

School of Doctoral Studies in Biological Sciences

University of South Bohemia in České Budějovice,
Faculty of Science

**Malacosporea and *Sphaerospora sensu stricto*:
Myxozoan clades with unique biology and evolution**

Ph.D. thesis

Sneha Patra M.Sc.

Supervisor: M.Sc. Astrid S. Holzer Ph.D.

Institute of Parasitology, Biology Centre CAS, České Budějovice
Faculty of Science, University of South Bohemia, České Budějovice

Co-supervisor: RNDr. Pavla Bartošová-Sojková Ph.D.

Institute of Parasitology, Biology Centre CAS, České Budějovice

České Budějovice
2017

This thesis should be cited as:

Patra S. (2017) Malacosporea and *Sphaerospora sensu stricto*: Myxozoan clades with unique biology and evolution. Ph.D. Thesis Series, No. 12. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 214 pp.

❖ Annotation

The Malacosporea and *Sphaerospora sensu stricto* clade are the two most basal myxozoan clades. While the evolutionary older malacosporeans possess unique features resembling cnidarians, sphaerosporids are known for special developmental features and unique long insertions in the small and large subunits of the ribosomal DNA. This thesis aimed at elucidating the hidden biodiversity, describing special biological traits and tracing the evolutionary history of these two clades based on both, morphological and molecular data. Host-parasite phylogenetic congruence was studied to understand the evolutionary patterns of cospeciation and host switching.

❖ Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dale s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, Date: 11.07.2017

Sneha Patra M.Sc.

This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Parasitology, Biology Centre CAS, supporting doctoral studies in the Parasitology study program.



Přírodovědecká
fakulta
Faculty
of Science



❖ Financial support

This study was supported by various grants including the Ministry of Education of the Czech Republic, the Czech Science Foundation (#505/12/G112, European Centre of Ichthyoparasitology), the European Commission (#634429, ParaFishControl), the Grant Agency of Jihočeská Univerzita (GAJU) (#140/2013/P awarded to Prof. RNDr. Václav Hypša, CSc., #147/2016/P awarded to RNDr. Jan Štefka, Ph.D.) and an international mobility scholarship from the University of South Bohemia for visiting Institute for Veterinary Medical Research, Hungarian Academy of Sciences, Budapest, Hungary.

❖ Acknowledgements

Firstly, I would like to thank, from the bottom of my heart, my supervisor Astrid S. Holzer for the immense support, encouragement, and for having faith in me. From bleeding fish to *Artemia* culture; from planning an experiment to acquiring new techniques; from negative results to manuscript writing - she has always maneuvered me to the right direction. I feel myself fortunate to be a part of her vocation, which itself is an achievement.

I would like to thank my co-supervisor Pavla Sojková, for her kind help and support in improving my manuscripts and thesis with valuable suggestions. Big thanks to Ivan Fiala, for solving my usual eleventh hour crises - from phylogenetic analyses to manuscript culmination.

Heartily thanks to all of my recent and ex- lab members: Ashlie, for her motivation and positivity, Ana, for standing beside me from the very beginning, Alena, for her valued suggestions, Hanka, for her kind assistance, Jirka, for quick discussions, Dariya and Anush, for translations, Gema, Milon, Bara, Anna, Tereza, Martina, Inga, and more for being a part of my wonderful PhD journey.

Special thanks to Prof. Julius Lukeš and Prof. Tomáš Scholz, for impelling me from the very first day.

All of my friends, from work and extended life, thanks a lot for cheering me up throughout the whole stay in the Czech Republic. You all are treasures of my life.

I am thankful to have a wonderful supportive family, my parents (maa and baba), In-laws, for supporting me in every stage of my life. The expression “thanks” is not enough when it comes to Som, my better half. Thanks for being my support system.

❖ List of papers and author’s contribution

Paper I: Holzer AS, Pecková H, **Patra S**, Brennan NP, Yanes-Roca C, Main KL (2013) Severe glomerular disease in juvenile grey snapper *Lutjanus griseus* L. in the Gulf of Mexico caused by the myxozoan *Sphaerospora motemarini* n. sp. *International Journal for Parasitology: Parasites and Wildlife* 2: 124–130. doi: 10.1016/j.ijppaw.2013.03.003.

- *SP performed DNA extraction, 18S rDNA amplification, morphological data documentation from scanning electron microscopy and contributed to the final draft of the manuscript.*

Paper II: Bartošová-Sojková P, Hrabcová M, Pecková H, **Patra S**, Kodádková A, Jurajda P, Tymi T, Holzer AS (2014) Hidden diversity and evolutionary trends in malacosporean parasites (Cnidaria: Myxozoa) identified using molecular phylogenetics. *International Journal for Parasitology* 44: 565–577. doi: 10.1016/j.ijpara.2014.04.005. [IF = 3.872].

- *SP participated in field sampling, fish dissection, DNA extraction and contributed to the final draft of the manuscript.*

Paper III: Holzer AS, Hartigan A, **Patra S**, Pecková H, Eszterbauer E (2014) Molecular fingerprinting of the myxozoan community in common carp suffering Swim Bladder Inflammation (SBI) identifies multiple etiological agents. *Parasites & Vectors* 7: 398. doi: 10.1186/1756-3305-7-398. [IF = 3.430].

- *SP performed DNA extraction, PCR amplification, in-situ hybridization, microscopic data documentation and contributed to the final draft of the manuscript.*

Paper IV: Hartigan A, Estensoro I, Vancová M, Bílý T, **Patra S**, Eszterbauer E, Holzer AS (2016) New cell motility model observed in parasitic cnidarian *Sphaerospora molnari* (Myxozoa: Myxosporidia) blood stages in fish. *Scientific Reports* 6: 39093. doi: 10.1038/srep39093. [IF = 4.259].

- *SP participated in fish sampling, light microscopic data documentation and contributed to the final draft of the manuscript.*

Paper V: Patra S, Hartigan A, Morris DJ, Kodádková A, Holzer AS (2017) Description and experimental transmission of *Tetracapsuloides vermiformis* n. sp. (Cnidaria: Myxozoa) and guidelines for describing malacosporean species including reinstatement of *Buddenbrockia bryozoides* n. comb. (syn. *Tetracapsula bryozoides*). *Parasitology* 144: 497–511. doi: 10.1017/S0031182016001931. [IF = 2.713].

- *SP performed field sampling, experimental setup and maintenance, fish and invertebrate screening, microscopic examination, movie editing, DNA extraction, PCR screening, sequencing, sequence alignments, morphological data documentation, preparation of line drawings, tables and supplementary data and drafted the manuscript.*

Paper VI: Patra S, Bartošová-Sojková P, Pecková H, Fiala I, Eszterbauer E, Holzer AS. Biodiversity and host-parasite cophylogeny of *Sphaerospora sensu stricto* (Cnidaria: Myxozoa). Manuscript in advanced preparation.

- *SP performed fish sampling and dissection, microscopic examination, DNA extraction, PCR screening, sequencing, sequence alignments, primer design, morphological data documentation, phylogenetic analyses, preparation of line drawings, figure plates, tables and supplementary data and drafted the manuscript.*

❖ Co-authors agreement

The senior and corresponding authors of the manuscripts included in this thesis, hereby confirm that Sneha Patra contributed significantly to these publications, according to the statement above:

Pavla Bartošová-Sojková

Ashlie Hartigan

Astrid S. Holzer

Malacosporea and *Sphaerospora sensu stricto*: Myxozoan clades with a unique biology and evolution

CONTENTS:

Chapter 1. General introduction of the Myxozoa	1
1.1. Myxozoan origins: From protists to cnidarians	1
1.2. Classification and phylogenetics	1
1.3. Life cycle and development	5
1.4. Pathology and host-parasite interactions	12
Chapter 2. The Malacosporea	14
2.1. History of the discovery	14
2.2. Life cycle specialties: Sacs and myxoworms in bryozoan hosts	16
2.3. Cryptic nature of parasitic stages	18
2.4. Biodiversity and phylogeny	18
Chapter 3. <i>Sphaerospora sensu stricto</i>	20
3.1. A clade with the short history but long rDNA inserts	20
3.2. Currently known diversity	21
3.3. Phylogeny of the <i>Sphaerospora sensu stricto</i> clade	30
3.4. Life cycle dilemma	31
3.5. Extrasporogonic proliferation in fish blood	32
Chapter 4. Objectives of the research	33
Chapter 5. Results and the research papers	34
5.1. Published paper I	34
5.2. Published paper II	42
5.3. Published paper III	71
5.4. Published paper IV	81
5.5. Published paper V	109
5.6. Unpublished paper VI	145
Chapter 6. Summary and general discussion	186
References	192
Appendix – Curriculum vitae	210

Chapter 1. General introduction of the Myxozoa

1.1. Myxozoan origins: From protists to cnidarians

The Myxozoa Grassé, 1970, are diverse metazoan parasite group with an extremely simplified and parasitism-adapted morphology. After the first report from a now extinct salmonid fish, *Coregonus fera* Jurine 1825, from Lake Geneva, Switzerland around 200 years ago (Jurine 1825), the Myxozoa today encompass approximately 2400 species (Zhang 2011).

Initially, myxozoans were grouped with different protist taxa (Microsporidia and Apicomplexa) within the former groups of Cnidospora Doflein, 1901 and Sporozoa Leuckart, 1879 (Bütchli 1882; Dogiel 1965). The multicellular nature of myxozoan spores recognized by Štolc (1899) was confirmed by ultrastructural studies (Grassé & Lavette 1978) and later by molecular data which placed the Myxozoa firmly into the Metazoa (Smothers et al. 1994; Katayama et al. 1995; Siddall et al. 1995; Schlegel et al. 1996).

Once rediscovered and identified as a myxozoan (Anderson et al. 1998) the peculiar vermiform and allegedly triploblast stage of *Buddenbrockia plumatellae* Schröder, 1910 containing four blocks of muscles (Schröder 1910, Jiménez-Guri et al. 2007a) was suggested to be the missing link between myxozoans and their bilaterian ancestors (Canning et al. 2002; Okamura et al. 2002; Okamura & Canning 2003). However, bilateral affinity of the Myxozoa based on the presence of four hox genes (Anderson et al. 1998) was later ruled out after confirming host contamination (Jiménez-Guri et al. 2007a). Moreover, mesodermal-like muscle cells typical for *Buddenbrockia* have also been reported from cnidarians (Seipel & Schmid 2006; Technau & Scholz 2003; Burton 2008). Morphologically similar myxozoan polar capsules and cnidarian nematocysts (Weill 1938) were later confirmed as homologous features (Siddall et al. 1995) as they both contain cnidarian-specific nematocyst proteins such as minicollagens (Holland et al. 2011; Feng et al. 2014) and nematogalectins (Shpirer et al. 2014; Kyslík 2016). Shared ancestry of genes as well as the tetra-radial symmetry of *B. plumatellae* firmly placed the Myxozoa within the Cnidaria (Jiménez-Guri 2007a; Gruhl & Okamura 2012). Based on multiple protein-coding gene analyses, the Myxozoa were placed sister to the Medusozoa (Jiménez-Guri et al. 2007a; Collins 2009; Holland et al. 2011; Nesnidal et al. 2013; Feng et al. 2014; Shpirer et al. 2014). A more recent analysis placed the Myxozoa as a sister group to Medusozoa, together with another enigmatic fish parasite of cnidarian origin, *Polypodium hydriforme* Ussov, 1885 (Chang et al. 2015; Okamura & Gruhl 2016).

1.2. Classification and phylogenetics

After the discovery of an indirect myxozoan life cycle, myxosporeans and actinosporeans (previously sister class organisms) were considered conspecific, representing two distinct phases of single life cycle (Wolf & Markiw 1984; Kent et al. 1994; Wolf et al. 1986). Myxosporea became the valid representative under phylum Myxozoa and Actinosporea was suppressed (Kent et al. 1994).

Presently, based on spore morphology and invertebrate host types, the Myxozoa are divided into two classes (Kent et al. 2001): the Myxosporea Bütschli 1881, with hard spore valves and annelid definitive hosts, and the Malacosporea Canning, Curry, Feist, Longshaw et Okamura 2000, with soft spore valves and bryozoan definitive hosts. In the two myxozoan classes, spore morphology in invertebrate (definitive) and vertebrate (intermediate) hosts differs considerably. Myxosporeans form two types of spores during their life cycle: myxospores in the vertebrate host and actinospores in the invertebrate host. In malacosporeans, insufficient records exist for soft-walled spores in the fish host since they are rarely detected (Kent & Hedrick 1986; Kent et al. 2000; Hedrick et al. 2004; Morris & Adams 2008; Bartošová-Sojková et al. 2014: Paper II). For this reason, malacosporean classification, in contrast to the myxosporean one is based mostly on morphological data from malacospores from their bryozoan host. The Myxozoa currently consist of 67 genera from 19 families (based on Lom & Dyková 2006; Fiala et al. 2015a and additional information included from Freeman & Kristmundsson 2015; Yang et al. 2017; Figure 1), with only two genera and one family described from malacosporeans.

