Evolution, Origins and Diversification of Parasitic Cnidarians

Beth Okamura*, Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom. Email: b.okamura@nhm.ac.uk

Alexander Gruhl, Department of Symbiosis, Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany

*Corresponding author

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Abstract

Parasitism has evolved in cnidarians on multiple occasions but only one clade – the Myxozoa – has undergone substantial radiation. We briefly review minor parasitic clades that exploit pelagic hosts and then focus on the comparative biology and evolution of the highly speciose Myxozoa and its monotypic sister taxon, *Polypodium hydriforme*, which collectively form the Endocnidozoa. Cnidarian features that may have facilitated the evolution of endoparasitism are highlighted before considering endocnidozoan origins, life cycle evolution and potential early hosts. We review the fossil evidence and evaluate existing inferences based on molecular clock and co-phylogenetic analyses. Finally, we consider patterns of adaptation and diversification and stress how poor sampling might preclude adequate understanding of endocnidozoan diversity.

1 Introduction

Cnidarians are generally regarded as a phylum of predatory free-living animals that occur as benthic polyps and pelagic medusa in the world's oceans. They include some of the most iconic residents of marine environments, such as corals, sea anemones and jellyfish. Cnidarians are characterised by relatively simple body-plans, formed entirely from two tissue layers (the ectoderm and endoderm), and by their stinging cells or nematocytes. Nematocytes are unique to Cnidaria and function primarily for prey capture and defense. Phylogenetic analyses identify cnidarians as early diverging metazoans and sister to Bilateria, with the Ctenophora and Porifera variously placed as earlier diverging sister lineages to Metazoa (e.g. Ryan et al. 2013; Simion et al. 2017; Whelan et al. 2017). Accordingly, cnidarians have a convincing fossil record dating from the early Cambrian (e.g. Dong et al. 2013) and, as discussed later, with probable representation even earlier in the Ediacaran Period.

Although mainly viewed as marine animals, a few cnidarians have invaded freshwater habitats (Jankowski et al. 2008), including the model organism *Hydra*. In addition, parasitic lifestyles have been adopted on several occasions by different cnidarian lineages. One parasitic group in particular – the Myxozoa – has undergone extensive radiation as endoparasites with complex life cycles, exploiting invertebrate and vertebrate hosts. According to the most recent estimate, myxozoans represent some 20% (2596/14,355) of all described cnidarian species (Okamura et al. 2018), a proportion expected to rise further in view of extensive undersampling.

Here we review the evolution, origins and diversification of parasitic cnidarians. We first describe the variety of cnidarian parasites known to date and highlight cnidarian features that may be generally conducive for adopting parasitic lifestyles. We then focus on the major clade of parasitic cnidarians, the Endocnidozoa, which contains the diverse Myxozoa and the monotypic *Polypodium hydriforme*. This leads us to consider more explicitly pathways to endoparasitism, origins and early hosts, and patterns and drivers of diversification within the Endocnidozoa.

2 Parasitic cnidarians other than endocnidozoans

According to current views of cnidarian systematics (Figure 2; Kayal et al. 2018) parasitic forms have evolved at least twice in Anthozoa (Rodriguez et al. 2014) and perhaps twice or more in Hydrozoa (Bentlage et al. 2018; Table 1). In all cases parasitic stages are associated with pelagic animal hosts. They have been described in the distantly-related burrowing anemone families, Edwardsiidae and Haloclavidae (Rodriguez et al. 2014), and in families belonging to the hydrozoan orders Narcomedusae and Anthoathecata (Collins et al. 2008; Bentlage et al. 2018). Infection is likely generally to occur via the larval (planula) stage (Boero and Bouillon 2005) with parasites then undergoing further development (Table 1). For example, larvae of the anthozoan *Peachia* develop to polyps on their medusa hosts which then drop off to take up benthic existence. Polyp stages of hydrozoan narcomedusae develop as endoparasites in medusa and polychaete hosts prior to assuming life as free-living medusae. Other hydrozoans develop as ectoparasitic colonies on fish, copepods and pteropods during the polyp phase of the life cycle. It is argued that the anthozoan *Edwardsiella lineata* develops in the digestive cavity of ctenophores as a novel life history stage. The latter is inferred on the basis of a unique combination of features (no cilia or tentacles but possessing a pharynx, retractor muscles and mesenteries), tissue remodelling (including apoptosis),

and a clear shift in the ecological niche between the parasitic and free-living life history stages (Reitzel et al. 2006).

The number of cnidarian species currently recognised to include parasitic stages (Table 1) is probably underestimated (Appeltans et al. 2012). Parasitic narcomedusans are particularly likely to be poorly known as their hosts are relatively infrequently sampled open ocean animals (often from the deep sea), they may be overlooked due to their inconspicuous nature, and they may occur at low prevalences of infection. It is possible that parasitic stages may be linked in future with narcomedusan species previously thought to be entirely free-living. Nor would it be surprising if further entirely new species with parasitic stages are detected, especially in poorly sampled habitats such as the deep sea and polar regions (Okamura et al. 2018). However, it may also be the case that our current understanding is compromised. For example, taxa described long ago as distinct genera may belong to a common genus (e.g. the pteropod-infecting taxa *Perigonella*, *Pandea* and *Kinetocodium*; Table 1). Alternatively, taxa currently recognised as different could be part of the same life cycle (e.g. if a broad range of hosts is exploited or distinct parasitic stages have evolved).

Despite caveats regarding sampling effort and taxonomic uncertainties, parasitic cnidarians other than endocnidozoans have apparently undergone little radiation and they are not associated with markedly long branches in phylogenetic trees (e.g. Rodriguez et al. 2014; Bentlage et al. 2018). In all cases the number of described species within lineages is ≤ 11. Thus even if we assume that all species of e.g. *Edwardsiella* incorporate a parasitic phase they would only amount to some 0.08% (11/14,355) of currently described cnidarian species diversity. The non-endocnidozoan lineages of cnidarians that incorporate parasitic stages in their life history therefore appear to have evolved fairly recently and to have undergone modest to minimal radiation.

3 The Endocnidozoa

As the name implies, the Endocnidozoa is comprised of endoparasitic cnidarians. This recently recognised clade incorporates the sister taxa *Polypodium hydriforme* (henceforth referred to as *Polypodium*) and the diverse Myxozoa (Collins 2009; Chang et al. 2015; Kayal et al. 2018). For a considerable time period long-branch attraction obscured phylogenetic placement of both Myxozoa and *Polypodium* in molecular phylogenetic analyses (Zrzavý and Hypša 2003; Foox and Siddall 2015, Okamura and Gruhl 2015). However, phylogenomic (Chang et al. 2015; Kayal et al. 2018) and some morphological (e.g. Siddall et al. 1995; and see below) evidence currently places these as sister taxa comprising the Endocnidozoa, which itself is sister to the Medusozoa (Figure 2).

3.1 General biology

Polypodium's one-host life cycle includes a free-living adult phase and parasitic larval stages in acipenseriform fish (sturgeon and paddlefish). Myxozoans have complex parasitic life cycles and require both invertebrate and vertebrate hosts for development. Invertebrate hosts include freshwater (phylactolaemate) bryozoans (exploited by the Malacosporea) and marine and freshwater oligochaetes and polychaetes (exploited by the Myxosporea) (Fiala et al. 2015a). Myxosporean infections (including spore production) reported in octopus (Yokoyama and Masuda 2001) and in a monogenean infecting fish (a case of hyperparasitism) (Freeman and Shinn 2011) suggest that other invertebrate hosts may at least occasionally be exploited. By far the greatest number of recognised vertebrate hosts of myxozoans are fish (including representatives of both

Table 1. Details for parasitic cnidarians (excluding Endocnidozoa) including higher taxonomy (Class, Order, Family according to WoRMS Editorial Board 2018), genus (and number of described species according to WoRMS Editorial Board 2018), hosts, examples of lifestyles, and inferred life history stages of parasitic forms (numbered references as superscripts). Note that the life cycles of many cnidarians are poorly known (Collins et al. 2008) thus it is unclear whether all species within the listed genera include parasitic stages. Also note that the narcomedusan families Cuninidae and Solmarisidae are polyphyletic (^{P}) and recent phylogenetic analysis (Bentlage et al. 2018) indicates that parasitic members of both families are each other's closest relatives.

Higher taxonomy	Genus	Hosts	Example lifestyles	Life history stages	References
Anthozoa, Actiniaria, Edwardsiidae	*Edwardsiella* (11)	Ctenophores	*E. lineata* positioned along pharynx or in ciliated region near esophagus as vermiform stages with oral end inside digestive cavity feeding on pre-digested material by ciliary currents[1]	Novel stage[1]	Reitzel et al. 2006
Anthozoa, Actiniaria, Haloclavidae	*Peachia* (11)	Hydrozoan and scyphozoan medusae	*P. quinquecapitata* initially parasitic in gastrovascular system (feeding on pre-digested material like *E. lineata*[1]) then moving to and replacing gonad of medusa host[2]; *P. parasitica* attached by expanded mouth to subumbrella or tissues of host[3]	Larvae and pre-adults (larvae mature to adult anemones that drop off host)[2]	Spaulding 1972; McDermott et al. 1982
Hydrozoa, Narcomedusae, Cuninidae^{P}	*Cunina* (11)	Hydrozoan medusae	Parasitic in gastrovascular system[4,5], also attached to and apparently replacing gonad[5]	Larvae[4]; Polyps[5]	Boero & Bouillon 2005; Bentlage et al. 2018
Hydrozoa, Narcomedusae, Solmarisidae^{P}	*Pegantha* (5)	Polychaetes (e.g. *Tomopteris*)	Polyps of *P. martagon* attached to peritoneum of *Tomopteris* and inferred to absorb nutrients from coelomic fluid (as there is no mouth), budded medusa released from polyps into coelomic cavity of host[5]	Larvae[4]; Polyps + early medusae[5]	Boero & Bouillon 2005; Bentlage et al. 2018

Table 1 (continued)

Higher taxonomy	Genus	Hosts	Example lifestyles	Life history stages	References
Hydrozoa, Anthoathecata, Pandeidae	*Hydrichthys* (6)	Fish, copepod	Attached to and eroding fish surface, tentacle-less polyps bend and mouth sucks in blood and tissues[4,6]; also attached to copepods parasitic on mesopelagic lanternfish[7]	Polyps[6]	Boero & Bouillon 2005; Boero et al. 1991; Moser & Taylor 1978.
	Larsonia (1)	Fish	Attached to and eroding fish surface, tentacle-less polyps bend and mouth sucks in blood and tissues[6]	Polyps[6]	Boero et al. 1991
	Perigonella (1)	Pteropods	*P. sulfurea* polyps attach to pteropod shells and feed on epithelia and embryos[4]	Polyps[4]	Boero & Bouillon 2005
	Pandea (4)	Pteropods	*P. conica* polyps attach to pteropod shells and feed on epithelia and embryos[4]	Polyps[4]	Boero & Bouillon 2005
Hydrozoa, Anthoathecata, Incertae sedis	*Kinetocodium* (1)	Pteropods	*K. danae* polyps attach to pteropod shells and feed on epithelia and embryos[4]	Polyps[4]	Boero & Bouillon 2005

subclasses of primitive cartilaginous fishes and a broad range of derived bony fish; Lom and Dykova 2006; Kodádková et al. 2015), but myxozoans also infect reptiles (turtles and tortoises), waterfowl (ducks), small mammals (shrews and probably moles) (Lom and Dykova 2006; Hallett et al. 2015) and all orders of amphibians (Hartigan et al. 2016).

