Myxozoa

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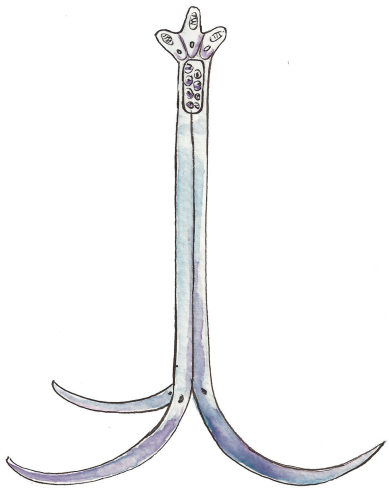

Although recognised as a subtaxon of Cnidaria by most recent phylogenetic analyses, the Myxozoa, with their highly aberrant life cycles, are covered separately herein.

Chapter vignette artwork by Brigitte Baldrian.

A. Gruhl
Natural History Museum,
Cromwell Road, London SW7 5BD, UK
e-mail: a.gruhl@nhm.ac.uk

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INTRODUCTION

Myxozoa are endoparasitic animals exhibiting complex life cycles that in most known cases involve two hosts: a vertebrate (usually fish, but also rarely amphibian, avian, or mammalian) intermediate host and an invertebrate, mostly annelid or freshwater ectoproct (bryozoan), definitive host. Direct fish-to-fish transmission has been demonstrated in only one species (Diamant 1997). About 2,200 species are known, but only about 100 life cycles have been completely resolved. Myxozoans occur in both marine and freshwater habitats; only few exclusively terrestrial life cycles are suspected. For general reviews on Myxozoa, see, e.g. Kent et al. (2001), Canning and Okamura (2004), Feist and Longshaw (2006), Lom and Dyková (2006), and Okamura et al. (2015).

Transmission between the two hosts is accomplished by microscopic spores (Figs. 7.1A–D and 7.2B, C). The spores can have diverse shapes, but the principal morphology is uniform: one or two sporoplasms, constituting the actual infective agent, are encased by a layer of flattened cells called valve cells, which can secrete protective surface coatings and form elaborate floatation appendages. Integrated into the layer of valve cells are two to four (in rare cases one or up to 15) specialised capsulogenic cells, each of which bears one polar capsule, an extrudable organelle that is used for host recognition, contact, and entry. Capsulogenic cells and valve cells are connected by cell junctions (septate and adherens junctions), thus forming a sealing epithelium.

With the revelation of the first complete life cycles (Markiw and Wolf 1983; Wolf and Markiw 1984), older classification schemes, which, based on spore morphology, differentiated actinosporeans (Fig. 7.1D), and myxosporeans (Fig. 7.1C), had to be revised. Instead of representing different taxa, these turned out to be different stages of the same life cycle: actinosporeans are the spores released from the definitive invertebrate host and myxosporeans from the intermediate vertebrate host (Fig. 7.3). In turn, only Myxosporea was continued as a taxon name (Kent et al. 1994); however, “actinosporeans” and “myxosporeans” are still used as technical terms for the invertebrate and vertebrate phases of Myxosporea, respectively. Recently, a further subtaxon was erected and shown to represent the sister group to Myxosporea: the Malacosporea, consisting of only few species so far, including *Tetracapsuloides bryosalmonae,* the causative agent of salmonid proliferative kidney disease (PKD) (Canning et al. 2000), the enigmatic worm *Buddenbrockia plumatellae* (Okamura et al. 2002), and few further lineages. However, malacosporean diversity appears highly underestimated (Hartikainen et al. 2014).

All known malacosporeans exclusively infect fresh water ectoprocts (Phylactolaemata) as their definitive hosts. Malacosporean spores (Figs. 7.1A, B and 7.2B, C) differ from those of myxosporeans (Fig. 7.1C, D) in the fact that they are uncuticularised with more or less similar morphology of both transmission phases. Spore characters are traditionally used for taxonomic purposes; however, molecular data increasingly demonstrate high levels of homoplasy in these traits. Thus, many traditional myxozoan genera and families are polyphyletic (Fiala 2006; Fiala and Bartosová 2010; Bartošová and Fiala 2011; Bartošová et al. 2013).

As opposed to the dormant spores, all myxozoan trophic stages (Fig. 7.1E–J) are exclusively found, either inter- or intracellularly, within the hosts, and exhibit extremely simple morphologies, lacking any form of gut, gonads, clearly recognisable gametes, and even nervous system and sensory organs. In fact, even some general metazoan cytological features such as cilia and centrioles are absent. In general, the trophic stages in invertebrate hosts (Fig. 7.1E, F, and H) are more complex, being delimited by at least one outer epithelial tissue layer, whereas stages in vertebrate hosts are syncytial plasmodia (large multinucleate cells, Fig. 7.1J) or pseudoplasmodia (large uninucleate cells, Fig. 7.1G, I). Uptake of nutrients from the host is in all cases facilitated by endocytosis via the external membrane. A characteristic phenomenon in many stages of the myxozoan life cycle is endogeny, where one cell (the primary cell) completely surrounds another cell (the secondary cell). Sometimes, even tertiary cells occur.