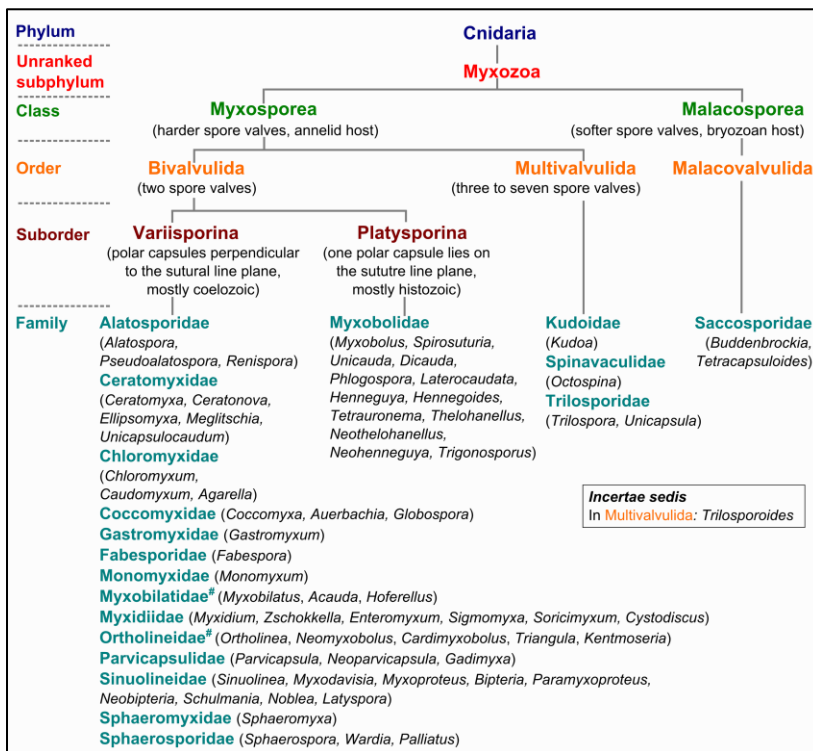


Figure 1: Schematic diagram of the classification of the Myxozoa up to the genus level based on Lom & Dyková (2006) and Fiala et al. (2015a). # = taxonomic transfer or demise of the family (Karlsbakk et al. 2017) is not included.

Kudo (1933) and Tripathi (1948) proposed the first systematic key for the classification of the Myxosporea. The most significant classification proposed by Shul'man (1966) is widely valid to date. Lom and Arthur (1989) provided the first guidelines for fishborne myxosporean descriptions, with focus on spore morphology. Revised classifications (Lom & Noble 1984; Lom & Dyková 1992; 2006; Canning & Okamura 2004) resulted in the selection of important morphological features for describing a new species: a) shape and size of spore, b) number of shell valves and sporoplasms, c) position and shape of suture line, d) presence or absence of surface ridges, projections, caudal appendages and mucous envelope, e) number, shape, orientation and size of polar capsules, f) number of polar filament turns within the polar capsule, g) characteristics of spore-forming plasmodia, and, h) final site of the infection.

Alongside morphological features, it is nowadays unacceptable to publish a new species description without providing small subunit ribosomal DNA (18S rDNA) sequence data. When the first 18S rDNA sequences of myxozoans were published in the 1990s (Smothers et al. 1994), it soon became clear that phylogenetic clustering conflicts traditional myxozoan taxonomy (Holzer et al. 2004; Fiala 2006; Bartošová & Fiala 2011; Rocha et al. 2013; Karlsbakk et al. 2017). At present, approximately 770 species have been characterized based on their 18S rDNA, which has become the most commonly used molecular marker (barcode) for myxozoans. According to the most recent phylogenetic analyses, 15 genera are para-/polyphyletic, though various genera are molecularly under-represented either without any molecular data or with single nominal representatives sequenced. As the morphology does not completely reflect phylogenetic clustering, other factors are responsible for shaping the evolution of the Myxozoa. Several biological traits correspond with the phylogenetic clustering to some degree: 1) localisation within the host tissue (Holzer et al. 2004; Fiala 2006; Hartigan et al. 2011; Shin et al. 2014; Rocha et al. 2013; 2015; Azizi et al. 2016), 2) aquatic habitat (marine, freshwater or brackish; Fiala 2006; Jirků et al. 2011; Bartošová et al. 2013; Azizi et al. 2016; Aguiar et al. 2017), 3) invertebrate host (bryozoan, polychaete or oligochaete; Holzer et al. 2007; Fiala et al. 2015a), 4) fish host family (Gunter & Adlard 2009; Gunter et al. 2009; Alama-Bermejo et al. 2011; Carriero et al. 2013; Naldoni et al. 2015; Leis et al. 2017), 5) sporogenesis (Morris & Adams 2008), and 6) geography (Whipps et al. 2003; Henderson & Okamura 2004; Whipps & Kent 2006; Liu et al. 2016).

According to 18S rDNA phylogenetic analyses, the Myxozoa are broadly divided into four major clades: 1) the most basal malacosporean clade and three myxosporean clades including 2) the *Sphaerospora sensu stricto* (*s. s.*) clade, 3) a predominantly marine, polychaete-infecting clade of myxosporeans and, 4) a predominantly freshwater, oligochaete-infecting clade of myxosporeans (Fiala 2006; Fiala et al. 2015a; Figure 2). *Sphaerospora s. s.* species have some of the longest 18S rDNA (up to 3.7 kbp) within eukaryotes and they predominantly cluster in a well-separated lineage at the base of all myxosporeans (Fiala 2006; Holzer et al. 2007; 2010; Bartošová et al. 2013; Fiala & Bartošová 2010; U-taynapun et al.

2012; Fiala et al. 2015a) though some analyses placed this clade sister to the marine myxosporean lineage (Karlsbakk & K oie 2009; Holzer et al. 2013a; Eszterbauer et al. 2013; Bartořova et al. 2013).

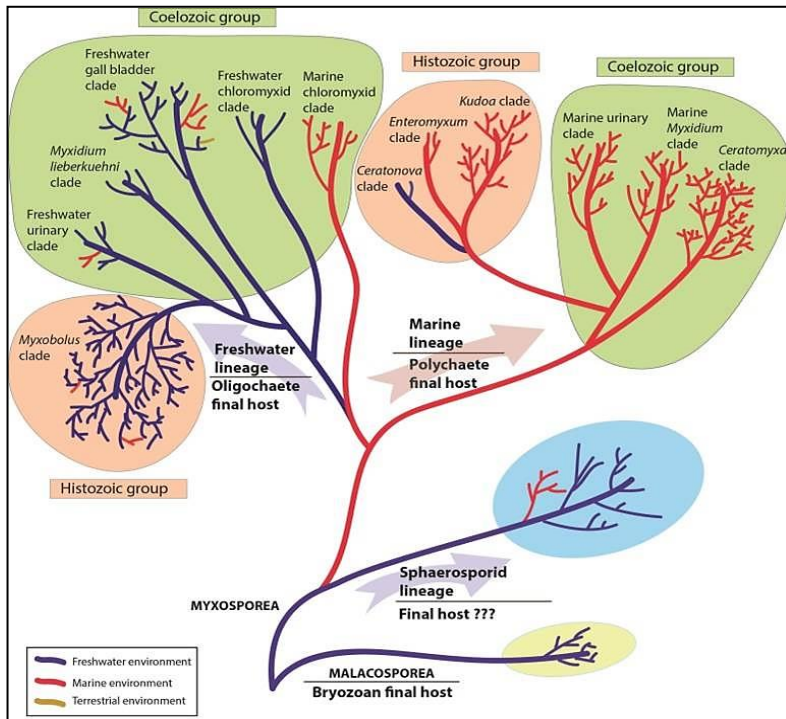


Figure 2: Summary of hypothetical evolution of the Myxozoa inferred from molecular data based on various studies (Fiala et al. 2015b).

High divergence of the 18S rDNA variable regions is indicative for a fast evolution of the myxozoans (Jimenez-Guri et al. 2007a, Evans et al. 2010, Chang et al. 2015, Takeuchi et al. 2015). This can result in problems during sequence alignments and potential errors in phylogenetic analyses. Other molecular markers like the large subunit ribosomal DNA (28S rDNA) and elongation factor 2 (EF2) supported the phylogenetic clustering of myxozoans based on the 18S rDNA (Bartořova et al. 2009; Fiala & Bartořova 2010; Hartikainen et al. 2014; Atkinson et al. 2015). Highly variable internal transcribed spacer region 1 proved as a suitable marker for inferring myxozoan relationships at the subspecies level, for example for phylogeographical studies (Henderson & Okamura 2004; Whipps & Kent 2006), whereas the more conservative heat shock protein 70 is appropriate for studies at the interspecific or phylum level (Andree et al. 1999; Whipps et al. 2004a).

With the increasing number of entries of myxozoan 18S rDNA sequences in Genbank and knowledge of their phylogenetic positioning, suppression or erection of genera, species or even families have been performed various times. The genera *Pentacapsula* (Naidenova et Zaika, 1970), *Hexacapsula* (Arai et Matsumoto, 1953)

and *Septemcapsula* (Hsieh et Chen, 1984) possessing five, six and seven spore valves, respectively, were transferred to a single genus *Kudoa* Meglitsch, 1947, originally defined to have four spore valves (Whipps et al. 2004b). The genus *Leptotheca* (Thélohan, 1895) harbouring 74 species was demised and its representatives were transferred to other genera, based on host tissue localisation and spore morphology: gall bladder-infecting species were absorbed in *Ceratomyxa* Thélohan, 1892, urinary system-infecting species were shifted to *Sphaerospora* Thélohan, 1892 and two other representatives were incorporated within *Ellipsomyxa* Køie, 2003 and *Myxobolus* Bütschli, 1882 (Gunter & Adlard 2010), despite lacking molecular data of most *Leptotheca* spp. including that of its type species *Leptotheca agilis* (Thélohan, 1892). However, solutions for many paraphyletic genera are not easily found and it is yet to be decided how to proceed with adapting a more realistic systematic system without having to rely on molecular data only.

1.3. Life cycle and development

Since the revolutionary discovery of the myxozoan two-host life cycle (Wolf & Markiw 1984; Wolf et al. 1986), Myxozoa are generally considered to have an indirect life cycle that alters between an invertebrate host (annelid or bryozoan) and a vertebrate host, mostly fish (Figure 3).

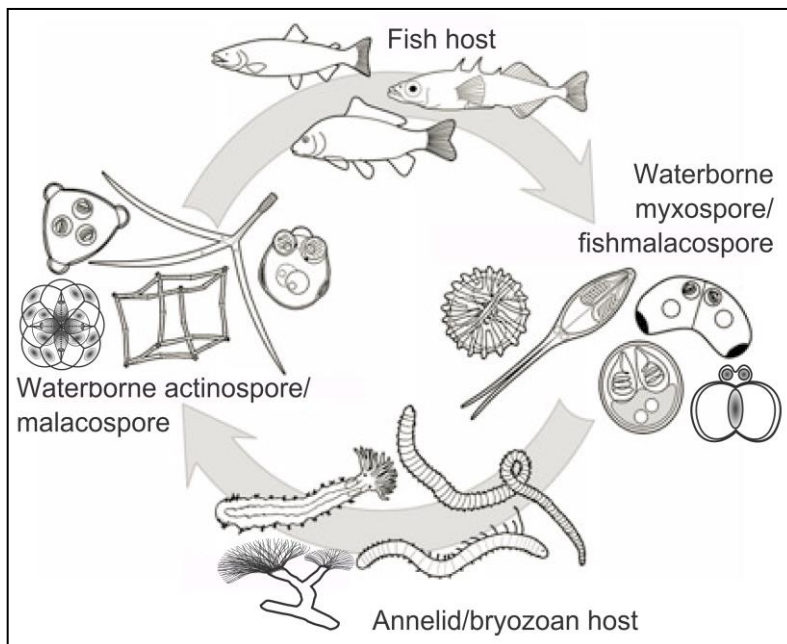


Figure 3: Schematic diagram of an indirect myxozoan life cycle between a fish host and an aquatic annelid or a bryozoan host (modified from Eszterbauer et al. 2015).

Actinospores are formed within the invertebrate host, by definition the definitive host (meiosis takes place within the gametocytes). Myxospores are formed within the vertebrate (intermediate) host. Known invertebrate hosts for myxosporeans are usually annelids: for myxosporeans in freshwater habitats generally oligochaetes, for those in marine environments usually polychaetes and rarely sipunculids (Ikeda 1912). Malacosporeans use freshwater Bryozoa belonging to the Phylactolaemata as invertebrate hosts and fish as vertebrate hosts. Teleost and cartilaginous fishes (Dyková & Lom 1982) are the most common vertebrate hosts for myxosporeans, while few records exist from amphibians, reptiles, birds and mammals including human (Eiras 2005; Jirků et al. 2007; Prunescu et al. 2007; Bartholomew et al. 2008; Kawai et al. 2012; Ohnishi et al. 2013; Székely et al. 2015, Hartigan et al. 2016a) and few exceptional invertebrates including Monogenea and Cephalopoda (Yokoyama & Masuda 2001; Freeman & Shinn 2011). To date 53 myxozoan life cycles are known, of which 38 are confirmed by molecular data from both hosts (Table 1). In contrast to 42 life cycles having oligochaete definitive hosts, only eight life cycles involve polychaetes and, three are known from bryozoans. It is unclear how myxozoans in terrestrial vertebrates complete their life cycle (Eiras 2005) but infection may occur by consumption of infected oligochaetes (earthworms) (Dyková et al. 2011) or spiders (Hallett et al. 2015).

Myxozoans are one of the supreme examples of morphological simplification to endoparasitism and reduction of body complexity to only a few cells throughout their life cycle. Spores are the most characteristic feature. Actinospores are usually larger, soft-shelled, with triradial symmetry, consisting of three polar capsules, three valve cells and one multinuclear sporoplasm (Kent et al. 2001; Lom & Dyková 2006; Morris 2010; 2012). Valve cells often form long floating appendages. Myxospores generally consist of one to four capsulogenic cells (exceptionally 13–15 in *Kudoa quadricornis* Whipps, Adlard, Bryant et Kent, 2003 or *Kudoa permulticapsula* Whipps, Adlard, Bryant et Kent 2003), two to four valvogenic cells (with same exceptions as before) and 2–12 uninucleated sporoplasms (Lom & Noble 1984; Sitjà-Bobadilla & Alvarez-Pellitero 1995; Bartošová et al. 2013), or a single binucleated sporoplasm (Lom & Dyková 1992; Sitjà-Bobadilla & Alvarez-Pellitero 1994). Polar capsules are formed from capsulogenic cells harbouring a coiled polar filament.

The cellular composition of the Malacosporea differs significantly from that of the Myxosporea. Malacospores found in the bryozoan coelom have eight shell valves, four polar capsules and two sporoplasms with secondary cells (Canning & Okamura 2004; Feist et al. 2015), while fish-malacospores have two or four valve cells, two polar capsules and one uninucleated sporoplasm devoid of any secondary cells (Kent & Hedrick 1986; Kent et al. 2000; Hedrick et al. 2004; Morris & Adams 2008; Feist et al. 2015).

