Adaptive Radiation and Evolution Within the Myxozoa

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Abstract

Myxozoans are endoparasites characterized by extensive morphological simplification and complex life cycles. Their definitive hosts are bryozoans—in the case of the more primitive and species-poor Malacosporea, or annelids—in the case of the more derived and speciose Myxosporea. This chapter reviews patterns of adaptive radiation within the Myxozoa and explores the drivers that may have promoted evolutionary change. Topics covered include: multiple transitions between worm-like and sac-like forms in the Malacosporea; undersampling that likely limits our appreciation of malacosporean diversity; and multiple shifts between marine and freshwater environments in the Myxosporea. We also examine morphological simplification that is observed in stages that produce spores and associated changes in the size of these sporogonic stages. This contrasts with the evolution of morphological complexity in spores. Features proposed to be involved in diversification include the acquisition of hardened spores and plasmodia, a high diversity of definitive hosts and invasion of novel hosts and host environments in the

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Myxosporea. The evolution towards higher complexity in spores can, in some cases, be linked with increasing transmission success. Convergence in spore form suggests that certain morphologies are highly adaptive. The significance of many features of spores, however, remains poorly known.

Keywords
Malacosporean diversification · Myxosporean diversification · Evolutionary transitions · Taxon sampling · Morphological simplification · Spore complexity

4.1 Introduction

The evolutionary trajectories of endoparasites are greatly influenced by interactions with their hosts. Thus, endoparasite diversification can be expected to reflect processes such as co-speciation and host switching along with the evolution of host specificity. In addition, parasites must achieve transmission to new hosts, a process that typically requires persistence outside their hosts. Finally, parasites with complex life cycles, such as myxozoans, require the ability to exploit distinctly different hosts. Drivers of diversification and evolution within the Myxozoa will therefore include biotic factors associated with host exploitation and abiotic factors associated with the environment of their free-living spores.

This chapter expands on these themes by focusing on patterns of evolution within the Myxozoa. We begin by comparing and contrasting diversification of the more primitive malacosporeans and more derived myxosporeans. We then explore why the malacosporeans have remained relatively species poor while the myxosporeans have undergone extensive diversification. Finally, we consider more specific adaptations displayed particularly by the myxosporeans that have enabled this group to exploit a variety of hosts and tissues and to survive in the environment when switching hosts. We refer readers to Chap. 2 for discussion of the evolution of parasitism, including discussion of the first myxozoan hosts and how myxozoan life cycles may have expanded to incorporate intermediate hosts. Chapter 5 reviews myxozoan taxonomy and systematics and discusses phylogenetic relationships within the Myxozoa.

4.2 The Malacosporean and Myxosporean Radiations

Although myxozoans have only recently been understood to be cnidarians (see Chap. 2), myxosporeans infecting fish have been recognized since the first half of the 19th century (Jurine 1825) and have had a relatively long period of study (Lom and Dyková 2006). Actinosporean stages of the Myxosporea had been described by the end of 19th century (Štolc 1899) and were regarded as a distinct group of endoparasites of annelids until it became clear that they share a common life cycle with fish-infecting counterparts (Wolf and Markiw 1984). Today there are some 2,200 myxosporean species (Lom and Dyková 2006). In contrast, the Malacosporea was described at the beginning of the 21st century as an early-diverging clade of myxozoans based on the distinctive features of sac-forming parasites of freshwater bryozoans (Canning et al. 2000). The enigmatic vermiform endoparasite of freshwater bryozoans, *Buddenbrockia plumatellae*, described in 1910 (Schröder 1910), was finally affiliated with the Malacosporea in 2002 (Monteiro et al. 2002; Okamura et al. 2002). As we show in this chapter there is currently evidence for some 16 malacosporean species, three of which have so far been described. The traditional taxonomy of myxozoans is based largely on spore morphologies and morphometrics but it is increasingly clear that molecular data are also

required for species discrimination due to convergence of spore morphotypes (see Sect. 4.4 and Chaps. 5 and 6). Below we review how molecular data combined with other data such as malacosporean body plans, patterns of host utilization and myxosporean infection sites are expanding our general understanding of myxozoan diversity.

4.2.1 Malacosporean Diversification

Unlike in myxosporeans, malacosporean diversity has been revealed by the discovery of stages in invertebrate hosts. These stages occur as sacs in *Tetracapsuloides bryosalmonae* (Canning et al. 2000) and *Buddenbrockia allmani* (Canning et al. 2007), and as worm-like stages (myxoworms; Canning et al. 2008) in *Buddenbrockia plumatellae* (Okamura et al. 2002). A striking result is that in some cases the sac-forming and vermiform malacosporeans are characterized by extremely low molecular sequence divergence (Monteiro et al. 2002). Indeed, this led Canning et al. (2002) to synonymise the sac-forming parasite of the gelatinous bryozoan, *Cristatella mucedo* (originally described as *Tetracapsula bryozoides*; Canning et al. 1996) with *Buddenbrockia plumatellae*, a myxoworm infecting tubular, branching species of *Plumatella* and *Hyalinella*. The two forms were proposed to represent alternate morphologies that developed in different bryozoan hosts (Canning et al. 2000). However, subsequent molecular phylogenetic studies (Tops et al. 2005; Jiménez-Guri et al. 2007; Bartošová-Sojková et al. 2014; Hartikainen et al. 2014) consistently separate these forms suggesting that the malacosporean infecting *Cristatella mucedo* is indeed a distinct species as originally described (Canning et al. 1996).

Recent molecular and morphological analyses show that the malacosporean clade includes parasites that develop as myxoworms (*Buddenbrockia plumatellae* and four undescribed species; Tops et al. 2005; Hartikainen et al. 2014), spherical sacs (*Tetracapsuloides bryosalmonae, Buddenbrockia allmani*, two undescribed *Tetracapsuloides* and *Buddenbrockia* species), oblong sacs (‘*Buddenbrockia plumatellae*’ in *C. mucedo*; see above), and elongate sacs with lobes (Hartikainen et al. 2014). Notably, sacs and myxoworms occur across the phylogeny and morphological transitions between sac- and worm-like forms have occurred repeatedly (Hartikainen et al. 2014). Further studies have discovered novel malacosporean diversity by detecting infections in fish kidney (Bartošová-Sojková et al. 2014). However, whether these novel isolates from fish kidneys identify true fish hosts requires confirmation of spore development and, ideally, demonstration of transmission back to bryozoans. Here, we combine the datasets from these two studies to present the most comprehensive analysis of malacosporean SSU rDNA data to date (Fig. 4.1). In addition to the three malacosporean species recognized before 2013, this analysis reveals 13 new malacosporean lineages representing new species or even genera (see Chap. 5). The analysis also shows, as previously demonstrated (Hartikainen et al. 2014), that sacs and myxoworms occur across the phylogeny.