The free-living *Polypodium* stage emerges from spawned eggs of acipenseriform fish as chains or stolons of budded but connected tentaculate individuals. The 'stolons' fragment into individual buds that take up benthic life, actively feeding and undergoing growth and fission during summer months (Figure 1). Reproductively mature individuals produce a specialised multicellular stage derived from gonadal tissue (Raikova 1994, 2008) that enables infection following direct contact with larval fish. Post-invasion infection dynamics are unknown until fish become reproductively mature, at which stage the development of *Polypodium* within fish eggs has been characterised (Raikova 1994). Larvae and budding stolons inside the eggs have inverted germ layers, an inner ectoderm and outer endoderm – a condition that is reversed prior to emerging from fish eggs. Stolons are liberated from eggs in the oviducts of spawning fish (Raikova 2002). *Polypodium* infections have been recorded in 78% of mature female sterlet in the Volga and Kama Rivers and up to 100% of eggs per female can apparently be infected (Raikova 1994) – a cause for concern given potential impacts on caviar production. The lineage is currently regarded as monotypic although sequence divergence in housekeeping genes has recently been revealed between North American and Russian isolates (Hartigan et al. unpublished data).

Myxozoans exploit invertebrate and vertebrate hosts that act as definitive and intermediate hosts, respectively (Figure 1). Multicellular spores released from hosts into the environment to achieve transmission are non-feeding and metabolically inactive. The early-diverging major myxozoan clades, the Malacosporea and Myxosporea, differ in invertebrate host use and morphological complexity (Fiala et al. 2015a, Gruhl 2015). In their freshwater bryozoan hosts, malacosporean sporogonic (spore-producing) stages develop in the host coelomic cavity as sacs (~ 300-700 μm in diameter) and 'myxoworms' (up to ~ 3 mm in length) that exhibit clearly recognisable epithelial layers and muscle systems (the latter only in myxoworms) (Feist et al. 2015, Gruhl 2015). Multicellular spores that achieve transmission to fish are produced within the hollow spaces of sacs and myxoworms. In contrast, malacosporean sporogonic stages that develop in fish kidney (called pseudoplasmodia) are comprised of a single cell (the so-called primary cell) within which multicellular spores infectious to bryozoans are produced. Malacosporeans exploit a broad range of freshwater bryozoans (Hartikainen et al. 2014) and infections have so far been detected in fish hosts in the families Salmonidae, Cyprinidae and Percidae (Grabner and El-Matbouli 2010; Bartošová-Sojková et al. 2014; Naldoni et al. 2019), however some fish in these families may be accidental hosts because spore development has not always been demonstrated.

Myxosporean sporogonic stages in annelids, called pansporocysts, are comparable to malacosporean sacs, but have an outer lining that is made up of only eight cells (El-Matbouli and Hoffmann 1998). These cells are extremely thin and show hardly any epithelial characteristics. Pansporocysts are very small (10-100 μm range) and develop in the epidermis, gut epithelium, and coelomic cavities of annelid hosts (Lom and Dykova 1997; Gruhl 2015). Myxosporean sporogonic stages in vertebrate hosts are either plasmodia (unicellular, multinucleate forms) or pseudoplasmodia (unicellular, uninucleate forms) within which multicellular spores develop. Spores produced by malacosporeans in invertebrate and vertebrate hosts (malacospores and fish malacospores, respectively) are

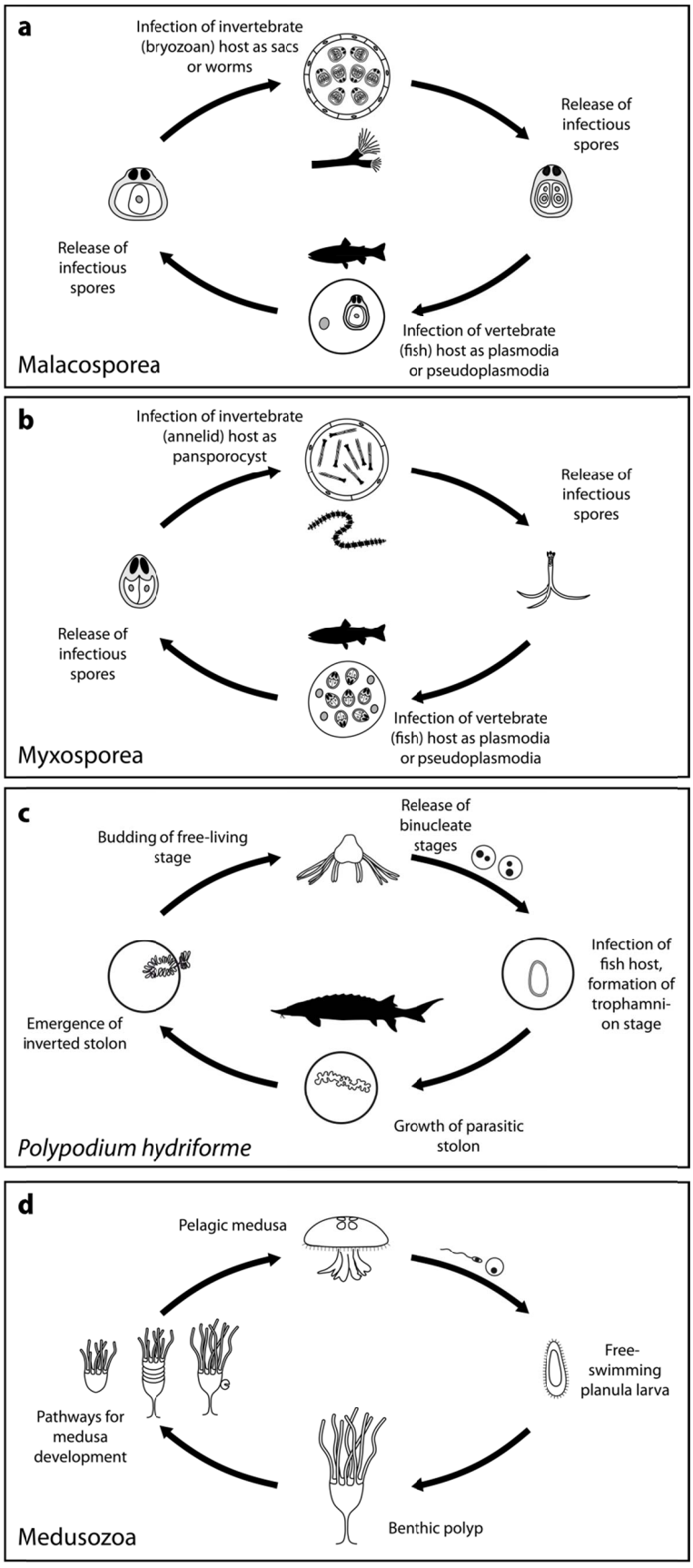

Figure 1. Life cycles of endocnidozoans and free-living cnidarians: A Malacosporea; B Myxosporea; C *Polypodium hydriforme*; D Medusozoa.

morphologically similar and short-lived (e.g. < 24 hr; de Kinkelin et al. 2002). Spores produced by myxosporeans (myxospores produced in fish hosts and actinospores produced in annelid hosts) show variable morphologies some of which can be useful taxonomically (Atkinson et al. 2015; Fiala et al. 2015b). Histozoic myxosporeans develop in small intercellular spaces within epithelia or connective tissues, whereas coelozoic species develop in the lumen of organs (e.g. bile duct, urinary bladder). The most common infection sites include gills, skin, fins, eyes, kidney, intestine, liver and gall bladder, nervous system, cartilage, musculature, and swim bladder (Molnár and Eszterbauer 2015). Myxosporean plasmodia and pseudoplasmodia are generally tiny, but some are mm to cm in dimensions. Annelid hosts of myxosporeans are mostly unknown – infections so far have been detected in 7 (Alexander et al. 2015) of approximately 120 families (Fauchald and Rouse 1997; Erséus 2005). Fish are widely exploited by myxosporeans with infections reported in many familes of both cartilaginous and bony fishes.

Myxozoans are causative agents of several diseases impacting aquaculture and wild fish populations. These include whirling disease, proliferative kidney disease and enteronecrosis in salmonids and pharyngeal myxosporidiosis in carp (Feist and Longshaw 2006; Jones et al. 2015).

3.2 Comparative development and body plans

There are noteworthy similarities and differences in morphology and life history stages between *Polypodium* and myxozoans consistent with the retention of a free-living stage in the life cycle of the former and the entirely endoparasitic life cycle of the latter (Table 2). In particular, myxozoans have lost many organs (e.g. no recognisable gut or gonads) and certain tissues (e.g. nerves, gonad) although proper epithelia characterise the early diverging malacosporeans. *Polypodium* and myxoworms both develop independent, sub-epidermal muscle systems (Raikova et al. 2007; Gruhl and Okamura 2012) in contrast to the typical cnidarian epitheliomuscular cells. Cilia, centrioles, and cnidocils are absent in myxozoans but present in *Polypodium*. Pinpointing the cells that act as gametes requires observing meiosis and the brief and transitory process of fusion and there are conflicting interpretations of these events in myxozoans (reviewed in Feist et al. 2015; Okamura et al. 2015b). Fusion may occur within spores that develop in myxozoan invertebrate hosts (resulting in self-fertilisation) or in fish hosts after cells released from spores invade and proliferate within fish (also potentially involving self-fertilisation). In *Polypodium* fusion has been inferred to happen at some time after fish hosts are invaded and to involve self-fertilisation of 'blastomeres' that develop within a nurse cell (the trophamnion) which becomes polyploid (Raikova 2008). This trophamnion-blastomere complex arises from the original binucleate cells that invade fish hosts. The complex is initiated by engulfment of one nucleus in the binucleate cell, resulting in a cell-in-cell organisation (Raikova 1994). It is apparent that fish hosts of both *Polypodium* and myxozoans support early stages of development. In addition, both *Polypodium* and myxozoans have similar nematocysts (organelles within nematocytes and referred to as polar capsules in myxozoans) that are used for attachment and are characterised by hollow tubes and an absence of spines. Such nematocysts, called 'atrichous isorhizae' are also found in free-living cnidarians (Okamura et al. 2015b). *Polypodium* additionally possesses nematocysts that penetrate prey.

The free-living *Polypodium* body plan is relatively consistent with that of other free-living cnidarians with an external ectoderm and internal endoderm (gastrodermis). We postulate that the independent sub-epidermal muscles may have evolved to facilitate unrestricted reversal of

Table 2. Similarities and differences in morphological and life history stages between *Polypodium* and myxozoans.

Feature	*Polypodium* (reference/s)	Myxozoans (reference/s)
Cilia/flagella	Flagellated endodermal cells (lining gut) (Raikova 2008)	Absent (Lom 1990; Feist et al. 2015)
Centrioles	Present (Raikova 2008)	Absent (Lom 1990; Canning et al. 2000; Feist et al. 2015)
Gametes	Absence of 'true' egg and sperm cells; 'egg' inferred to be small cell enclosed within another cell that will become a trophamnion (forming cell-in-cell complex); this complex results from 2nd meiotic division with trophamnion arising from polar body; diploidy proposed to be restored by fusion of blastomeres (Raikova 1994, 2002, 2008)	Not evident or recognisable; conflicting interpretations of which cells represent gametes during spore development; meiosis during sporogony in invertebrate hosts results in production of spores inferred to be haploid; when fusion (diploidy) is achieved is unknown (Feist et al. 2015; Okamura et al. 2015a)
Nematocysts	Atrichous isorhizae (enable attachment of walking tentacles); holotrichous isorhizae (penetrants for prey capture) (Raikova 1990; Ibragimov & Raikova 2004)	Nematocyst homologues (polar capsules) equated to atrichous isorhizae; function in attachment to hosts (Siddall et al. 1995)
Cnidocil (a modified cilium)	Present in unusual apical location above nematocyst lid (Raikova 1990)	Absent; apical plug-like structure similar to that in medusozoans in equivalent position (Canning & Okamura 2004; Okamura et al. 2015a)
Mitochondria with tubular cristae	Present (Raikova 2008)	Present but variability of this trait suggests it is not particularly noteworthy (Canning & Okamura 2004)
Epithelia	Present (Raikova 1994)	Present in sporogonic stages of malacosporeans developing in invertebrate hosts (Canning et al. 2000; Gruhl & Okamura 2015)

Table 2 (continued).