Host recognition in myxozoans is based on the molecule inosine present in fish mucus (Kallert et al. 2005a; 2011). Actinospores expel their polar filaments and consequently the spore ruptures along the suture between the shell valves initiating the release of the sporoplasm and its entry into fish via mucus cells or

epithelia in general (El-Matbouli et al. 1999; Kallert et al. 2011). Following hosts' invasion by the sporoplasms, asexual multiplication produces numerous presporogonic stages before reaching the target organ where sporogony takes place. Myxozoans show a unique cell-in-cell development where a primary cell contains one to many secondary and later tertiary cells. Primary cells break up and liberate secondary-tertiary cell doublets, which start proliferation over.

Limited knowledge exists about the complete intrapiscine development of myxozoans (El-Matbouli et al. 1995a; Holzer et al. 2003; Morris & Adams 2008; Bjork & Bartholomew 2010). Extrasporogonic, proliferative blood stages are known from a number of *Sphaerospora* s. s. spp. (Baska & Molnár 1988; Lom et al. 1985; 1991; Molnár 1994; McGeorge et al. 1997; Paperna & Cave 2001; Holzer et al. 2003). In contrast to proliferation in the blood which seems to be limited to sphaerosporids, the blood stream appears to be commonly used by myxozoans as means of transport to the target organ for sporogenesis (Johnson 1980; Kent & Hedrick 1985; Moran et al. 1999a; b; Bjork & Bartholomew 2010; Holzer et al. 2013b: Paper I; Holzer et al. 2014: Paper III). In contrast, *Myxobolus cerebralis* Hofer, 1903 migrates to the cartilage of brain and vertebrae via nerve fibers, avoiding an immunological reaction of the host (El-Matbouli et al. 1995a).

In general, myxozoan sporogony is initiated within plasmodia in the target organ. These are categorized into large plasmodia (multiple vegetative nuclei, numerous spores) or pseudoplasmodia (single vegetative nuclei, 1–2 spores) (Kent et al. 2001; Canning & Okamura 2004) and are either histozoic (in tissues, e.g. muscle, gills, liver, skin, kidney and testes) or coelozoic (in body/organ cavities, e.g. renal tubules, urinary bladder, gall bladder and biliary ducts). Sporogonic cells within plasmodia further divide into the different spore-forming cell types: capsulogenic, valvogenic and sporoplasmogenic cells. Fully formed mature spores are then released from the vertebrate host and settle in aquatic sediments until taken up by the invertebrate host.

After ingestion of myxospores by the invertebrate host, the polar filaments are extruded thus attaching the spore to the hosts' surface, valve cells split along the spore's suture line to release sporoplasms and initiate proliferation, similar as in the vertebrate host. After initiation of sporogenesis following proliferation, haploid sporoplasms multiply and later fertilize to produce pansporocysts within which haploid α somatic cell and β gametic cell unite to form four or more often eight diploid zygotes (Morris 2012). Further multiplication produces capsulogenic, valvogenic and sporoplasmogenic cells. At the end of the cell division, four (e.g. *Tetraspora discoidea* Hallett et Lester, 1999 and *Tetraspora rotundum*, Hallett et Lester, 1999) (Hallett & Lester 1999) or mostly eight actinospores are formed within one pansporocyst (Kent et al. 2001; Lom & Dyková 2006). Actinospores are produced within different areas of the host body (body wall; Bartholomew et al. 1997; digestive epithelium; El-Matbouli & Hoffmann 1998; coelomic cavity; Rangel et al. 2009). In contrast to myxosporeans, malacosporian development occurs within the coelom of freshwater bryozoans. Spore-forming stages are relatively

large and of three types: 1) spherical/sub-spherical sacs, 2) elongate motile vermiform stages and 3) lobe-like stages (Hartikainen et al. 2014).

A few exceptions are known to the general actinosporean-myxosporean life cycle of myxozoans. The genus *Enteromyxum* Palenzuela, Redondo et Alvarez-Pellitero, 2002, is the only genus known to be transmitted from fish-to-fish by dislodging host cells with intracellular developmental stages from the digestive epithelia of the fish host that are released with faeces and can infect receptor fish (Diamant 1997; Redondo et al. 2002). This direct and fast fish-to-fish transmission of the parasite causes huge economic losses in aquaculture (Diamant et al. 2006; Fleurance et al. 2008). However, the alternative existence of an invertebrate host as a part of the *Enteromyxum* life cycle cannot be ruled out (Redondo et al. 2004) due to the relatively recent finding of an actinospore type belonging to the *Enteromyxum* clade (Rangel et al. 2011).

Vertical propagation of myxozoans was reported in few other species and only from their invertebrate hosts. An undescribed myxozoan (triacinomyxon actinospore) and *Myxobilatus gasterostei* (Parisi, 1912) Davis, 1944 from the oligochaetes *Lumbriculus variegatus* (Müller, 1774) and *Nais communis* Pignet, 1906, respectively, produced new individuals with actinospores by fragmentation (asexual reproduction) of infected hosts (Morris & Adams 2006a; Atkinson & Bartholomew 2009). Colonial fragmentation of the bryozoans *Lophopus crystallinus* (Pallas, 1766) and *Fredericella sultana* (Blumenbach, 1779) infected with *Buddenbrockia allmani* Canning, Curry, Hill, Okamura, 2007 and another malacosporean, respectively, were able to produce malacosporean infection in the new colonies (Morris & Adams 2006a; Hill & Okamura 2007). Moreover, vertical transmission by statoblasts (asexual unit of bryozoans for surviving during adverse conditions) was also reported for *L. crystallinus* and *F. sultana* infected with *B. allmani* and *Tetracapsuloides bryosalmonae* (Canning, Curry, Feist, Longshaw et Okamura, 1999), respectively (Hill & Okamura 2007; Abd-Elfattah et al. 2014). However, these findings still require confirmation as several transmission experiments using statoblasts from infected colonies failed to infect the emerging colonies (Grabner & El-Matbouli 2008; 2010a).

Table 1: List of complete and partial myxozoan life cycles available to date. Abbreviations used in this table: X = not experimentally transmitted, \leftrightarrow = complete life cycle, \rightarrow and \leftarrow = partial transmission from one host to another (direction of the arrow shows the transmission pathway), blue = oligochaete host, green = polychaete host and orange = bryozoan host, * = later proven wrong by Eszterbauer et al. (2006), # = later proven wrong by Holzer et al. (2004). Accession numbers are only for 18S rDNA available in GenBank, N/A = data not available.

Myxozoan species	Invertebrate host	Transmission	Vertebrate host	Acc. no.	Reference
<i>Ceratomyxa auerbachii</i> Kabata, 1962	<i>Chone infundibuliformis</i>	X	<i>Clupea harengus</i>	EU616730, EU616733	Køie et al. 2008
<i>Ceratonova shasta</i> Noble, 1950	<i>Manayunkia speciosa</i>	\leftrightarrow	<i>Oncorhynchus</i> spp.	AF001579	Bartholomew et al. 1997
<i>Chloromyxum auratum</i> Hallett, Atkinson, Holt, Banner et Bartholomew, 2006	Freshwater oligochaete	\leftarrow	<i>Carassius auratus</i>	AY971521	Hallett et al. 2006; Atkinson et al. 2007
<i>Chloromyxum schurovi</i> Shul'man et Ieshko, 2003	<i>Eiseniella tetraedra</i>	X	<i>Salmo salar</i> , <i>S. trutta</i>	AJ582007	Holzer et al. 2006a
<i>Chloromyxum truttae</i> Leger, 1906	<i>Stylodrilus heringianus</i>	X	<i>Salmo salar</i> , <i>S. trutta</i>	AJ582006, AJ581916	Holzer et al. 2004
<i>Ellipsomyxa gobii</i> Køie, 2003	<i>Nereis</i> spp.	\rightarrow	<i>Pomatoschistus microps</i>	AY505126-27	Køie et al. 2004
<i>Ellipsomyxa mugilis</i> (Sitjà-Bobadilla et Alvarez-Pellitero, 1993)	<i>Nereis diversicolor</i>	X	Mugilidae spp.	N/A	Rangel et al. 2009
<i>Gadimyxa atlantica</i> Køie, Karlsbakk et Nylund, 2007	<i>Spirorbis</i> spp.	\rightarrow	<i>Gadus morhua</i>	EU163412-13, EU163416, EU163418	Køie et al. 2007
<i>Henneguya exilis</i> (Kudo, 1929)	<i>Dero digitata</i>	X	<i>Ictalurus punctatus</i>	AF021881	Lin et al. 1999
<i>Henneguya ictaluri</i> Pote, Hanson et Shivaji, 2000	<i>Dero digitata</i>	\rightarrow	<i>Ictalurus punctatus</i>	AF195510	Pote et al. 2000
<i>Henneguya nuesslini</i> Schuberg et Schröder, 1905	<i>Tubifex tubifex</i>	\rightarrow	<i>Salmo trutta</i> , <i>Salvelinus fontinalis</i>	AY669810	Kallert et al. 2005b
<i>Henneguya mississippiensis</i> Rosser, Griffin, Quiniou, Khoo, Greenway, Wise et Pote, 2015	<i>Dero digitata</i>	X	<i>Ictalurus punctatus</i>	AF021878	Rosser et al. 2015
<i>Hoferellus carassii</i> Achmerov, 1960	<i>Nais</i> sp. <i>Branchiura sowerbyi</i> <i>Tubifex tubifex</i>	\leftrightarrow \rightarrow \rightarrow	<i>Carassius auratus</i> <i>Carassius auratus</i> <i>Carassius auratus</i>	N/A N/A N/A	Trouillier et al. 1996 Yokoyama et al. 1993 El-Matbouli et al. 1992
<i>Hoferellus cyprini</i> (Doflein, 1898)	<i>Nais</i> sp.	\leftarrow	<i>Cyprinus carpio</i>	N/A	Grossheider & Körting 1992
<i>Myxidium giardi</i> Cepede, 1906	<i>Tubifex</i> sp.	\leftarrow	<i>Anguilla anguilla</i>	N/A	Benajiba & Marqués 1993
<i>Myxidium truttae</i> Léger, 1930	<i>Tubifex tubifex</i>	X	<i>Salmo trutta</i>	AJ582009	Holzer et al. 2004
<i>Myxobilatus gasterostei</i> (Parisi, 1912)	<i>Nais communis</i> , <i>Nais pseudobtusa</i>	X	<i>Gasterosteus aculeatus</i>	EU861209	Atkinson & Bartholomew 2009
<i>Myxobolus arcticus</i> Pugachev et Khokhlov, 1979	<i>Stylodrilus heringianus</i> , <i>Lumbriculus variegatus</i>	\rightarrow \rightarrow	<i>Onchorhynchus nerka</i> , <i>Onchorhynchus masou</i>	HQ113228 AB353128	Kent et al. 1993a

Table 1 continued

					Urawa 1994, (Sequences from Urawa et al. 2011)
<i>Myxobolus bramae</i> Reuss, 1906	<i>Tubifex tubifex</i>	←	<i>Abramis brama</i>	AF507968	Eszterbauer et al. 2000
<i>Myxobolus carassii</i> Klokacewa, 1914	<i>Tubifex tubifex</i>	←	<i>Leuciscus idus</i>	N/A	El-Matbouli & Hoffmann 1993
<i>Myxobolus cerebrealis</i> Hofer, 1903	<i>Tubifex tubifex</i>	↔	<i>Onchorhynchus mykiss</i>	N/A	Wolf & Markiw 1984
<i>Myxobolus cotti</i> El-Matbouli et Hoffmann, 1987	<i>Tubifex tubifex</i>	←	<i>Cottus gobio</i>	N/A	El-Matbouli & Hoffmann 1989
<i>Myxobolus cultus</i> Yokoyama, Ogawa et Wakabayashi, 1995	<i>Branchiura sowerbyi</i>	→	<i>Carassius auratus</i>	N/A HQ613407, HQ613409	Yokoyama et al. 1995 Xi et al. 2013
<i>Myxobolus dispar</i> Thélohan, 1895	<i>Tubifex tubifex</i>	←	<i>Cyprinus carpio</i>	N/A	Molnár et al. 1999a
<i>Myxobolus diverscapsularis</i> Slukhai, 1984	<i>Tubifex tubifex</i>	X	<i>Rutilus rutilus</i>	AY495708, AY325285	Molnár et al. 2010
<i>Myxobolus drjagini</i> (Akhmerov, 1954)	<i>Tubifex tubifex</i>	←	<i>Hypophthalmichthys molitrix</i>	N/A	El-Mansy & Molnár 1997a
<i>Myxobolus erythrophthalmi</i> Molnár, Eszterbauer, Marton, Cech et Székely, 2009	<i>Isochaetides michaelsoni</i>	X	<i>Scardinius erythrophthalmus</i>	KF515727-8	Székely et al 2014
<i>Myxobolus fundamentalis</i> Molnár, Marton, Székely et Eszterbauer, 2010	<i>Isochaetides michaelsoni</i>	X	<i>Rutilus rutilus</i>	KF515725	Székely et al 2014
<i>Myxobolus hungaricus</i> Jaczó, 1940	<i>Tubifex tubifex</i>	←	<i>Abramis brama</i>	AF448444	El-Mansy & Molnár 1997b
<i>Myxobolus intimus</i> Zaika, 1965	<i>Tubifex tubifex</i>	←	<i>Abramis brama</i>	N/A	Rácz et al. 2004
<i>Myxobolus lentisuturalis</i> Dyková, Fiala et Nie, 2002	<i>Branchiura sowerbyi</i>	X	<i>Carassius auratus auratus</i>	AY119688	Caffara et al. 2009
<i>Myxobolus macrocapsularisi</i> Reuss, 1906	<i>Tubifex tubifex</i>	←	<i>Abramis brama</i>	N/A	Székely et al. 2002
<i>Myxobolus parviformis</i> Kallert, Eszterbauer, Erséus, El-Matbouli et Haas, 2005	<i>Limnodrilus hoffmeisteri</i> , <i>Tubifex tubifex</i>	↔	<i>Abramis brama</i>	AY836151	Kallert et al. 2005c
<i>Myxobolus pavlovskii</i> (Akhmerov, 1954)	<i>Tubifex tubifex</i> <i>Limnodrilus sp.</i>	↔ →	<i>Hypothalmichtys molitrix</i> <i>Hypothalmichtys molitrix</i>	N/A HM991164	Ruidisch et al. 1991 Marton & Eszterbauer 2011
<i>Myxobolus portucalensis</i> Saraiva et Molnár, 1990	<i>Tubifex tubifex</i>	→	<i>Anguilla anguilla</i>	N/A	El-Mansy et al. 1998
<i>Myxobolus pseudodispar</i> Gorbunova, 1936	<i>Limnodrilus hoffmeisteri</i> , <i>Psammoryctides spp.</i> , <i>Tubifex tubifex</i>	↔	<i>Rutilus rutilus</i>	N/A	Székely et al. 1999; Marton & Eszterbauer 2012
<i>Myxobolus rotundus</i> Nemeček, 1911	<i>Tubifex tubifex</i>	↔	<i>Abramis brama</i>	FJ851448	Székely et al. 2009