Despite problems with taxon undersampling, the molecular phylogeny reveals several potentially notable patterns. These include the apparent association of species in the *Buddenbrockia* clade with single bryozoan hosts compared with the diversity of bryozoan hosts utilized by the species of the *Tetracapsuloides* clade (Fig. 4.1). This suggests that these clades may be characterized by specific versus generalist bryozoan host exploitation strategies. Similarly, some malacosporean infections are associated with a broad fish host range. Thus, some are detected in kidney tissues of both cypriniform and perciform fish (i.e. Malacosporea sp. A and the vermiform *Buddenbrockia plumatellae*), while others have been detected only in kidneys of fish belonging to a single family (Fig. 4.1). The degree of host specificity that malacosporeans exhibit at either invertebrate or fish host level requires further investigation and understanding of malacosporean life cycles. The current molecular phylogeny also demonstrates the utilization of fredericellids as bryozoan hosts in the two earliest diverging lineages (Malacosporea sp. E and F) as well as in the early diverging lineage of the *Buddenbrockia* clade

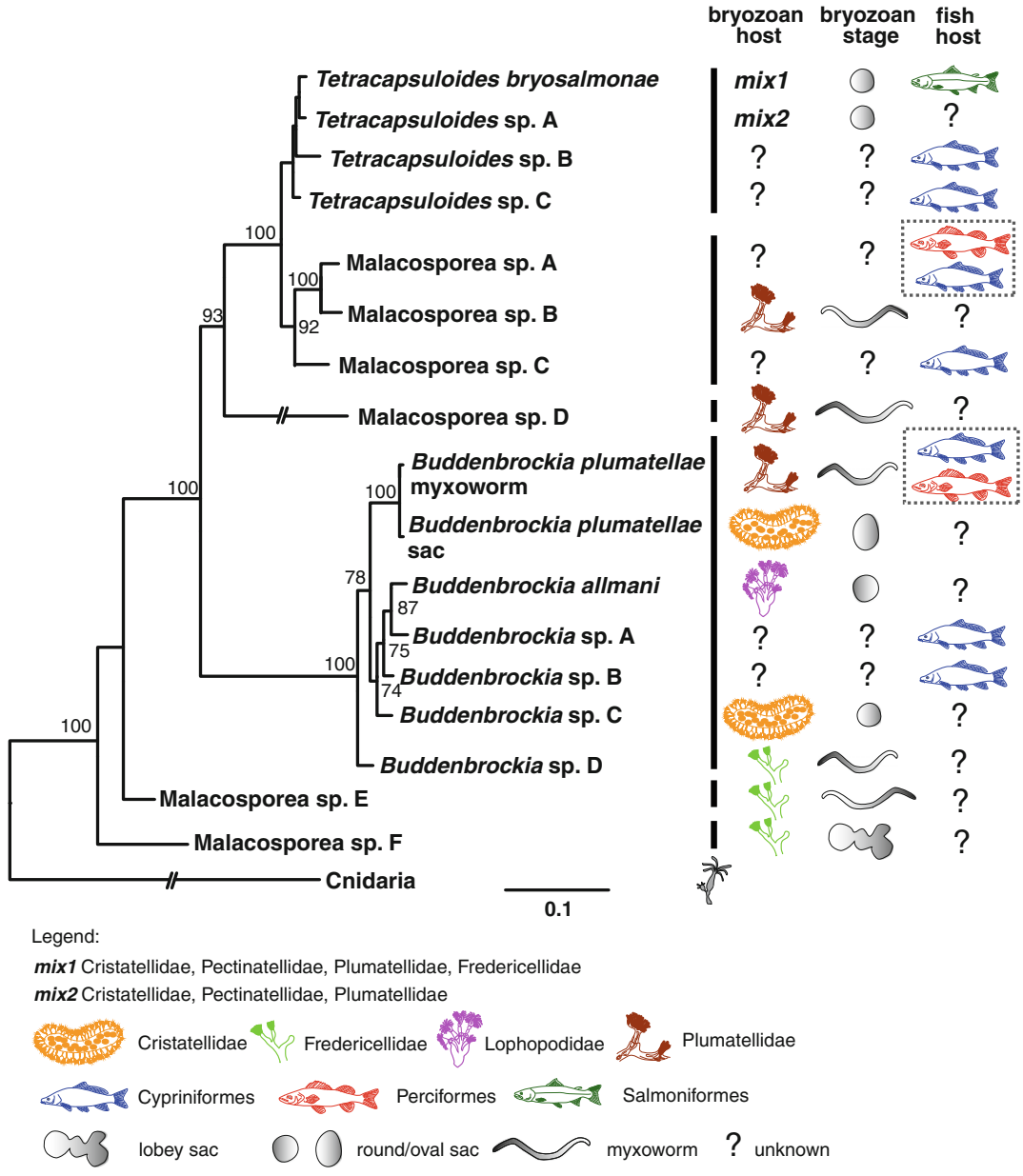

Fig. 4.1 The phylogeny of the Malacosporea based on the maximum likelihood analysis of SSU rDNA data. Bootstrap supports with >50 % shown at nodes. Cnidaria and Malacosporea sp. D branches shortened to 50 % of original length. *Tetracapsuloides* sp. A = *Tetracapsuloides* sp. 1 in Bartošová-Sojková et al. (2014) (BS) and *Tetracapsuloides* spp. in Hartikainen et al. (2014) (H); *Tetracapsuloides* sp. B = *Tetracapsuloides* sp. 4 in BS; *Tetracapsuloides* sp. C = *Tetracapsuloides* sp. 5 in BS; Malacosporea sp. A = *Tetracapsuloides* sp. 3 in BS; Malacosporea sp. B = novel lineage 3 in H; Malacosporea sp. C = *Tetracapsuloides* sp. 2 in BS; Malacosporea sp. D = novel malacosporean lineage in BS and *Buddenbrockia* sp. 4 in H; *Buddenbrockia* sp. A = *Buddenbrockia* sp. 3 in BS; *Buddenbrockia* sp. B = *Buddenbrockia* sp. 2 in BS and *Buddenbrockia* sp. 3 in H; *Buddenbrockia* sp. C = *Buddenbrockia* sp. 1 in H; *Buddenbrockia* sp. D = *Buddenbrockia* sp. 1 in BS and *Buddenbrockia* sp. 2 in H; Malacosporea sp. E = novel lineage 2 in H; Malacosporea sp. F = novel lineage 1 in H