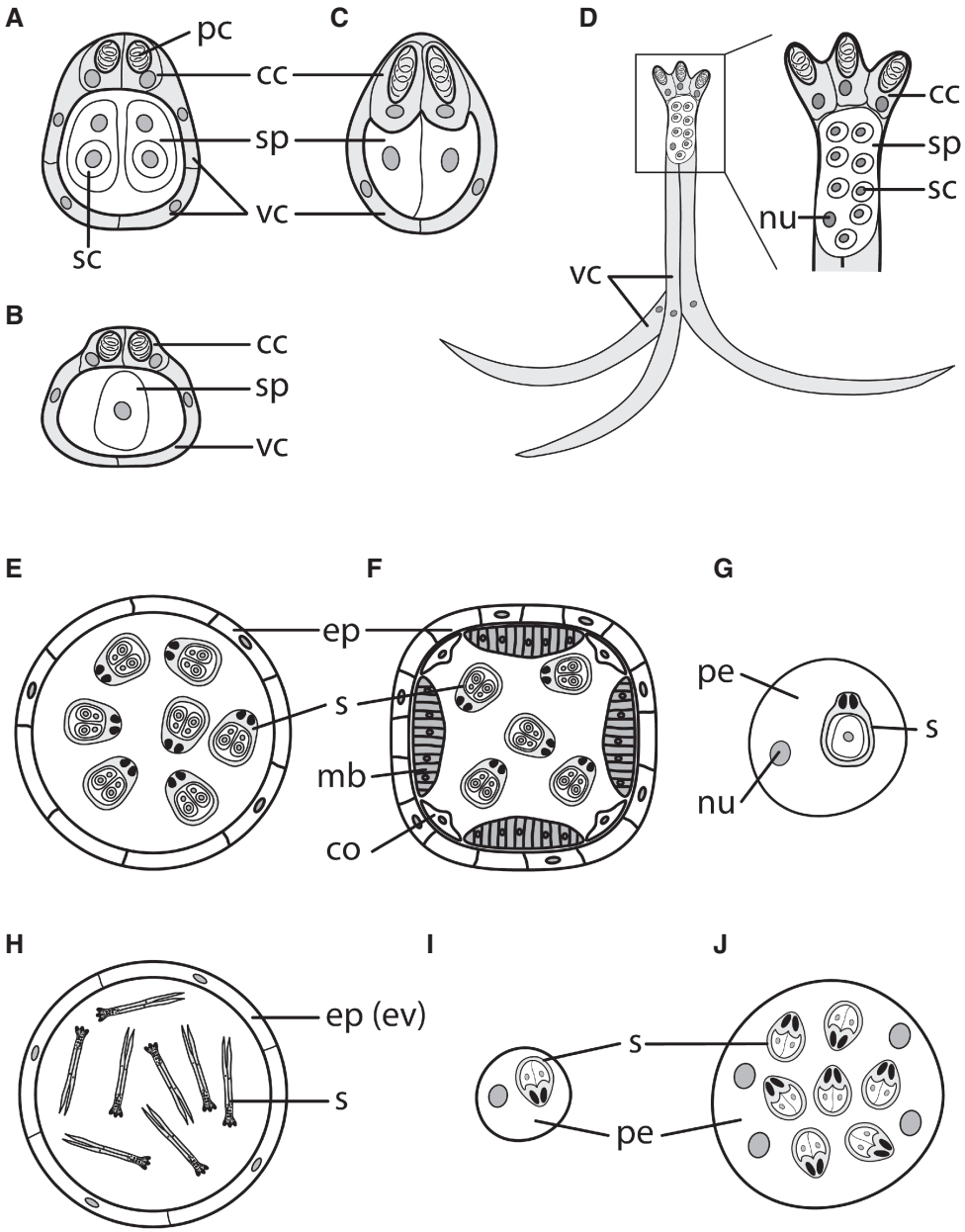


Fig. 7.1 Morphology of myxozoan life cycle stages. (**A**–**D**) Spores. (**A**) Malacospore (malacosporean spore produced in ectoproct host). The spore wall is formed by capsulogenic cells and flattened valve cells. These spores typically contain two sporoplasms, each of which encloses one secondary cell. (**B**) Fishmalacospore containing one uninucleate sporoplasm. (**C**) Myxospore (myxosporean spore produced in fish host) containing two uninucleate or one binucleate sporoplasm(s). (**D**) Actinospores (myxosporean spore produced in annelid host) are usually triradiate with three valves and three capsulogenic cells. Sporoplasms are large uninucleate cells harbouring numerous secondary (germ) cells. (**E**–**J**) Trophic stages, schematic cross sections. (**E**) Saclike malacosporean (e.g. *Tetracapsuloides bryosalmonae*) with epidermis and spores. (**F**) Wormlike malacosporeans (*Buddenbrockia*) with four longitudinal muscle blocks and four rows of connecting cells. (**G**) Malacosporean pseudoplasmodium producing one single spore. (**H**) Myxosporean pansporocyst with eight envelope cells and eight actinospores (the valve cells gain turgescence upon release and acquire the shape depicted in (**D**)). (**I**) Myxosporean pseudoplasmodium producing one single spore. (**J**) Myxosporean plasmodium with multinuclear pericyte producing multiple spores. *cc* capsulogenic cell, *co* connecting cell, *ep* epidermis, *ev* envelope cell, *mb* muscle block, *nu* nucleus, *pc* polar capsule, *pe* pericyte, *s* spore, *sc* secondary cell, *sp* sporoplasm, *vc* valve cell