Feature	*Polypodium* (reference/s)	Myxozoans (reference/s)
Independent muscles (non-epitheliomuscular cells)	Present: Muscles sub-epidermal, underlying the ectoderm (Ra ikova et al. 2007)	Only present in vermiform malacosporeans: 4 longitudinal, sub-epidermal muscle blocks with chiral pattern (Gruhl & Okamura 2012)
Digestive tract	Present (Raikova 1994)	Absent (Canning & Okamura 1994)
Nervous system	Present (Raikova & Raikova 2016)	Absent (Canning & Okamura 1994)
Gonads	Two sequentially developing gonads (Type I and II); the first possesses gonoducts and degenerates (Raikova 1994, 2002, 2008)	Absent (Canning & Okamura 1994)
Transmission stage	Modified gonad that attaches to larval acipenseriform fish (Raikova 1994, 2008)	Multicellular spores released into the water column (Canning & Okamura 2004; Feist & Longshaw 2006)
Early presporogonic stages	Binucleate cells that leave gonad attached to larval fish (Raikova 2002, 2008)	Invertebrate hosts: amoeboid uninucleate cells in malacosporeans; binucleate cells in myxosporeans (Feist et al. 2015); Vertebrate hosts: primary cell with enclosed secondary cell/s in malacosoreans and in myxosporeans, respectively (Feist et al. 2015)
Cell-within-cell development and proliferation	Trophic cell (referred to as 'trophamnion' and acting as 'nurse cell') surrounds developing larvae in fish eggs and becomes polyploid (Raikova 1994, 2008)	Characterises sporogonic stages with primary and secondary cells in myxosporeans and in malacosporeans developing in fish hosts (but not in invertebrate hosts); primary cells become multinucleate forming plasmodia in myxosporeans but are uninucleate in pseudoplasmodia (Feist et al. 2015)
Intracellular parasitism	Present: larval stages develop in eggs of acipenseriform fishes	Present in some myxozoans (Canning & Okamura 2004; Sitjà-Bobadilla et al. 2015)

ectoderm and endoderm positions when these germ layers invert during formation of the mature stolon. Two Type I gonads inferred to be female (with 'oviducts' opening into the gastric cavity) develop, but mature eggs have not been observed and the gonadal complexes degenerate (Raikova 2008). Four Type II gonads (without gonoducts) support gametogenesis which is described as first resembling spermatogenesis but subsequently becoming more similar to oogenesis (entailing 'reorientation of male into female gonad'; Raikova 2008). Fertilisation would be achieved at a much later stage given Raikova's conclusion that the binucleate cells (produced by Type II gonads) that go on to invade fish are haploid. Cleavage and early morula-like embryonic stages are observed within fish eggs that may not develop for years (e.g. sterlet and beluga require up to 10 and 16 years to become reproductive, respectively) indicating prolonged arrested development of *Polypodium* stages following infection of juvenile fish (Raikova 1994). It is unclear whether early invading binucleate cells multiply within fish and thus eventually contribute to high percentages of infected eggs, however Raikova (2002) notes that in sections of ovary from young fish, cells resembling binucleate stages were repeatedly observed. Nourishment from the fish egg yolk is provided indirectly by the surrounding trophamnion whilst larvae develop to the adult stolon. Further yolk incorporated during the inversion of germ layers just prior to egg spawning supports several days of free-living existence after the stolon is released and undergoes fragmentation in the environment.

The endo- and ectodermal nature of myxozoan epithelia remains unknown, but, if homologous with that of *Polypodium,* the outer epithelium of malacosporean sacs and worms and the wall of myxosporean pansporocysts in invertebrate hosts would be endodermal (see also below discussion). In contrast, sporogonic stages developing in fish hosts (plasmodia of myxosporeans and pseudoplasmodia of malacosporeans) are characterised by development within an outer cell – an arrangement similar to the trophamnion-enclosed larval stages of *Polypodium* (Table 2).

Endocnidozoans have repeatedly evolved stages that retain multiple copies of nuclear content within a single cell. These include cell-in-cell complexes of myxozoans, binucleate stages of *Polypodium*, syncytial plasmodia of myxosporeans, and polyploidy in the trophamnion stage of *Polypodium* (the latter inferred in an early cytophotometry study [Raikova 1965]). All cell-in-cell myxozoan stages appear to originate from engulfment of one cell by another forming cell complexes (Feist et al. 2015). These have mistakenly been described as binucleate stages similar to those of *Polypodium* (Raikova 1994, 2008; Holzer et al. 2018). However, Morris (2012) showed that the cell membrane surrounding engulfed myxozoan cells becomes indistinct (suggesting a binucleate condition), but this membrane reappears later in sporoplasm development. Binucleate cells in *Polypodium* are regarded as long-lived haploid stages that have not subsequently divided after the second meiotic division (i.e. cytokinesis is postponed; Raikova 2008).

4 Evolution and life cycles of endocnidozoans

4.1 Preadaptations to parasitism

Cnidarians possess several traits that may predispose them for endoparasitic lifestyles (Okamura et al. 2015b). Their diploblastic body plan is manifested by the extensive development of external and internal epithelial layers across which resource capture, uptake and excretion are performed. An inherent capacity for uptake of dissolved organic material across these surfaces (e.g. Grover et al. 2008) may particularly facilitate adopting endoparasitic lifestyles (Okamura et al. 2015b). These

combined cnidarian features are similarly likely to support the intimate relationships cnidarians have repeatedly evolved with endosymbionts (Kayal et al. 2018) based on nutrient exchange (e.g. in sea anemones, corals, green *Hydra*, and stalked jellyfish).

Nematocysts are triggered to discharge filaments in response to specific chemical or mechanical environmental stimuli. These filaments function variously in prey capture or defense, achieved by penetration and injection of venoms or digestive enzymes, attachment, and entanglement. Nematocysts have been co-opted in endoparasitic cnidarians. For example, the filaments discharged from myxozoan polar capsules anchor transmission stages (spores) to host surfaces to enable the initiation of infection (Kallert et al. 2015). Cnidarians secrete, either via nematocysts or standard secretory pathways, a wide variety of compounds such as toxins and antimicrobial peptides as well as cytolytic, proteolytic and other digestive enzymes (Balasubramanian 2012; Dunlap et al. 2013). These substances have a high potential of becoming co-opted for host-parasite interactions such as host invasion, tissue modification or defence against host immune system elements. Furthermore, soft-bodied cnidarians have evolved specific epithelial defence mechanisms manifested in a high structural and molecular diversity of the glycocalyx, the apical membrane surface layer (Bosch 2016). The glycocalyx forms the outer layer of myxozoan spores, but also covers endoparasitic stages possibly functioning in immune evasion or other host-parasite interactions (Gruhl and Okamura 2015).

Cnidarians are characterised by a high degree of asexual reproduction in the form of e.g. fission, fragmentation and budding (Fautin 2002). The ability to proliferate asexually is a hallmark of many parasites, enabling them to exploit host resources, to produce new stages, to overwhelm host immune responses, and to facilitate horizontal transmission (Poulin 2007). Examples of such asexual replication in endoparasitic cnidarian stages include evidence for fission (Figs. 3.2 c, d; in Okamura et al. 2015a) and budding (Okamura 1996; McGurk et al. 2006) in sac-forming myxozoans in the body cavity of bryozoan hosts, and budding of medusae from polyp-like endoparasitic narcomedusans within the body cavity of polychaete hosts (Bentlage et al. 2018). Parasitic polyp stages also bud off medusae in the ring canal system of parental medusae (Bigelow 1909). However, the proliferation of early stages of endocnidozoan infections and of parasitic larval narcomedusans is achieved by binucleate cells and cell-within-cell stages (Bigelow 1909; Feist et al. 2015) that, as far as we are aware, have no ready homology with cell complexes in free-living cnidarians.

There is clearly substantial plasticity in cnidarian life histories and the ability to develop novel stages (Cartwright and Nowracki 2010; Okamura et al. 2015b). For example, medusa stages have been lost in many hydrozoans, including in *Hydra*, with progenesis enabling sexual reproduction (Boero et al. 1992) and some medusozoans incorporate parasitic stages inferred to be specialised polyps (Table 1). Novel stages of cnidarians can undergo dormancy, regeneration and transdifferentiation (reviewed in Lai and Aboobaker 2017) – processes that are intimately related to asexual reproduction. For example, planulae or polyps shrink and persist as cysts and dormant hydrorhizae with low metabolic costs during stressful periods. When favourable conditions return, cell proliferation and morphogenesis ensue (Boero et al. 1992). Polyp stages of the hydrozoan genus, *Turritopsis*, can be reformed from regressed tissues of sexual stages (medusa) via reverse ontogeny – a process mediated by proliferation of interstitial stem cells and cell transdifferentiation (Piraino et al. 2004). Medusa buds of the hydrozoan, *Sarsia tubulosa*, transform back to polyp buds when exposed to different temperatures (Werner 1963). This phenomenon could be more common in

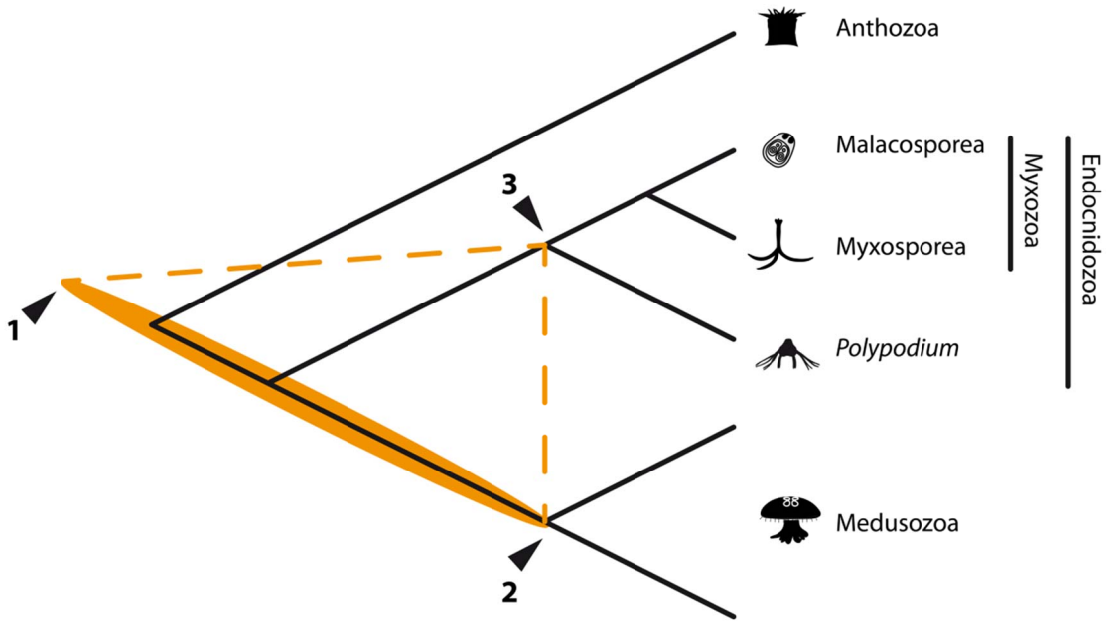

Figure 2. Cnidarian phylogenetic tree with mapped time constraints for the evolution of Endocnidozoa. 1) Maximum age of crown Cnidaria (~ 900 Ma); 2) Minimum age of crown Medusozoa (505 Ma); 3) Minimum age of crown Endocnidozoa (not calibrated by fossils).