(*Buddenbrockia* sp. D). This suggests that derived freshwater bryozoans (Okuyama et al. 2006) may have served as the first invertebrate hosts of malacosporeans which co-diversified with their hosts. An undescribed lobey sac-like species (Malacosporea sp. F) with an unknown fish host is predicted at the base of the phylogeny (Fig. 4.1). Further sampling is clearly required to substantiate or refute these patterns and predictions.

In view of poor taxon sampling, we can certainly expect malacosporean diversity to be greater than the 16 species revealed so far. The southern hemisphere is particularly undersampled with only one myxoworm found in freshwater bryozoans in Borneo (Hartikainen et al. 2014; Fig. 4.1). Vast regions of the northern hemisphere also remain unsampled. Indeed, early reports of vermiform parasites in freshwater bryozoans, identified at the time as *Buddenbrockia plumatellae,* in Brazil (Marcus 1941), Japan (Oda 1980) and Turkestan (present day Kyrgyzstan; Braem 1911) provide evidence for a global distribution of malacosporeans, many of which are likely to represent new species. The detection of novel malacosporean species with our limited sampling largely within Europe (Bartošová-Sojková et al. 2014; Hartikainen et al. 2014) suggests that greater diversity may even be expected in relatively well-sampled regions. Furthermore, as discussed in Chap. 2 we cannot discount the possibility that there may be undetected malacosporeans in marine habitats in suspension or deposit-feeding marine hosts, such as phoronids or brachiopods, whose body cavities could support the development of the relatively large spore-forming sacs and myxoworms. Hastings (1943) commented on the vermiform bodies found in some marine bryozoan zooids, and although no illustrations are provided, the descriptions do not match closely with myxoworms as seen in freshwater bryozoans. Chapter 2 reviews further studies suggesting the presence of worm-like endoparasites within marine bryozoans, none of which appear to be myxozoans. Marine bryozoans, seem unlikely hosts in view of their compartmentalized and miniaturized zooids that offer little space for parasites to develop.

There are several explanations for the lack of detection of malacosporeans. First, infections in freshwater bryozoans remain covert for much of the year with parasites occurring as cryptic, single cell stages within host tissues that cannot be detected unless polymerase chain reaction (PCR) or histology is conducted (see Chap. 11). Second, infections may be asymptomatic, with infected fish exhibiting no external signs of disease or compromised health. Finally, presporogonic and sporogonic stages in fish (single cells in blood and kidney interstitium and very small pseudoplasmodia in kidney tubules, respectively) may be easily overlooked or not recognised.

4.2.2 Myxosporean Diversification

Reconstruction of myxosporean evolution reveals three well supported lineages: a marine lineage that utilises polychaetes as final hosts, a freshwater lineage that utilises oligochaetes as invertebrate hosts and a lineage comprised of sphaerosporids whose invertebrate hosts remain unknown (Fig. 4.2; Fiala and Bartošová 2010; Bartošová et al. 2013). The position of the sphaerosporid lineage is unclear: it may be sister to all myxosporeans, to the freshwater lineage or to the marine lineage (Holzer et al. 2007; Jirků et al. 2007; Bartošová et al. 2009, 2013; Karlsbakk and Køie 2009). The marine and freshwater myxosporean lineages contain a relatively large number of species and a variety of myxospore morphotypes (see Sect. 4.4.1). In contrast, uniform myxospore morphology of an inferred ancestral sphaerosporid morphotype (Fiala and Bartošová 2010) characterises the sphaerosporid lineage. The number of species in the sphaerosporid lineage is expected to be relatively high due to the high number of nominal *Sphaerospora* species that lack molecular data (Bartošová et al. 2013).

Molecular phylogenetic analyses suggest some correspondence between the main myxosporean lineages with fish host environment and also demonstrate that myxosporeans have shifted between hosts occupying freshwater and marine environments on multiple occasions (e.g.