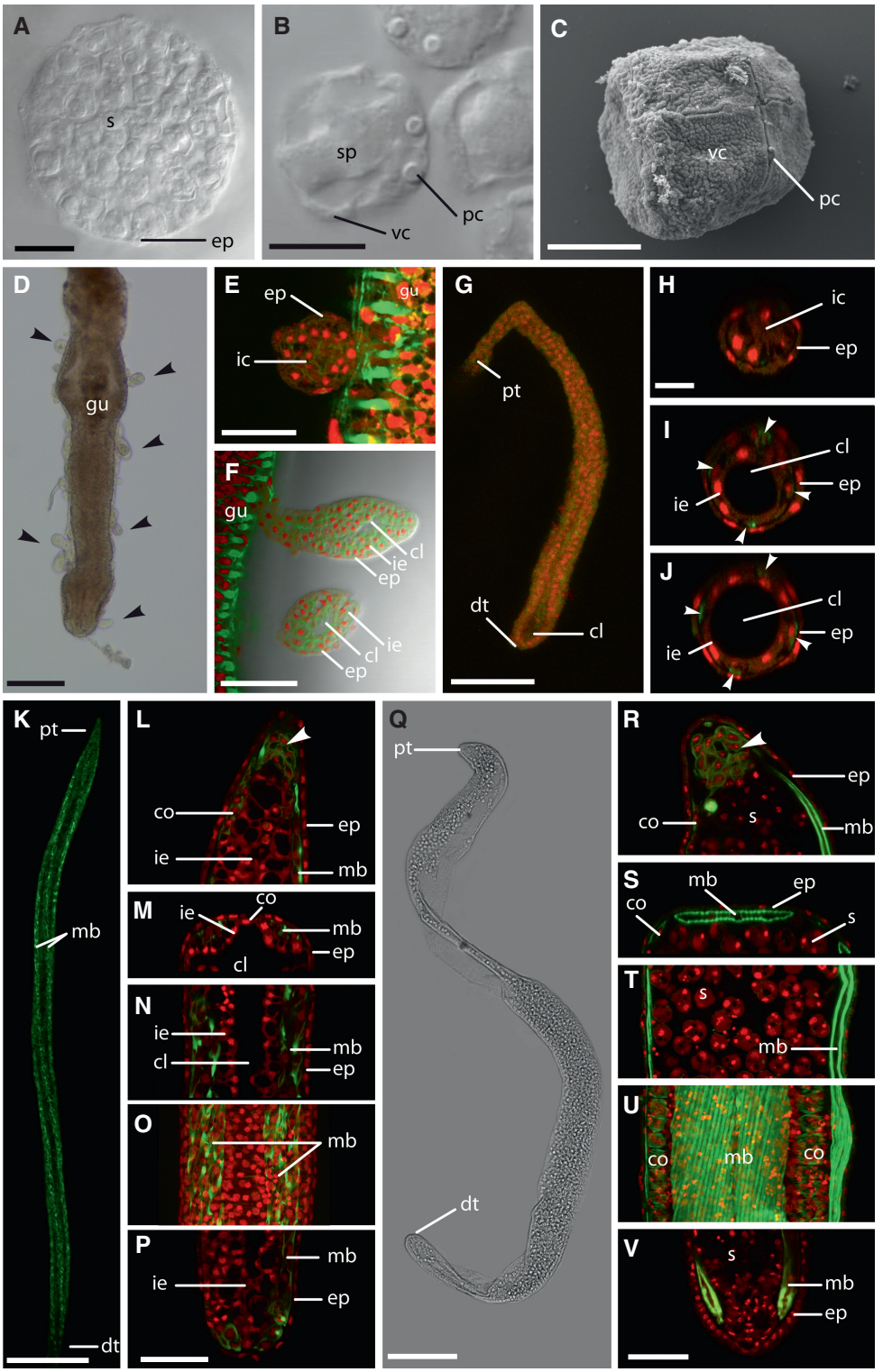
Pansporocysts (Fig. 7.1H), the intra-invertebrate stages of myxosporeans, represent simple, more or less spherical sacs delimited by two to eight cells, the envelope cells, which are flat and interconnected by cell junctions, thus constituting an epithelial layer. In some species, surface extensions of the envelope cells' apical and/or basal membranes occur, indicating transcytotic uptake of nutrients and potential secretion of waste products.

Malacosporean intra-invertebrate trophic stages occur inside the fluid-filled body cavity of fresh water ectoprocts, either as large sacs (*T. bryosalmonae, Buddenbrockia allmani,* Figs. 7.1E and 7.2A) or as worms (*Buddenbrockia plumatellae,* Figs. 7.1F and 7.2Q). Both sacs and worms are lined by an outer epithelium. In *Buddenbrockia* species, a further, internal epithelium exists in juveniles. *Buddenbrockia* worms (*B. plumatella* and a few further, undescribed lineages) exhibit, between the inner and outer epithelium, four longitudinal muscle blocks which span the entire length of the worm (Fig. 7.2K) and are used to facilitate helicoidal movements of the worm. The muscle blocks consist of individual, obliquely arranged elongate muscle cells (Fig. 7.2U). At least two cell types are discernible in the inner epithelium: connecting cells, which reside in a single line between the muscle blocks, and remaining cells, which are considered sporogonic. When the worm matures (Fig. 7.2Q–V), the inner epithelium disintegrates, with the connecting cells remaining in place between the muscle blocks and the sporogonic cells detaching from each other and beginning to float freely in the internal cavity to form spores, a process similar to that in saclike malacosporeans.