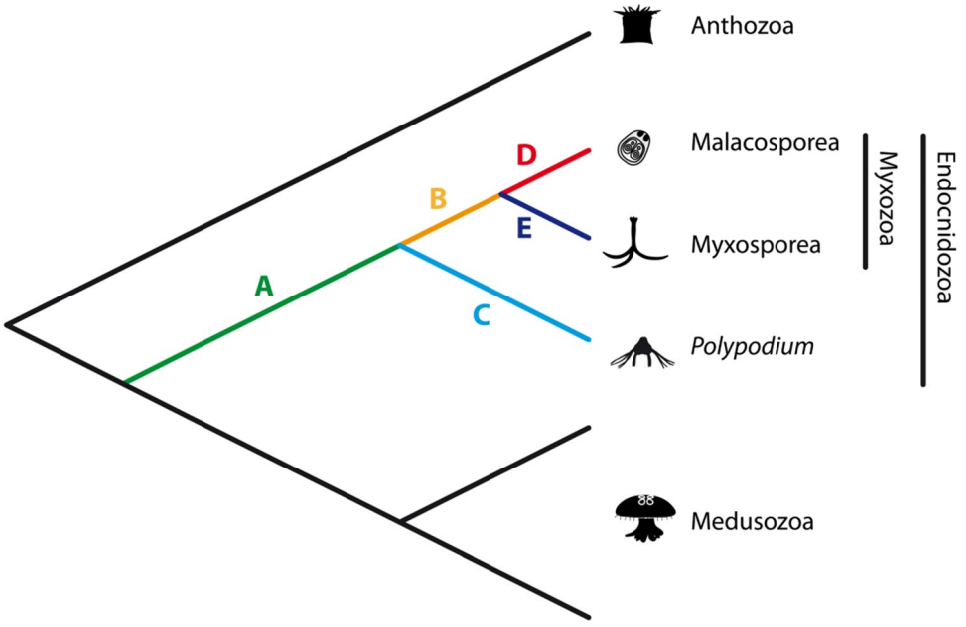

Figure 3. Cnidarian phylogeny with A-E delineating time periods during which various hosts were acquired. Note that because we lack fossil evidence, host acquisition could have occurred at nodes or at any subsequent time period prior to later nodes or the present-day. Scenarios for host acquisition are: 1) Gain of fish host during A, gain of invertebrate host during B; 2) Gain of fish host during A, gain of bryozoan host at D, gain of annelid host at E; 3) Gain of both fish and invertebrate hosts during A, loss of invertebrate host at C; 4) Gain of invertebrate host during A, gain of vertebrate host at B, switching from invertebrate to vertebrate host at C; 5) Gain of invertebrate host at B, gain of fish host at C; 6) Gain of fish host at C; gain of bryozoan host at B.

cnidarians than previously anticipated (e.g. He et al. 2015). These features may have aided precursors of parasitic cnidarians initially to survive within hosts. For example, cryptic dormant stages of myxozoans are viable and present in dormant propagules produced by invertebrate hosts (freshwater bryozoans) (Abd-Elfattah et al. 2014). Such capacities of regeneration and transdifferentiation may have facilitated the evolution of novel stages during transition to parasitism.

4.2 Life cycle speculations

Sexual reproduction, evidenced by meiosis, occurs when myxozoans develop in their invertebrate hosts. This suggests that trophic stages that develop in invertebrates are extremely morphologically simplified stages that may be equivalent to the free-living, sexual stages of *Polypodium* (Okamura et al. 2015b). Although the typical medusa umbrella is lacking, the free-living stage of *Polypodium* has been inferred to be a modified medusa (Raikova 1994; Raikova and Raikova 2016). This inference is primarily based on the assumption that sexual reproduction invariably is expressed in the medusa stage. The developing stolon enclosed within fish eggs therefore is proposed to correspond to a polyp stage. However, molecular phylogenetic placement and life cycle plasticity arguments (see above) would equally suggest that the free-living stage could be equivalent to a polyp stage. Regardless of the unresolved nature of *Polypodium's* free-living stage (medusa vs. polyp), it is achieved by larval development in fish eggs, albeit this is delayed until fish reach sexual maturity. In contrast, myxozoans incorporate a second life cycle phase that develops in vertebrate intermediate hosts. The lack of a biphasic life cycle in *Polypodium* would suggest this could have been absent in the common ancestor of *Polypodium*/Myxozoa and thus that myxozoan stages in vertebrate hosts are novel.

5 Origins and fossil records of Endocnidozoa and their recognised major host groups

The fossil record of endoparasites is poor for many reasons (De Baets and Littlewood 2015; Leung 2017). Although endocnidozoan parasites are diverse and abundant and have probably existed on earth for hundreds of millions of years, there is so far neither a direct nor indirect fossil record for this group. The fossilization potential of within-host life-cycle stages of endocnidozoans is arguably low. Such stages are soft, microscopic and most often reside within soft host tissues which themselves are only rarely preserved. However, mature myxozoan spores, especially myxospores (myxosporean spores produced in vertebrate hosts) can withstand adverse conditions like desiccation, extreme temperatures, chemical treatments, and gut passage (El-Matbouli and Hoffmann 1991; Hedrick et al. 2008). Their outer layer consists of a hardened, cuticle-like, extracellular material secreted by the underlying valve cells. This material may be elaborated as ridges and other surface features across the spore surface (reviewed in Gruhl and Okamura 2015). In some species a pronounced cytoskeleton inside the valve cells adds further mechanical reinforcement, and chitin has been identified in the periphery of myxospores (Munoz et al. 1999). Myxospores in mud have been shown to remain viable for several months (El-Matbouli and Hoffmann 1991). Thus, there is a good chance that these could be present along with other palynomorphs in sedimentary deposits or in peat. Such preservation has been shown for other parasite remains (e.g. nematode and trematode eggs), but recovery may be heavily influenced by the extraction method used (Dufour and Le Bailly 2013). It is unclear, however, how easily such remains could be identified as myxospores. Although spore shape and surface structure are of some

use, the fossilization potential of the most important diagnostic myxozoan characters, the polar capsules, is unclear. Clusters of nematocysts (for example so-called “nematocyst batteries“ in jellyfish) are recognisable in fossil cnidarians with soft-tissue preservation (Han et al. 2016), but the individual nematocysts are, to our knowledge, hardly ever retained. As of yet, no experimental studies on the taphonomy of myxospores exist. Such data would inform on the potential recognition of fossilized myxozoan spores.

Indirect fossil evidence of endocnidozoan parasitism, i.e. pathological changes in their hosts, could theoretically be recognised, particularly if such pathologies occur in skeletal tissues. Soft tissue pathologies such as cysts or swollen organs require exceptional preservation, but even in such cases it will still be difficult to unambigously identify myxozoan infection. This is because infections by many other organisms can produce similar disease symptoms, as exemplified by fossilized fish skin nodules (Petit 2010, Petit and Khalloufi 2012) from the Monte Molca and Solnhofen deposits. In addition, many diagnostic techniques, such as histological staining, are inapplicable to fossilized material.

Because of the inherent restrictions of the fossil record, current reconstructions of endocnidozoan evolutionary history have to use other sources of information (reviewed in De Baets and Littlewood 2015, Martinez-Aquino 2016). These can include extant parasite and host phylogenies, the fossil record of closely related free-living cnidarian taxa as well as that of hosts, and paleo-environmental data. Such data can be analysed and combined in different ways. Ancestral character states (such as host preference) can be reconstructed using standard phylogenetic techniques. Phylogenetic bracketing, for example, postulates the occurrence of the parasite in the last common ancestor of all recent hosts (except for those that were clearly acquired by recent host switching). Molecular clock analyses can also provide age estimates for origins of groups that lack fossil data when based on both a well-resolved phylogeny and a reliable fossil calibration of a wide set of nodes (Parham et al 2012; Benton et al 2009). A common practice for age estimates of a taxon is to use the oldest reliable fossil as a minimum age estimate and either the earliest molecular clock or the stratigraphic maximum estimate as a (soft) maximum age estimate (De Baets et al. 2015, Parham et al 2012).

Molecular clock analyses, however, can be particularly challenging for parasitic groups for many reasons. First, evolutionary rates have repeatedly been shown to be increased in parasite lineages (Bromham 2009, Bromham et al. 2013, Paterson et al. 2010). In addition, reconstructions and calibrations of inferred parasite-host associations are only reliable if host switching is very rare. The latter can be detected independent of molecular clock analyses, by co-phylogenetic analyses, which, however have certain pitfalls (de Vienne et al. 2013; see also discussion in later section). For example, parasites, including many endocnidozoans, can often exploit a range of hosts and this introduces complications to co-phylogenetic analyses that assume strict host use (Banks and Paterson 2005). Finally, any inferences of parasite evolution, including molecular clock methods and host-parasite cophylogenies, can be highly compromised when parasite diversities are poorly known. As we argue here and elsewhere (Okamura et al. 2018) endocnidozoan diversities are certainly grossly underestimated and pivotal taxa may be overlooked due to lack of sampling in many environments.

An additional and important conceptual point to stress in any discussion of evidence for fossil remains is appreciation that evolution is a continuous process. Thus, at some period endocnidozoans

will have diverged from an entirely free-living common ancestor. However, whether this divergence was simultaneously linked with adoption of parasitism or whether parasitism evolved subsequently is an open question. This question is of course relevant to a timeframe when fossils of parasites might be expected. Later we will review how initial and further hosts may have been adopted in simple and complex parasite life cycles, but complications arising from the continuous nature of evolution should be born in mind as we discuss the origins of endocnidozoans and of their potential early hosts in this section.

Below we review the current state of knowledge of the origins of cnidarians and of hosts known to be used by endocnidozoans based on the fossil record and other evidence (e.g. molecular clock analyses). We also assess the potential for recognizing myxozoan infections in host fossil material. We then critically evaluate conclusions of a recent study of the origin and evolution of endocnidozoans deriving from molecular clock and co-phylogenetic analyses.

5.1 Cnidarian origins and fossil record

Phylogenomic analyses of multi-gene datasets across the animal kingdom have in recent decades resolved many questions. Basal metazoan relationships, however, including those of cnidarians, remain especially controversial and several equally well supported hypotheses exist (discussed in Dohrmann and Wörheide 2013; Dunn et al. 2014; Halanych 2015). The two most prominent groups of hypotheses mainly differ in the placement of Placozoa and Ctenophora: either united with Cnidaria to form the “Diploblastica” (e.g. Schierwater et al. 2009) or as basal metazoan branches leaving Cnidaria as sister to bilaterians (e.g. Simion et al. 2017; Whelan et al. 2017). Although most molecular clock analyses predict both metazoan and bilaterian origins in the Neoproterozoic (900 – 600 Ma) (e.g. Dohrmann and Wörheide 2017), reliable fossil evidence from these times is extremely scarce (reviewed in Cunningham et al. 2017). Both the oldest reliable crown-bilaterian and crown-cnidarian fossils, *Kimberella* (555 Ma) and *Corumbella* (543 Ma), respectively, are dated to the uppermost Ediacaran (Grazhdankin 2004, Warren et al. 2012). Other Ediacaran fossils, including the classic Vendobionta (soft-bodied Ediacaran macrofossils like *Dickinsonia*) and the Weng’an and Lantian biota are difficult to interpret as to both their exact age and affinities.