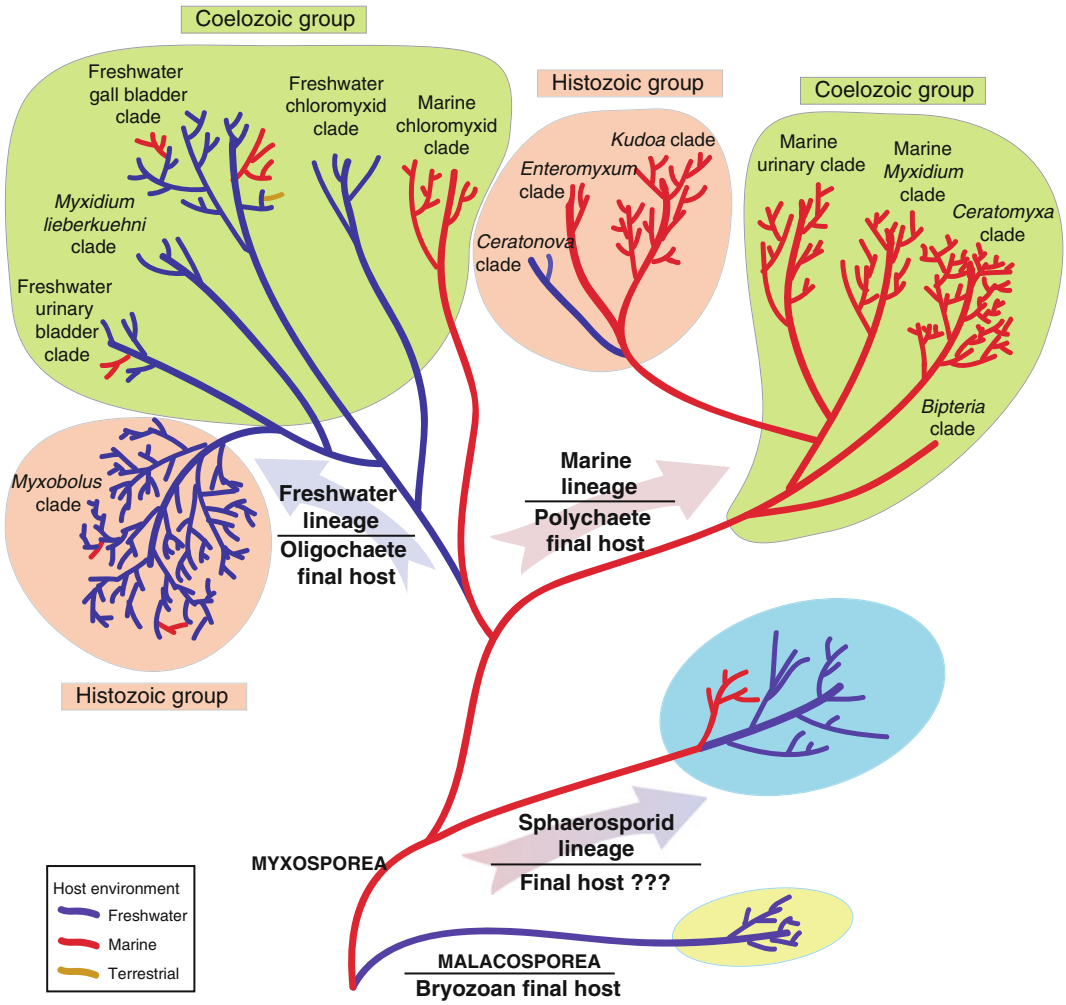

Fig. 4.2 Summary of hypothetical evolution of the Myxozoa inferred from molecular data based on studies of Fiala and Bartošová (2010), Fiala et al. (submitted), Kodádková et al. (submitted)

Kent et al. 2001; Fiala and Bartošová 2010). Figure 4.2 provides an up-to-date summary of hypothetical evolutionary trends of myxosporeans as revealed by mapping host and environmental characters onto molecular phylogenetic data (phylogenies based on those in: Fiala 2006; Fiala and Bartošová 2010; Jirků et al. 2011; Gleeson and Adlard 2012; Bartošová et al. 2013; Fiala et al. 2014, submitted; Kodádková et al. 2015). The early-diverging Malacosporea radiated in freshwaters while early-diverging myxosporeans (the sphaerosporid lineage and the marine chloromyxid and *Bipteria* clades) inhabited the marine environment. Myxosporeans then radiated into many species that comprise the large marine lineage which utilises polychaete worm hosts. One clade of the marine lineage, which currently contains two *Ceratonova* spp., has invaded freshwaters, perhaps via stickleback hosts (Fiala et al., submitted). Myxosporeans in the freshwater lineage use oligochaetes as invertebrate hosts, and we can infer that the ancestor of this lineage invaded freshwaters after the split from the marine chloromyxid clade. Reinvasion of the marine environment has happened several times independently by taxa in this lineage.

Examples include the large clade of typical marine *Sphaeromyxa* species (Kristmundsson and Freeman 2013), two marine *Myxidium* species (Fiala 2006; Kalavati et al. 2013), several *Myxobolus* and *Henneguya* spp. from marine or brackish fish (e.g. Li et al. 2012; Carriero et al. 2013), the marine zschokkellids (Heiniger and Adlard 2014), and the marine *Ortholinea* spp. (Karlsbakk and Køie 2011). Moreover, freshwater myxosporeans have invaded the terrestrial environment with *Soricimyxum fegati* infecting shrews (Prunescu et al. 2007; Dyková et al. 2007, 2011).

Whilst the major myxosporean lineages follow the freshwater-marine and invertebrate host separation (Kent et al. 2001; Holzer et al. 2007), divergences within these lineages appear to be related to tissue tropism in the intermediate vertebrate host (Eszterbauer 2004; Holzer et al. 2004, see also Chap. 16). For instance, clades within both the freshwater and marine lineages contain species that are exclusively coelozoic (infecting the gall bladder or the urinary bladder and kidney tubules) or histozoic (infecting muscles or other tissues) (Fig. 4.2). Unlike myxospore morphology, the site of infection can be linked with myxosporean phylogenies (see later discussion and Chap. 5). For example, species classified as belonging to the genus *Zschokkella* clearly cluster in molecular phylogenies according to their site of infection (gall or urinary bladder) irrespective of their classification according to myxospore morphology. There are no strong phylogenetic affinities within particular myxosporean clades, however some myxosporean radiations have occurred within host families and genera e.g. in the *Ceratomyxa* and *Myxobolus* clade (Gunter et al. 2009; Carriero et al. 2013).

4.2.3 Drivers of Radiations

Although myxozoan diversity is, in general, underestimated, myxosporeans are clearly more speciose than malacosporeans. There are several key factors that may have enabled myxosporeans to establish themselves in new environments and to subsequently radiate. These include: (1) the acquisition of hardened, environmentally-resistant spore valves (soft valves characterise malacosporean spores; Canning and Okamura 2004); (2) reduced rates of uptake and excretion across epithelia present in malacosporean stages in invertebrate hosts; (3) the acquisition of plasmodia that may be better suited to sporulation in organs and tissues of diverse fish hosts and, in some cases, retaining spores until host death (malacosporeans are limited to sporulation in renal tubules of certain hosts and release spores from living hosts via urine); (4) a high diversity of primary hosts which promotes diversification i.e. 3,500 species of oligochaetes and 8,000 species of polychaetes (Ruppert et al. 2004) versus 94 species of freshwater bryozoans (Massard and Geimer 2008); (5) incorporation of additional vertebrate host groups by myxosporeans (to date malacosporeans are only known to infect fish).