Vertebrate stages of both malacosporeans and myxosporeans are plasmodia or pseudoplasmodia bearing internal proliferative or sporogonic cells within them. Plasmodia can become large and often show differentiation into an outer and inner layer. The former is specialised in secretion, endocytotic uptake of nutrients, and defence against host attacks. It differs in cytoplasmic composition from the inner compartment which bears the nuclei, most of the other organelles and the endogenous cells.

For a long time, myxozoans had been considered as protists, due to their simple morphologies. However, with the advent of better microscopic techniques, their metazoan nature became obvious. Connections to both Bilateria and Cnidaria were suggested; recent molecular phylogenetic and phylogenomic analyses along with morphological and protein data now provide convincing evidence that Myxozoa are an ingroup of Cnidaria. Potentially due to their

Fig. 7.2 Malacosporean stages. (**A**) *Tetracapsuloides bryosalmonae* spore sac. Light micrograph. Scale bar: 30 µm. (**B**) *T. bryosalmonae* spore. Light micrograph. Scale bar*:* 10 µm. (**C**) Scanning electron micrograph of a *T. bryosalmonae* spore*.* Scale bar*:* 5 µm. (**D**) Dissected ectoproct gut with attached early stages of *Buddenbrockia* (*arrowheads*). Scale bar*:* 150 µm. (**E–J**) Confocal images (optical sections) of early *Buddenbrockia* developmental stages. *Green* – F-actin, *red* – nuclei. (**E**) Attached spherical stage with beginning segregation into outer epidermis and inner compact tissue. Scale bar: 30 µm. (**F**) Attached elongate stage, developing cavity and inner epithelium at distal end. Scale bar: 50 µm. (**G**) Detached early worm. Scale bar: 50 µm. (**H–J**) Optical cross sections through (**G**), from proximal to distal. Scale bar: 10 µm. (**K–P**) Immature *Buddenbrockia* worm with developing muscle blocks, confocal images. (**K**) Whole mount specimen. Scale bar: 200 µm. (**L**) Proximal tip with cluster of undifferentiated cells (*arrowhead*). (**M**) Cross section through central region. (**N**) Horizontal optical section of central region. (**O**) Maximum intensity projection of central region. (**P**) Horizontal optical section of distal tip. Scale bar for l-p: 30 µm. (**Q–V**) Light and confocal micrographs of a mature *Buddenbrockia* worm. The inner epithelium has disintegrated, and spores are fully developed. (**Q**) Whole-mount specimen. Scale bar: 200 µm. (**R**) Proximal tip with cluster of undifferentiated cells (*arrowhead*). (**S**) Partial optical cross section through central region showing muscle block. (**T**) Optical horizontal section showing inner cavity filled with spores. (**U**) Maximum intensity projection of the central region showing arrangement of muscle fibres. (**V**) Optical horizontal section of distal tip. Scale bar for **R–V**: 30 µm. *cc* capsulogenic cell, *cl* central lumen, *co* connecting cell, *dt* distal tip, *ep* epidermis, *ev* envelope cell, *gu* ectoproct gut, *ic* inner compact cells, *ie* inner epithelium, *mb* muscle block, *nu* nucleus, *pc* polar capsule, *pt* proximal tip, *s* spore, *sp* sporoplasm, *vc* valve cell (Images (**D–V**) from Gruhl and Okamura (2012))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aberrant morphology and high rates of sequence evolution, the exact position within the Cnidaria is not well resolved, the most parsimonious assumption being a sister group relationship to Medusozoa (Staurozoa, Cubozoa, Hydrozoa, Scyphozoa) (Nesnidal et al. 2013; Chapter 6). However, rDNA data still support alternative positions at the base of Bilateria with a sister group relationship to *Polypodium hydriforme*, a further parasitic cnidarian that, however, is likely to be related to leptomedusans (Evans et al. 2008, 2010). Many morphological characters support the metazoan nature of myxozoans, but only few correspondences to Cnidaria exist. Myxozoan polar capsules exhibit striking resemblance to cnidarian nematocysts (Weill 1938; Siddall et al. 1995) that, apart from ultrastructure and genesis, includes molecular architecture, with the presence of cnidarian-specific proteins (minicollagens) (Holland et al. 2011). Further evidence comes from the myoarchitecture of *Buddenbrockia plumatellae*, which shows tetraradial symmetry, a pattern unique to medusozoans (Gruhl and Okamura 2012).

DEVELOPMENT

Due to the complex life cycle and the still unclear state of sexual reproduction, several phases of development can be identified that might correspond to either embryological stages or forms of asexual development found in related cnidarians. Most studies of developmental stages do not have a high temporal resolution, thus, many inferences about developmental processes are based on observations of a few stages only and have to be considered with care.