Extant Cnidaria comprise the taxa Anthozoa, Staurozoa, Endocnidozoa, Scyphozoa, Cubozoa and Hydrozoa. Phylogenetic relationships among these taxa have been subject to extensive debate. The most recent phylogenomic analyses (Kayal et al. 2018) support a sister group-relationship between Anthozoa and the clade comprising Medusozoa and Endocnidozoa (Figure 2). Previously regarded as sister group to Medusozoa, Kayal et al. (2018) recover Staurozoa within Medusozoa, as sister to a clade uniting Cubozoa and Hydrozoa. The earliest generally accepted cnidarian polyps, *Corumbella* and *Paraconularia*, are from the uppermost Ediacaran or earliest Cambrian (<550/540 Ma) (Van Iten et al. 2014). Several Neoproterozoic cnidarian candidates remain questionable, including *Haootia,* a 560 Ma impression from the Ediacaran of Newfoundland interpreted as a staurozoan polyp. *Lantianella* bears similarity to a scyphozoan medusa, but the Lantian formation is not dated precisely, with estimates ranging from 590 – 635 Ma (Wan et al 2016). According to molecular clock analyses the origin of cnidarians and their initial diversification most likely date to the Cryogenian or even earlier (Park et al. 2012, dos Reis et al. 2015, Cunningham et al. 2017, Dohrmann and Wörheide 2017). Given its phylogenetic position, the endocnidozoan stem lineage must have diverged at some point between the basal branching of Cnidaria and the emergence of crown medusozoans. The

earliest unambiguous cnidarian medusae are from the Middle Cambrian Burgess Shale (Epoch 3, 505 Ma) (Young and Hagadorn 2010 and references therein). Stem medusozoans, perhaps still lacking the medusa stage could of course be older, but recognising these forms could be highly challenging. Molecular clock analyses date crown medusozoans to the late Cryogenian to early Ediacaran (~700-600 Ma) (Park et al. 2012, Dohrmann and Wörheide 2017, Holzer et al. 2018). Holzer et al. (2018) infer ages of 651 Ma (601–700 Ma) and 588 Ma (540–642 Ma) for the origins of Endocnidozoa and Myxozoa, respectively.

5.2 Vertebrate origins and fossil record

Vertebrates, especially "fish", are the most speciose and best studied endocnidozoan hosts, and they have a relatively extensive fossil record (Friedman and Sallan 2012). A number of soft-bodied potential vertebrates are known from Cambrian strata. A particular problem in the interpretation of these fossils, however, is the lack of crown-vertebrate characters. Among the more convincing fossil vertebrates are *Metaspriggina* from the Burgess Shale and *Haikouichtys* from Chengjiang. These fossils show definitive chordate characters, including gill slits, fins, and metameric musculature. The provenance of many other proposed vertebrate fossils is more controversial. For example, *Haikouella* or *Yunnanozoon* could be fossil hemichordates, acranians (vertebrates lacking skulls), stem deuterostomes or stem vertebrates (Donoghue and Keating 2014). Several attempts have been made to date the vertebrate phylogeny using molecular clocks. Most studies, however, focus on the gnathostomes for which more fossil calibration points exist, leaving the origin of the vertebrates relatively uncertain. Dohrmann and Wörheide (2017) place the deuterostome origin before the Marinoan glaciation and chordate and vertebrate origins into Cambrian and Ordovician, respectively. The origin of (crown) vertebrates has been variously placed at 490 (504-476) Ma (Delsuc et al. 2018), 490 (504-476) Ma (dos Reis et al. 2015) and 460 -533 Ma (Holzer et al. 2018).

Recent craniate vertebrates include the jawless lampreys and hagfishes, the chondrichthyans (cartilaginous fishes), and the osteichthyans (bony fishes), the latter comprising sarcopterygians and actinopterygians. The Agnatha (or Cyclostomata) (jawless fishes) are currently considered to be monophyletic, together forming the sister group to Gnathostomata (vertebrates with jaws, including most present-day fish). Because of the complete lack of hard skeletons the origin of agnathans is difficult to trace; the earliest reliable fossils are from the Devonian and Carboniferous. Molecular clock studies converge on ~460 Ma for the gnathostome divergence (Irissari et al. 2017; dos Reis et al. 2015; Broughton et al. 2013).

Two further exclusively fossil groups are of relevance for early vertebrate relationships: the conodonts and the ostracoderms. Conodonts are known by their characteristic tooth-like elements from the late Cambrian until the Late Triassic when they apparently went extinct. Currently conodonts are mostly considered to branch at the base of vertebrates, either as stem agnathans or stem gnathostomes (Goudemand et al. 2011; Murdock et al. 2013; Turner et al. 2010). The ostracoderms (Placodermi, Osteostraci, Acanthodii, with origins estimated from 467-295 Ma) are interpreted as a paraphyletic assemblage of gnathostome stem-lineage taxa that mostly lacked jaws (Janvier 2001). The earliest lobe-fin fossils are from 423 Ma (Brazeau and Friedman 2015), marking the putative chondrichtyan/osteichthyan (crown-gnathostome) divergence. Chondrichthyan-like scales are known from as early as the late Ordovician (458 Ma; Andreev. et al 2016). Vertebrates with chondrichthyan body fossils, however, do not appear in the fossil record before 400 Ma

(Brazeau and Friedman 2015). The first definitive actinopterygian, *Cheirolepis,* is from the early Middle Devonian (~ 390 Ma), although isolated scales of ray-fins were present from ~427 Ma (Friedman 2015). Molecular clock analyses suggest crown Actinopterygii to have been present by 400 Ma (Broughton et al. 2013), 384 Ma (Near et al 2012), or 320 Ma Irissarri et al. 2017) and ages for teleosts to range from 300-200 Ma. Recent Actinopterygii include the three sequentially branching taxa Cladistia (bichirs and ropefish), Chondrostei (sturgeon and paddlefish), and Holostei (gars and bowfin). The most derived actinopterygians, the Teleostei, includes the majority of the ~30,000 actinopterygian species (Friedman 2015). Only scattered actinopterygian fossils are known from the Devonian indicating relatively cryptic evolution until several radiations took place after the Devonian/Carboniferous boundary. Further teleost diversification occurred in the Triassic, including transitions from marine to freshwater and back. As a result the majority of recent marine actinopterygians derive from freshwater ancestors (Carrete Vega and Wiens 2012). These radiations are of potential significance, especially for the diversification of myxosporeans, which show a distinct subdivision into freshwater and marine clades (Fiala et al. 2015a,b; Holzer et al. 2018). Co-phylogenetic analyses that take these habitat changes into account may better resolve myxozoan radiations and could additionally serve to explain switches of invertebrate hosts. Moreover, node calibrations for fish radiations following invasions of freshwater or marine habitats could be directly transferred to the corresponding parasite groups. For example, the hypothesis could be examined that the marine myxosporean clade diverged from a freshwater clade that infected oligochaetes following the first transition of teleosts to the marine environment.

Myxozoans infect a range of organs and tissues of their vertebrate hosts causing diverse disease symptoms. The site of sporogony usually demonstrates the greatest pathology and is often quite specific (Molnár and Eszterbauer 2015). The fossilization potential of myxozoan stages in lumina and soft tissues is probably very low. Fish immune responses can, however, lead to encapsulation of parasites by dense connective or cartilaginous tissues, which may have a higher probability of preservation. Furthermore, a common reaction against myxozoan infection is the formation of granulomata with melanomacrophage centres – dense accumulations of immune cells containing melanin (Sitjà-Bobadilla et al. 2015; Steinel and Bolnick 2017), a substance which has been characterised from well-preserved fossil vertebrates (Colleary et al. 2015). Cysts occurring in gills, skin or muscle tissue are the most common form of tissue alteration and may be recognisable in fossils with exceptional soft-tissue preservation, but recognition of spores (see above) within these structures is crucial to unequivocally link to myxozoan infection. Myxozoan infection symptoms most likely to be preserved in the fossil record would be expected to result from species that infect cartilage and cause skeletal deformation. This phenomen is best studied in salmonid whirling disease caused by *Myxobolus cerebralis,* which infects cartilage prior to ossification leading to malformations of spine, skull, jaw or fin rays (Sarker et al. 2015).

Due to their high phosphate content and rapid fossilization potential, vertebrate faeces could provide a further means of detecting myxozoans via analyses of coprolite contents. Coprolites can sometimes offer exceptionally well-preserved soft tissue remains comparable to those of a Konservat-Lagerstätte (Qvarnström et al. 2016). Accordingly, coprolites have been found to contain parasite remains (Poinar and Boucot 2006; Hunt et al. 2012; Dentzien-Dias et al. 2013; Hugot et al. 2014; Brachaniec et al. 2015; Dentzien-Dias et al. 2018) along with preserved hair, feathers, muscles, bones, chitinous exoskeletons, bacteria, and fungi. Coprolites could therefore have a high potential to yield myxozoan spores. These spores may derive from species infecting sites where spores are

released into the digestive tract (e.g. infections in the bile ducts, gall bladder or intestine), or they could derive from infected prey. The latter possibility is supported by the detection of myxozoan DNA in faeces collected from fish-eating birds (cormorants) (Briscoe et al. in press) and evidence for viable spores excreted from great blue herons (Koel et al. 2010). Not all coprolites have equal preservation potential. Shark coprolites are often well-preserved (Hunt et al. 2012) and have yielded tapeworm eggs (e.g. Dentzien-Dias et al. 2013). Smaller actinopterygian coprolites have also been successfully analyzed for microfossils (e.g. from the Lake Messel deposit; Richter and Baszio 2001; Richter and Wedmann 2005).

5.3 Lophotrochozoan origins and fossil record

The most important invertebrate host groups of myxozoans are annelids and phylactolaemate bryozoans, both of which are members of the large superphylum Lophotrochozoa. The internal relationships of lophotrochozoans have proven notoriously difficult to resolve. Lophotrochozoan stem-group members are among the classic fossils of the Cambrian Lagerstätten (Sirius Passet, Burgess Shale, Chengjiang). Many of these show character combinations that have led to varying assignments as stems of extant phyla (e.g. molluscs, annelids, brachiopods).

5.4 Annelid origins and fossil record

The traditional annelid phylogeny with subdivision into polychaetes and clitellates has undergone major changes given recent phylogenomic data (Struck et al. 2011; Weigert et al. 2014; Struck et al. 2015; Weigert and Bleidorn 2016). Polychaetes now have to be considered paraphyletic with Clitellata being an ingroup of a large taxon embracing most of the classical “sedentarian” polychaetes. Thus, the ancestral annelids were polychaete-like and marine. Clitellates (which include the oligochaetes), in contrast, originated in freshwater or terrestrial habitats as shown by adaptations such as direct development inside a cocoon, and reduction of palps and parapodia. Recent marine clitellates (mostly tubificids) have clearly invaded the sea secondarily. Inferred basal splits within Annelida are still somewhat unstable and lack robust support, but generally suggest a motile or errant ancestor. The earliest annelid fossils are reported from the Cambrian Sirius Passet formation (Conway Morris and Peel 2008; Vinther et al. 2011). Later Cambrian annelid fossils have been found in the Burgess Shale (Conway Morris 1979) and Chengjiang Lagerstätten (Liu et al. 2015). These animals were in the mm-cm size range and had homonomous segmentation, parapodia and palps (Parry et al. 2014, 2015). If Sipunculida, which are also reported from Chengjiang (Huang et al. 2004), are really an annelid ingroup, the initial annelid radiation must have happened in the Early Cambrian or before. Although Clitellata are now consistently placed within polychaetous taxa, their sister group is not yet identified unambigously (Weigert and Bleidorn 2016). The oldest clitellate fossils are leech cocoons from the Triassic (Manum et al. 1991; Bomfleur 2015) suggesting a late Paleozoic origin of this group. Most modern polychaete groups first appear in the Carboniferous, but some are already known from the Devonian (Parry et al. 2014).

