

**ORIGINAL ARTICLE**

# The joint evolution of the Myxozoa and their alternate hosts: A cnidarian recipe for success and vast biodiversity

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**Abstract**

The relationships between parasites and their hosts are intimate, dynamic and complex; the evolution of one is inevitably linked to the other. Despite multiple origins of parasitism in the Cnidaria, only parasites belonging to the Myxozoa are characterized by a complex life cycle, alternating between fish and invertebrate hosts, as well as by high species diversity. This inspired us to examine the history of adaptive radiations in myxozoans and their hosts by determining the degree of congruence between their phylogenies and by timing the emergence of myxozoan lineages in relation to their hosts. Recent genomic analyses suggested a common origin of *Polypodium hydriforme*, a cnidarian parasite of acipenseriform fishes, and the Myxozoa, and proposed fish as original hosts for both sister lineages. We demonstrate that the Myxozoa emerged long before fish populated Earth and that phylogenetic congruence with their invertebrate hosts is evident down to the most basal branches of the tree, indicating bryozoans and annelids as original hosts and challenging previous evolutionary hypotheses. We provide evidence that, following invertebrate invasion, fish hosts were acquired multiple times, leading to parallel cospeciation patterns in all major phylogenetic lineages. We identify the acquisition of vertebrate hosts that facilitate alternative transmission and dispersion strategies as reason for the distinct success of the Myxozoa, and identify massive host specification-linked parasite diversification events. The results of this study transform our understanding of the origins and evolution of parasitism in the most basal metazoan parasites known.

**KEYWORDS**

Cnidaria, host–parasite codiversification, life history evolution, molecular clock analyses, Myxozoa, *Polypodium hydriforme*

## 1 | INTRODUCTION

Biodiversity is to a great extent the result of the evolutionary history of species interactions, and major events in the diversification of life can be traced back to the appearance of new relationships (Margulis & Fester, 1991; Szathmáry & Smith, 1995). Organisms with a

parasitic lifestyle show the most spectacular species radiations (Price, 1980), accounting for more than half of the Earth's biodiversity and more than 10% of metazoan taxa (Dobson, Lafferty, Kuris, Hechinger, & Jetz, 2008; Poulin & Morand, 2004; Windsor, 1998). In parasitism, mutual evolutionary pressures fuel host and parasite genetic diversification (Pilosof, Morand, Krasnov, & Nunn, 2015; Thompson,

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1999; Yoder & Nuismer, 2010) and scenarios such as the acquisition of new hosts or new niches as well as within-host competition foster parasite speciation (Morand, Krasnov, & Littlewood, 2015).

Despite the central importance of species interactions to the diversification of life, the knowledge about the processes by which species undergo reciprocal evolutionary change through natural selection is patchy (Carmona, Fitzpatrick, & Johnson, 2015). An explicit framework to understand the role of species diversification in co-evolution is still required, and authors repeatedly caution against a direct link of the two (Althoff, Segraves, & Johnson, 2014; Brockhurst & Koskella, 2013; Poisont, 2015). However, investigating the extent of codivergence or parallel speciation of parasites and their hosts under strict analytical conditions (timing, sympatry, etc.) allows for insights into the joint evolutionary history of two organisms. This provides a basis for studies uncovering underlying processes that govern the nature of their interactions (Charleston & Perkins, 2006) and can help us to better understand the dynamics and history of biodiversity.

Myxozoans are a group of diverse cnidarians, representing almost one-fifth of presently known Cnidarian species (Zhang, 2011). Extremely reduced as an adaptation to parasitism, myxozoans are composed of only a few cell types throughout their life cycle. Characteristic spores (Figure 1, centre) serve as transmission stages between invertebrate and vertebrate hosts, predominantly in aquatic habitats. Myxozoans are strongly opportunistic and show a high degree of flexibility with regard to exploring hosts of different animal groups. Annelids and bryozoans are known as definitive hosts with 55 life cycles elucidated to date (Supplementary file S1). Fish compose the largest number of known intermediate hosts, but amphibians, reptiles, birds and mammals are also exploited (Fiala, Bartošová-Sojtková, Okamura, & Hartikainen, 2015; Fiala, Bartošová-Sojtková, & Whipps, 2015). Some fish-parasitic myxozoans even evolved into hyperparasites of platyhelminth endo- and ectoparasites of fishes (Freeman & Shinn, 2011; Overstreet, 1976; Siau, Gasc, & Maillard, 1981), and a muscle-dwelling species was found in an octopus (Yokoyama & Masuda, 2001). The history of these host-parasite associations is largely unexplored, but their interactions likely represent important drivers of the evolution of parasitism at the base of the Metazoa.

Based on a limited number of life cycle discoveries, in 2007, it was first suggested that the invertebrate host type mirrored large-scale myxozoan phylogeny as well as 18S rRNA secondary structure (Holzer, Wootten, & Sommerville, 2007). Most recently, Kodádková, Bartošová-Sojtková, Holzer, and Fiala (2015) determined that cartilaginous fish represent ancestral states for a number of phylogenetic lineages and these basal parasite lineages and their cartilaginous hosts likely co-originated in the Silurian. These results prompted us to document the common evolutionary history of myxozoans and their vertebrate and invertebrate hosts by cophylogenetic analyses, study the patterns of cospeciation and diversification in different host groups and investigate temporal congruence of these evolutionary events, using molecular clock analysis. Cospeciation has rarely been observed in fish parasites (summarized in Vanhove et al., 2015), and studies analysing codivergence patterns of heteroxenous parasites in more than one of their host groups are still missing. Our analyses cover all

myxozoan host-parasite associations for which sequence data are presently available and decipher the series of events that led to the distinct success of this enigmatic parasite group at the base of the Metazoa.

## 2 | MATERIALS AND METHODS

### 2.1 | Sequence data and phylogenetic analyses

A prerequisite for cophylogenetic studies is the reconstruction of reliable phylogenetic trees at both family and species levels. Large-scale relationships among myxozoan taxa are relatively well established based on 18S rDNA sequences, and topologies are congruent with those based on the limited data available from other genes (recently reviewed by Fiala, Bartošová-Sojtková, Okamura, et al. (2015) and Fiala, Bartošová-Sojtková, and Whipps (2015)). We used a comprehensive collection of 18S rDNA sequences from 633 taxa (1,638 bp; Supplementary file S2), predominantly from vertebrate (563 taxa) and to a much lesser extent from invertebrate (110 taxa) hosts (with 40 species overlapping between the two data sets). For construction of invertebrate phylogenetic trees, 18S rDNA sequence data were used (23 taxa, 1,859 bp) while for vertebrates, mitochondrial 16S rRNA gene sequences were compiled (245 taxa, 1,572 bp), as the latter is the most comprehensively sampled gene in fishes. For vertebrate hosts, a second data set was compiled focusing on a considerably larger data set (full mitogenomes, 105 taxa, 15,913 bp) and a higher taxonomic level, that is, host families. This data set allows for analyses of the early history of cophylogeny signatures in vertebrate hosts, independent from recent events.