4.2.4 Incorporation of Novel Hosts

The common ancestor of the freshwater and marine myxosporeans may have exploited cartilaginous fish as the first vertebrate hosts. This inference is supported by utilisation of a chimaera (*Chimaera monstrosa*) by the early diverging *Bipteria vetusta* (Kodádková et al. 2015) and the utilisation of elasmobranchs by the early diverging marine *Ceratomyxa* clade (which is sister to other members of the marine myxosporean lineage) and the marine chloromyxids (which form a sister group to the freshwater clade) (Fig. 4.2).

There are several cases where myxosporeans adopted vertebrate hosts other than fish and elasmobranchs. Eiras (2005) reported 15 myxosporean species belonging to 6 genera that infect amphibians. Since that time at least six other amphibian-infecting species have been described and the genus *Cystodiscus*, whose members also infect amphibians, has been resurrected (Hartigan et al. 2011). Switching to amphibian hosts has occurred at least three times independently (Kodádková et al. 2015): once in the sphaerosporid lineage (Jirků et al. 2007; Bartošová et al. 2013) and two times in the freshwater myxosporean lineage—once in the *Cystodiscus* clade (Hartigan et al. 2011) and once

in a single species, *Chloromyxum careni* (Jirků et al. 2011). Although only a small number of myxosporean species that infect Amphibia has been described so far, myxosporeans appear to exploit a broad range of amphibian species. For example, *Myxidium serotoninum* is recorded from 37 amphibian species (Eiras 2005). At present it is unclear whether amphibian-infecting myxosporeans are truly generalist parasites or whether they may represent cryptic species assemblages. Also, no complete life cycles are known and potential invertebrate hosts and transmission pathways remain a mystery. Nevertheless, since relatively little research has been conducted on myxozoans parasitic in amphibians, their diversity is likely to be underestimated. This may change as conservation biologists attempt to understand drivers of global declines in amphibian populations (Hartigan et al. 2013).

There are four described myxosporean species from aquatic reptiles (Eiras 2005). Like the amphibian-infecting myxosporeans, at least some of these may be generalists or they may represent cryptic species assemblages. Thus, Johnson (1969) found *Myxidium chelonarum* in 14 of the 21 North American turtle species. Only a single myxosporean species has been recorded so far from birds and is described in ducks (*Myxidium anatidum*; Bartholomew et al. 2008). Similarly, a single species has been encountered in mammals and infects three species of shrews (Prunescu et al. 2007; Dyková et al. 2007, 2011). Myxozoan-like developmental stages, causing xenomas, have been detected in the brain of the mole *Talpa europaea* (Friedrich et al. 2000). However, no spores that would enable parasite identification, were observed. Despite the fact that Myxozoa are not human pathogens the consumption of raw fish meat with myxozoan infection is associated with diarrhoea and *Kudoa septempunctata* was identified as the etiological agent (Kawai et al. 2012). The pathogenicity of *K. septempunctata* was demonstrated in an in vitro experiment on human intestinal cells, which were rapidly invaded by sporoplasms (Ohnishi et al. 2013). Notably, all of these myxosporeans recorded in reptile, bird and mammal hosts appear to have originated independently within the gall bladder clade of the freshwater lineage. Since many myxozoan infections are innocuous there is a reasonable possibility that these myxosporeans are diverse and widespread endoparasites of a variety of vertebrate hosts and are therefore extremely undersampled. In all cases the invertebrate hosts remain unknown (see Chap. 7 for further discussion of myxozoans infecting homeotherms).

Finally, we note that there are several reports of myxozoans in invertebrate hosts other than worms and freshwater bryozoans. For instance, a species of *Kudoa* has been discovered in muscles of giant octopus and produces spores in these molluscan hosts (Yokoyama and Masuda 2001) and a species of *Myxidium* has been described which is capable of infecting and producing spores in monogenean parasites of fish gills (Freeman and Shinn 2011). Observations of myxozoan infections in other gill monogeneans (reviewed in Freeman and Shinn 2011) suggest that hyperparasitism of fish parasites may be an overlooked strategy of myxozoans. However, the dynamics of such infections require further investigation, for instance to determine if monogeneans acquire myxozoan infections through infected fish or vice versa. Early reports of parasites inferred to be myxozoans include *Chloromyxum diploxys* in the lepidopteran *Tortrix viridana* (Thélohan 1895), but inferences based on early studies that lack molecular or ultrastructural confirmation should be viewed with some caution. As argued above for vertebrate hosts, the possibility that myxozoans exploit a diversity of invertebrate hosts remains unclear and merits further investigation. For instance, exploitation of shrews as hosts suggests the possibility that myxosporeans may have radiated to exploit terrestrial oligochaetes. Infections may then be transmitted when vertebrates consume earthworms (see Chap. 7).

4.3 Morphological Simplification and Changes in Body Size

As discussed in Chap. 2, myxozoans demonstrate the most extreme example of morphological simplification relative to their ancestors in any

group of parasites—a trait commonly but not universally associated with parasitism (Poulin 2007). The great reduction in body size that characterises myxozoans is likely to be adaptive for living within restricted host environments, much as occurs in meiofaunal organisms that live interstitially between sand grains (e.g. as in meiofaunal sea anemones; Giere 2009). However, once the plasmodial level of organisation was obtained, 'body size' in some cases has also subsequently increased. Below we examine more specifically the patterns of morphological simplification and variation in body size in the two myxozoan clades.

4.3.1 Malacosporeans: From Worms to Sacs

The *Buddenbrockia* myxoworm displays tetraradial symmetry, characterized by four blocks of longitudinal muscles that are enclosed by external and internal epithelial layers during pre-sporogonic stages of development (Canning et al. 2002; Okamura et al. 2002). The chiral pattern of muscle fibre orientation in *Buddenbrockia* and the connecting cells that are anchored to the extracellular matrix and link muscle blocks are novel myxozoan features that result in helical swimming (Gruhl and Okamura 2012). The sac-forming malacosporeans demonstrate morphological simplification, as they lack muscles and connecting cells as well as the internal epithelial layer that develops in the pre-sporogonic stages of myxoworms. Mature *Buddenbrockia* myxoworms are larger (up to 3.7 mm in length and 100 μm in width; Canning and Okamura 2004) than mature sacs whose longest dimensions are 350, 300 and 700 μm in *Tetracapsuloides bryosalmonae* (Canning et al. 2000), *Buddenbrockia allmani* (Canning et al. 2007) and '*Tetracapsula bryozoides*' (Canning et al. 1996), respectively.