Development in the Invertebrate Host

Malacosporea

Usually referred to as "sacculogenesis", the development of malacosporeans in the invertebrate host (Fig. 7.3A–G) has been described ultrastructurally in a few publications (Canning et al. 1996, 2000, 2007, 2008; Okamura et al. 2002; McGurk et al. 2006; Morris and Adams 2007a, b; Gruhl and Okamura 2012). The development differs between the species.

The simplest form of development is found in *Tetracapsuloides bryosalmonae,* where unicellular stages firstly occur in the body cavity or attached to the apical surface of the peritoneum of the ectoproct host. These can remain relatively inactive inside the host, facilitating a long-term cryptic infection or, potentially triggered by host condition, begin to proliferate rapidly by division initiating an acute overt infection. Early multicellular stages are clusters of several cells, similar in their ultrastructure to the unicellular stages. These clusters are believed to arise either by aggregation (thus potentially being chimaeras of different genotypes) or by mitosis. Subsequent stages have developed an outer epithelial layer embracing a compact mass of inner cells. Vegetative growth leads to sacs of up to 300 µm in diameter. At some stage, the inner cells undergo sporogony, which, in contrast to the growth, happens rather synchronously. Thus, usually sacs of different sizes but at a similar stage of sporogony are found within one host.

The early development in sac-forming *Buddenbrockia* is essentially similar; however, the inner compact mass differentiates into a transitory epithelium, encompassing a central lumen. Prior to sporogony, this epithelium disintegrates, and cells detach and float freely inside the sac.

The most complex trophic stages are found in the wormlike *Buddenbrockia.* In contrast to the other malacosporeans, early unicellular stages are not free in the host coelom, but within the extracellular matrix (ECM), usually between gut epithelium and peritoneum, but also, more rarely, between epidermis and peritoneum. These stages proliferate by division and form clusters of cells which seem rather unstructured, invading large portions of the ECM. At some point, parts of the multicellular masses penetrate the peritoneum towards the coelom (Fig. 7.2D–F). The part that lies in the coelom becomes bilayered (similar to early stages in the sac-forming species), with an outer epithelial layer and an inner compact mass of cells.