Nucleotide sequences were aligned applying the MAFFT version 7.017 alignment (Katoh, Misawa, Kuma, & Miyata, 2002) implemented in GENEIOUS version 9 (Kearse et al., 2012), using the E-INS-i algorithm, with a gap opening penalty (-op) 1.5 (myxozoans)/1.5–4.0 (vertebrates and invertebrates) and gap extension penalty (-ep) 0.0. The alignments were edited, and highly variable sections were removed. Maximum parsimony (MP) analyses were performed in PAUP\* version 4.b10 (Swofford, 2003), using a heuristic search with random taxa addition, the ACCTRAN option, TBR swapping algorithm, all characters treated as unordered and gaps treated as missing data. JModelTest (Posada, 2008) was used to select the best-fitting model of evolution, using the corrected Akaike information criterion. Maximum-likelihood (ML) heuristic searches were performed in RAXML version 7.0.3 (Stamatakis, 2006), using the GTR +  $\Gamma$  model of nucleotide substitution. Bootstrap searches included 500 replicates for both MP and ML analyses. Bayesian inference (BI) analyses were performed in MRBAYES version 3.0 (Ronquist & Huelsenbeck, 2003), using the GTR +  $\Gamma$  model of evolution. Posterior probabilities were estimated from 1 million generations with four simultaneous MCMC chains, sampled at intervals of 100 trees, with burn-in set to 10%. To ensure convergence and an effective sample size, results were verified with TRACER version 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). Problems associated with single-gene phylogenies of rDNA and mtDNA sequences (Jeffroy, Brinkmann, Delsuc, & Philippe, 2006; Rokas & Carroll, 2006) were disrupted by comparison of the resultant host phylogenetic trees with

those of other studies employing large molecular data sets (Andrade et al., 2015; Betancur-R et al., 2017; Near et al., 2012; Nesnidal et al., 2013; Struck et al., 2011) and by exclusion of taxa with a strongly divergent position from these studies, for example, if a fish species did not cluster with other members of the same host family.

## 2.2 | Diversification estimates

Overall rates of diversification for myxozoans were estimated in R version 3.2.4 (R Core Team, 2013), using the APE package version 3.4 (Paradis, Claude, & Strimmer, 2004) and the GEIGER package version 2.0.6 (Pennell et al., 2014). Diversification estimates were calculated based on a pure birth process and on a speciation:extinction rate of 2:1. The constant rate test (Pybus & Harvey, 2000) was used to test for even rates of cladogenesis through time. The Monte Carlo CR (MCCR) test was used to account for incomplete taxon sampling. A lineages through time (LTT) plot was produced, and the relative cladogenesis (RC) statistic (Pennell et al., 2014) was used to detect unusually rapid diversification shifts leading to lineage-rich clades in the tree.

## 2.3 | Cophylogeny analyses

Two methodological approaches were used to compare host and parasite phylogenetic relationships: (i) event-based tree reconciliation and (ii) global Fit analyses. The reconciliation of tree topologies interprets co-evolution as a stochastic process with cospeciation (concomitant host and parasite speciation), duplication (parasite speciation on one host lineage), host switching (colonization of a new host) and lineage sorting (disappearance of a parasite lineage from a host) as discrete events. Partially resolved ML trees with polytomies for clades with bootstrap support <50% were used for cophylogeny analyses. In some phylogenetic trees where polytomies affected >35% of all taxa, fully resolved ML trees were used (indicated in results in Table 1). To eliminate the risk of maximizing cospeciation events by tree reconciliation (de Vienne et al., 2013), we performed a parameter-adaptive approach in CORE-PA version 0.5 (Merkle, Middendorf, & Wieseke, 2010), testing  $10^4$  cost sets on the quality function, using the simplex method and avoiding an a priori cost assignment. Global fit estimates were implemented in PARAFIT (Legendre, Desdevises, & Bazin, 2002; APE package version 3.4 in R), which uses a permutation test on a matrix of raw patristic distances to evaluate codivergence and thereby overcomes the need for well-resolved tree topologies. Distance-based cophylogenetic analyses are hence considered less biased than topology-based methods. Multiparasite host species as well as multihost parasite taxa are anomalous under a codivergence paradigm (Banks & Paterson, 2005). Most myxozoans are highly host specific with regard to their vertebrate hosts (Molnár & Eszterbauer, 2015). Only a few marine histozoic species, that is, *Kudoa* spp. (Burger & Adlard, 2011; Gleeson, Bennett, & Adlard, 2010; Whipps & Kent, 2006) and *Enteromyxum leei* (Sitjà-Bobadilla & Palenzuela, 2012), are able to infect a broad range of hosts from different fish families. Myxozoans appear to be less host specific with regard to their invertebrate hosts, with, for example, *Tubifex tubifex* as a known host for >30 different taxa. It is unlikely that

such host-parasite associations are the result of true cophylogeny but rather, for example, host switching to a more abundant alternative host (Lootvoet, Blanchet, Gevrey, Buisson, & Tudesque, 2013; Poulin, 2011). As the mechanisms leading to the generation of such associations are unknown, independent from the data set, species with >8 hosts or parasites were excluded from analyses. This affected only a small percentage of the data, namely 0.8% of myxozoan species and 2.9% of hosts (detailed in Supplementary file S3).

All myxozoans and their invertebrate hosts were analysed together. Due to the large number of taxa sequenced from their vertebrate hosts and the respective size of trees, the data set was split into subsets, representing the major clades in the parasite tree (Figure 1) but excluding malacosporans, as uncertainty exists with regard to “true” fish hosts in this group, that is, hosts that can form spores, which are infective to an invertebrate host (Patra, Hartigan, Morris, Kodádková, & Holzer, 2017). Analyses were also performed after reduction in the large subclades “Biliary tract I,” “Histozoic I” and “Histozoic III”; Figure 1) and of these subclades themselves, allowing assessment of recent and ongoing cospeciation in closely related myxozoans and their hosts. Results from diversification and cophylogeny analyses were considered statistically significant at  $\alpha = 0.05$ .

## 2.4 | Molecular clock analyses

To provide meaningful information on the timing and divergence of the major clades of myxozoans in relation to that of their hosts and other metazoans, concatenated alignments of six protein-coding genes, that is, aldolase (200 aa), triosephosphate isomerase (217 aa), phosphofructokinase (175 aa), methionine adenosyltransferase (348 aa), elongation factor 1 alpha (418 aa) and ATP synthase beta chain (430 aa) were used. Using an existing data set (Erwin et al., 2011), we mined published and new genomic and transcriptomic parasite and host data sets resulting in the final alignment of 2,444 aa, including 10 myxozoans + 128 other metazoan taxa (Supplementary file S4). Divergence times were estimated using BEAST version 2.4.7 (Bouckaert et al., 2014; Drummond, Suchard, Xie, & Rambaut, 2012). The ML tree topology computed using RAXML under the WAG+  $\Gamma$  model of evolution was used as a user-specified starting tree. The best model of evolution for this analysis was selected by PROTTEST 3.4.2 (Darriba, Taboada, Doallo, & Posada, 2011), using the Akaike information criterion. The same model was used for molecular clock analysis. The BEAST input file was constructed using BEAUTI (within BEAST package). The lognormal relaxed molecular clock model, which accounts for independent rates of heterogeneity from lineage to lineage (Thorne & Kishino, 2002), and the Yule speciation prior set were used to calculate divergence times and corresponding credibility intervals. Fourteen fossil calibration points (Supplementary file S5; adapted from Dohrmann & Wörheide, 2017) were used. MCMC analyses were run for 10,000,000 generations, and after 10% burn-in sampled every 1,000 generations. The convergence of chains was confirmed using TRACER version 1.6 (Rambaut et al., 2014) and chronograms generated in TREEANNOTATOR version 2.4.6 (within BEAST