Molecular phylogenetic analyses indicate a striking pattern of repeated transitions between vermiform and sac-like taxa within the Malacosporea (Hartikainen et al. 2014). At present this has apparently occurred at least in: the lineage leading to the *Tetracapsuloides* clade; the *Buddenbrockia plumatellae* clade, and the lineage leading to the clade containing *Buddenbrockia allmani* and three novel *Buddenbrockia* species (species A, B, C) (Fig. 4.1). The low levels of genetic divergence between myxoworms and sacs (Tops et al. 2005; Hartikainen et al. 2014; Fig. 4.1) suggests that the evolution of morphologically simplified sacs may be achieved readily, possibly by modifications of regulatory gene networks, the drivers of which are unclear, but may be associated with e.g. host switching.

4.3.2 Myxosporeans: From Coelozoic to Histozoic Forms

Reductions in body size and complexity reach an extreme level in the Myxosporea, which have entirely lost tissues (but see Chap. 9 regarding apparent tissue loss) and consist of tiny stages comprised of only a few cells that then develop into the sporogonic plasmodial and pseudoplasmodial (both spore-producing) stages. Myxosporeans of the sphaerosporid lineage and myxosporeans of the marine and freshwater lineages associated with early splits in molecular phylogenies are coelozoic and occur in the cavities of organs in fish hosts (Fig. 4.2; Fiala and Bartošová 2010; Bartošová et al. 2013). Coelozoic plasmodia of the early-diverging sphaerosporids are usually very small (10–20 μm) and are mono- or disporic (producing one or two spores), exceptionally tetrasporic (Jirků et al. 2007). Plasmodia of intermediate size (e.g. tens to hundreds of micrometres) may be mono-, di- or polysporic and are produced by coelozoic myxosporeans of both marine and freshwater lineages (e.g. *Ceratomyxa*, *Chloromyxum,* and *Parvicapsula*). Some coelozoic plasmodia can be large (up to several millimetres) (e.g. *Sphaeromyxa*; Kristmundsson and Freeman 2013, *Myxidium* from amphibians; Jirků et al. 2006). Species that infect tissues as histozoic forms evolved from coelozoic species independently in both freshwater and marine lineages. Plasmodia of histozoic myxosporeans often grow to enormous size (up to several millimetres). These large plasmodia can be encased within a fibroblast envelope and are visible as large

cysts in infected tissues (e.g. *Myxobolus*, *Henneguya* and *Kudoa*).

4.4 Diversification of Spores

In this section we review how spores have diversified to display a variety of morphologies, the considerable plasticity of these morphologies, and how spores may be adapted to their environments. Apart from the presence of one versus two sporoplasms and two versus four polar capsules in spores that develop in fish (Hedrick et al. 2004; Morris and Adams 2008) and bryozoan (Canning et al. 2000) hosts, respectively, the soft-bodied spores produced by malacosporeans so far investigated are morphologically indistinguishable. In addition, only a few malacosporean species have been described. Our discussion therefore focuses on spores produced by myxosporeans.

4.4.1 Myxosporean Spore Morphotypes, Drivers of Diversification and Plasticity in Form

Before it was demonstrated that myxozoans are characterised by a complex two-host life cycle (Wolf and Markiw 1984), actinospores and myxospores were regarded as belonging to independent groups of parasites (Actinosporea and Myxosporea). This classification was based on the exploitation of invertebrate and vertebrate hosts and by the morphologically distinct actinospores and myxospores that are produced in these invertebrate and vertebrate hosts, respectively. In the typically triradiate actinospores, valve cells inflate osmotically upon release into the environment producing caudal processes that diverge in different directions. These processes likely reduce sinking rates. Actinospores possess three polar capsules and numerous sporoplasms in a region anterior to the caudal processes. In myxospores the valve cells are hardened and joined by a conspicuous suture. One to four polar capsules and one or two sporoplasms are generally produced in myxospores (Lom and Dyková 2006). The taxonomy of both 'groups' was largely based on variation in spore morphology. As a result, genera or collective groups (morphotypes) of myxosporeans and actinosporeans were recognised (Lom and Dyková 2006). Despite the fact that only a small fraction of myxosporean life cycles (see Chap. 10) has been resolved, it is now clear that several myxospore morphotypes share the same actinospore morphotype (e.g. in *Ceratomyxa auerbachi*, *Ceratonova shasta* (syn. *Ceratomyxa shasta*), *Gadimyxa atlantica*, *Parvicapsula minibicornis,* and *Ellipsomyxa gobii*; Fig. 4.3). This suggests that myxospores may have undergone greater morphological differentiation than actinospores, although further sampling of actinospores is required to confirm this speculation.

The production of morphologically distinct actinospores and myxospores within the same life cycle demonstrates considerable plasticity in spore design and may be related to maximising transmission from fish to invertebrate hosts (myxospores) and from invertebrate to vertebrate hosts (actinospores). Furthermore, it may be inappropriate to equate actinospores and myxospores as homologous stages that are reiterated within a life cycle. For instance, since myxozoans are cnidarians, the two spore types could reflect highly modified medusa and polyp stages and the sporogonic stages that produce them may represent specialised propagative forms such as frustrules (see Chap. 3). Unravelling the molecular basis for the striking morphological variation displayed by myxospores and actinospores is of great interest and could be achieved by transcriptomic studies to identify variation in gene expression repertoires.