<i>Myxobolus shaharomae</i> Molnár, Eszterbauer, Marton, Cech et Székely, 2009	<i>Isochaetides michaelsoni</i>	X	<i>Alburnus alburnus</i>	KF515726, KF515729-30	Székely et al. 2014
<i>Myxobolus woottenii</i> Molnár, Marton, Székely et Eszterbauer, 2010	<i>Tubifex tubifex</i>	X	<i>Rutilus rutilus</i>	DQ231157, DQ231146	Eszterbauer et al. 2006; Molnár et al. 2010
<i>Ortholinea auratae</i> Rangel, Rocha, Borkhanuddin, Cech, Castro, Casal, Azevedo, Severino, Székely et Santos, 2014	<i>Limnodriloides agnes</i>	X	<i>Sparus aurata</i>	KR025868 KF703856	Rangel et al. 2015
<i>Parvicapsula minibicornis</i> Kent, Whitaker et Dawe, 1997	<i>Manayunkia speciosa</i>	←	<i>Oncorhynchus</i> spp.	DQ231038	Bartholomew et al. 2006
<i>Sigmomyxa sphaerica</i> (Thélohan, 1895)	<i>Nereis pelagica</i>	X	<i>Belone belone</i>	JN033225	Karlsbakk & Kjøie 2012
<i>Sphaerospora dicentrarchi</i> Sitjà-Bobadilla et Alvarez-Pellitero, 1992	<i>Capitella</i> sp.	X	<i>Dicentrarchus labrax</i>	KT970639	Rangel et al. 2016
<i>Sphaerospora dykova</i> (Lom et Dyková, 1982)	<i>Branchiura sowerbyi</i>	←	<i>Cyprinus carpio</i>	N/A	Molnár et al. 1999b*
<i>Sphaerospora truttae</i> Fischer-Scherl, El-Matbouli et Hoffmann, 1986	<i>Lumbriculus variegatus</i> , <i>Tubifex tubifex</i>	→	<i>Salmo salar</i>	N/A	Özer & Wootten 2000 [#]
<i>Thelohanellus hovorkai</i> Akhmerov, 1960	<i>Branchiura sowerbyi</i>	←	<i>Cyprinus carpio</i>	DQ231155	Yokoyama 1997; Székely et al. 1998; Anderson et al. 2000; Eszterbauer et al. 2006
<i>Thelohanellus kitauei</i> Egusa et Nakajima, 1981	<i>Branchiura sowerbyi</i>	X	<i>Cyprinus carpio</i>	JQ690367	Zhao et al. 2016
<i>Thelohanellus nikolskii</i> Akhmerov, 1955	<i>Tubifex tubifex</i> <i>Nais</i> spp.	← X	<i>Cyprinus carpio</i> <i>Cyprinus carpio</i>	N/A N/A	Székely et al. 1998 Borkhanuddin et al. 2013
<i>Thelohanellus wangi</i> Yuan, Xi, Wang, Xie et Zhang, 2015	<i>Branchiura sowerbyi</i>	X	<i>Carassius auratus gibelio</i>	JX458816	Xi et al. 2015
<i>Zschokkella nova</i> Klokacewa, 1914	<i>Tubifex tubifex</i>	←	<i>Carassius carassius</i>	N/A	Uspenskaya 1995
<i>Buddenbrockia plumatellae</i> Schröder, 1910	<i>Plumatella repens</i>	→	<i>Phoxinus phoxinus</i>	FJ939289, FJ939291	Grabner & El-Matbouli 2010a
<i>Tetracapsuloides bryosalmonae</i> (Canning, Curry, Feist, Longshaw et Okamura, 1999)	<i>Fredericella sultana</i> , <i>Plumatella repens</i>	↔	<i>Oncorhynchus mykiss</i> , <i>Salmo trutta</i>	FJ939294	Feist et al. 2001; Morris & Adams 2006b; Grabner & El-Matbouli 2008; 2010b
<i>Tetracapsuloides vermiformis</i> Patra, Hartigan, Morris, Kodádková et Holzer, 2017	<i>Fredericella sultana</i>	→	<i>Cyprinus caprio</i>	KX013242-43	Patra et al. 2017: Paper V

1.4. Pathology and host-parasite interactions

A number of Myxozoa have received attention due to the pathology and disease-related mortalities they cause in both marine and freshwater fish worldwide. One of the most important and best studied myxozoan pathogens is *Myxobolus cerebralis* which causes whirling disease in salmonids by destruction of cartilage and subsequent pressure on the central nervous system, leading to an erratic swimming behaviour. The disease occurs in juveniles prior to ossification and leads to high mortality rate (>90% in net-pen-reared smolts) (Shinn et al. 2015). Another important fish pathogen is *T. bryosalmonae* causing proliferative kidney disease (PKD) in salmonids (Hedrick et al. 1993), reaching up to 85% mortality in infected fish (Sterud et al. 2007). Infection of the digestive tract by *Enteromyxum leei* Redondo et Alvarez-Pellitero, 2002 (enteromyxosis) (Diamant 1992; Yasuda et al. 2005) and *Ceratomyxa shasta* (syn. *Ceratomyxa shasta*) (Noble, 1950) (enteronecrosis, ceratomyxosis) can lead to 100% mortality in affected stocks (Wales & Wolf 1955; Ching & Munday 1984). *C. shasta* has been made responsible for massive salmonid declines in the Klamath River basin at the US West coast (Ray et al. 2012). Reproductive castration is caused by myxozoans invading the testes of their fish hosts, e.g. *Sphaerospora testicularis* Sitjà-Bobadilla et Alvarez-Pellitero, 1990 in European seabass *Dicentrarchus labrax*, (Sitjà-Bobadilla & Alvarez-Pellitero 1990; Toledo-Guedes et al. 2012), *Henneguya testicularis* Azevedo, Corral et Matos, 1997 in *Moenkhausia oligolepis* (Günther, 1864) (Azevedo et al. 1997), and *Myxobolus testicularis* Tajdari, Matos, Mendonta et Azevedo, 2005 in *Hemiodopsis microlepis* Kner, 1859 (Tajdari et al. 2005). Well-known fish-pathogenic myxozoans of carp are *Sphaerospora dykoveae* (Lom et Dyková, 1982) and *Sphaeropora molnari* Lom, Dyková, Pavlásková et Grupcheva, 1983 (swim bladder inflammation of carp and gill- and skin sphaerosporosis) (Kovács-Gayer 1983, Lom et al. 1983a), *Parvicapsula pseudobranchicola* Karlsbakk, Sæther, Høstlund, Fjellsøy et Nylund, 2002 (parvicapsulosis) (Karlsbakk et al. 2002; Sterud et al. 2003), and *Henneguya ictaluri* Pote, Hanson et Shivaji, 2000 (proliferative or 'hamburger' gill disease) (Pote et al. 2000). There is only one record of the Myxozoa acting as a human pathogen, i.e. *Kudoa septempunctata* Matsukane, Sato, Tanaka, Kamata et Sugita-Konishi, 2010 which was associated with gastroenteritis after consumption of raw flounder sushi *Paralichthys olivaceus* Temminck et Schlegel, 1846 in Japan (Kawai et al. 2012; Ohnishi et al. 2013; Yahata et al. 2015).

Similar to other parasitic infections, after establishing contact with fish mucosal surfaces, rejection of myxozoans by immune molecules present in fish mucus is a common phenomenon (Gonzalez et al. 2007). If not eliminated, myxozoan stages encounter an array of cell-mediated and humoral immune responses that arise in the hosts' epithelial tissues. For counter attack, myxozoans produces various molecules such as proteases for successful host tissue invasion (Martone et al. 1999; Kelley et al. 2003; 2004; Dörfler & El-Matbouli 2007; Funk et al. 2008). Blood is one of the most common routes for myxozoans to reach target tissues though some myxozoans are eradicated within the blood by an array of cellular and humoral immune factors (Bjork & Bartholomew 2010). In the target host tissues, presence of myxozoan parasites activates host immune mechanisms to regulate the interaction of both immunoactivating and immunosuppressive cytokines (Gorgoglione et al.

2013; Bjork et al. 2014). Establishment of acute or chronic disease pathology depends on the host-myxozoan model, target tissue, age and immunity of the host. Immunological data are available from the most important pathogenic myxozoan species, i.e. *C. shasta* (Bjork & Bartholomew 2010; Bjork et al. 2014), *Enteromyxum* spp. (Bermúdez et al. 2006; Cuesta et al. 2006a; b; Alvarez-Pellitero et al. 2008; Muñoz et al. 2007; Davey et al. 2011; Estensoro et al. 2012; 2013a; b; 2014a; b; Ronza et al. 2016) and *M. cerebralis* (Kelley et al. 2003; 2004; Baerwald 2013). Other species have received less attention in the past but are becoming more important as environmental parameters change in different aquatic habitats.

Innate immune responses represent the first line of defence that is based on cellular and humoral factors. The most common reaction is the formation of granulomas, resulting in the elimination of the parasite by encapsulation (Koehler et al. 2004; Fleurance et al. 2008; Hallett & Bartholomew 2012). A more severe case is extensive proliferation of leucocytes (mainly macrophages and lymphocytes), leading to hyperplasia of the haematopoietic organs (Al-Samman et al. 2003; Schmidt-Posthaus et al. 2012). Phagocytes play important roles in the hosts' fight against parasites but their activity can be modulated by myxozoans (Sitjà-Bobadilla et al. 2006; 2008). Humoral innate responses include peroxidases, lysozyme or complement in fish serum, and these are variably involved in myxozoan infection, showing increasing levels (Muñoz et al. 2007) or depletion (Cuesta et al. 2006a) upon infection.

Adaptive immune responses have been reported from hosts, infected with a number of myxozoan species, e.g. with specific antibodies being formed against *M. cerebralis* (Hedrick et al. 1998), *T. bryosalmonae* (Saulnier et al. 1996), *C. shasta* (Bartholomew et al. 2001) and *E. leei* (Estensoro et al. 2010), though production and efficiency of antibodies vary depending on hosts and parasites (Hedrick et al. 1991; Ryce 2003).

Transcriptional profiling during myxozoan infection (RNA-seq) is a powerful tool providing new insights into the immune mechanisms shaping protective immunity and chronic disease (Robledo et al. 2014; Ronza et al. 2016). Such data have been produced for *T. bryosalmonae* in trout (Kumar et al. 2014) and *E. leei* infections in gilthead seabream (Robledo et al. 2014; Ronza et al. 2016). In all of these molecular data sets, the prominent involvement of macrophages suggests that innate immune responses play a key role in disease susceptibility and resistance. Both IgM and IgT transcripts are upregulated during PKD (Gorgolione et al. 2013) and in the intestines of trout surviving infection with *C. shasta* (Zhang et al. 2010). The elevation of IL10 in IgM+ and IgT + B cells indicate the presence of regulatory B cells in fish (Takizawa et al. 2013) and their involvement in myxozoan specific immunity.

Myxozoans evolved various additional host evasion strategies to escape the host immune system for successful establishment within the host. Some histozoic myxozoans provoke minimal host immune system reaction by invading immunologically privileged sites of the host body (for e.g. *M. cerebralis* and *Myxobolus fryeri* Ferguson, Atkinson, Whipps et Kent, 2008 in brain and the central nervous system, *Myxobolus* and *Sphaerospora* spp. in the eye and *S. testicularis* in testes) where host can abide parasitic antigen without any inflammatory response (Lom & Dyková 1992; Sitjà-Bobadilla & Alvarez-Pellitero 1993; El-Matbouli et al.

1995a; Ferguson et al. 2008). Intracellular development is an advantageous strategy for some myxozoan species (Kabata & Whitaker 1989; Lom et al. 1989a; Amandi et al. 1985; Swearer & Robertson 1999; Casal et al. 2008). Due to adaptive immunity, fish hosts were found to acquire higher resistance against previous encountered pathogenic Myxozoa (Foott & Hedrick 1987; Sitjà-Bobadilla et al. 2007).

In the case of sphaerosporid blood stages in carp, their massive proliferation is at race against the fish' cellular, humoral and potentially adaptive immune responses. Hence, only carp fry are sensitive to gill and skin sphaerosporosis caused by *S. molnari* or swim bladder inflammation (SBI) caused by *S. dykova* and *S. molnari* (Holzer et al. 2014: Paper III). Blood stages are engulfed by macrophages (Figure 4) but their number is often far superior to that of macrophages. We determined that the unique motility of these stages in the blood is a method of evading contact-based immune mechanisms of macrophages, independent of phagocytosis (Hartigan et al. 2016b: Paper IV).