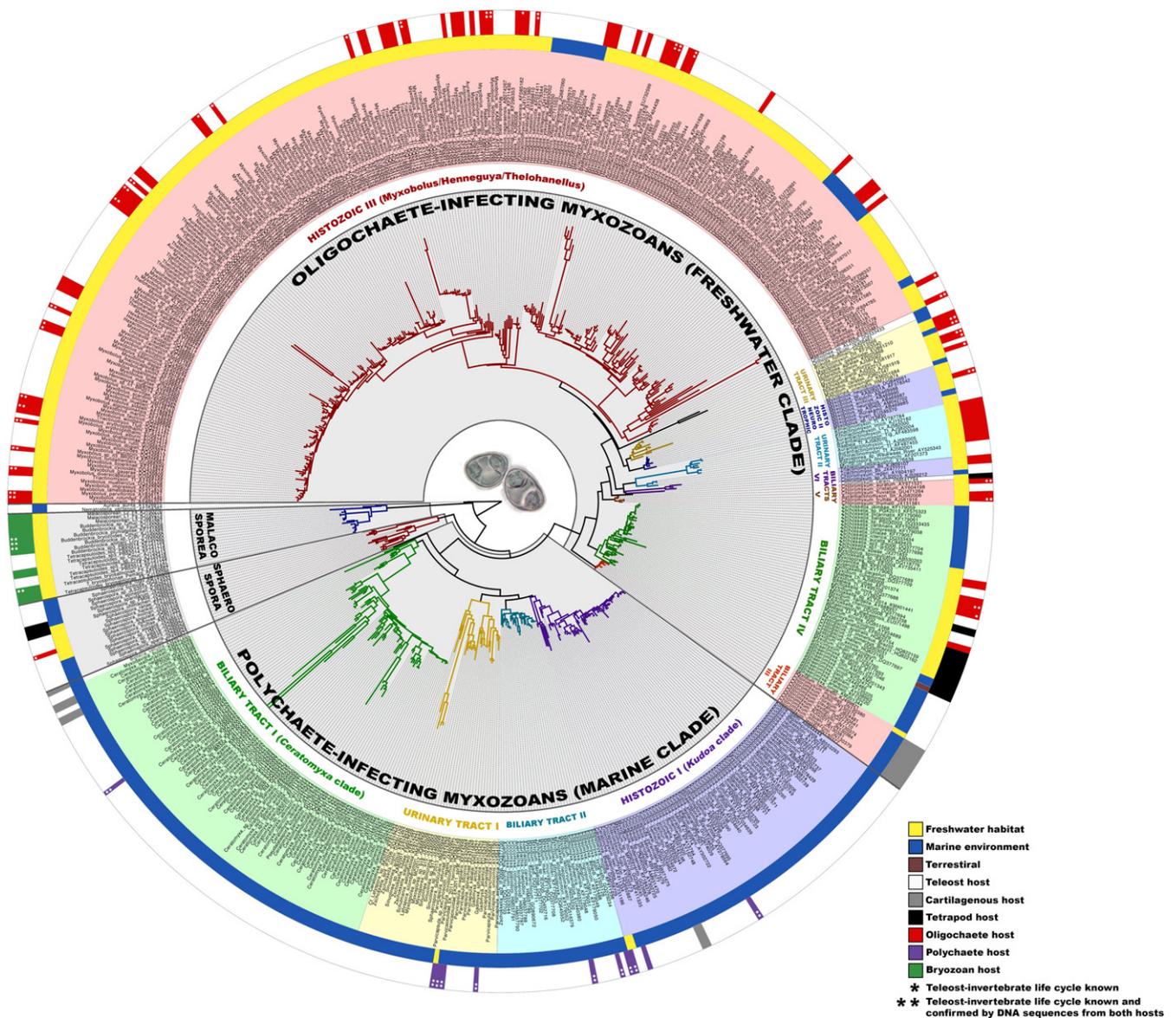
package). *Polypodium hydriforme* is a sister taxon of the Myxozoa (Chang et al., 2015; Okamura, Gruhl, & Reft, 2015) with high nucleotide substitution rate and assumed long-branch attraction (LBA) to the Myxozoa (Evans, Lindner, Raikova, Collins, & Cartwright, 2008). To estimate reciprocally independent divergence times of the Myxozoa and *P. hydriforme* and ascertain the influence of these taxa on the calculated origin of the Cnidaria, we performed BEAST analyses excluding either or both of the taxa from the data set. To confirm whether phylogenetic placement of the two long-branching taxa as sister lineages is correct, the position of each long-branching taxon was examined separately within a large metazoan phylogenomic data set (51,940 aa; Chang et al., 2015; data re-analysed).

### 3 | RESULTS

#### 3.1 | Host and parasite phylogenies

##### 3.1.1 | Myxozoa

In accordance with published records on smaller data sets (Bartošová, Fiala, & Hypša, 2009; Fiala, 2006; Fiala, Bartošová-Sojtková, Okamura, et al., 2015; Fiala, Bartošová-Sojtková, & Whipps, 2015), the present large-scale phylogenetic analysis of 633 18S rDNA sequences of myxozoans produced a tree topology composed of four major lineages (Figure 1): Malacosporea, *Sphaerospora* sensu



**FIGURE 1** Maximum-likelihood phylogenetic tree of the Myxozoa (633 taxa; RAXML), indicating different biological characteristics important for phylogenetic clustering: infection site in vertebrate host, aquatic habitat (freshwater vs. marine) as well as vertebrate and invertebrate host groups. The number of species per subclade is indicative for their known diversity; notice massive radiation of histozoic myxozoans in freshwater teleosts, especially cyprinids. Center: typical myxozoan spores as produced in fish [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

stricto (s. str.; referred to as sphaerosporids) and two large clades, previously termed “freshwater” and “marine” clades (Fiala, 2006). All four lineages are highly supported (bootstrap values 95%–100%) in both MP and ML analyses and maximum posterior probabilities in BI (not shown). Mapping of host and habitat characteristics to the parasite tree (Figure 1) provided new insights into the evolution and classification of the Myxozoa. While the taxa from the “marine” clade rarely inhabit freshwater environments (<2%), almost a quarter of the species clustering in the “freshwater” clade were collected in marine hosts and habitats. Twelve years after the original definition of these clades by Fiala (2006), we show that they are less defined by their environment (freshwater vs. marine) but rather by their invertebrate host type, oligochaete-infecting myxozoans (OIM) vs. polychaete-infecting myxozoans (PIM), without exceptions. With malacosporeans using bryozoans as hosts, all major clades but sphaerosporids, for which molecular data from invertebrate hosts have not been obtained yet, are characterized by a distinct invertebrate host group. Subclades (colour coded in Figure 1) are supported by bootstrap values between 82 and 100 and posterior clade probabilities of 0.9–1.00 and appear to be defined by the organ system or tissue they infect in their vertebrate hosts. 18S rDNA sequences available from cartilaginous fish (17 taxa) cluster in various basal positions (Figure 1): (i) *Bipteria vetusta* as the most basal lineage of PIM, (ii) basal lineages of biliary tract I clade (*Ceratomyxa* spp.), (iii) most basal representatives of *Kudoa* spp. in Histozoic I clade, and (iv) *Chloromyxum* spp. of Biliary tract III clade, the most basal lineage of OIM. Tetrapod hosts are known from some members of *Sphaerospora* s. str. as well as from a number of OIM and represent relatively early lineages in each clade.

### 3.1.2 | Invertebrate hosts

The phylogenetic analysis of invertebrate hosts in myxozoan life cycles shows four major clades (Figure 2): Bryozoans belonging to the Phylactolaemata represent the most basal lineage, and the members of respective families (Lophopodidae, Fredericellidae, Pectinatellidae, Cristatellidae and Plumatellidae) cluster in accordance with previous analyses based on large genomic data sets (Waeschenbach, Taylor, & Littlewood, 2012). Annelid hosts are represented by three clades, two of which belong to the polychaetes and separate the more basal Errantia from Sedentaria, while the last one is composed of oligochaetes, in agreement with phylogenomic and biological studies on annelids (Zattara & Bely, 2015; Zrzavý, Říha, Piálek, & Janouškovec, 2009).

### 3.1.3 | Vertebrate hosts

Estimating fish (vertebrate) phylogeny based on mitochondrial 16S rRNA gene sequences produced less robust trees than those of full mitogenomes. However, the use of 16S rRNA gene data allowed for the inclusion of a maximum number of host sequences and the resultant topologies were overall found congruent with the most recent comprehensive phylogenetic analysis of bony fishes based on

21 molecular markers (Betancur-R et al., 2017). Full mitogenome phylogeny consistently resolved into correctly positioned taxa. Due to the spectacular radiation of percomorph fishes (Percomorpha = Percomorphaceae) but restricted taxon sampling with regard to myxozoan hosts, the subclades Carangaria, Ovalentaria and Eupercaria were often polyphyletic, but were maintained as these lineages cluster closely together and their relationship is presently *incertae sedis* (Betancur-R et al., 2017). The phylogeny of cyprinid hosts corresponds to published reports (Gilles et al., 2001; Stout, Tan, Lemmon, Lemmon, & Armbruster, 2016).