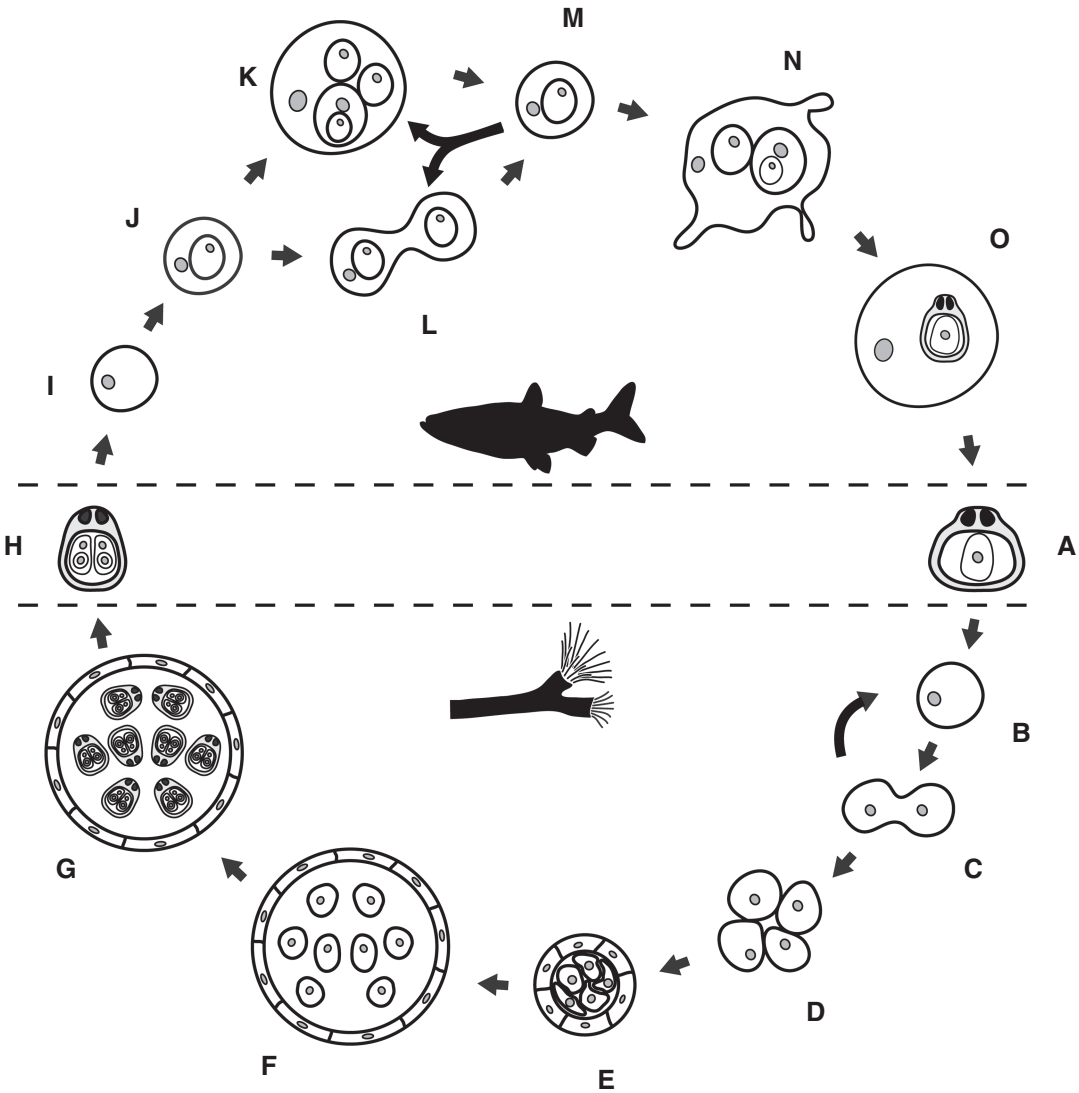

Fig. 7.3 Malacosporean life cycle (exemplified by *Tetracapsuloides bryosalmonae*; see main text for deviations in other malacosporeans). (**A**) Fishmalacospore. (**B**) Sporoplasm enters the ectoproct host via gut epithelium and epidermis. (**C**) Proliferation in the host coelom via mitoses. (**D**) Early cell cluster. (**E**) Early compact bilayered stage. (**F**) Immature sac, sporogonic cells floating freely in inner cavity. (**G**) Mature sac, filled with spores. (**H**) Malacospore. (**I**) Sporoplasm germ enters the fish host via epidermis. (**J**) Endogenic stage with secondary cell. (**K**) Proliferation by mitotic division and release of secondary cells. (**L**) Proliferation by mitotic division of primary and secondary cells. (**M**) Endogenic stage with secondary cell. (**N**) Sporogonic pseudoplasmodium. (**O**) Mature sporogonic pseudoplasmodium ()

The inner cells differentiate further into an inner epithelium and muscle precursor cells, which reside between the two epithelial tissue layers (Fig. 7.2G–J). At some stage, the worms detach from the cell mass in the ECM and swim freely in the host coelom, retaining their initial body polarity. Further growth leads to elongation, differentiation of the musculature, and differentiation of the inner epithelium into connecting cells and sporogonic cells (Fig. 7.2K–P).

Sporogony has not been described in sufficient detail, but so far seems to be similar in all malaco-

sporean species. It starts from individual cells that float more or less freely in the internal cavity and are derived either from a compact mass or from the disintegrating inner epithelium. Two types of cells can be distinguished: larger cells with electron-lucent cytoplasm, and high content of membrane-bound vesicles and smaller, more electron-lucent cells. Meiosis is visible ultrastructurally by the presence of synaptonemal complexes in nuclei of the larger cells. Expulsion of polar bodies has not been directly observed, but in *Tetracapsuloides bryosalmonae*, tiny cells remain within the sac even after spore formation has been completed. Sporogony continues with the smaller cells forming the spore hull and differentiating into capsulogenic cells and valve cells. The larger cells become the sporoplasms. Mature spores comprise two sporoplasms, each consisting of one larger outer and one smaller inner cell (endogenic cell, secondary cell), four capsulogenic cells and eight valve cells. It is currently not clear if fusion of the meiotic products happens during sporogony, i.e. whether some or all spore cells are haploid.

Myxosporea

The development of pansporocysts (Fig. 7.4A–H), especially of the early stages, has been followed in detail only in very few cases (Marques 1986; Lom and Dyková 1992, 1997; Lom et al. 1997; El-Matbouli and Hoffmann 1998; Hallett et al. 1998; Oumouna et al. 2002). Most other studies focus on spore formation; however, occasional reports of early developmental stages are given (e.g. Bartholomew et al. 1997; El-Mansy et al. 1998; Hallett and Lester 1999; Özer and Wootten 2001; Alvarez-Pellitero et al. 2002; Meaders and Hendrickson 2009; Rangel et al. 2009; Morris 2010, 2012a; Marton and Eszterbauer 2012), adding bitwise information to the whole picture.

The most comprehensive study so far is by El-Matbouli and Hoffmann (1998) on *Myxobolus cerebralis* development in the oligochaete *Tubifex tubifex.* Tubificid worms get infected by ingestion of myxospores from decaying fish. Upon contact with the gut lining, the polar capsules discharge, open, and release amoeboid binucleate sporoplasms, which penetrate the gut epithelium and undergo presporogonic proliferation (schizogony) by nuclear division, resulting in multinucleated stages. These undergo plasmotomy and, later, mostly unicellular stages are present. Subsequent stages are complexes of two uninucleate cells which are interpreted as beginning to fuse. Next, binucleate cells are formed which undergo karyotomy, resulting in tetranucleate stages. The initial pansporocysts are complexes of four uninucleate stages thought of having arisen from the tetranucleate condition by plasmotomy. Two of these cells are in a more peripheral position and envelope the other two cells by formation of membrane protrusions and cell-cell junctions. Mitotic divisions lead to pansporocysts, which are lined by eight envelope cells and contain eight α- and eight β-cells. α- and β-cells differ slightly in size and each population is interpreted as deriving from one of the two initial internal cells. Subsequently, they undergo meiosis (as evidenced by occurrence of synaptonemal complexes), expelling three polar bodies each. Eight complexes of each one α- and one β-cell form which fuse and result in eight zygotes.