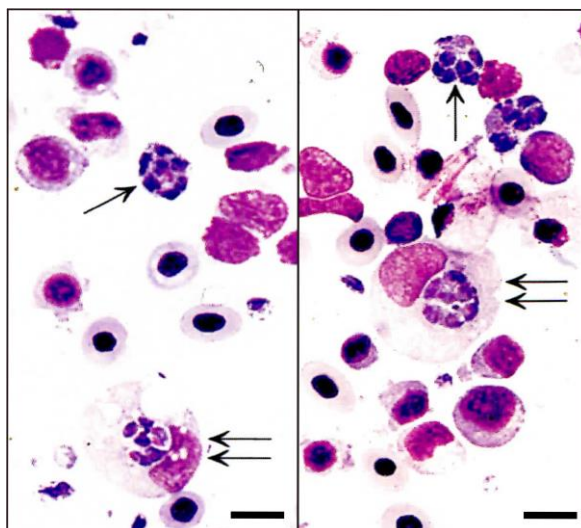


Figure 4: Giemsa stained blood smear of common carp showing extrasporogonic blood stages of *Sphaerospora dykova* consisting multiple cells inside (arrows) engulfed by host macrophages (double arrows) (scale = 10 μ m) (Dyková & Lom 2007).

Chapter 2. The Malacosporea

2.1. History of the discovery

The Malacosporea represent the most basal primitive myxozoans, with a limited number of nominal taxa. Malacosporeans differ from myxosporeans in various aspects e.g. bryozoans as definitive hosts, soft spore shell valves, unique bar-like electron dense sporoplasmosomes in primary cells of sporoplasm and unique developmental (triploblast, vermiform) stages observed in the bryozoan hosts (Schröder 1910; Canning et al. 1996; 2000) (Figure 5).

Malacosporean vermiform parasites were first discovered in the middle of nineteenth century (Dumortier & van Beneden 1850) and later named *Buddenbrockia plumatellae* (Schröder 1910). This parasite was suggested to be a mesozoan, a nematode or a platyhelminth (Schröder 1910; 1912; Braem 1911). Correct identification of *B. plumatellae* took almost a century (Monteiro et al. 2002; Okamura et al. 2002).

Presently, the Malacosporea are composed of only five nominal species from two genera: *B. plumatellae*, *B. allmani*, *Buddenbrockia bryozoides* (Canning, Okamura et Curry, 1996), *T. bryosalmonae* and *Tetracapsuloides vermiformis* Patra, Hartigan, Morris, Kodádková et Holzer, 2017 (Schröder 1910; Canning et al. 2002; 2008; Patra et al. 2017: Paper V). Recently, molecular identification of malacosporeans from bryozoan and fish hosts considerably increased their diversity account to a total of 17 taxa (Hartikainen et al. 2014; Bartošová-Sojtková et al. 2014: Paper II). The discovery of this previously hidden diversity is explained by the cryptic nature of malacosporean infections in both host groups (Hartikainen et al. 2014; Bartošová-Sojtková et al. 2014: Paper II; Patra et al. 2017: Paper V).

Only one malacosporean species, *T. bryosalmonae* is recognized as economically important as it causes PKD in salmonid fish. Being originally described as PKX organism (Seagrave et al. 1980), later it was determined as the etiological agent of the PKD (Kent & Hedrick 1985; Feist & Bucke 1987) and even later as a malacosporean (Anderson et al. 1999; Canning et al. 1999; Feist et al. 2001). This was the first discovery of a malacosporean indirect two-host life cycle including a bryozoan and a fish host (Table 1).

Initially, malacosporeans were considered a single genus, *Tetracapsula* (Canning, Okamura et Curry, 1996) in a single family, Saccosporidae (Canning et al. 2000), with two species: *Tetracapsula bryosalmonae* (Canning et al. 1999) and *Tetracapsula bryozoides* (Canning, Okamura et Curry, 1996), both forming sac-like stages in the invertebrate host. Later, based on highly similar 18S rDNA sequences, *T. bryozoides* (Canning et al. 1996) was incorporated into *B. plumatellae*, which has vermiform stages. This made *T. bryozoides* a junior synonym of the earlier described *B. plumatellae*, with both vermiform and sac-like stages in the bryozoan host (Monteiro et al. 2002; Canning et al. 2002). Additionally, due to differences in developmental and molecular data, *Tetracapsula bryosalmonae* was renamed under a new genus: *Tetracapsuloides bryosalmonae* (Canning et al. 2002). As part of the present work, due to clear phylogenetic clustering in two clades, the use of different hosts for the formation of vermiform vs. sac-like stages, the vermiform stages retained the previous name *B. plumatellae* while the sac-like stages were ascribed to *Buddenbrockia bryozoides* (n. comb., Patra et al. 2017: Paper V).

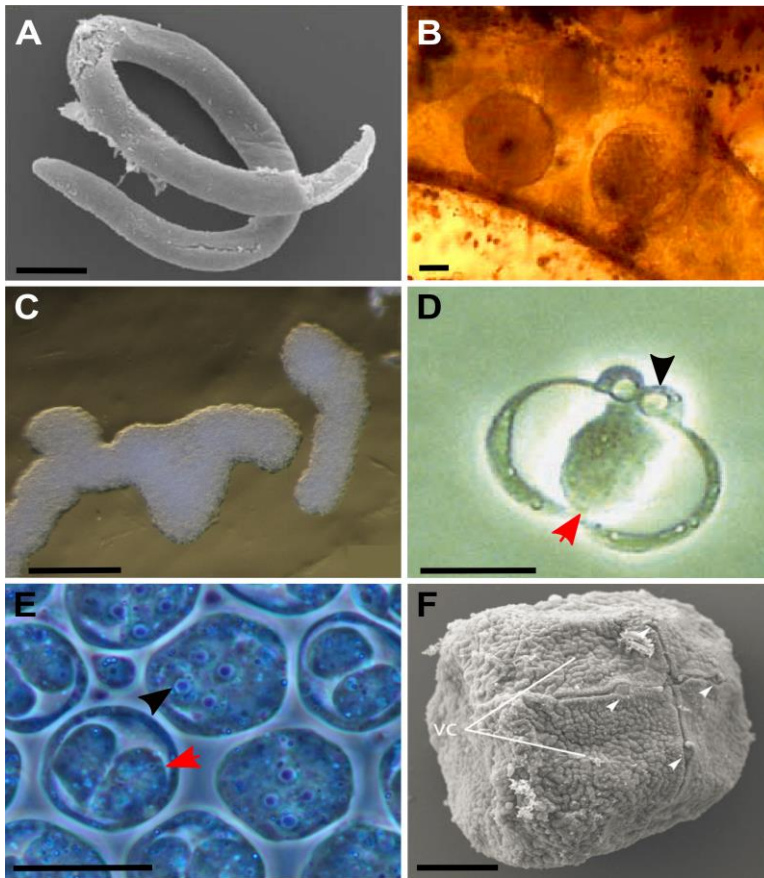


Figure 5: Different malacosporan spore-forming stages (A–C) and spores (D–F), produced in bryozoan (A–C, E–F) and fish (D) hosts. A: vermiform stage of *Buddenbrockia plumatellae* (scale = 100 μm) (Jiménez-Guri et al. 2007a); B: sac-like stages of *Tetracapsuloides bryosalmonae* (scale = 50 μm) (McGurk et al. 2006a); C: lobed stage (Hartikainen et al. 2014); D: fish malacospore (scale= 10 μm) (Hedrick et al. 2004); E: malacospores of *T. bryosalmonae* from the bryozoan host (scale= 20 μm) (<http://www.nhm.ac.uk/our-science/our-work/sustainability/emerging-diseases.html>); F: malacospore of *T. bryosalmonae* with valve cells (vc) and polar capsule openings (white arrowheads) (scale = 3 μm) (Okamura et al. 2015). Red arrow = sporoplasm, black arrowhead = polar capsule.

2.2. Life cycle specialties: Sacs and myxoworms in bryozoan hosts

Malacosporans have been reported exclusively from freshwater bryozoans of all phylactolaemate (Phylactolaemata) families, i.e. Cristatellidae, Fredericellidae, Lophopodidae, Pectinatellidae and Plumatellidae (reviewed by Patra et al. 2017: Paper V). Fish belonging to three families, i.e. Salmonidae, Cyprinidae and Percidae (reviewed by Patra et al. 2017: Paper V) have been reported to harbour malacosporans. *B. plumatellae*, *T. vermiformis* and an unidentified *Buddenbrockia* sp. were successfully transmitted from bryozoan to cyprinid hosts (Table 1), but only the life cycle of *T. bryosalmonae* was fully completed in aquaria systems, using specific pathogen free (SPF) hosts (Table 1).

As mentioned above (Chaper 1.3), apart from the traditional indirect life cycle, vertical transmission of malacosporeans to the new colonies by colonial fragmentation of bryozoans and via infected statoblasts have been described for malacosporeans (Hill & Okamura 2007; Abd-Elfattah et al. 2014). Worm-like stages (= myxoworms, Canning et al. 2008) were observed to be released from a statoblast of *Plumatella fungosa* (Pallas, 1768) on their hatching (Taticchi et al. 2004). Parasitic stages within the statoblasts of the bryozoan host could solve the problem of limited longevity (Kinkelin et al. 2002) of the infective soft-shelled malacospores from bryozoan hosts, which supposedly cannot survive the winter in the environment. During favourable environmental conditions when zooids hatch to form a new colony, malacosporeans simultaneously begin their proliferation to establish infection within the newly grown bryozoan colony.

Malacosporeans produce different types of sporogonic stages within the bryozoan hosts (Figure 5A-C). Outer walls of the sac-like stages of *B. allmani*, *B. bryozoides* and *T. bryosalmonae* are composed of two simple layers (outer epithelial layers with mural cells and inner basal lamina) (Okamura 1996; Canning et al. 2007; Morris & Adams 2007a). In contrast, vermiform stages are triploblastic, and possess an additional muscle cell layer with visible muscle fibers (McGurk et al. 2006b; Morris & Adams 2007b; Canning et al. 2008; Gruhl & Okamura 2012; Okamura & Gruhl 2016; Figure 6). Vermiform stages of *B. plumatellae* were found to have four tetraradially arranged muscle blocks (Figure 6). The muscle cells cause rhythmic sinusoidal or spiralling movement of the myxoworm (Gruhl & Okamura 2012). A third type of trophozoite is a non-motile, lobed irregular structure, devoid of muscle fibres and being similar to sac-like stages in *F. sultana* (Hartikainen et al. 2014; Figure 5C). Development of the trophozoite stages starts within the bryozoan epithelium where cryptic single cell stages are formed. These covert stages can endure for years until switching to an overt infection after which the production of multicellular trophozoite stages in the coelom of bryozoans occurs (Hartikainen et al. 2013; Abd-Elfattah et al. 2014). Worm-like stages of *B. plumatellae* develop very fast within the bryozoan host where their development from single cell stages to mature myxoworms takes only six days. Release of mature myxoworms from host is accomplished overnight (Canning et al. 2002).

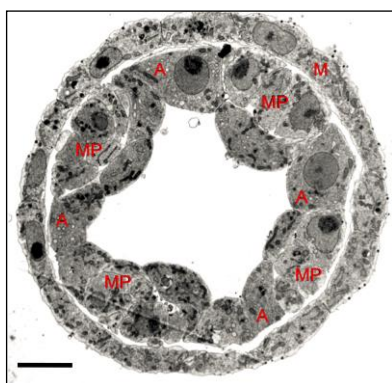


Figure 6: Transverse section of a vermiform stage of *Buddenbrockia plumatellae* showing two-layer arrangement of the body wall consisting of outer most epithelial layer made of mural cells (M) and inner four sets of muscle cells (MP) separated by basal lamina. Four primary type A cells (A) are separating four MP surrounding the central cavity (scale = 4.5 μ m) (Canning et al. 2008)

Speculations exist about the multiplication of whole sac-/vermiform stages which were mentioned as 1) budding of sacs (Okamura 1996), 2) budding of worm-like stages of *Buddenbrockia* sp. (Morris et al. 2002) and 3) division of myxoworms, (Canning et al. 2002).

2.3. Cryptic nature of parasitic stages

Very little is known about the development of malacosporans in their fish hosts, with comprehensive data existing only for *T. bryosalmonae*. Skin and gills were identified as portals of entry into fish (Morris et al. 2000, Longshaw et al. 2002; Holzer et al. 2006b; Grabner & El-Matbouli 2010b) while extrasporogonic stages were detected in fish blood and in the interstitial kidney tissue (Ferguson & Needham 1978; Lom et al. 1983b; 1991; Kent & Hedrick 1985; 1986; Clifton-Hadley et al. 1987; Morris et al. 2000; Holzer et al. 2006b). Presporogonic stages invade the renal tubules where amoeboid monosporic pseudoplasmodia are produced (Morris & Adams 2008). Similarity of early malacosporan pseudoplasmodia with those of other myxozoans (*Sphaerospora* spp., *Hoferellus* spp., *Ortholinea* spp.) often impedes the recognition of malacosporans in fish. Ambiguity of the fish malacosporan valve cell number and arrangement in *T. bryosalmonae* in different fish hosts *Salmo trutta* L. (four) and *Oncorhynchus mykiss* Walbaum, 1792 (two) (Kent & Hedrick 1986; Kent et al. 2000; Hedrick et al. 2004; Morris & Adams 2008) still requires further verification. Early infections with low infection intensity can easily be overlooked by microscopy and sporogonic stages within the kidney tubules require an expert's eye, even when spores are present, as the soft spore valves are difficult to discriminate (Bartošová-Sojtková et al. 2014: Paper II).

Similar to spore-forming stages in fish hosts, malacosporans within bryozoan host are difficult to recognize. Initial unicellular stages are indistinguishable until multicellular spore-forming stages are visible. Sac- or vermiform stages of distantly related malacosporans can be morphologically identical (Hartikainen et al. 2014; Patra et al. 2017: Paper V). *T. bryosalmonae* spores are the only ones for which the complete data of cellular arrangement in the bryozoan is known (McGurk et al. 2005).