## 3.2 | Cophylogenetic comparisons

### 3.2.1 | Myxozoa—vertebrate hosts

Our analyses strongly rejected random associations between myxozoans and their invertebrate hosts, with a highly significant outcome of global fit and tree reconciliation analyses (18 cospeciation events determined by CORE-PA and PARAFIT; Table 1). The basal branching pattern in the myxozoan tree is highly congruent with that of their invertebrate host groups (Figure 2). Bryozoans as the phylogenetically oldest host group accommodate the Malacosporea which show lower support for codivergence with their bryozoan hosts, with four (CORE-PA) and five (PARAFIT) cospeciation events (11 parasites/7 hosts), likely explained by high parasite diversity and low host specificity in the few presently known bryozoan hosts. In contrast, support for cophylogeny between myxozoans and their annelid hosts is considerable with 14 cospeciation events detected in 16 annelid hosts (28 parasites, Figure 2). Different scenarios of host acquisition exist in annelids, with similarly supported placements: The evolutionary older errant polychaetes were conquered either by host switching from the ancestor of Sedentaria (Figure 2, main tree and A, best solution by CORE-PA) or multiple lineages emerged in polychaete ancestors (Figure 2b, second-best solution by CORE-PA). Another solution is a switch from errant polychaetes to more recent sedentary ones (Figure 2c). The uncertainty about the common evolutionary history of myxozoans and the basal annelid branches is likely based on the high number of divergent polychaete lineages and limited parasite data from these. Duplications and host switches happened frequently in oligochaetes from which a high diversity of taxa is known.

### 3.2.2 | Myxozoa—vertebrate hosts

Significant cophylogenetic signal was detected between myxozoans and their vertebrate hosts, in all major clades; however, the outcome of the analyses varied depending on the data set (Table 1). For all data sets but sphaerosporids, tree reconciliation methods ascribed a higher number of cospeciation events to higher-level taxa (host families and above) while PARAFIT detected more associations in lower level taxa (host species; Table 1). As an example, 46 of 65 (71%, CORE-PA) vs. 8 of 32 (25%, PARAFIT) codiversification events in the PIM data set were ascribed to family- and higher-level associations. The sphaerosporid data set lacks lineages of numerous closely related

**TABLE 1** Cophylogenetic reconciliations by CORE-PA and PARAFIT

Data set	#P	#H	Tree reconciliation (CORE-PA)				Global fit (PARAFIT)			
			#C/D/HS/S	Cost	Quality	Sign.	#C	ParaFitGlobal	Sign.	
Invertebrate hosts										
Complete set	39	23	18/30/15/5	11	0.000000	Yes	18	0.0945601	<b><i>p</i> = .001</b>	
Vertebrate host species										
<i>Sphaerospora</i> s. str.	17/19	17/19 <sup>†</sup>	10/3/3/10 <sup>‡</sup>	5	0.009468	Yes	5	0.01670787	<i>p</i> = .092	
PIM full data set	161 <sup>†</sup>	121 <sup>†</sup>	65/67/28/515	60	0.003426	No	32	7.88407522	<b><i>p</i> = .001</b>	
PIM reduced data set	76	71 <sup>†</sup>	27/33/14/72 <sup>‡</sup>	25	0.002306	Yes	18	0.58972102	<b><i>p</i> = .001</b>	
OIM full data set	259	120	85/119/36/459	81	0.000439	No	113	20.3755961	<b><i>p</i> = .001</b>	
OIM reduced data set	106 <sup>†</sup>	69 <sup>†</sup>	47/36/16/182	34	0.001385	No	46	8.79035722	<b><i>p</i> = .003</b>	
PIM Biliary tract I	63 <sup>†</sup>	50 <sup>†</sup>	23/30/9/65	21	0.000285	Yes	28	0.56145651	<b><i>p</i> = .008</b>	
PIM Histozoic I	47 <sup>†</sup>	42 <sup>†</sup>	22/15/8/148	16	0.001288	Yes	4	0.03684647	<i>p</i> = .165	
OIM noncyprinids	135	88	46/69/19/258	44	0.005878	No	57	3.58548745	<b><i>p</i> = .001</b>	
OIM cyprinids	109 <sup>†</sup>	31	30/38/12/93	26	0.000000	No	1	0.06933227	<i>p</i> = .895	
Vertebrate host families										
<i>Sphaerospora</i> s. str.	19	14	9/8/1/20 <sup>‡</sup>	6	0.011448	Yes	6	0.01443587	<b><i>p</i> = .043</b>	
PIM	158	46	46/84/27/319	55	0.027099	No	8	0.81350365	<i>p</i> = .532	
OIM <sup>§</sup>	128	51	29/74/24/151	40	0.056951	Yes	15	0.49897181	<b><i>p</i> = .005</b>	
PIM Biliary tract I	61	26	16/33/11/67	20	0.022055	Yes	5	0.14785371	<i>p</i> = .284	
PIM Histozoic I	46	22	10/27/8/100 <sup>‡</sup>	16	0.036960	Yes	3	0.01248965	<i>p</i> = .904	

#P = number of parasite taxa, #H = number of host taxa used for analysis (in case data sets differ due to malpositioning of host taxa in phylogenetic trees these are stated "tree reconciliation/Global fit"); #C/D/HS/S = number of cospeciation/duplication/host switching/sorting events, cost and quality (CORE-PA) and ParaFitGlobal value as well as significance (yes/no for CORE-PA, probability for PARAFIT). Reconciliations of myxozoans and invertebrate hosts based on 18S rDNA sequences, of vertebrate hosts based on 16S rRNA (vertebrate host species) or full mitogenomes (vertebrate host families). Topology-based cophylogenetic analyses used ML trees with nodes supported by a bootstrap value under 50 collapsed and fully resolved ML trees if >35% of taxa affected by polytomy (marked<sup>†</sup>). <sup>‡</sup>second-best solution in CORE-PA chosen as best result due to unrealistically high costs for certain events, which are not biologically plausible (Desdevises, Morand, Jousson, & Legendre, 2002), clade names as in Figure 1 with PIM = polychaete-infecting myxozoans and OIM = oligochaete-infecting myxozoans, <sup>§</sup>excludes data from cyprinids. Significant outcomes are listed in bold.