The next phase is sporogony: the envelope cells remain unchanged, and the zygotes divide twice to form clusters (sporoblasts) of one central and three peripheral cells. The peripheral cells undergo one further division, and of these six cells, three become capsulogenic and three valve cells. The central cell is the prospective sporoplasm and divides asymmetrically (endogenically) to form a complex of one outer and one inner (generative cell). The inner cell gives rise to up to 64 germ cells. Spores are released by rupture into the intestinal tract of the tubificid worm.

Deviations from the above pattern have been reported. In many species, only two or four cells constitute the sporoplasm envelope. Early stages have been described differently. For an *Aurantiactinomyxon,* Lom et al. (1997) described no tetranucleate or four cell stages, but the pansporocyst starting from an endogenic (primary/secondary) cell. However, tetranucleate stages have been described for *Aurantiactinomyxon* and *Raabeia* (Oumouna et al. 2002). Hallett and Lester (1999) described the genus *Tetraspora* in which only four spores develop in one pansporocyst.

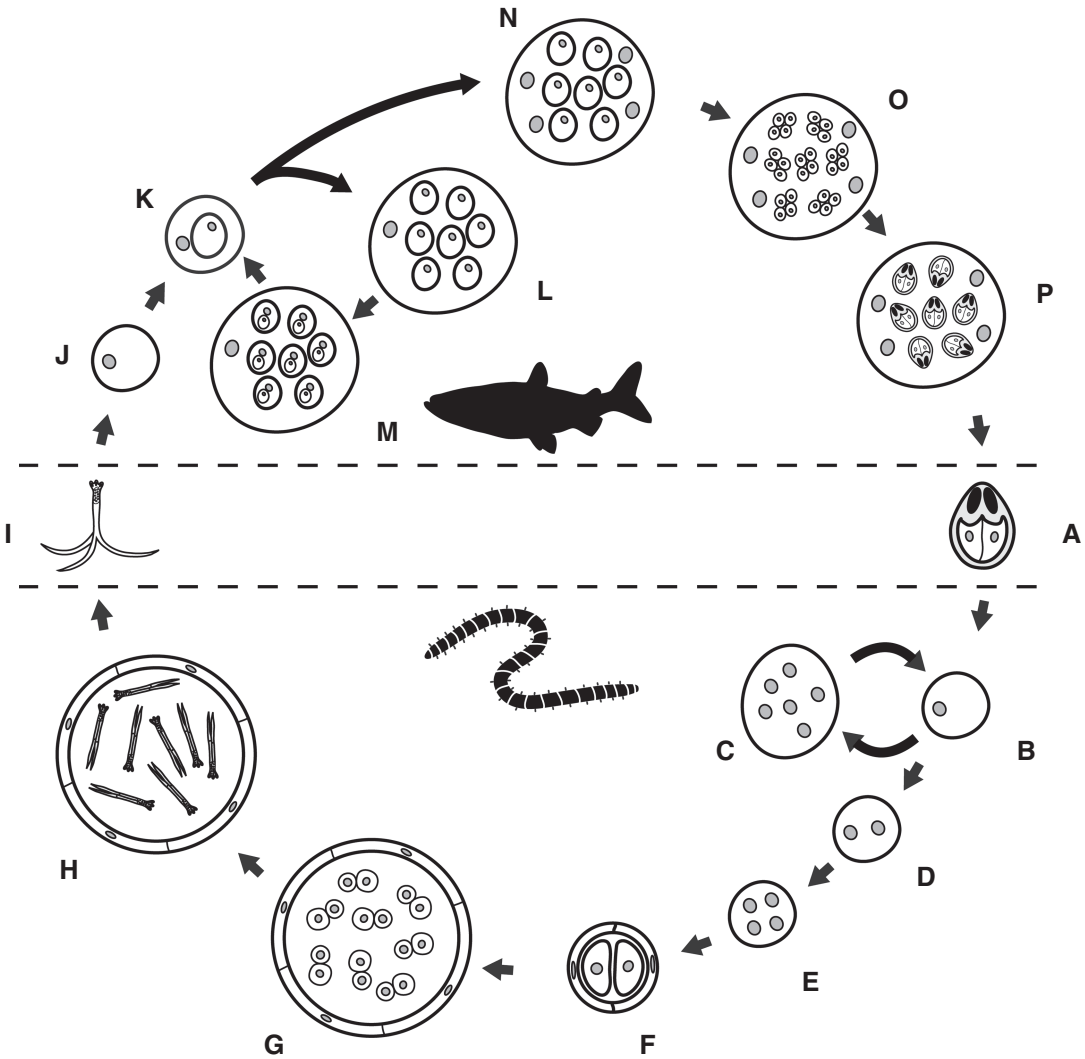

Fig. 7.4 Myxosporean life cycle (mostly adopted from *Myxobolus cerebralis*; see main text for deviations in other myxosporeans). (**A**) Myxospore. (**B**) Sporoplasm enters the annelid host via gut epithelium or epidermis. (**C**) Extrasporogonic proliferation by nuclear divisions followed by plasmotomy. (**D**) Binucleate cell. (**E**) Tetranucleate cell. (**F**) Early pansporocyst consisting of two internal and two envelope cells. (**G**) Pansporocyst with fusing α- and β-cells, resulting in eight zygotes. (**H**) Mature pansporocyst containing eight actinospores. (**I**) Actinospore. (**J**) Sporoplasm germ cell released from sporoplasm after penetration of host epidermis. (**K**) Endogenic (primary/secondary) stage. (**L**, **M**) Extrasporogonic proliferation by division of secondary cells and formation and release of secondary/tertiary cell doublets. (**N**) Sporogonic multinucleate plasmodium containing several secondary cells. (**O**) Formation of sporoblasts by division of secondary cells. (**P**) Mature sporogonic plasmodium