2.4. Biodiversity and phylogeny

In comparison to the Myxosporia, the Malacosporia have always been considered a species-deficient group. However, the hosts, freshwater bryozoans show a worldwide distribution (Wood 2001; Massard & Geimer 2008). To date, approximately 94 bryozoan species are known (Massard & Geimer 2008) and it can hence be expected that the biodiversity of malacosporans is higher than known from morphological reports. Using molecular methods, Hartikainen et al. (2014) discovered five new malacosporan species from bryozoan hosts from Borneo, Germany, Italy and USA. Similarly, Bartošová-Sojtková et al. (2014: Paper II) detected an unexpected biodiversity of malacosporans in fish with another five new species in four different countries (Czech Republic, Hungary, Italy and Slovakia). While this likely indicates high diversity also in other regions, sampling from both host groups is currently strongly biased (predominantly from North America and Europe) and reflects only a punctual distribution. Limited records from vast areas of Asia (Braem

1911; Hartikainen et al. 2014) and South America (Marcus 1941) and no record from other continents (Africa and Australia) clearly indicate that malacosporean diversity is likely much higher than presently known. Though all malacosporeans were found in freshwater bryozoans, vermiform parasite stages were also recorded from marine bryozoans (Hastings 1943), implying potential existence of marine malacosporeans. Bryozoans are predominantly marine group of organisms (Gordon 1999) including around 8000 species worldwide (Ryland 2005) and marine bryozoans as potential hosts for malacosporeans have not yet been explored sufficiently. Other invertebrate groups closely related to bryozoans (phoronids or brachiopods) or even echinoderms, hemichordates or molluscs were also suggested to serve as potential invertebrate hosts (Bartošová-Sojková et al. 2014: Paper II; Okamura & Gruhl 2015).

Similar to myxosporeans, the majority of malacosporean sequence data is available from 18S rDNA though there are few records of 28S rDNA (Hartikainen et al. 2014; Bartošová-Sojková et al. 2014: Paper II) and protein-coding genes (Jiménez-Guri et al. 2007a; b; Bartošová-Sojková et al. 2014). Based on 18S rDNA phylogeny, malacosporeans are divided into four clades: *Buddenbrockia* spp., *Tetracapsuloides* spp. and two novel basal lineages (Hartikainen et al. 2014; Bartošová-Sojková et al. 2014: Paper II; Fiala et al. 2015b; Patra et al. 2017: Paper V). Phylogenetic analysis based on 28S rDNA data also strongly supported this outcome (Bartošová-Sojková et al. 2014: Paper II; Hartikainen et al. 2014). Vermiform stages were considered to represent a more primitive character than sac-like stages which were suggested to be a result of morphological simplification due to endoparasitism (Bartošová-Sojková et al. 2014: Paper II). However, with increasing molecular data gained on the two morphotypes this hypothesis fell (Hartikainen et al. 2014; Patra et al. 2017: Paper V). It appears that the complex bauplan including muscle strands was gained and lost multiple times during the evolution (Hartikainen et al. 2014). The most basal clade has lobed stages which opened a new dimension to this debate (Hartikainen et al. 2014; Fiala et al. 2015b).

Spore morphology is the most commonly used feature for myxozoan classification. While this is feasible to a large extent in myxosporeans, despite many para-/polyphyletic genera (see section 1.2), malacosporean spores from bryozoans are virtually indistinguishable between taxa as they are of identical size and shape. To differentiate the two nominal malacosporean genera, *Buddenbrockia* and *Tetracapsuloides*, Canning et al. (2002) and Canning & Okamura (2004) proposed several distinguishing characters: 1) only the former one has radially striated lucent layer on the mature polar capsule, 2) sporogenesis and ploidy of the cells are different in both genera, 3) fish host is known only from *Tetracapsuloides*, 4) vermiform stages are present only for *Buddenbrockia*, 5) shape and size of bryozoan-related stages differs (spherical with up to 250 µm in *Tetracapsuloides*; ellipsoid, elongate, irregular or constricted with up to 700 µm in *Buddenbrockia*). and, 6) up to 20% of 18S rDNA divergence exists between the two genera. Later, with the discovery of new species, developmental studies and life cycles, many points were either found to be subtle or overruled (McGurk et al. 2006a; b; Grabner & El-Matbouli 2010a; Bartošová-Sojková et al. 2014: Paper II; Hartikainen et al. 2014; Patra et al. 2017: Paper V) and it was shown that both, *Buddenbrockia* and *Tetracapsuloides* can have sac- and vermiform stages (Hartikainen et al. 2014;

Bartošová-Sojková et al. 2014: Paper II; Patra et al. 2017: Paper V). DNA sequence divergence is presently the strongest differential character in malacosporean systematics and is an absolute requirement for new species descriptions and for designing a new taxonomic system. However, the synthesis of molecular data with biological characters and data on plasmodia morphology in the bryozoan host appear to be the best combination for species differentiation at present, though confirmation is urgently required for more taxa (Patra et al. 2017: Paper V).

Chapter 3. *Sphaerospora sensu stricto*

3.1. A clade with the short history but long rDNA inserts

The etymology of “*Sphaerospora*” was adopted from the typical spherical or subspherical shape of the spores formed by *Sphaerospora* spp. in the vertebrate host (Lom & Dyková 2006). Spores consist of two shell valves with two spherical or subspherical polar capsules situated perpendicular to the suture plane. Sphaerosporids produce mostly disporic, sometimes monosporic and very rarely polysporic plasmodia (Kudo 1919; Lom et al. 1983a; Hedrick et al. 1990; Supamattaya et al. 1991; Lom & Dyková 2006; Jirků et al. 2007; Bartošová et al. 2013).

Only in 2004, the first *Sphaerospora* 18S rDNA sequences, i.e. that of *Sphaerospora truttae* Fischer-Scherl, El-Matbouli et Hoffmann, 1986 and that of the type species *Sphaerospora elegans* Thélohan, 1892, became available, and it was shown to be much longer (2541 bp; Holzer et al. 2004) than that of other myxozoans (generally 1500–2000 bp; Fiala 2006). When further sphaerosporid sequences became available, sequence alignments showed that all members have unique extensive nucleotide insertions in the variable regions of the 18S and 28S rDNA (Jirků et al. 2007; Holzer et al. 2007; Bartošová et al. 2013). So far, the longest insertions were found in *Sphaerospora molnari* Lom, Dyková, Pavlaskova et Grupcheva, 1983, producing one of the longest 18S rDNA sequences amongst eukaryotes with 3.7 kb (Eszterbauer et al. 2013). The uniqueness of the rDNA insertions of *Sphaerospora* spp. might indicate special behavioural or biological adaptations, potentially related to invertebrate host switching (Holzer et al. 2007; Bartošová et al. 2013) or a special type of proliferation and motility in the blood (Hartigan et al. 2016b: Paper V). To date, 18S rDNA sequence data from 19 *Sphaerospora s. s.* species (15 described and four undescribed species) are available in GenBank. Additionally, we amplified new 18S rDNA sequences of 13 species (Patra et al. In Prep.: Paper VI). All are characterized by large expansion segments in their rDNAs and cluster together with the type species *S. elegans* in a separate phylogenetic group coined *Sphaerospora s. s.* (Jirků et al. 2007). This clade is basal or sister to the other two myxosporean clades (see section 1.2; Figure 2). Five other sphaerosporid species are present in Genbank and cluster outside these “true” sphaerosporids now considered *Sphaerospora sensu lato* (Jirků et al. 2007; see below).

The vertebrate host spectrum of *Sphaerospora* spp. expands from marine, freshwater and brackish fish to amphibians (Table 2). Sequenced *Sphaerospora s. s.* members are predominantly coelozoic (except histozoic *S. molnari* and *Sphaerospora fugu* Tun, Yokoyama, Ogawa et Wakayabashi, 2000), affecting mostly the urinary system and often causing severe disease (Lom et al. 1983a; Sitjà-

Bobadilla & Alvarez-Pellitero 1992; Tun et al. 2000; Lom & Dyková 2006; Holzer et al. 2013b: Paper I). Sphaerosporids are generally host species-specific (Lom et al. 1989b). They have been reported to cause maximum pathogenicity in juvenile (0+ age) fish (McGeorge et al. 1997; Holzer et al. 2013b: Paper I). Some economically important pathogenic species are: *S. dykova*, the causative agent of SBI in common carp (Kovács-Gayer 1983), *S. molnari*, the agent of gill and skin sphaerosporosis in common carp and an important cofactor or precondition of SBI (Lom et al. 1983a; Holzer et al. 2014: Paper III), *Sphaerospora motemari* Holzer, Pecková, Patra, Brennan, Yanes-Roca et Main, 2013, and *Sphaerospora sparis* (Sitjà-Bobadilla et Alvarez-Pellitero, 1995) which cause severe glomerular disease in grey snapper *Lutjanus griseus* (L.) (Holzer et al. 2013b: Paper I) and gilthead sea bream *Sparus aurata* L. (Palenzuela et al. 1999), respectively.

3.2. Currently known diversity

The genus *Sphaerospora* is currently composed of 103 described nominal species (Table 2). Molecular data is available for a total of 24 previously reported (Jirků et al. 2007; Bartošová et al. 2013, Eszterbauer et al. 2013; Holzer et al. 2013b: Paper I; Sanders et al. 2015) and 13 new *Sphaerospora* spp. (Patra et al. In Prep.: Paper VI) while nothing is known about the phylogenetic origin of most sphaerosporids which are rendered *incertae sedis* (Jirků et al. 2007). Eighteen *Sphaerospora* species were renamed from *Leptotheca* (Gunter & Adlard 2010), based on morphological and in few cases molecular data. The genus *Polysporoplasma* (Sitjà-Bobadilla et Alvarez-Pellitero, 1995) was demised and its representatives were included into genus *Sphaerospora* as the two genera differed only in the number of sporoplasms (four to twelve in *Polysporoplasma* spp. vs. one to two in *Sphaerospora* spp.) (Sitjà-Bobadilla & Alvarez-Pellitero 1995; Lom & Dyková 2006; Bartošová et al. 2013). This taxonomic revision was supported by phylogenetic studies, which placed all four polysporoplasmid species including the type species *Polysporoplasma sparis* (Sitjà-Bobadilla et Alvarez-Pellitero, 1995) within the *Sphaerospora s. s.* clade. For similar reasons *Bipteria formosa* (Kovalova et Gaevskaya, 1979), was renamed *Sphaerospora formosa* though it possesses short, wing-like appendages (Bartošová et al. 2013).

Based on 18S rDNA data, only five out of total 37 sphaerosporids i.e. *S. testicularis*, *Sphaerospora dicentrarchi* Sitjà-Bobadilla et Alvarez-Pellitero, 1992, *Sphaerospora oncorhynchi* Kent, Whitaker et Margolis, 1993, *Sphaerospora elwhaiensis* Jones, Fiala, Prosperi-Porta, House, Mumford, 2011, and *Sphaerospora* sp. from *Mugil curema* are currently designated to *Sphaerospora sensu lato* (Figure 7; Table 2) due to a) uncommon ancestry with “true” sphaerosporids though being morphologically similar or, b) erroneous DNA sequencing of co-occurring myxosporean infections (Diamant et al. 2005; Fiala 2006; Bartošová et al. 2009; 2011; Jones et al. 2011). *S. testicularis* clusters within the *Parvicapsula* subclade of the marine urinary clade (Figure 2; Bartošová et al. 2011) and has atypical sphaerosporid features like a sporogony resembling the disporic development of members of the freshwater myxosporean lineage (Morris & Adams 2008) and spores with soft shell valves, a binucleate sporoplasm and polar capsules with a burred stopper structure (Sitjà-Bobadilla & Alvarez-Pellitero 1993) similarly as in

malacosporians (Morris & Adams 2008). *S. dicentrarchi* and *Sphaerospora* sp. from *Mugil curema* are truly histozoic and cluster within the marine *Kudoa* clade (Diamant et al. 2005; Fiala 2006; Bartošová et al. 2009; 2011) with other histozoic species of the genera *Kudoa* or *Unicapsula* Davis, 1924 (e.g. Diamant et al. 2005). *S. dicentrarchi* also shares several other features with multivalvulids such as baglike polysporous plasmodia, a single binucleated sporoplasm and miniature spores with overlapping shell valves (Sitjà-Bobadilla & Alvarez-Pellitero 1992). *S. elwhaiensis* and *S. oncorhynchi* cluster within the freshwater *Myxidium* clade (Jones et al. 2011) and *Myxidium lieberkuehni* clade (Fiala 2006), respectively, but recent re-sequencing of these sphaerosporids has revealed misidentification due to mixed infection and true affinity to the *Sphaerospora s. s.* clade (Atkinson et al. 2015). Co-infections and preferential amplification of shorter amplicons without rDNA inserts in non-sphaerosporid species led to misidentifications of several other myxozoans as *Sphaerospora* spp. but these sequencing errors have already been corrected. For example, a *Zschokkella* species was wrongly identified as *Sphaerospora angulata* Fujita, 1912 (Acc. no. AY735411) in goldfish *Carassius auratus* (L.) (Eszterbauer & Székely 2004). Two different *Myxobolus* spp. had been ascribed to *S. molnari* (Acc. no. AF378345; Kent et al. 2001) and *S. dykova* (Acc. no. AY735410; Eszterbauer & Székely 2004) due to mixed infection in the host organ. The ambiguities were resolved after the amplification of the complete 18S rDNAs of *S. angulata*, *S. molnari* and *S. dykova*, which were found to cluster within *Sphaerospora s. s.* (Eszterbauer 2011; Eszterbauer et al. 2013; Holzer et al. 2013a). Table 2 summarizes all *Sphaerospora* spp. reported to date. Based on sequence corrections and taxonomic changes, it is likely that *Sphaerospora s. s.* will become a well-defined monophyletic clade.