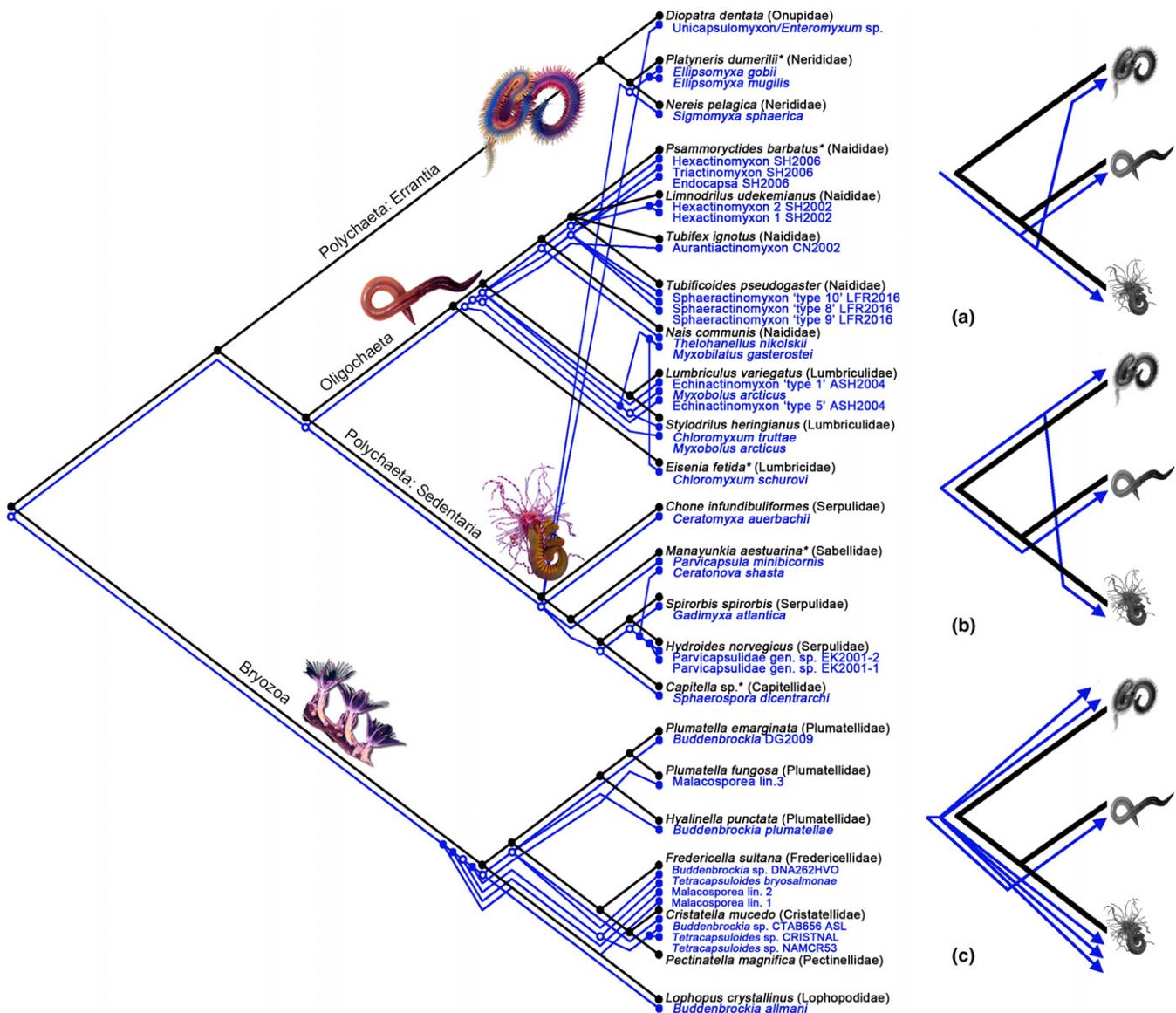
species (both, host and parasites); hence, the presently known codiversification events are historic in nature. Overall, we conclude that older codiversifications in myxozoans and their fish hosts are likely equally common than those occurring at species level, indicating similar levels of historic and recent cospeciation events in fish.

The *Sphaerospora* s. str. data set (17/19 parasites, depending on analysis), as composed of distantly related hosts, compromises global fit analyses, which revealed only individual associations ( $n = 5$ ) but no significant overall fit ( $p = .092$ ). In contrast, Co-Re-PA estimated 10 cospeciation events and rejected random associations between hosts and parasites (Supplementary file S6\_Figure 1). The use of the full mitogenome data set for vertebrate hosts considerably improved the analyses (better range of sequence divergences) and resulted in a significant cophylogenetic signal in both analyses, although global fit was only marginally significant ( $p = .043$ ).

The PIM data set (161 parasites) contains two extremely diverse subclades of closely related taxa infecting the biliary tract and the muscle (biliary tract I and histozoic I in Figure 1). Their inclusion resulted in significant global fit ( $p = .001$ ), but tree reconciliation by CORE-PA rejected a cophylogeny scenario of myxozoans and their fish hosts unless these two subclades were collapsed, resulting in a database of 76 vs. 161 parasite taxa for the PIM clade (Table 1,

Supplementary file S6\_Figure 2, PIM reduced data set). CORE-PA is sensitive to analyses of large phylogenetic trees where host switches become more and more likely while, simultaneously, cospeciation events become less likely (Merkle et al., 2010). Independent analysis of the two species-rich subclades resulted in cophylogenetic signal only by tree reconciliation but no significant outcome by distance-based methods (Table 1). Relationships based on host families (mitochondrial data set) showed insufficient phylogenetic congruence in all methods (Table 1).

The largest data set of myxozoans and vertebrate hosts is available from OIM (259 taxa). Only global fit produced significant support for cophylogenetic scenarios. CORE-PA detected random associations in the full and two reduced data sets (106 taxa, random selection of representatives from all subclades, and 135 taxa, only noncyprinid hosts; Table 1 and Supplementary file S6\_Figure 3). This is explained by frequent host switches from the evolutionary older cypriniform to percomorph fishes, both hyperdiverse fish groups that show a maximum diversity and number of myxozoan lineages. The large data set of 109 myxozoan parasites from Cypriniformes (part of OIM) is characterized by extremely frequent within-group host switches (Supplementary file S6\_Figure 4), as a consequence all methods rejected a cophylogenetic scenario in this group. The



**FIGURE 2** Significant phylogenetic congruence of myxozoans and their invertebrate hosts (Bryozoa and Annelida), with cospeciation points indicated by open circles. Parasite tree (blue), host tree (black); multiparasite hosts *Tubifex tubifex*, *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi* excluded. a, b and c indicating potential history of host acquisition: (a) errant polychaetes were settled by host switch from sedentary polychaetes (CORE-PA, best reconstruction), (b) Errantia and Sedentaria were settled independently various times (CORE-PA, second-best reconstruction), (c) Sedentary polychaetes were settled from the ancestor of errant taxa (other likely scenario) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

analysis of phylogenetic congruence on host family level (mitochondrial genome data) produced a significant outcome in both methods (Table 1), indicating that historic codivergence scenarios in the OIM clade are obscured by more recent host switching events.

### 3.3 | Relative timing of divergence of myxozoans and their hosts

Our molecular clock analysis (Figure 3) estimated the origin of the Cnidaria as early as the Cryogenian Period, 786 Ma (723–848 Ma, 95% HPD). This is in accordance with recent conulariid fossil records (Van Iten, Leme, Marques, & Simoes, 2013) and supported by molecular data (e.g., Park et al., 2012), suggesting that the major cnidarian

lineages originated 720–635 Ma (Van Iten et al., 2013), or even earlier (784–772 Ma; Dohrmann & Wörheide, 2017). The basal divergence of the Myxozoa occurred in the late Cryogenian, 651 Ma (601–700 Ma), and the most recent common ancestor (MRCA) of all myxozoans is dated in the Ediacaran, 588 Ma (540–642 Ma), which is when annelid-infecting myxosporeans diverged from bryozoan-infecting malacosporeans. The origin of sphaerosporid myxosporeans was estimated for the beginning of the Cambrian, 534 Ma (487–580 Ma) and the split of PIM and OIM for the late Cambrian and early Ordovician, 495 Ma (447–537 Ma). The basal divergence of the Bryozoa was dated 575 Ma (514–627 Ma) and overlaps with the split of Malacosporea and Myxosporea. The basal divergence of Annelida is estimated later in the Ediacaran, 549 Ma (518–579 Ma),



**FIGURE 3** Timing of the evolutionary history of myxozoans and their invertebrate (bryozoan, annelid) and vertebrate (fish and tetrapod) hosts, using six independent loci, 138 taxa and 14 fossil calibration points (Supplementary file S5). Lognormal molecular clock method implemented in BEAST. Horizontal bars indicate 95% credible intervals of the divergence time estimates. For simplicity, node ages (million years ago) are only labelled for taxa relevant to this study. Clitell. = Clitellata (Oligochaeta+Hirudinea), Cam = Cambrian, Ordo = Odovician, Sil = Silurian, Devon = Devonian, Carb = Carboniferous, Per = Permian, Tria = Triassic, Jur = Jurassic, Cretac = Cretaceous, Pal = Paleogene, Neo = Neogene [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

slightly predating but overlapping in range with the emergence of sphaerosporids. The estimated origins of myxozoan invertebrate hosts agree with previously published data (e.g., Erwin et al., 2011; Simakov et al., 2015). Chondrichthyes, the intermediate hosts of the most basal lineages of PIM and OIM (see Sections 3 and 3.1), emerged in the Early Devonian, about 363 Ma (304–427 Ma), confirmed slightly earlier, 411–417 Ma (Licht et al., 2012) and 410–447 Ma (Inoue et al., 2010).