Myxospore morphotypes are distinguished by e.g. the number and shape of spore valves, the shape, position and number of polar capsules, the relative position of the suture line and polar capsules, the presence of surface ridges and appendages, and the number of polar filament coils (Feist and Longshaw 2006; Lom and Dyková 2006). Figure 4.4 shows the main myxospore morphotypes that are associated with the majority of myxosporean diversity (i.e. those produced in species of *Myxobolus*, *Henneguya*, *Ceratomyxa*, *Myxidium*, *Zschokkella*, *Chloromyxum*, *Sphaerospora*,

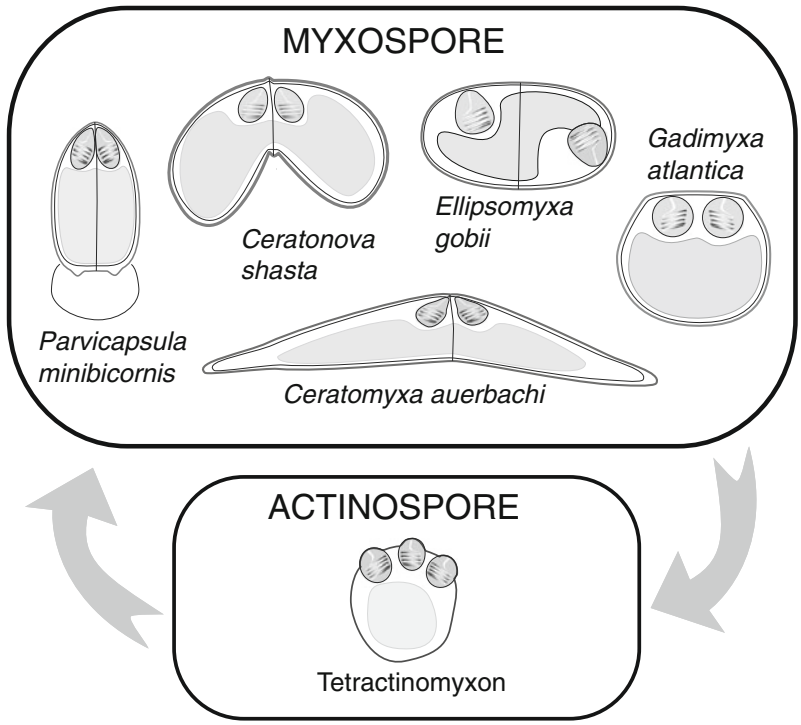

Fig. 4.3 Example of myxosporean species characterised by different myxospore morphotypes but the same actinospore morphotype

Kudoa, *Thelohanellus* and *Sphaeromyxa*) and thus can be considered as most evolutionarily successful. The remaining morphotypes are associated with some 255 species in about 50 genera (Lom and Dyková 2006). These rare morphotypes represent only 10 % of all described myxosporeans but they illustrate the broad range of myxospore morphologies that have evolved (detailed description of all myxozoan genera/morphotypes is provided in the taxonomic key of Chap. 5).

The most common myxospore morphotype is that of *Myxobolus,* a genus which has diversified to more than 800 species histozoic in fish (Liu et al. 2013). Many myxospore morphotypes associated with other tissue-dwelling genera appear to be modifications of this relatively simple morphotype, varying in only minor ways (e.g. loss of one polar capsule, development of spore caudal appendages) (i.e. *Henneguya*, *Hennegoides*, *Unicauda*, *Dicauda*, *Tetraauronema*, *Thelohanellus*, *Neothelohanellus* and *Phlogospora*). The *Myxobolus* morphotype and its variations are thus associated with over 1,100 species—some 50 % of myxosporean species described to date (Lom and Dyková 2006; Liu et al. 2013). The success of the *Myxobolus* morphotype may relate to the lateral flattening of spores that enabled invasion of tissues from precursors that lived in organ cavities and then subsequently radiated to exploit a range of niches offered by different tissues. According to Shulman (1964), tissue-dwelling myxozoans experience mechanical pressures that favour flattened spores (as e.g. in *Myxobolus*) or spores of decreased size and which incorporate strengthening features (e.g. multiple valve cells forming an arch in e.g. *Kudoa*). Such designs were proposed to avoid premature opening of shell valves. However, mechanical pressures in tissues versus organ cavities may not be sufficiently different to drive such variation in form. This is because tissues are comprised of cells and water contributes 70 % to total cell weight. Although water will contribute to a greater percentage of the fluid in organ cavities the pressures experienced in tissues versus organ cavities

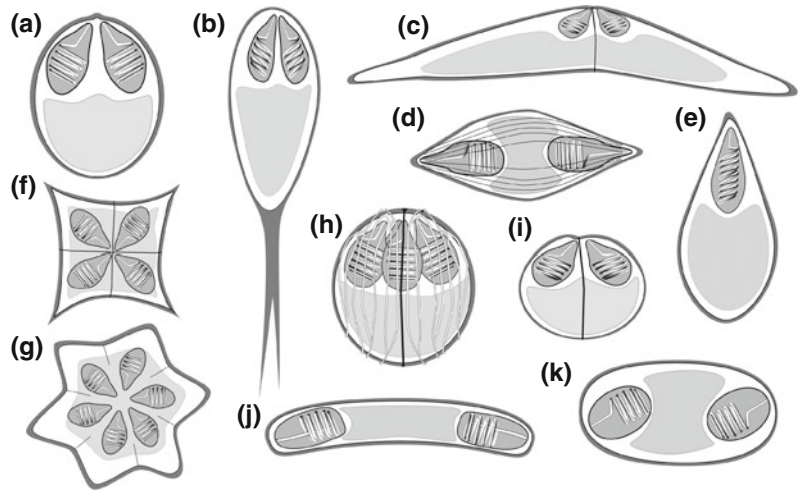

Fig. 4.4 Representatives of genera of major myxospore morphotypes. **a** *Myxobolus*, **b** *Henneguya*, **c** *Ceratomyxa*, **d** *Myxidium*, **e** *Thelohanellus*, **f** *Kudoa* (four valves), **g** *Kudoa* (six valves), **h** *Chloromyxum*, **i** *Sphaerospora*, **j** *Sphaeromyxa*, **k** *Zschokkella*

must be quite similar. The significance of morphologies of spores in tissue-dwelling species may relate more to the maintenance of spore integrity during release from decaying histozoic environments (when fish hosts die) and subsequent spore survival in sediments prior to ingestion by worms.