Table 2: List of *Sphaerospora* species along with the information about their synonyms (if present), vertebrate hosts, organ infected within the vertebrate host, spore measurements, Genbank accession numbers and the references. Abbreviations – BL: blood, EY: eye, GB: gall bladder, GL: gill, GM: glomeruli, IN: intestine, KD: kidney, LV: liver, MS: muscle, NS: nose, OC: oral cavity, OV: ovary, PR: peritoneum, RT: renal tubules, RI: renal interstitial tissue, SK: skin, SP: spleen, SW: swim bladder, TS: testis, UB: urinary bladder, UR: ureter, X: unknown, L: spore length, W: spore width, T: spore thickness, D: spore diameter, PC: polar capsule length x width. All spore measurements are in μm . Genbank accession numbers are specified for 18S rDNA (black), 28S rDNA (red) and EF2 (purple). Amphibian hosts are shown in green colour, Blue = *Sphaerospora sensu stricto* spp., orange = *Sphaerospora sensu lato* spp. and, bold= the type species.

Species name	Vertebrate host	Organ	Spore measurements	Access no.	Reference
<i>Sphaerospora aldrichettae</i> Su et White, 1994	<i>Aldrichetta forsteri</i>	GL	L: 6.3-7.0, T: 6.2-7.0, PC: 2.1-2.8x1.5-1.8	X	Su & White 1994
<i>Sphaerospora amurensis</i> Akhmerov, 1960	<i>Hypophthalmichthys molitrix</i>	RT	L: 9-11, T: 8-10, PC: 3.5-4x3-3.5	X	Shul'man 1966
<i>Sphaerospora angulata</i> Fujita, 1912	<i>Carassius auratus auratus</i> , <i>Carassius gibelio</i>	RT	L: 6.53-7.1, T: 5.78-6.2, PC: 2.76-3.32x2.18-2.72	JQ801525-30; JQ801534-38; JQ801537	Fujita 1912
<i>Sphaerospora araii</i> Arthur et Lom, 1985	<i>Raja rhina</i>	RT	L: 15.2, T: 14.6, PC: 6.5	X	Arthur & Lom 1985
<i>Sphaerospora armatura</i> (Yoshino et Moser, 1974) (Syn: <i>Leptotheca armatura</i>)	<i>Albatrossia pectoralis</i> , <i>Coryphaenoides leptolepis</i>	UB, RT	L: 12.9, T: 20.9, PC: 5.7x5.7	X	Yoshino & Moser 1974
<i>Sphaerospora bramae</i> El-Matbouli, Hoffmann et Kern, 1995	<i>Abramis brama</i>	RT	L: 4.4-5.4, T: 4.3-5.4, PC: 2.1	X	El-Matbouli et al. 1995b
<i>Sphaerospora brevis</i> (Polyanskii, 1955) (Syn: <i>Leptotheca brevis</i>)	<i>Myoxocephalus scorpius</i>	UB	L: 10-12, T: 11.7-12.6, PC: 3.6-4.5	X	Polyanskii 1955; Shul'man 1966
<i>Sphaerospora brevoides</i> (Zhao et Song, 2009) (Syn: <i>Leptotheca brevoides</i>)	<i>Chirolophis japonicus</i>	UB	L: 6-8, T: 7-10, PC: 2.1x1.7	X	Zhao & Song 2009
<i>Sphaerospora carassii</i> Kudo, 1919	<i>Hypophthalmichthys molitrix</i> , <i>Ctenopharyngodon idella</i> , <i>Carassius auratus auratus</i> , <i>C. gibelio</i> , <i>Rutilus rutilus</i>	GL	D: 8-13, PC: 4-5	X	Kudo 1919
<i>Sphaerospora chagasi</i> (Nemeczcek, 1926) (Syn: <i>Leptotheca chagasi</i>)	<i>Leptodactylus ocellatus</i>	RT	L: 10-11, T: 15, PC: 8-8.5x8-8.5	X	Nemeczck 1926
<i>Sphaerospora chinensis</i> (Lee et Nie in Chen, 1973) (Syn: <i>Sphaerospora branchialis</i>)	<i>Cyprinus carpio</i>	GL	L: 7.4, T: 7	X	Lom et al. 1983a
<i>Sphaerospora colomani</i> Baska, 1990	<i>Acipenser ruthenus</i>	RT	L: 8.6, T: 10.2	X	Baska 1990

Table 2 continued

<i>Sphaerospora compressa</i> (Noble, 1939) (Syn: <i>Leptotheca compressa</i>)	<i>Rimicola eigenmanni</i>	UB	L: 10.5, T: 12.2, PC: 3.3x4	X	Noble 1939
<i>Sphaerospora coregoni</i> El-Matbouli, Hoffmann et Kern, 1996	<i>Coregonus lavaretus</i>	RT	L: 8.2, T: 11.3	X	El-Matbouli et al. 1996
<i>Sphaerospora corsulae</i> Sarkar et Ghosh, 1991	<i>Rhinomugil corsula</i>	GL	L: 4.9, T: 4.76, PC: 1.74x1.7	X	Sarkar & Ghosh 1991
<i>Sphaerospora cristata</i> Schulman, 1962	<i>Lota lota</i>	UB, UR	L: 9-10, W: 8.5-10, T: 8.3-10, PC: 3.5- 4x2.5-3	X	Shul'man 1966
<i>Sphaerospora ctenopharyngodonii</i> Chen, 1998	<i>Ctenopharyngodon idella</i>	GL	L: 14.7, W: 12.4, T: 12, PC: 5.9x4.8	X	Chen & Ma 1998
<i>Sphaerospora danubialis</i> Molnár, 1991	<i>Gymnocephalus schraetzer</i> , <i>G. cernua</i> , <i>Sander lucioperca</i>	RT	L: 10.1, W: 9.1, T: 8.7, PC: 4.4	X	Molnár 1991
<i>Sphaerospora dicentrarchi</i> Sitjà-Bobadilla et Alvarez-Pellitero, 1992	<i>Dicentrarchus labrax</i>	GB, IN, other tissue	L: 4.5, T: 3.9, PC: 1.78x 1.4	KT970638-39, KC516864, AY278564; KC516866-67, FJ417074; JX286642	Sitjà-Bobadilla & Alvarez-Pellitero 1992
<i>Sphaerospora diminuta</i> Li et Desser, 1985	<i>Lepomis gibbosus</i>	RT, UR	D: 7.5, PC: 2.5	X	Li & Desser 1985
<i>Sphaerospora dissostichi</i> Brickle, Kalavati et MacKenzie, 2001	<i>Dissostichus eleginoides</i>	GB	L: 13, T: 11.5, PC: 3.6	X	Brickle et al. 2001
<i>Sphaerospora dogieli</i> Schulman, 1962	<i>Silurus soldatovi</i> <i>Parasilurus asotus</i>	UB	L: 7-7.5, W: 7-7.5, T: 5.7-6, PC: 2.8- 3x1.7-2	X	Shul'man 1966
<i>Sphaerospora dubinini</i> Schulman, 1962	<i>Pseudobagrus ussuriensis</i>	UB	L: 8-10.2, T: 7.3-8.5, PC: 4.2-4.6x2.5-2.8	X	Chen & Ma 1998
<i>Sphaerospora dykova</i> (Dyková et Lom, 1982) (Syn: <i>Sphaerospora renicola</i>)	<i>Cyprinus carpio</i>	RT, BL, SB	L: 7, T: 7.18, PC: 3.11x2.42	JQ801531-33, JF758875; JQ801540-46	Dyková & Lom 1982
<i>Sphaerospora elegans</i> Thélohan, 1892	<i>Gasterosteus aculeatus</i> , <i>Pungitius pungitius</i> , <i>Pungitius</i> <i>platygaster</i> , <i>Platichthys flesus</i> , <i>Phoxinus phoxinus</i>	RT, UB, OV	L: 10.2, T: 10.1, PC: 3.9	AJ609590, JX286618; JX286627	Thélohan 1892
<i>Sphaerospora elwhaiensis</i> Jones et Fiala, 2011	<i>Oncorhynchus nerka</i>	RT	L: 10.3, T: 11.2, PC: 3.5	EU371498; HQ450772	Jones et al. 2011

Table 2 continued

<i>Sphaerospora epinepheli</i> Supamattaya, Fischer-Scherl, Hoffmann et Boonyaratpalin, 1991	<i>Epinephelus malabaricus</i> , <i>Epinephelus coioides</i>	RT, GM	L: 8.7, T: 13.4, PC: 3.7	HQ871152-3, KJ939364	Supamattaya et al. 1991
<i>Sphaerospora formosa</i> (Kovalova et Gaevskaya, 1979) (Syn: <i>Bipteria formosa</i>)	<i>Merlangius merlangus</i>	RT	L: 6.6, T: 6.6, PC: 3.6	FJ790307-9, GQ374533,	Karlsbakk & Kjøie 2009
<i>Sphaerospora fugu</i> (Tun, Yokoyama, Ogawa et Wakayabashi, 2000) (Syn: <i>Leptotheca fugu</i>)	<i>Takifugu rubripes</i>	IN	L: 9, T:14, PC: 2.8x2.8	AB195805	Tun et al. 2000
<i>Sphaerospora galinae</i> Evlanov, 1981	<i>Tinca tinca</i>	RT	L: 8.8, T: 8.4, PC: 4.8x4	X	Evlanov 1981
<i>Sphaerospora gasterostei</i> Schuurmans-Stekhoven, 1920	<i>Gasterosteus aculeatus</i>	RT, UB, OV	L: 6.7, T: 7, PC: 3.5	X	Schuurmans-Stekhoven 1920
<i>Sphaerospora gobionis</i> Lom, Pavlovskova et Dyková, 1985	<i>Gobio gobio</i>	RT	L: 7.1, T: 6.9, PC: 3.4x2.9	X	Lom et al.1985
<i>Sphaerospora glomerata</i> (Davis, 1917) (Syn: <i>Leptotheca glomerata</i>)	<i>Paralichthys albigutta</i>	UB	L: 4.5, T: 9, PC: 2x2	X	Davis 1917
<i>Sphaerospora hankai</i> (Lom, Desser et Dyková 1989)	<i>Ameiurus nebulosus</i>	RT	L: 6.1, T: 6, PC: 2.7-2.8x2-2.4	JX286623; JX286632	Lom et al. 1989b
<i>Sphaerospora hangzhouensis</i> Li et Wu, 1983	<i>Cyprinus carpio</i>	UB	L: 6-6.4, T: 6.4-6.9, PC: 2.4-2.8x1.6-2.4	X	Chen & Ma 1998
<i>Sphaerospora hunanensis</i> Chen, 1998	<i>Carassius auratus auratus</i>	GL	L: 9.1, W: 10, T: 6, PC: 4.8x3.6	X	Chen & Ma 1998
<i>Sphaerospora hupehensis</i> Li et Nie, 1973	<i>Acathorhodeus</i> sp.	GL	L: 7.8, W: 7.4, T: 7.5, PC: 3.3x2.8	X	Chen & Ma 1998
<i>Sphaerospora hypophthalmichthydis</i> (Chen et Hsieh, 1984) (Syn: <i>Podospora hypophthalmichthydis</i>)	<i>Hypophthalmichthys molitrix</i>	IN	L: 9.6, T: 12.0	X	Arthur & Lom 1985
<i>Sphaerospora inaequalis</i> Landsberg, 1987	<i>Clarias gariepinus</i>	RT	L: 7.4, T: 6.9, PC: 4.2x3.6 & 3.3x2.9	X	Landsberg 1987
<i>Sphaerospora johnae</i> Sarkar, 2010	<i>Johnius belangerii</i>	RT	L: 10.2, T: 10, PC: 3.9	X	Sarkar 2010
<i>Sphaerospora koreana</i> (Cho et Kim, 2001) (Syn: <i>Leptotheca koreana</i>)	<i>Sebastes schlegelii</i>	RT	L: 8.59, T: 13.42, PC: 3.86x3.86	X	Cho & Kim 2001
<i>Sphaerospora krogjusi</i> (Shul'man, 1966) (Syn: <i>Leptotheca krogjusi</i>)	<i>Oncorhynchus keta</i> , <i>O. nerka</i> , <i>O. gorbusha</i> , <i>O. mykiss</i> , <i>O. tshawytscha</i> , <i>Salvelinus alpinus</i> , <i>S. leucomaenis</i>	UB	L: 5.8-7.5, T: 7.5-11, PC: 2.7-3.6x2.7-3.3	X	Shul'man 1966
<i>Sphaerospora kwangtungensis</i> Chen, 1998	<i>Ctenopharyngodon idella</i>	GL	L: 9.5, T: 7.6, T: 7.6, PC: 3.1x2.9	X	Chen & Ma 1998
<i>Sphaerospora leuciscusi</i> Longshaw, 2004	<i>Leuciscus leuciscus</i>	RT	L: 5.56, T: 5.87, PC: 2.35	X	Longshaw 2004
<i>Sphaerospora lieni</i> Feng et Wang, 1990	<i>Hypophthalmichthys molitrix</i>	RT	L: 11.75, T: 10, PC: 5.4x5	X	Chen & Ma 1998