To investigate the effect of the high rate of nucleotide heterogeneity on the position and divergence time estimate for *Polypodium hydriforme* and the Myxozoa and on potential long-branch attraction (LBA) phenomena, we performed independent analyses. The analysis of our and Chang's data set (Chang et al., 2015) proved that, excluding reciprocal influence, Myxozoa and *P. hydriforme* both cluster as sister groups to the Medusozoa (trees not shown). In our timed data set, the resulting estimated node ages for the MRCA of Cnidaria were generally younger, 768 Ma (670–833 Ma) when *P. hydriforme* was excluded, and 735 Ma (682–801) when *P. hydriforme* and Myxozoa were excluded, than the node estimate including these taxa, which was 786 Ma (723–848 Ma) (Supplementary file S7). Timing of the emergence of vertebrate and invertebrate hosts of myxozoans was only minimally influenced by the elimination of the long-branching cnidarian taxa (Supplementary file S7).

### 3.4 | Diversification rate and lineages through time

Under a pure birth process, the MCCR-adjusted gamma statistic shows minor evidence of deceleration of cladogenesis over time ( $\gamma = -1.710481$ ,  $p = .032$ ; significant negative gamma statistic defined as  $\gamma < -1.645$ , one-tailed test). The lineages through time plot (Figure 4) clearly demonstrate that massive radiation took place only once teleost hosts had been acquired, although increased recent diversification may to some degree be linked to the extinction of earlier lineages (Pybus & Harvey, 2000), which is presently unknown for Myxozoa. The estimated overall net diversification rate within the Myxozoa is 24.8 per Ma (pure birth model of diversification) and 23.6 per Ma (2:1 speciation:extinction rate). The RC statistic indicated a significant difference in cladogenesis rate between lineages with unusually rapid shifts in diversification at the main three basal nodes defining the myxozoan tree (see Section 3.1) and at 25 nodes within the OIM lineage (Supplementary file S8). Applying the RC statistic with Bonferroni correction resulted in a stricter sampling of hyperdiverse clades of myxozoans only in cypriniform hosts (Supplementary file S8).

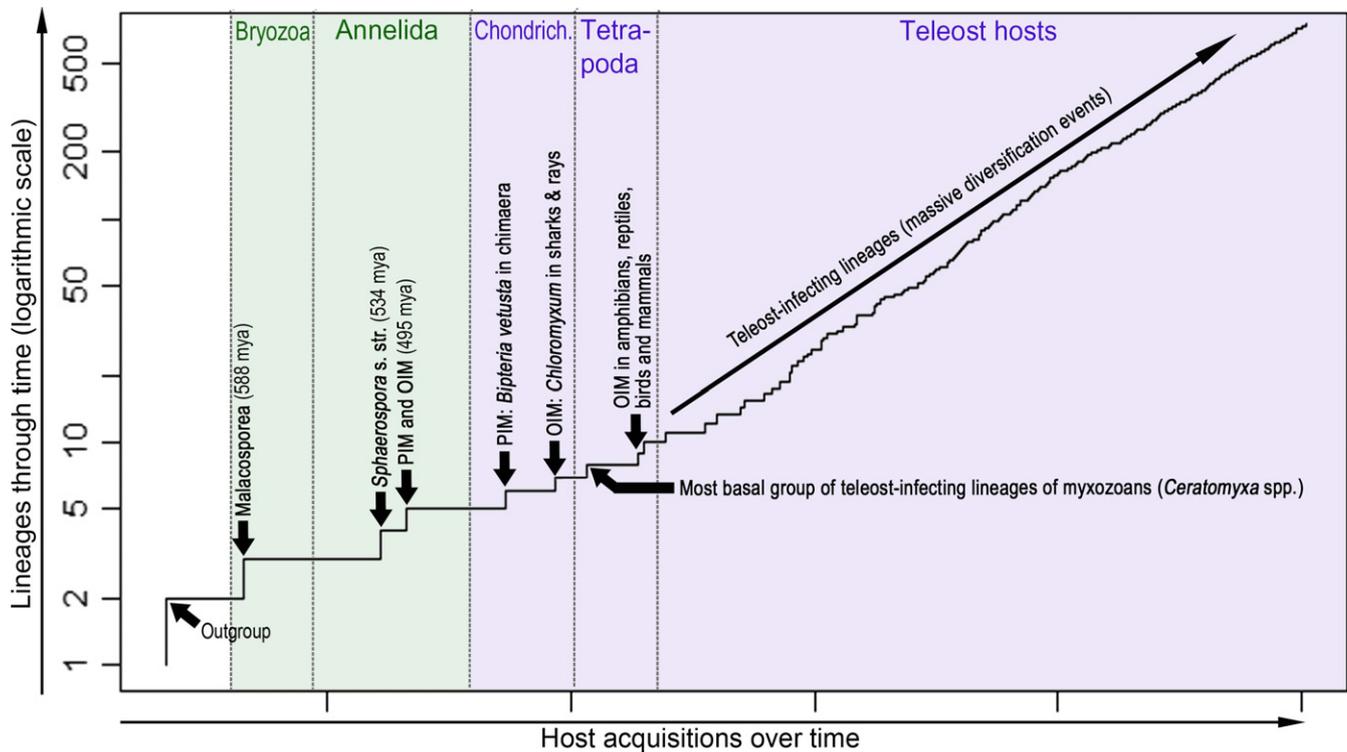
## 4 | DISCUSSION

### 4.1 | Myxozoan origin, host acquisition and timing of events

For the first time, we investigated the birth age of parasitic cnidarians belonging to the Myxozoa, using six independent nuclear genes for the reconciliation of molecular divergence time estimates of 10

myxozoans in relation to 128 metazoan taxa. Parasitic taxa are likely to have a faster rate of molecular evolution in order to win the "arms race" against their hosts (e.g., Bromham, Cowman, & Lanfear, 2013; Paterson et al., 2010). The rate heterogeneity across genes within the Myxozoa can be as high as that between myxozoans and other organisms (Hartigan et al., 2016; this study), and mitochondrial gene order and organization is highly variable (Takeuchi et al., 2015; Yahalomi et al., 2017), indicating a considerably accelerated rate of molecular evolution, possibly the fastest known among eukaryotes. This may well be explained by the extraordinary level of radiation that occurred within this group (Castro, Austin, & Dowton, 2002; Eo & DeWoody, 2010). The accelerated clock compromises molecular dating analyses of the Myxozoa, which are further limited by a missing fossil record. Despite compensating for this by the use of multiple genes and "relaxed clock" methods, at present, the enormous rate heterogeneity in the Myxozoa, further enforced by that of the parasitic sister taxon *Polypodium hydriforme*, remains a potential source of error and variation when dating the origin of the Myxozoa (588–616 Ma) and the Cnidarian crown lineages (735–796 Ma). Independent from exact dates, Cnidaria originated in the Early- to Mid-Cryogenian, suggesting a "slow burn" rather than a "Cambrian explosion" scenario for the evolution of the Cnidaria, a view supported by palaeontological (Budd, 2008; Fedonkin, 2003; Seilacher, Bose, & Pflüger, 1998; Van Iten et al., 2013) and molecular data (Cartwright & Collins, 2007; Peterson, Cotton, Gehling, & Pisani, 2008). Our analyses suggest that the basal divergence of the Myxozoa occurred in the late Cryogenian 651 Ma (601–700 Ma), during long-term global glaciations ("Snowball Earth", ~720–635 Ma). Crown lineages diverged in the Ediacaran and are older than previously estimated based on 18S rDNA sequences and excluding *P. hydriforme* (Precambrian and Cambrian, Kodádková et al., 2015).