A typical example of convergence in myxospore morphotypes is exhibited by the myxospores of *Myxidium* and *Zschokkella*, which are both characterised by polar capsules situated at opposite ends of an elongate myxospore (Fig. 4.4d, k). Species of these genera, which parasitise the gall and urinary bladder of marine and freshwater fish, have evolved similar myxospore morphotypes in freshwater and marine lineages several times independently. Perhaps there is some aspect of their convergent myxospore morphologies that suits development in cavity organs although what this is remains obscure. The *Chloromyxum* morphotype represents another case of remarkable convergent evolution with evolutionary reconstruction suggesting multiple origins of this morphotype (Fiala and Bartošová 2010). The success of the *Chloromyxum* morphotype may derive from the development of a large number of polar capsules that may facilitate attachment of spores to hosts and thus enhance transmission.

Many convergent events have been suggested for the *Sphaerospora* myxospore morphotype since distant positions of *Sphaerospora* spp. in molecular phylogenies were indicative of extensive polyphyly (Fiala and Bartošová 2010). However, it has subsequently been determined that PCR amplification of SSU rDNA of a group of sphaerosporids with long inserts (Jirků et al. 2007; Holzer et al. 2007; Bartošová et al. 2013) is problematic. This has led to erroneous results for PCR of samples with mixed myxozoan infections, which, in turn, led to misinterpretations of sphaerosporid evolutionary history. Corrected and additional molecular data have revealed *Sphaerospora* as separate myxosporean evolutionary lineage (Bartošová et al. 2013; Eszterbauer et al. 2013; Holzer et al. 2013). However, there still appear to be a few cases of convergent evolution of myxosporeans with *Sphaerospora* myxospore morphotypes. These include similar spores of *S. testicularis* (which clusters in the marine urinary clade; Bartošová et al. 2011) and of *S. dicentrarchi* (which clusters within multivalvulids; Kent and Palenzuela 2001). A convergent origin of the latter would have entailed loss of the multivalvulid character in some kudoid ancestor giving rise to the sphaerosporid morphotype of extant *S. dicentrarchi*.

Above we have explored how myxosporeans exhibit plasticity in spore morphologies in terms of producing highly distinct spore types at different stages in the life cycle (actinospores and myxospores) and in terms of convergence of spore morphologies to similar myxospore morphotypes. Thus, there appears to be considerable flexibility in the development of spores resulting in morphological variation, which is likely to have some functional significance (see Sect. 4.4.2). Furthermore, it is apparent that morphological change may evolve within very short evolutionary timescales since closely related species can demonstrate substantial variation in spore morphologies. For example, each of the closely related species in the freshwater urinary bladder clade (*Acauda*, *Chloromyxum*, *Hoferellus*, *Myxidium*, *Myxobilatus*, *Ortholinea* and *Zschokkella*) represents highly distinct spore morphotype (Fiala 2006; Karlsbakk and Køie 2011; Whipps 2011) and similar levels of variation amongst myxospore morphologies characterise species in the marine urinary clade (Bartošová et al. 2011; Kodádková et al. 2014). This is in contrast to rather morphologically uniform myxospores produced in the *Ceratomyxa*, *Kudoa* and *Sphaerospora* clades. Further research is required to examine why morphological variation in myxospores may occur in some myxosporeans but not in others.

4.4.2 Morphological Adaptations of Myxosporean Spores as Free-Living Stages

As parasites with complex life cycles, myxozoans have not only evolved to exploit two hosts but during transmission they must be adapted to abiotic factors that spores experience when exposed to the external environment. For instance, hardening of myxospore valves is associated with dormancy. Thus, frog sphaerosporids produce robust myxospores that may have evolved for protection during the period of frog hibernation (Jirků et al. 2007) and the myxospores of *M. cerebralis* may remain viable for many years before they are ingested by oligochaetes (Halliday 1976). As mentioned above, the caudal processes of actinospores that inflate upon release from annelid hosts almost certainly provide a large surface area that prolongs the period of time that spores remain in the water column to enhance transmission to fish. Shulman (1964) suggested that characters that influence spore sinking rates may be some of the most important adaptive features of myxospores, acting similarly to the caudal processes and anchor-like structures of actinospores. Thus, surface ridges and projections such as tails, posterior or lateral protuberances, or bumps increase the surface area of myxospores and may reduce sinking rates thereby enabling the spores to disperse longer distances. Evidence that such features have evolved several times independently (e.g. multiple origins of caudal appendages of *Henneguya* spores) supports the hypothesis that they play an important functional role. Particularly notable surface elaborations include the membranaceous veils on myxospores of deep sea ceratomyxids (e.g. *Palliatus*, *Myxodavisia*; Fiala et al., submitted), and the keel-like or wing-like extensions of five rare myxospore morphotypes produced by *Bipteria*, *Neobipteria*, *Noblea*, *Paramyxoproteus* and *Schulmania* (see review of Lom and Dyková 2006) which may serve as floats for better dispersal.

4.5 Conclusions

Myxozoans adapted to the parasitic way of life by evolving complex two-host life cycles, simplifying their morphology and introducing evolutionary novelties, such as hardened spores and using polar filaments to attach to hosts. During their evolution from cnidarian ancestors, myxozoans fundamentally transformed from highly organised multicellular organisms to very simple myxoworms and sacs, in the case of malacosporeans, and to even simpler plasmodial forms, in the case of myxosporeans. However, in parallel with this reduction in body complexity, myxosporeans have evolved different types of plasmodia and myxospore morphotypes. Myxosporeans appear to have first invaded fish body cavities and later adapted to exploit host tissues. The remarkable

variability of myxospore morphotypes, reflects adaptations to achieve transmission in different host (biotic) and abiotic environments. Further research on myxozoan phylogeny will enable more detailed reconstruction of myxozoan evolution, allowing further inference of some of the key drivers of the adaptive radiation of these extraordinary endoparasitic cnidarians.

4.6 Key Questions for Future Study

- To what degree is the diversity of malacosporeans and myxosporeans underestimated?
- How will genetic data for unsequenced morphotypes influence molecular phylogenies and our understanding of myxozoan radiations?
- How diverse are myxozoans that exploit hosts other than fish?
- Are there special physiological adaptations that enable myxozoans to develop in homeothermic hosts (e.g. birds, shrews)?
- Is the development of actinospores and myxospores controlled by a common gene expression repertoire?
- How can the extensive occurrence of convergence in spore morphologies be explained?

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