Few studies have investigated myxozoan infections in annelids (e.g. El-Matbouli and Hoffmann 1998; Bartholomew et al. 1997). Pathological effects on annelid hosts include tissue damage, reduced fecundity and hypertrophic growth (Elwell et al. 2009; Alexander et al. 2015) – as occurs in infected phylactolaemate bryozoans (see below). In general, myxozoan stages are unlikely to be preserved in fossil annelids, however, soft-body features are clearly recognisable in several exceptional preservations (e.g. Briggs and Bartels 2010; Timm et al. 2016). A particularly stunning example is the

preservation of spermatozoa – structures comparable in size to myxozoan spores – from Eocene clitellate cocoons (Bomfleur et al. 2015). Considering the vast diversity of annelids, it is clear that only a minor proportion of recent annelid taxa is currently known to serve as hosts of myxozoans. This could argue for a late acquisition of this host group, but might also reflect serious undersampling, as definitive hosts of the nearly 2,200+ myxosporean species described from fish are yet to be resolved (Eszterbauer et al. 2015).

5.5 Bryozoan origins and fossil record

Recent Bryozoa comprise the taxa Phylactolaemata, Stenolaemata and Gymnolaemata. Phylactolaemata is considered sister to the other two groups (Waeschenbach et al. 2012) and includes < 100 described species (Massard and Geimer 2008). In contrast to the vast majority of bryozoans, phylactolaemates are uncalcified and occur exclusively in fresh water. Relationships amongst phylactolaemates are mostly unresolved in molecular phylogenetic analyses (Waeschenbach et al. 2012; Hartikainen et al. 2013b). The oldest bryozoan fossils occur in the lowest Ordovician (Xia et al. 2007). The diversity of the calcified stenolaemates present at that time was already high (six major groups, of which only the cyclostomes survived the later Permian and Triassic extinctions), suggesting an earlier Cambrian radiation. However, neither calcified nor soft-bodied bryozoans have been found so far in the Cambrian (Taylor and Waeschenbach 2015). The lack of bryozoan soft body fossils hampers reconstruction of the origin and history of the uncalcified Phylactolaemata. Stem members of this group theoretically should have co-occurred with stenolaemates and ctenostomatous gymnolaemates. The oldest chitinous statoblasts (asexual propagules and resting buds of phylactolaemates) are found in the Permian (Vinogradov 1996). Statoblasts are interpreted as clear adaptations to fresh water, indicating that this lifestyle had evolved by then. Earlier stem phylactolaemates are likely to have been marine. Other recent marine bryozoan groups (the cheilostomes and cyclostomes) diversified in the mid-Mesozoic (Taylor and Waeschenbach 2015).

So far phylactolaemates are the only known bryozoan hosts of myxozoans. Hartikainen et al. (2013a) have shown that malacosporeans can affect phylactolaemate host morphology when the development of spore-producing infectious stages results in larger zooids, malformed statoblasts and reduced statoblast production. Recognition of such effects in fossil material would require good preservation, lack of post-preservation deformation, and comparative assessment of many individuals. Several extinct bryozoan groups could be promising candidates as hosts of ancient myxozoans.

A predisposing trait of phylactolaemates as myxozoan hosts is the relatively large and confluent colony-wide coelomic cavity. The continuous action of cilia lining this coelomic cavity ensures that metabolites are distributed throughout the colony. In addition the unobstructed coelomic space allows malacosporean sacs and myxoworms to move freely amongst the zooids of a colony, with the circulation of sacs being assisted by ciliary beating. Cyclostome and gymnolaemate zooids, in contrast, are separated by walls, which have communication pores that are either very small (cyclostomes) or filled with tissue (gymnolaemates), thus preventing myxozoan stages from migrating between zooids. Furthermore, nutrient transfer in cyclostomes and gymnolaemates is achieved largely via tissue connections (the funicular system) – leaving the coelomic fluids with relatively low concentrations of nutrients. The Paleozoic stenolaemate *Corynotrypida*, however,

completely lacked interzooidal walls. In addition, some representatives of other Paleozoic stenolaemate groups (Cystoporata, Esthonioporata, Trepostomata and Cryptostomata) possessed zooids with larger communication pores while others (the free-walled stenolaemates) lacked calcified exterior walls and thus are assumed have had confluent hypostegal coelomic cavities (Boardman 1998; Ernst and Schäfer 2006. These ancient bryozoan taxa could, thus potentially have served as malacosporean or stem myxozoan hosts.

5.6 Other potential ancient invertebrate hosts

Discoveries of myxosporeans in octopus (a species of *Kudoa*; Yokoyama and Masuda 2001) and as hyperparasites in three monogenean species (*Myxidium* or *Myxidium*-like species) and in two digenean species (*Fabespora vermicola* and *Fabespora* sp.; Freeman and Shinn 2011) suggest that a greater range of invertebrate hosts may be routinely used. Notably *Kudoa*, *Myxidium* and *Fabespora* are all derived lineages within the Myxosporea (Fiala 2006; Freeman and Shinn 2011). These host findings are, however, not straightforward. Thus, at least in some cases monogenean 'hosts' could be incidentally exploited by myxozoans infecting fish and *Kudoa* infection has only been observed in a single octopus. It is conceivable that the latter could have developed if infection was transmitted from infected fish prey (perhaps if injury was sustained in catching and subduing the fish). In addition, the spores that develop in both octopus and the platyhelminth species are typical myxospores (Freeman and Shinn 2011). This implies that these invertebrates may either be used as alternative hosts to fish or have been adopted as novel hosts via host switching. There is no evidence so far of a further range of invertebrate hosts associated with actinospore- or malacospore-producing stages of myxozoans.

Annelids and phylactolaemate bryozoans share traits that might predispose them to parasitism. These include collecting small particles for ingestion by screening relatively large environmental samples (via suspension or deposit feeding) and large body cavities in which parasites can develop. Other invertebrate groups with such traits (for example bivalves, echinoderms or phoronids), might act or have acted as hosts but have yet to be detected. Infection of fish may also have predisposed myxosporean hyperparasitism of platyhelminths enabling diversification in host use over time.

5.7 Summary of origins and ancient hosts

Due to the complete lack of fossils, we have no means of calibrating the age of the last common ancestor of endocnidozoans. We can, however, constrain the divergence of the endocnidozoan stem lineage by using both the maximum age estimate for crown Cnidaria and the minimum age estimate for Medusozoa (505 Ma) (Figure 2). Endocnidozoan characters, however, could have evolved at any point along the endocnidozoan stem lineage – between their divergence from the rest of the Cnidaria and the presence of the last common ancestor of all living endocnidozoans. Thus we cannot currently assess the traits of endocnidozoan stem lineage members, including whether they were parasitic or which hosts they parasitised. Molecular clock estimates so far undertaken propose ages of 651 Ma (601–700 Ma) for Endocnidozoa and 588 Ma (540–642 Ma) for Myxozoa (Holzer et al. 2018) and divergence of Malacosporea and Myxosporea at 540 $\pm$ 73 Ma (Kodádková et al. 2015). Although the crown groups of the main hosts of Recent endocnidozoans are mostly estimated to be slightly younger than endocnidozoans (see above) it is entirely possible that stem group members acted as hosts.

6 Inferring endocnidozoan origins and acquisition of early hosts

The lack of a fossil record and the possibility of host switching over time may highly constrain our understanding of endocnidozoan origins and what hosts were acquired when. Any insights, however flawed they may be, must be gained by evaluating data on extant taxa. Here we consider in general how hosts may be acquired and, by extension, host acquisition by endocnidozoans. We then go on to examine more closely some of the pitfalls of inferring parasite origins and patterns of host use over time on the basis of molecular clock and co-phylogenetic analyses.

6.1 The process of host acquisition

Adoption of a parasitic life style would have required frequent proximity of future hosts and parasites leading to enhanced fitness of parasite precursors. Transitions to parasitism could be based on precursors depending on future hosts for e.g. food or dispersal. Alternatively, precursors may have been regularly ingested by future hosts (Poulin 2007; Schmid-Hempel 2011). Precursor pre-adaptations may have facilitated benefitting from such an association (Poulin 2007). For example, as outlined above, cnidarian traits such as uptake of dissolved organic matter across relatively extensive epithelial surfaces, the ability to persist as dormant stages, regeneration, and nematocysts may have variously facilitated host exploitation, survival in adverse host environments and attachment to hosts.

Exploitation of fish hosts by both *Polypodium* and myxozoans along with their sister relationship status suggest that their common ancestor could have exploited fish (Figure 3; Scenario 1 - gain of fish host during A, gain of invertebrate host during B; Scenario 2 - gain of fish host during A, gain of bryozoan host at D, gain of annelid host at E). It can be further argued that fish hosts were exploited either by a larval or novel pre-adult stage of this ancestor because sexual reproduction occurs in the free-living stage of *Polypodium* and when myxozoans are exploiting invertebrate hosts. Alternative more complicated scenarios include loss of primary invertebrate hosts in *Polypodium* (a scenario that would require the re-acquisition of an adult free-living stage in *Polypodium*) (Figure 3; Scenario 3 - gain of both fish and invertebrate hosts during A, loss of invertebrate host at C), switching from vertebrate to invertebrate host in *Polypodium* (Figure 3; Scenario 4 - gain of invertebrate host during A, gain of vertebrate host at B, switching from invertebrate to vertebrate host at C) and independent transitions to parasitism in *Polypodium* and Myxozoa (Figure 3; Scenario 5 - gain of invertebrate host at B, gain of fish host at C). The latter scenario implies a unique transition resulting in the single host life cycle of *Polypodium* and another unique transition resulting in the initial single host life cycle of myxozoans. Precedence for this scenario is provided by the multiple independent origins of parasitism across the animal kingdom (Weinstein and Kuris 2016) and also within taxa, including within the Cnidaria (as described above) and the Nematoda (Blaxter and Koutsovoulos 2014). Flexibility in cnidarian life histories and the capacity to produce novel stages are features that would support the more complicated scenarios described here, but we must also appreciate that unknown host switching events potentially complicate any interpretations about the identity of ancestral hosts.

The evolution of complex life cycles is linked with the great radiation of myxozoans and was achieved by the incorporation of a second host. For helminths it has been argued that secondary hosts may be acquired when parasites evolve to exploit predators or prey of the definitive first host (Choisy et al. 2003; Parker et al. 2003). For example, frequently ingested original hosts may become

intermediate hosts when new, larger hosts are exploited by 'upward incorporation' – an outcome that may be associated with increased parasite fecundity, increased probability of finding a sexual partner (Brown et al. 2001; Parker et al. 2015) or a decrease in inbreeding because of multiple infections of larger hosts (Rauch et al. 2005). Alternatively, 'downward incorporation' could occur when prey of the original host frequently ingest parasite propagules and become intermediate hosts, thus enhancing transmission to the original host (Parker et al. 2003). However, these scenarios are based on helminth life cycles that initially involved sexual reproduction in the first hosts.

If fish (or their ancestors) were first hosts of ancestral larval myxozoan stages then invertebrates could have been adopted as hosts of adult forms by a kind of downward incorporation (Figure 3; Scenarios 1, 2). This would require release of larval forms in sufficient numbers from fish (or ancestral fish) hosts that they were frequently consumed by invertebrates, with sexual reproduction then being undertaken in invertebrate hosts. Alternatively, if a stem lophotrochozoan (some ancestral precursor to bryozoans and annelids) served as first host of ancestral myxozoans then upward incorporation may have enabled e.g. a stem chordate or vertebrate to be adopted as a secondary host (Figure 3; Scenarios 4, 5). The retention of primitive features in malacosporeans (e.g. recognisable epithelia, musculature) suggests that ancestral invertebrate hosts may have been more similar to present day freshwater bryozoans than annelids. Subsequently, annelids (or their ancestors) may have been incorporated as invertebrate hosts by the common ancestor of the more derived Myxosporea via host-switching. It is also possible that first hosts were acquired much later and that present-day patterns of host use are directly representative of host group acquisition. In this case, an argument could be made against annelids (or their relatively recent ancestors) as first hosts because this scenario would require the unlikely re-acquisition of primitive traits in the malacosporeans. However, if *Polypodium* and myxozoans acquired parasitism independently and relatively recently (Figure 3; Scenario 6) then bryozoans or fish as first myxozoan hosts seem equally feasible scenarios.