Multigene analyses firmly place *P. hydriforme* as a sister taxon to the Myxozoa (present study; Chang et al., 2015). *P. hydriforme* uses acipenseriform fishes as hosts during larval development of a free-living and free-reproducing adult life cycle, and it had been suggested that both, *Polypodium* and the Myxozoa first exploited early primitive fish and thereafter only the myxozoans adopted invertebrate hosts for the development of their adult stages (Okamura & Gruhl, 2016; Okamura et al., 2015). In contrast, the results of the present study show compelling evidence identifying invertebrates as original hosts of myxozoans before the acquisition of fish as secondary/intermediate hosts: (i) Although molecular proof for sphaerosporids is still lacking, all other major clades of myxozoans are characterized by different invertebrate host groups (Bryozoa, Polychaeta and Oligochaeta; representing two to three independent host acquisition events), with invertebrate and parasite phylogenies congruent down to the most basal branches, while the history of cophylogeny of myxozoans and their fish hosts repeats itself along the time axis of each major clade indicating multiple acquisitions of fish as secondary hosts. (ii) According to our estimates, the Myxozoa and *P. hydriforme* diverged 651 Ma (601–700 Ma), long before the origin of the oldest fish lineages, with the first record of prehistoric Chondrichthyes, *Tantalepis gatehousei* (Sansom, Davies, Coates, Nicoll, &



**FIGURE 4** Lineages through time plot of myxozoans (18S rDNA sequences of 633 taxa), based on an ultrametric tree produced by BEAST, with time on the x-axis and the number of new lineages on the y-axis (logarithmic scale). Acquisition and diversification in different host taxa and specific events (lineages) indicated [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Ritchie, 2012) from the late Darrivilian (467–458 Ma). Divergence dates of Malacosporea, PIM and OIM mirror that of basal stem lineages of their respective invertebrate hosts. Ancient myxozoans may have initially parasitized extinct lineages of Bryozoa and Annelida. In fact, of the many bryozoan groups, only the evolutionary oldest group, Phylactolaemata, serves as known hosts of today's malacosporeans.

While the acquisition of an invertebrate followed by a vertebrate host is a likely scenario for myxozoans, this would imply that the ancestor of the Myxozoa and *P. hydriforme* was free-living and that *P. hydriforme* (emergence 601–700 Ma including Myxozoa, 581–699 Ma excluding Myxozoa) acquired its fish host only once Acipenseriformes appeared on Earth (Devonian, 419–359 Ma; Near et al., 2012); hence, parasitism in the Myxozoa and *P. hydriforme* would have evolved twice independently. In the past, support for a common origin of the Myxozoa and *P. hydriforme* was found in their development, where a binucleate cell represents the invasive stage and cell-in-cell development occurs, at least in initial stages. However, the binucleate cell in *P. hydriforme* infects fish (Raikova, 1994), while in myxozoans, it infects annelids (summarized in Morris, 2010) in contrast to first stages in fish that are multicellular (El-Matbouli, Hoffmann, & Mandok, 1995; Morris & Adams, 2008). The binucleate cell in *P. hydriforme* is a larval, diploid stage (Raikova, 1994), while in myxozoans, it is represented by a haploid binucleate cell that merges to form a zygote in the annelid host (Morris, 2012). Their homology is hence unlikely. Cell-in-cell development has been interpreted as another common feature of early *P. hydriforme* and most of

myxozoan development, but it also occurs convergently in parasitic protists of marine invertebrates, the Paramyxida (Ward et al., 2016). Recent genomic data further support an independent origin of Myxozoa and *P. hydriforme*: the genome size of the myxozoan *Kudoa iwatai* amounts to only 4% of that of *P. hydriforme* (22.5 Mb vs. 561 Mb), and rigorous reductions depleted genes related to development, cell differentiation and cell–cell communication, only in the Myxozoa (Chang et al., 2015). The overlap of exclusive orthologous groups of genes (OGs) between *K. iwatai* and *P. hydriforme* is lower than between *K. iwatai* and free-living *Nematostella* or *Hydra* (Chang et al., 2015), indicating a somewhat closer relationship to these free-living taxa than to the parasitic sister taxon. Finally, *P. hydriforme* is a monotypic species that appears to be an evolutionary blind lineage, partially parasitic in old acipenseriform fishes, a living fossil that failed to evolve and radiate into modern fish lineages, while the Myxozoa, parasitic throughout their life cycle, successfully established in different invertebrate hosts, and thrived and diversified after multiple entries into fishes. The analysis of presently available developmental and genome-based features in the light of a dated origin and host–parasite phylogenetic convergence strongly suggests that the Myxozoa and *P. hydriforme* represent not one but two independent routes to endoparasitism in the Cnidaria and that the “Endocnidozoa” (Zrzavý & Hypša, 2003) are an invalid taxon uniting the two. Sharing the same habitat but being highly mobile makes fish ideal hosts for parasitic cnidarians, and multiple origins of parasitism in fishes can also be found in hydrozoans (summarized in Weinstein & Kuris, 2016). Despite similar phylogenetic positioning of *P. hydriforme* and

the Myxozoa as sister groups to the Medusozoa, it is likely that *P. hydriforme* and the Myxozoa represent independent lineages drawn together by long-branch attraction of their highly divergent genes and genomes. The lack of other, closely related taxa in combination with fossils (Wiens, 2005) presently prevents resolving this relationship.

Within the Myxozoa, phylogenetic clustering in the tree based on six molecular genes was in accordance with 18S rDNA tree reconstructions, with sphaerosporids consistently positioned basal to the PIM and OIM lineages, while, in the past, they had sometimes been reconciled as sister to the PIM clade (Bartošová et al., 2013; Karlsbakk & K  ie, 2009). Basal to the known annelid hosts of myxozoans two groups can be found, Haplodrili (= Archiannelida, five families) and Sipuncula (Andrade et al., 2015; Dunn et al., 2008; Struck et al., 2011). Based on congruent myxozoan and invertebrate host trees, these offer themselves as ancestral hosts of sphaerosporids, while modern taxa may well have adapted more recent annelid lineages. The most basal sphaerosporid clade (Bartošová et al., 2013) is represented by isolates exclusively from fishes in marine habitats, which also home these evolutionary old annelids (Struck et al., 2007). According to the present reconstruction of the history of myxozoan host acquisitions, sipunculids are excellent candidates for invertebrate hosts of basal sphaerosporids and are worthy of in-depth study including 18S rDNA screening, especially because infected specimens were found to harbour more than one spore phenotype (Ikeda, 1912). In contrast to previous reports suggesting a different host for sphaerosporids (Bartošová et al., 2013; Holzer et al., 2007), based on the present analysis, we are able to pinpoint specific taxa.

Findings of myxozoans in fish-parasitic flatworms (Freeman & Shinn, 2011; Overstreet, 1976; Siau et al., 1981) and a free-living mollusc (Yokoyama & Masuda, 2001) describe spores exhibiting a bilateral symmetry, in contrast to the triradial symmetry of spores from annelid hosts, and represent generic morphotypes known from fishes (*Kudoa*, *Fabespora* and *Myxidium*). Further supported by their close phylogenetic relationship to histozoic taxa from marine fishes (Freeman & Shinn, 2011), it is likely that they were acquired from fish hosts by blood and tissue feeding helminths and settled as hyperparasites within them. These invertebrates hence replace the intermediate vertebrate host, but their myxozoans likely retained a two-host life cycle involving an additional invertebrate definitive host. The high potential for adaptation to different host groups throughout their life cycle, and the relatively recent rediscovery of bryozoans as intermediate hosts of myxozoans (Canning, Okamura, & Curry, 1996) provide exciting perspectives regarding additional host discoveries that may further improve our understanding of the evolutionary history of the Myxozoa.