Development in the Vertebrate Host

Malacosporea

Malacosporean development within the vertebrate (fish) host (Fig. 7.3H–O) has only been studied in *Tetracapsuloides bryosalmonae* (Kent and Hedrick 1986; Morris and Adams 2008). The entry portals seem to be thin parts of the epidermis and mucus cells (Grabner and El-Matbouli 2010). The earliest stages visible are unicellular stages, most likely representing the inner (secondary) cells of the sporoplasms. Shortly after infection, typical cell doublets consisting of one primary and one internalised

secondary cell occur. Multiple extrasporogonic proliferation cycles prior to spore formation seem to be the norm and take place in the kidney interstitium with the parasite stages engulfed by host phagocytes. Proliferation involves division of secondary cells followed by division of primary cells. The maximum number of secondary cells found in these stages is three (Morris and Adams 2008), with rare findings of tertiary cells.

Sporogonic stages migrate into the kidney tubules, where larger so-called pseudoplasmodia are formed. These are essentially large pericytes, which harbour several secondary cells and secondary/tertiary cell doublets. The cytoplasm of the outer primary cell differs from that of the extrasporogonic stage in the absence of sporoplasmosomes, secretory vesicles characteristic for many myxozoan cells. Sporogony commences by the secondary cells developing into sporoplasms and the secondary/tertiary cell complexes differentiating into capsulogenic cells and valve cells. Each pseudoplasmodium produces a single so-called fishmalacospore consisting of one sporoplasm cell, two capsulogenic cells and four valve cells. The spores are released from the fish via the urine.

Myxosporea

Early development and sporogony of myxosporeans (Fig. 7.4J–P) have been described extensively especially in economically important species. However, many studies are based on pathological results, and exact developmental sequences are sometimes difficult to infer.

The fish host is infected by the actinospore attaching to the host and the sporoplasm penetrating the epidermis. In most cases, the outer cell disintegrates soon to release the sporoplasm (germ) cells, which then spread into the species-specific target tissues. In most cases, extrasporogonic proliferation cycles precede spore formation. These can happen in a range of different ways: one extreme would be represented by the way as described for malacosporeans (i.e. by mitotic division of secondary cells, followed by division of the primary cells). The other extreme would be the formation of large complex stages which harbour large numbers of secondary cells and secondary/tertiary cell doublets which are either released successively or by breakdown of the large pericyte. Many species undergo several extrasporogonic proliferative cycles, each in a different host tissue.

Sporogony takes place in small pseudoplasmodia that produce only one or few spores, or in larger multinucleate plasmodia. In the latter, two pathways are common. In the first, the spores are produced individually within so-called pansporoblasts, which are essentially cell doublets where the secondary cell undergoes several mitotic divisions to form sporoplasm, valve, and capsulogenic cells. Alternatively, a large population of secondary cells is present, which separately develop into sporoplasms, valve cells, or capsulogenic cells. The differentiated cells then aggregate to form the spores.

EXPERIMENTAL AND GENE EXPRESSION STUDIES

In situ hybridization studies focussing on developmental gene expression are lacking so far for myxozoans. However, a survey of genes expressed during spore activation has been conducted (Eszterbauer et al. 2009), the first myxozoan genome has been published (Yang et al. 2014), and several transcriptome and a few genome sequencing projects are currently on the way. Light and electron microscopic techniques are well established and other procedures, such as confocal microscopy, fluorescence staining, and antibody labeling, have been successfully applied (e.g. Alama-Bermejo et al. 2012; Gruhl and Okamura 2012). At least for a few species, partial replication of the life cycle in the laboratory is possible (Tops and Okamura 2003; Tops et al. 2004; Hartikainen et al. 2009; Kumar et al. 2013), and attempts for in vitro culture have been made (Morris 2012b). Thus, although myxozoans are comparatively difficult to access, possibilities to tackle more EvoDevo questions using experimental and molecular techniques are within reach.

OPEN QUESTIONS

- How is the myxozoan life cycle related to that of free-living cnidarians/medusozoans?
- Where and how does sexual reproduction and outcrossing happen in the myxozoan life cycle?
- What are the mechanisms that have caused extreme body simplification and loss of cytological features in myxozoans?
- How do mechanisms of tissue specification differ within myxozoans and between myxozoans and other cnidarians?
- Is myxozoan body plan diversity underestimated?
- When and where are key developmental genes such as Hox, ParaHox, and other homeobox genes expressed in the various myxozoan life cycles, and what are their roles?

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