparasitic in marine bryozoans suggests a possible identity for some of the vermiform bodies that have been observed in bryozoans (Hochberg and Kruse 2009).

The apparent absence of malacosporeans in marine bryozoans could of course reflect low or patchy infection prevalence, and relatively little research focusing on what are regarded as ‘minor phyla’ such as the Bryozoa. Nevertheless, we believe that marine bryozoans are unlikely hosts because the presence of walls between constituent zooids in colonies results in a very small space in which sacs or worms could develop—the body cavity of a single zooid. In phylactolaemates (freshwater bryozoans) the lack of walls between constituent zooids in colonies produces a voluminous, colony-wide, fluid-filled body cavity that supports the proliferation of numerous sacs and worms whose maximum dimensions range from 0.3 (for sacs) to 3 mm (for worms). Even at maturity these malacosporean stages are bathed by host fluids and undergo active movements (worms) or are passively circulated (sacs) within the common, colony-wide body cavity. In contrast, a single *Buddenbrockia* worm or a couple of sacs would completely pack the volume of a single zooid of a marine bryozoan, entailing difficulty in spore release and probably in nutrient uptake. We further suggest that the

size constraints associated with developing in marine bryozoan zooids would drastically reduce transmission due to extremely low concentrations of spores released in marine environments from small stages. More plausible candidates for marine invertebrate malacosporean hosts might therefore be found among groups that exhibit similar features, i.e. deposit or suspension feeding life styles allowing contact with infectious spores in combination with large body cavities providing space and nutrients for parasite trophic stages. These would include potential relatives of bryozoans such as phoronids or brachiopods, but also invertebrates such as echinoderms, hemichordates or molluscs.

2.7 Conclusions

The cnidarian nature of myxozoans is increasingly supported by evidence from multiple independent sources. However, both morphological and molecular markers appear to be highly divergent in myxozoans and thus currently do not provide confidence in a more precise phylogenetic hypothesis regarding their closest cnidarian relatives. This picture is likely to be resolved in the near future as many studies are now focussing on these questions by searching for new phylogenetically informative characters.

The evolution of the complex myxozoan life cycle is a fascinating but inherently difficult topic to evaluate. The close association of a free-living precursor with what would become the first myxozoan hosts would have led to myxozoans with simple life cycles. The occurrence of meiosis in invertebrate hosts, the more derived nature of myxozoans that infect annelids and the fact that fish are hosts for most members of all major myxozoan clades suggest that either freshwater bryozoans or fish (or their precursors) acted as such ancestral hosts. The morphological complexity of malacosporeans in freshwater bryozoans renders a scenario of fish as first hosts perhaps less probable. However, cnidarian characters that likely pre-adapted them to endoparasitism, including life cycle plasticity and a

capacity to evolve novel propagative stages, can also be invoked to explain many alternative scenarios for the evolution of complex parasitic life cycles.

The diversity of myxozoans as currently recognised is no doubt underestimated due to the biased focus on a narrow range of economically-important hosts. Further research on myxozoan diversity and life cycles will enable greater insights into the phylogeny and evolution of this group.

2.8 Key Questions for Future Study

- Which (cnidarian) group is sister to myxozoans?
- Are there other invertebrate taxa that act as primary hosts?
- Have *Polypodium* and Myxozoa independently evolved endoparasitism?
- How might we gain insights into when endoparasitism evolved and what hosts were first acquired?

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