Another actual debate is that of a marine vs. freshwater origin of myxozoans. Cnidarians live predominantly in marine environments, and Kent et al. (2001) suggested that myxozoans first became endoparasitic in old marine annelid worms. However, this interpretation predates the rediscovery of malacosporeans, the oldest clade of myxozoans, in bryozoan hosts. Malacosporeans infections have only

been detected in Phylactolaemata, the radix group of bryozoans that occurs exclusively in freshwater habitats (reviewed in Taylor & Waeschenbach, 2015). This, together with the fact that *P. hydriforme* occurs in freshwater fish, prompted some authors to suggest a freshwater origin of the Myxozoa. However, the last common ancestor of today's Phylactolaemata first evolved in marine environments and only secondarily occupied freshwaters habitats (Koletic, Novosel, Rajevic, & Franjevic, 2015). The oldest known annelid hosts (polychaetes) and the oldest known vertebrate hosts (Chondrichthyes) of myxozoans are predominantly or even exclusively marine, with myxozoan species from cartilaginous fishes clustering in basal positions (Figure 1; Gleeson & Adlard, 2012; Kod  tkov   et al., 2015). Furthermore, an origin of the Myxozoa towards the end of the "Snowball Earth" episode, when terrestrial and freshwater habitats were under permanent frost, would strongly point to an origin of the Myxozoa in the slushy marine realm.

## 4.2 | Myxozoan cophylogeny, diversification and success

Myxozoans entered fish as their second hosts at least once (possibly many more times) in each major clade, and the history of the common evolution of fish and myxozoans repeats itself in these parallel evolving branches, confirmed by ancient myxozoan lineages in Chondrichthyes, followed by those in tetrapods and finally mirroring teleost emergence patterns. However, cophylogenetic methods show varying significance for the congruence of myxozoans and fish host phylogenies than of myxozoans and phylogenies of invertebrates. This is likely because after the first conquest of fishes, cophylogenetic signatures with vertebrates are received as mixed signals together with those from invertebrate hosts. While an interdependent cophylogeny-estimating model for the two co-evolutionary events parallel to each other would be desirable to optimize the outcome of the present analyses, reciprocal influence of myxozoan-vertebrate host phylogenies is still significant for all major clades, when estimated by existing methods.

Myxozoans are some of the most spectacular examples of parasite radiation and hold a largely unexploited potential for speciation research. Although parasitism has originated seven times independently in the Cnidaria (Weinstein & Kuris, 2016; considering a common origin for Myxozoa and *P. hydriforme*), myxozoans are the only group that is parasitic throughout their whole life cycle, has developed an indirect life cycle and shows extremely successful diversification, currently constituting about one-fifth of all cnidarian taxa (Zhang, 2011), with diversity estimates from eDNA accounting for an approx. thirteen times higher number than presently known (Hartikainen et al., 2016). We showed that myxozoans diversified massively predominantly after the acquisition of their second hosts, fishes, approx. 300 Ma. It is without doubt that, the acquisition of fish as second hosts brought along alternative transmission and dispersion strategies that allowed for the conquest of new habitats. But what explains massive diversification events in some subclades but not in others? Ray-finned fishes include half of the entire species

richness of vertebrates (Eschmeyer, Fricke, & Van der Laan, 2017; Nelson, Grande, & Wilson, 2016), with two hyperdiverse but relatively recent clades (Ostariophysi and Percomorpha; Vega & Wiens, 2012). These clades together host 49% of all myxozoans sequenced to date, with the highest diversity in Cypriniformes (28% of sequenced taxa). The present study appears to support the hypothesis that a pronounced potential for parasite diversification exists in hosts that underwent explosive speciation themselves (Gao et al., 2013; Pariselle, Morand, Deveney, & Pouyaud, 2003). Parasite diversification is likely fuelled by the high host specificity of myxozoans in their vertebrate hosts and, consequently, a greater possibility for successful host switching to, and radiation among closely related host species, especially sympatric Cypriniformes (Supplementary file S6\_Figure 4; Forró & Eszterbauer, 2016; Shin et al., 2014). Hence, apart from niche specialization in the host (clustering according to organ system) myxozoans clearly show host-associated diversification mechanisms. It appears that many biological aspects and likely an important sampling bias (Tedersoo, Bahram, & Dickie, 2014) explain myxozoan hyperspeciation in some clades, and for a better understanding of the ecological and evolutionary principles than impact on diversity in this parasite group much research is still required. However, the evolutionary history of their hosts is presently an essential predictor of myxozoan diversity. Thus, our study clearly highlights the importance of host phylogenetic information for explaining parasite speciation. From an evolutionary perspective and based on the early birth age of the Myxozoa, it is possible that myxozoan parasites themselves contributed to promoting host diversification. Parasites have often been considered a threat to biodiversity because of their negative impact on host population persistence (de Castro & Bolker, 2005; Valenzuela-Sanchez et al., 2017), but they may actually play important roles in maintaining and promoting host biodiversity (Buckling & Rainey, 2002; Karvonen & Seehausen, 2012). In myxozoans, the oldest metazoan parasites on Earth, the evolutionary history of host interactions is key for their distinct success, leading to an extraordinary richness of modern taxa in all aquatic habitats and fish lineages, whose contribution to total levels and patterns of aquatic biodiversity on Earth is expected to be considerable.

## 5 | CONCLUSIONS

Our data suggest an origin of the Myxozoa in extinct marine Phylactolaemata and archiannelids, around 651 Ma. Myxozoans first evolved in invertebrates and their main lineages split before a two-host life cycle was acquired, with the members of three major clades of myxozoans strictly using specific invertebrate groups (Bryozoa, Polychaeta, Oligochaeta). Fish were conquered as second hosts multiple times, firstly when prehistoric cartilaginous fish became available (approx. 450 Ma). Chimaeras, sharks and rays still serve as hosts for the oldest lineages of the two major annelid-infecting clades. Myxozoans clearly show a common evolution with their vertebrate hosts, with lineages over time in (i) cartilaginous fishes, (ii) tetrapods and (iii) following the

emergence pattern of modern ray-finned fishes. Due to their origin in invertebrates prior to the emergence of vertebrates, invertebrate and myxozoan phylogenies are highly congruent, while the co-evolutionary signatures of myxozoans and fish depend on the tested data set. Despite a sister lineage relationship of *Polypodium hydriforme* and the Myxozoa, the origin of *P. hydriforme* in old acipenseriform fishes likely represents an independent conquest of fish as cnidarian hosts. We point out important differences in the development of the two parasite lineages and demonstrate that characteristics considered developmental homologies represent divergences. Myxozoans are parasitic throughout their life cycle and, in contrast to *P. hydriforme* thrived and diversified massively, especially after the acquisition of fish as secondary hosts, to whose diversification they may well have contributed, with massive bursts of speciation occurring only in highly diverse host lineages.

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## CONFLICT OF INTEREST

The authors declare no competing interests.

## DATA ACCESSIBILITY

Tree files and alignments used for cophylogeny and for molecular clock analyses are available on Dryad <https://doi.org/10.5061/dryad.8f4b9> (review: <http://datadryad.org/review?doi=doi:10.5061/dryad.8f4b9>).

## AUTHOR CONTRIBUTIONS

A.S.H. and I.F. were responsible for conceptualization; P.B.-S., A.S.H. and I.F. performed data mining; A.S.H., P.B.-S. and A.B.-T. carried out phylogenetic and cophylogenetic analyses; I.F. performed molecular dating analyses; A.S.H. created visualizations; A.S.H. and I.F. wrote the original draft of the manuscript; All authors reviewed and edited the manuscript.

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## SUPPORTING INFORMATION

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