6.2 Molecular clock and co-phylogenetic investigations

1) Some general pitfalls. Investigations of parasite origins are inherently difficult due to their extremely poor fossil record. Some researchers have therefore adopted the use of molecular clock and co-phylogenetic analyses involving parasites and their hosts in order to infer when parasites may have originated. A general constraint in these approaches is the central assumption that parasite origins can be imputed from host origins while at best they can only be constrained by these (De Baets et al. 2015). In addition, there are a number of other assumptions typically involved in such analyses that should be appreciated. For example, patterns of host use by parasite taxa that we recognise today may have arisen from a complex history of host switching (De Baets et al. 2015). Myxozoans clearly do undergo host switching and indeed the acquisition of new hosts in their complex life cycles provides a case example. Furthermore, identifying first hosts based on those used in the present day may be erroneous. Presently known hosts (or their ancestors) may have been acquired at any time during the potentially very long history of lineages observed today. We appreciate that it can be tempting to propose that early-diverging parasites associated in the present day with early-diverging hosts represent ancient parasite-host associations. For instance, Kodádková et al. (2015) make this argument for the early-diverging myxosporeans *Bipteria* and *Ceratomyxa* in cartilaginous fish hosts. However, species of both *Bipteria* (albeit these may be

currently mis-assigned) and *Ceratomyxa* also exploit teleost fish hosts (Lom and Dykova 2006; Adriano and Okamura 2017), an observation suggesting that cartilaginous fishes could have been adopted much later when teleost-infecting lineages switched hosts. Presentation of such alternative scenarios would be helpful to promote more balanced interpretations of results.

Extinctions may also obscure inferences about parasite origins and patterns of diversification by influencing the present-day distributions of parasites. For example, if parasitism first evolved when stem endocnidozoans began to exploit a stem lophotrochozoan or craniate host in the late Cryogenian, then extinctions over time (of hosts or parasites) could explain gaps in the distribution of parasites across the range of present-day hosts (De Baets et al. 2015). Any inferences concerning the origins and evolutionary histories of parasitic lineages should therefore acknowledge the possibility of a potentially rich complexity of host-parasite interactions that may have been impacted over time by gradual and mass extinctions. Indeed, it is fascinating to contemplate what parasites may have been lost during mass extinction events and what host lineages may have survived and subsequently diversified having lost those parasites. It should be noted that there is a growing appreciation that co-exinction of parasites (affiliate taxa) along with taxa they depend on (hosts) may be the most common form of species loss (Dunn et al. 2009; Strona 2015). However, as far as we are aware, the modelling approaches developed so far to estimate parasite extinctions are constrained to estimating the number of extinctions of affiliate species as a function of host extinctions retrospectively, based on knowledge of present-day patterns (Colwell et al. 2012), or by modelling dynamics of digitally evolving organisms (reviewed in Strona 2015). Parasite extinctions have also been inferred by detection of ancient DNA (e.g. host-specific heterokoid nematodes in coprolites of moa; Boast et al. 2018), but this technique cannot be applied to reveal extinctions over deep time.

In addition, limited sampling of the diversity of many parasites will constrain insights about patterns of host use. Current undersampling of both host and parasite diversities may thus compromise current views, for example that all major myxozoan clades are characterised by different invertebrate host groups. This limitation is particularly relevant when cophylogenetic analyses attempt to characterise patterns of parasite co-evolution relative to focal host groups. Such analyses require reliable phylogenetic trees – otherwise congruence of phylogenies may be compromised by poor phylogenetic resolution.

2)Endocnidozoan origins and host use over time. The first attempt to characterise the timing and divergence of endocnidozoans (Holzer et al. 2018) provides a useful platform for interpreting endocnidozoan evolution as well as to illustrate how inferences may be compromised by the various pitfalls of such analyses. We variously elaborate on these issues below.

To estimate divergence times Holzer et al. (2018) analysed concatenated alignments of six protein-coding genes in endocnidozoans, their hosts, and other metazoans (10 myxozoans, *Polypodium* and 127 other metazoan taxa [from Erwin et al. 2011]). Molecular clock analyses estimated divergence times of endocnidozoans and myxozoans to be in the late Cryogenean at 651 Ma (601-700 Ma) and the Ediacaran at 588 Ma (540-642 Ma), respectively. As outlined earlier, such divergence times are consistent with other molecular clock analyses which predict cnidarian origins in the Cryogenean (720-625 Ma) and the presence of crown cnidarians in the uppermost Ediacaran or earliest Cambrian. However, one of the main conclusions, that endocnidozoans diverged when invertebrates

were incorporated as first hosts in the Cryogenian, is not well supported. Parasitism of stem hosts other than invertebrates is also possible. Although crown group vertebrates are estimated to be much younger, the vertebrate stem lineage extends well back into the early Cambrian or even Ediacaran. The tunicate-vertebrate divergence, for instance, is resolved at 575 and 550 Ma (by Dohrmann and Wörheide 2017 and dos Reis et al. 2015, respectively), close to the Cryogenian estimate for the inferred endocnidozoan divergence. This provides evidence that fish ancestors were present and could equally have been first hosts. The scenario that very ancient endocnidozoans may have been free-living is also not recognised. So far there is no fossil evidence to demonstrate when and in what hosts endocnidozoans or myxozoans first appeared. Further processes that are not considered include host switching and extinction. Myxozoans clearly do undergo host switching and presently known hosts may have been acquired at any time since they have evolved. However, under Holzer et al.'s scenario, myxozoans have consistently only exploited those host lineages in which they are currently parasites.

Cophylogenetic studies conducted by Holzer et al. (2018) are employed along with their molecular clock analyses to argue that invertebrates were adopted as first hosts. However, the inferences proposed as a result of these analyses are potentially problematic. Thus, Holzer et al. (2018) infer that invertebrate host and parasite phylogenies are congruent 'down to the most basal branches' and highlight that all major myxozoan clades are associated with different invertebrate acquisition events (phylactolaemates, oligochaetes and polychaetes) (apart from *Sphaerospora* whose invertebrate hosts are unknown). Yet all major clades (including *Sphaerospora*) are also associated with fish hosts and, as argued above, fish (or their ancestors) cannot necessarily be excluded as first hosts. Furthermore, relationships among phylactolaemate bryozoans are largely unresolved in molecular phylogenetic analyses (most genera and families in this small group comprise a polytomy; Waeschenbach et al. 2012), and recent studies reveal new higher level taxa in undersampled environments (e.g. Amazonia; Wood & Okamura 2017). Thus the congruence of phylogenies may be anomalous and compromised by both limited sampling and poor phylogenetic resolution of invertebrate hosts. Finally, their multiple examples of congruent cophylogenies of myxozoans and fish hosts could arise if fish served as primary hosts followed by host switching, rather than being multiply acquired as secondary hosts as proposed. Quite apart from these specific issues are more general concerns about what can be inferred by such cophylogenetic investigations. These include the problem of non-independence of phylogenies using PARAFIT as done by Holzer et al. (2018) (Felsenstein 1985; de Vienne et al. 2013), testing for congruence on the basis of estimated phylogenies without accounting for uncertainty in the inference, and underestimation of the high probability of host-shift speciations (de Vienne et al. 2013).

3) The Endocnidozoa. Despite their molecular clock evidence for a common origin of Myxozoa/*Polypodium* in the late Cryogenian, Holzer et al. (2018) propose that *Polypodium* and myxozoans are actually not sister taxa and that Endocnidozoa is thus an invalid taxon. They suggest that the apparently contradictory evidence for the Devonian appearance of acipenseriform fish hosts of *Polypodium* (419-395 Ma) and their molecular clock estimate of a much earlier origin for the divergence of *Polypodium*/Myxozoa (651 Ma) is a result of long branch attraction. This view ignores the possibilities of host switching or prolonged existence as free-living forms. Furthermore, none of the specific arguments raised in support of these scenarios are convincing. These include: large differences in genome sizes of *Polypodium* and the myxosporean *Kudoa iwatai*; differences in gene content in myxozoans and *Polypodium*; comparative differences in orthologous genes of *K. iwatai*,

Polypodium and free-living cnidarians; putative differences in stages that invade hosts ('binucleate cells'), and; contrasting diversities of *Polypodium* and myxozoans. We consider these in order as follows:

- Genome sizes are notoriously variable and they are often reduced in parasitic organisms (e.g. Tsai et al. 2013). The larger genome size of *Polypodium* may thus reflect inclusion of a free-living stage in the life cycle. Furthermore, the genome size of the myxozoan *Thelohanellus kitauei* is 8x larger (188.5 Mb) than that of *K. iwatai* (and is some 33% of the genome size of *Polypodium*).

- Described differences in gene content between *Polypodium* and myxozoans (e.g. reductions of genes related to development, cell differentiation and cell-cell communication in myxozoans) can be explained by retention of a free-living stage in *Polypodium*. In addition, the comparison so far only includes myxosporeans, which show a higher degree of simplification, whereas the morphologically more complex malacosporeans may have retained more of these genes.

- The highlighted lower overlap in exclusive orthologous genes (OGs) between *K. iwatai* and *Polypodium* than between *K. iwatai* and two free-living cnidarians (*Nematostella vectensis* and *Hydra magnipapillata*) is based on relatively low numbers (24 with *Polypodium*, 44 with *N. vectensis*, 31 with *H. magnipapillata*). Such variation could reflect any number of processes (e.g. patterns of gene loss, gene assembly, contamination [e.g. see Kayal et al. 2018], etc.).

- Binucleate cells in *Polypodium* are described as 'a larval, diploid stage' that invades fish hosts and those in myxzoans as haploid cells that invade annelid hosts. Both the descriptions and juxtapositions are flawed. *Polypodium* binucleate cells that invade fish are haploid (Raikova 1994; 2008; and see earlier discussion) and myxozoan stages that invade both fish and annelid hosts are cell-in-cell complexes (Feist et al. 2015; and see earlier discussion). Furthermore, in malacosporeans unicellular haploid stages invade bryozoan hosts.

- There are precedents for extensive radiation in one parasitic lineage and little in its sister taxon within parasite clades. For example, the Aspidogastrea (with some 80 species) is sister to the Digenea (with >10,000 species) (Cribb et al. 2003). Such patterns of radiations are likely explained by unique, lineage-specific key innovations (see later section on diversification patterns). The argument that different patterns of diversification signify independently evolved lineages is therefore not necessarily convincing. In addition, the monotypic status of *Polypodium* may partly be an artefact arising from lack of investigation of parasites in eggs of non-commercial fish. New evidence (divergence in housekeeping genes; Hartigan et al. unpublished data) also demonstrates cryptic species within acipenseriform hosts.

In summary, we propose that various shared aspects of development and life history that we highlight above (e.g. sub-epidermal muscles, development within cells, cell-mediated invasion of fish hosts) are consistent with the repeated identification of *Polypodium* and myxozoans as sister taxa in phylogenomic analyses (Chang et al. 2015; Kayal et al. 2018). In contrast, none of the arguments for the demise of Endocnidozoa forwarded by Holzer et al. (2018) are well supported. We therefore

conclude that the available evidence continues to support *Polypodium* and Myxozoa as sister taxa forming the Endocnidozoa.

6.3 Scenarios of endocnidozoan evolution and recommendations for future study

We now have evidence in the form of molecular clock analysis (Holzer et al. 2018) that endocnidozoans are an ancient lineage that originated during a period when ancestors to all their currently recognised hosts were also present. Parasitism could have evolved at that time (or later) when first hosts were acquired that belonged to groups currently recognised as endocnidozoan hosts (or their precursors). Alternatively, first hosts may have belonged to an unrecognised or extinct group. Extrapolation from the life cycle of *Polypodium* suggests that early endocnidozoans may have been free-living benthic animals that used tentacles for food capture and to gain purchase to and move across the substratum using nematocysts and muscles. In view of the phylogenetic placement of Endocnidozoa, this suggests a beguiling scenario of a transitional form that shares a benthic habitat with attached basal anthozoans and an unattached mobile adult form with its sister medosozoans. Inferring patterns of host acquisition over time (Figure 3) are difficult in view of the many issues we discuss above, including undersampling, lack of fossil evidence, extinctions, and plasticity in cnidarian life cycles. However, if these various issues are not problematic, we propose that the more convincing scenarios are that fish or their precursors served as first hosts (Figure 3; Scenarios 1, 2) or that parasitism was acquired independently in lineages leading to *Polypodium* and Myxozoa (Figure 3; Scenario 6). The scenario of invertebrates as first hosts acquired by both lineages (Figure 3; Scenario 4) is more unlikely because it requires re-acquisition of a complex free-living adult stage in *Polypodium*. Because parasitism generally entails a transition to host dependency associated with specialized and reductive evolution (e.g. simplified metabolism, loss of digestive tracts, expansive uptake surfaces) reversion back to the more complex features of free-living ancestors is often regarded as nearly impossible - in keeping with Dollo's law (a complex trait cannot re-evolve in the same form) (Cruickshank & Paterson 2006). There is evidence for such reversions in taxa that are not strongly modified for parasitism (e.g. mites and strongyloid nematodes; Cruickshank & Paterson 2006). Reversion to a free-living form has also been inferred for a diplomonad flagellate via acquisition of bacterial genes coding for prey-degrading enzymes needed by a free-living phagotroph (Xu et al. 2016). We contend that reversibility is unlikely for highly modified metazoans like cestodes or myxozoans.

De Baets et al. (2015) outlined various recommendations for future work on discerning the origins and patterns of host acquisition by parasite taxa, and proposed that the most conservative and least circular approach would be to use robust relaxed molecular clock estimates for the origin of free-living ancestors and the earliest certain appearance of parasites in the fossil record. It is just conceivable that fossil myxozoan spores may be preserved along with the soft and small remains of e.g. embryos in ancient deposits (such as the Douchantuo Formation) and these would be extremely valuable as calibrations in molecular clock analyses to constrain age estimates. Furthermore, as myxozoan diversity and ecology becomes better understood other calibrations, such as unique radiations associated with geological events whose timing is well constrained, may be adopted (De Baets et al. 2016; Ho et al. 2015). An exciting possibility here would be successful sequencing of ancient DNA from chitinised myxosporean spores preserved in sediment cores. Calibration of the molecular clock is the most important factor influencing divergence dates (Inoue et al. 2010; dos Reis et al. 2015), thus incorporating myxozoan-specific calibration points would greatly improve

future molecular clock investigations. This could also help to control for their notoriously rapid rates of molecular evolution that may remain problematic even in analyses that accommodate rate variation. We also note that improved understanding of host use would enable greater confidence in patterns revealed by any phylogenetic analyses. Future studies of endocnidozoan origins and host acquisition should be judicious in selecting data for analysis and consider how conclusions may be compromised and constrained by current knowledge of endocnidozoan diversity and patterns of host use in the present-day.

7 Adaptation and diversification of endocnidozoans

7.1 Adaptations to a parasitic life style

Many endoparasites exhibit morphologies that are simplified in comparison to those of their free-living relatives. These may reflect processes such as selection against features that are no longer functional, increased allocation of resources for reproduction, or adaptation to confined spaces within hosts (Okamura et al. 2015b). Malacosporeans exhibit considerable tissue loss, but this morphological simplification is taken to the extreme in myxosporeans which develop exclusively as amorphous plasmodia and pseudoplasmodia. Myxozoans have also undergone miniaturisation, perhaps as a consequence of adapting for life in confined host spaces, such as body cavities, blood vessels and between or within cells (Okamura et al. 2015b). Simplification along with miniaturisation has enabled myxozoans to converge on patterns of host exploitation similar to those of protists (Okamura et al. 2015a), involving extensive multiplication as unicellular forms within hosts. In myxozoans these forms comprise invasive cells and cell-in-cell complexes that multiply within hosts whilst journeying to target infection sites, as well as sacs, myxoworms, pansporocysts, pseudoplasmodia and plasmodia that support spore development. Free-living cnidarians produce relatively few and large eggs and numerous tiny sperm (anisogamy). Even though there is some confusion over which myxozoan cells are gametes, it is clear that both *Polypodium* and myxozoans are isogamous (Raikova 2008; Okamura et al. 2015b). This may be adaptive for parasitism if self-fertilisation is undertaken because finding a mate is difficult within hosts (Okamura et al. 2015b). Other adaptations to parasitism in *Polypodium* include inversion of germ layers and a polyploid trophamnion (Raikova 1994) functioning as a nurse cell during larval development and the multinucleate plasmodia that support sporogony in myxosporeans.

Parasites, however, may also evolve innovations involving complex and specialised designs. Examples include arrays of hooks and spines for host attachment in the scolex of cestodes and the proboscis of acanthocephalans, and the outer syncytial tegument specialised for absorption, secretion and protection in trematodes and cestodes (Ruppert et al. 2004). Multicellular spores of myxozoans and the modified detachable gonads (gonophores) of *Polypodium* are both specialised endocnidozoan innovations for transmission using anchoring nematocysts (Ibragimov and Rakova 2004). In addition, myxosporean spores display further morphological and structural innovations in the form of inflatable caudal processes in actinospores and hardening of the outer wall of myxospores. Caudal processes enable actinospores to be carried away from the benthic habitats of worm hosts, presumably facilitating transmission to fish hosts. Hardening confers environmental resistance and hence spore longevity, presumably facilitating eventual ingestion of myxospores by worms. Myxospores also notably vary in form with some morphotypes arising from convergence. The drivers and functional significance of variation in myxospore morphology remain obscure.

Similar actinospore morphotypes are produced in species that develop different myxospore morphotypes suggesting that myxospores have undergone greater morphological diversification than actinospores (Fiala et al. 2015a). However, it must be stressed that actinospores are very poorly sampled (see below).

7.2 Patterns of diversification

A meta-analysis study has revealed that parasite species richness is strongly correlated with that of their hosts – richer host clades harbour richer parasite assemblages (Kamiya et al. 2014). This pattern is reflected in the invertebrate host diversities of the relatively species-poor malacosporeans and the highly speciose myxosporeans. Malacosporeans exploit freshwater bryozoans belonging to the Class Phylactolaemata, a notably depauperate group of 74 extant species (Massard and Geimer 2008), whilst myxosporeans exploit freshwater and marine oligochaetes and polychaetes which comprise 3500 and 8000 extant species, respectively (Ruppert et al. 2004). Coincident with myxosporean radiations in polychaete and oligochaete radiation is residency in marine and freshwater environments, respectively (Fiala et al. 2015a). The relatively limited radiation of malacosporeans (despite their apparent capacity to infect diverse fish families) suggests that invertebrate host diversity may primarily explain the contrasting diversification patterns of malacosporeans and myxosporeans. Congruence in molecular phylogenies of some myxosporeans and fish provides evidence for co-diversification with certain fish (Holzer et al. 2018).

In the present day the highly speciose myxosporeans infect vertebrate host gills, skin, fins, eyes, kidney, intestine, liver, gall bladder, nervous system, cartilage, muscles, swimbladder and gonad to produce infectious spores (Feist & Longshaw 2006; Molnár and Eszterbauer 2015) whilst the malacosporeans exploit fish kidney for spore production. The acquisition of plasmodia suited for sporulation in organs and tissues may have been critical for myxosporeans to exploit tissues (Fiala et al. 2015a). Phylogenetic analyses have provided evidence that patterns of ‘tissue tropism’ (site-preference) are lineage-specific and that the ability to exploit particular host environments may support radiations in at least some myxosporeans (e.g. Eszterbauer 2004; Holzer et al. 2004; Heiniger et al. 2013).

Several other traits have been identified as potential drivers of the spectacular myxosporean radiation or as constraints on malacosporeans to undergo radiation (Fiala et al. 2015a). These include: acquisition of hardened, environmentally resistant spore valves in myxosporeans; improved uptake of metabolites by syncytial myxosporean stages (vs. epithelial malacosporean stages), and; incorporation of additional vertebrate host groups (e.g. amphibians, waterfowl and shrews) by myxosporeans. Some non-fish hosts (e.g. shrews) are entirely terrestrial thus terrestrial oligochaetes are highly likely to serve as primary hosts with trophic transmission achieved when infected worms are ingested. This raises the spectre of a diversity of fully terrestrialised myxozoans with life cycles involving vertebrate hosts that eat earthworms.

A current hindrance to understanding the significance of host diversity and environments for myxozoan diversification is our considerable ignorance of parasite diversity in general and of myxozoans in particular (Okamura et al. 2018). Even in relatively well-sampled regions, novel myxozoan diversity is being detected by eDNA sampling (Hartikainen et al. 2016). Particularly poorly sampled regions include the deep sea, polar and tropical environments. Ongoing research suggests that extrapolation of diversity in tropical fish may lead to predictions of myxozoan species richness

that rivals or exceeds that of their free-living cnidarian relatives. For instance, the high host specificity of many ceratomyxids (Gunter et al. 2010, Heiniger and Adlard 2013) suggests that Australia's coral reef fish will be exploited by over 1,500 species of *Ceratomyxa* of which < 1% have been described (Queensland Museum Network, 2010). Recall that the currently described 2596 myxozoans represent some 20% of cnidarian species diversity already. While new free-living cnidarians species can also be expected (Appeltans et al. 2012) the hidden nature of endoparasites is likely to make myxozoans particularly undersampled. Further impediments to understanding myxozoan diversification include: limited study of invertebrates as parasite hosts; a research focus on economically important parasites; inapparency of many myxozoan infections; and difficulties in sampling and identification (including low infection prevalences, lack of expertise, and requirement of microscopy and molecular sequence data) (Okamura et al. 2018).

Finally, it is clear that endocnidozoans are characterised by rapid rates of molecular evolution. How this trait may relate to diversification remains unclear. We predict that this issue will begin to be resolved with the development and analysis of new transcriptome, genome and proteome datasets with larger taxonomic coverage.

8 Conclusions

Cnidarians have evolved parasitism on multiple occasions but only in the Myxozoa has this entailed substantial radiation. There is evidence that myxozoan diversification is linked with host diversity and it is likely that various cnidarian traits served as preadaptations for assuming parasitic lifestyles. However, our review raises more questions than answers. Was parasitism adopted as an ancient lifestyle in the cnidarians when the Endocnidozoa first appeared in the Ediacaran or was there a substantial period when early endocnidozoans were free-living? Does undersampling and our current lack of knowledge of endocnidozoan diversity and host use greatly compromise insights on host groups and co-phylogenetic analyses? To what extent might host switching preclude inferences about ancient hosts and how have mass extinctions driven patterns of endocnidozoan diversity over time? The holy grail of gaining insights into endocnidozoan stem lineages may begin to be addressed if relevant data can be gathered. Such data include fossil evidence that would strengthen molecular clock analyses. Thus, systematic investigation of host pathologies and attempts to retrieve remains of myxozoan spores in palynological samples or from coprolites could be fruitful, particularly from sites with exceptional soft-tissue preservation. Investigations of myxozoan spore taphonomy would be complementary and informative for such research. Meanwhile, more convincing cophylogenetic analyses require better understanding of myxozoan diversity and better resolution of host phylogenies. This could be gained by systematic sampling of further potential host groups, eDNA sampling (especially from severely undersampled habitats), screening of existing archival databases, and incorporation of more comprehensive data in molecular phylogenies of host groups. We expect that advances will be made on at least some of these fronts and could thus help to reveal how endocnidozoans have evolved and diversified to become one of the major cnidarian clades in the present day. We also expect that further work will provide a better understanding of the origins and diversification of the other more limited radiations of parasitic cnidarians.

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