Table 2 continued

<i>Sphaerospora lobosa</i> (Davis, 1917) (Syn: <i>Leptotheca lobosa</i>)	<i>Paralichthys dentatus</i>	UB	L: 9-10, T: 16-18, PC: 3x3	X	Davis 1917
<i>Sphaerospora luciopercae</i> Moshu, 1992	<i>Sander lucioperca</i>	OV	L: 7.6 (7.2-9.6), T: 6.2 (5-7), PC: 3.8 (3-4)x 3.0 (2.5-3.6)	X	Moshu 1992
<i>Sphaerospora lutjani</i> (Kpatcha, Diebakate et Toguebaye, 1996) (Syn: <i>Leptotheca lutjani</i>)	<i>Lutjanus fulgens</i>	KD	L: 6.06, T: 8.95, PC: 2.12x2.12	X	Kpatcha et al. 1996
<i>Sphaerospora mackenzii</i> (Kalavati et Rao, 2005) (Syn: <i>Leptotheca mackenzii</i>)	<i>Fejervarya limnocharis</i>	UB	L: 10.2, T: 15.6, PC: 6.2x4.8	X	Kalavati & Rao 2005
<i>Sphaerospora markewitschi</i> Donets, 1962	<i>Gymnocephalus acerina</i> , <i>Gymnocephalus cernua</i>	RT	L: 10.8-11.2, W: 10.8-11.7, T: 9.5-10.8, PC: 5.5x4.2	X	Shul'man 1966
<i>Sphaerospora masovica</i> Cohn, 1902	<i>Abramis brama</i>	GB, IN	D: 8 x 8	X	Kudo 1919
<i>Sphaerospora mayi</i> Moser, Kent et Dennis, 1989	<i>Atherinomorus lacunosus</i>	GB	L: 4-6, W: 4-5, T: 3-3.5, PC: 2x1	X	Moser et al. 1989
<i>Sphaerospora melenensis</i> Fomena, Marques et Bouix, 1993	<i>Oreochromis niloticus</i>	KD	L: 9-10, T: 7.5-9.5, PC: 3-4.5x 2.5-4	X	Fomena et al. 1993
<i>Sphaerospora minima</i> Kaschkovsky, Razmashkin et Skripchenko, 1974	<i>Rutilus rutilus</i>	UB	L: 6-7.7, T: 5.4-6, PC: 2.8-3x 2.3-2.8	X	Kaschkovsky, Razmashkin & Skripchenko 1974
<i>Sphaerospora minuta</i> Konovalov, 1967	<i>Chanodichthys mongolicus</i>	UB	L: 6.2-7.3, W: 5.8-6.8, T: 5, PC: 2.9-3.3x 2.6-2.8	X	Konovalov 1967
<i>Sphaerospora molnari</i> Lom, Dyková, Pavlaskova et Grupcheva, 1983	<i>Cyprinus carpio</i>	BL, GL, SW, SK	L: 10.3, T: 10.5, PC: 4.5x 4.3	JX431510-11, AF378345	Lom et al. 1983a
<i>Sphaerospora motemarinii</i> Holzer, Pecková, Patra, Brennan, Yanes-Roca et Main, 2013	<i>Lutjanus griseus</i>	GM, RT, RI	L: 16.31, T: 19.85, PC: 6.47x 6.43	KC526873	Holzer et al. 2013b
<i>Sphaerospora mugili</i> Yurakhno et Maltsev, 2002	<i>Mugil cephalus</i>	GB	L: 4.5-5.8, W: 3.8-4.3, T: 4.1-6.2, PC: 1.7-2.5x1.0-1.6	X	Yurakhno & Maltsev 2002
<i>Sphaerospora mugilis</i> (Sitjà-Bobadilla et Alvarez-Pellitero, 1995) (Syn: <i>Polysporoplasma mugilis</i>)	<i>Liza aurata</i>	RT, GM	L: 23.97, T: 20.5, PC: 6.37	X	Sitjà-Bobadilla & Alvarez-Pellitero 1995
<i>Sphaerospora mystus</i> Xiao et Feng, 1997	<i>Hemibagrus macropterus</i>	KD	L: 10, W: 8.9, T: 8.7, PC: 4x 2.6	X	Xiao & Feng 1997
<i>Sphaerospora notropis</i> Fantham, Porter et Richardson, 1939	<i>Notropis cornutus</i> , <i>Catostomus commersoni</i>	MS, OC	L: 9-11.4, T: 9-9.5, PC: 2.7-5.5x1.8-3.6	X	Fantham et al. 1939

Table 2 continued

<i>Sphaerospora ohlmacheri</i> Whinery, 1893	<i>Rana catesbeiana</i>	RT	L: 12.6, T: 10.9, PC: 4.4	JX286619; JX286628	Desser et al. 1986
<i>Sphaerospora ojiroveci</i> Dyková et Lom, 1997	<i>Pangasius sutchi</i>	X	L: 8, T: 5.2, PC: 2.8x 3.5	X	Dyková & Lom 1997
<i>Sphaerospora oncorhynchi</i> Kent, Whitaker et Margolis, 1993	<i>Oncorhynchus nerka</i>	RT	L: 8.2-10.2, T: 7.8-9.2, PC: 2.9-3.3	AF201373	Kent et al.1993b
<i>Sphaerospora olsoni</i> Sanders, Jaramillo, Ashford, Feist, Lafferty et Kent, 2015	<i>Atherinops affinis</i>	RT	L: 6, T: 5.8, PC: 2	KJ526213	Sanders et al. 2015
<i>Sphaerospora ousei</i> Longshaw, 2004	<i>Rutilus rutilus</i>	RT	L: 8.46, T: 8.21, PC: 3.22	X	Longshaw 2004
<i>Sphaerospora ovophila</i> Xiao et Desser, 1997	<i>Lepomis gibbosus</i>	OV	L: 8.2, W: 6.2, T: 7.9, PC: 3x 2.8	X	Xiao & Desser 1997
<i>Sphaerospora paulini</i> Lom, Desser et Dyková, 1989	<i>Semotilus atromaculatus</i>	RT	D: 8-8.5, PC: 6.1x6	X	Lom et al.1989b
<i>Sphaerospora pectinacea</i> Bocharova et Donetz, 1974	<i>Perca fluviatilis</i>	GB, KD, UB, LV, MS	L: 8.5, T: 10	X	Bocharova & Donetz 1974
<i>Sphaerospora periophthalmi</i> Fantham et Porter, 1943	<i>Boleophthalmus dussumieri</i> , <i>Periophthalmus barbarus</i>	GB	D: 6.6x10.4-11.5	X	Fantham & Porter 1943
<i>Sphaerospora perlata</i> (Gurley, 1893) (Syn: <i>Leptotheca perlata</i>)	<i>Gymnocephalus cernuus</i>	X	X	X	Gurley 1894; Gunter & Adlard 2010
<i>Sphaerospora petruschewskii</i> Schulman, 1962	<i>Misgurnus fossilis</i> , <i>Misgurnus anguillicaudatus</i>	UB	L: 9- 12.5, W: 7-7.5, T: 5.5-6, PC: 2.7-2.9x2	X	Shul'man 1966
<i>Sphaerospora plagiognathopsis</i> (Chen et Hsieh, 1984) (syn: <i>Podospora plagiognathopsis</i>)	<i>Plagiognathops microlepis</i>	OV	L: 7.2, T: 7.6, PC: 2.4-2.5	X	Chen & Hsieh 1984
<i>Sphaerospora platessae</i> Woodcock, 1904	<i>Pleuronectes platessa</i>	EY	D: 8-9	X	Kudo 1919
<i>Sphaerospora poljanskii</i> Kulemina, 1969	<i>Rutilus rutilus</i>	RT	L: 9.5-10, T: 9-10, PC: 3-4x 2.5-3	X	Kulemina 1969
<i>Sphaerospora ranae</i> (Morelle, 1929)	<i>Rana dalmatina</i> , <i>Rana temporaria</i>	RT	L: 10.1, T: 11.7, PC: 4-5	EF211975; FJ417075	Jirků et al. 2007
<i>Sphaerospora reichenowi</i> Jacob, 1953	<i>Anguilla anguilla</i>	IN	L: 9, T: 10, PC: 4	X	Jacob & Bremen 1953
<i>Sphaerospora renalis</i> Bond, 1938	<i>Fundulus heteroclitus</i>	RT	L: 9.5, T: 9, PC: 4.3x 3.5	X	Bond 1938
<i>Sphaerospora renicola</i> (Thélohan, 1895) (syn: <i>Leptotheca renicola</i>)	<i>Scomber scombrus</i>	RT	L: 10, T: 8	X	Thélohan 1895

Table 2 continued

<i>Sphaerospora rostrata</i> Thélohan, 1895	<i>Mugil</i> sp.	GM	L: 10, T: 12, PC: 1x 2	X	Thélohan 1895
<i>Sphaerospora rota</i> Zaika, 1961	<i>Brachymystax lenok</i> , <i>Leuciscus leuciscus baicalensis</i> , <i>Cobitis taenia</i>	RT, UB, UR	L: 8.4-11, T: 9.1-9.8, PC: 4.2-5.6x 2.8-4.2	X	Shul'man 1966
<i>Sphaerospora sangmelimaensis</i> Fomena et Bouix, 1994	<i>Brienomyrus brachyistius</i> , <i>Petrocephalus simus</i> , <i>Hepsetus odoe</i>	RT	L: 8-9, T: 6.5-9, PC: 2.5-4x 2-3	X	Fomena & Bouix 1994
<i>Sphaerospora sapae</i> Donets, 1962	<i>Abramis sapa</i>	RT	L: 6-6.7, W: 5.7-6.3, T: 5.4-7.2, PC: 3.6-4.3x3	X	Shul'man 1966
<i>Sphaerospora saurogobii</i> Ma, 1998	<i>Saurogobio dabryi</i>	GL, SK, NS	L: 10.7, W: 9.5, T: 8.7, PC: 4.1x 3.3	X	Chen & Ma 1998
<i>Sphaerospora scardinii</i> El-Matbouli et Hoffmann, 1992	<i>Scardinius erythrophthalmus</i>	RT, UR	L: 6.01, T: 5.79	X	El-Matbouli & Hoffmann 1992
<i>Sphaerospora schulmani</i> (Rumyantsev, 1997) (Syn: <i>Leptothecha Schulmani</i>)	<i>Coregonus lavaretus</i> , <i>C. albula</i>	UB	L: 6.5-7.5, T: 7.5-8.5, PC: 3.5x 3.5	X	Rumyantsev 1997
<i>Sphaerospora sebasta</i> (Zhao et Song, 2003) (Syn: <i>Sphaerospora sebastis</i>)	<i>Sebastes schlegelii</i>	GB	L: 15.3, T: 15, PC: 2.9	X	Zhao et al. 2015
<i>Sphaerospora siluri</i> Molnár, 1993	<i>Silurus glanis</i>	RT	L: 6.8, T: 6.8, PC: 3.1	X	Molnár 1993a
<i>Sphaerospora simplex</i> (Kovalova et Zubchenko, 1984) (Syn: <i>Leptothecha simplex</i>)	<i>Hippoglossus hippoglossus</i>	UB	L: 10.6-12.5, T: 13.3-15.8, PC: 4x4	X	Kovalova & Zubchenko 1984
<i>Sphaerospora sparidarum</i> (Sitjà-Bobadilla et Alvarez-Pellitero, 2001) (Syn: <i>Leptothecha sparidarum</i>)	<i>Dentex dentex</i> , <i>Sparus aurata</i>	GM, RT, RI, UB, IN	L: 6.02, T: 10.65, PC: 2.91x2.75	JX286620; JX286629	Sitjà-Bobadilla & Alvarez-Pellitero 2001
<i>Sphaerospora sparis</i> (Sitjà-Bobadilla et Alvarez-Pellitero, 1995) (Syn: <i>Polysporoplasma sparis</i>)	<i>Sparus aurata</i>	RT, GM	L: 19.83, T: 18.14, PC: 6.25x5.9	JX286624; JX286634	Sitjà-Bobadilla & Alvarez-Pellitero 1995
<i>Sphaerospora sphaerica</i> Dogiel, 1948	<i>Takifugu pardalis</i>	UB	L: 10, W: 10, PC: 3	X	Dogiel 1948
<i>Sphaerospora sphaerula</i> (Noble, 1939) (Syn: <i>Leptothecha sphaerula</i>)	<i>Gibbonsia metzi</i>	UB	L: 13, T: 13, PC: 4.6x 5	X	Noble 1939
<i>Sphaerospora subsphaerica</i> (Zaika, 1963) (Syn: <i>Leptothecha subsphaerica</i>)	<i>Coregonus autumnalis migratorius</i> , <i>Thymallus arcticus</i>	UR, RT	L: 7-8.4, T: 9.8-10.5, PC: 3.5-4.2x 3.5-4.2	X	Shul'man 1966
<i>Sphaerospora testicularis</i> Sitjà-Bobadilla et Alvarez-Pellitero, 1990	<i>Dicentrarchus labrax</i>	TS	L: 11-15, T: 13-18, PC: 2.5-4.5x3.5-4.2	HM230825	Sitjà-Bobadilla & Alvarez-Pellitero 1990

Table 2 continued

<i>Sphaerospora tilapiae</i> Fomena, Marques et Bouix, 1993	<i>Oreochromis niloticus</i>	KD, SP	L: 7-8.5, T: 7-8.5, PC: 2.5-3.5	X	Fomena et al. 1993
<i>Sphaerospora tincae</i> Plehn, 1925	<i>Tinca tinca</i>	PR	L: 8, T: 7, PC: 2.7x2.5	X	Plehn 1925
<i>Sphaerospora tongrenensis</i> Xiao et Feng, 1997	<i>Siniperca scherzeri</i>	KD	L: 6, W: 6, T: 6, PC: 3x2	X	Xiao & Feng 1997
<i>Sphaerospora toxabramis</i> Wu et Li, 1986	<i>Toxabramis swinhonsi</i>	OV	L: 5.9, W: 5.8, T: 5.85, PC: 2.2x 1.75	X	Chen & Ma 1998
<i>Sphaerospora truttae</i> Fischer-Scherl, El-Matbouli et Hoffmann, 1986	<i>Salmo trutta</i> , <i>Salmo salar</i> , <i>Thymallus thymallus</i>	RT	L: 6.84, T: 8.81	AM410773, AJ512829, AJ581915, AJ512831, AJ512830; JX286633; JX286639	Fischer- Scherl et al. 1986
<i>Sphaerospora umbrae</i> Moshu et Trombitsky, 2007	<i>Umbra krameri</i>	GM, RT	L: 6.25, W: 6.2, T: 6.25, PC: 1.2-2.5	X	Moshu & Trombitsky 2007
<i>Sphaerospora xenocypris</i> Xiao et Feng, 1997	<i>Xenocypris macrolepis</i>	GL, GB	L: 8.9, W: 8.7, T: 8.8, PC: 4x 3	X	Xiao & Feng 1997
<i>Sphaerospora zingeli</i> Moshu et Trombitsky, 2008	<i>Zingel zingel</i>	GM, RT, RI,	L: 10, T: 7.5, PC: 4.3x3.7	X	Moshu & Trombitsky 2008
<i>Sphaerospora</i> sp.	<i>Chelon labrosus</i>	RT	X	JX286625; JX286635	Bartošová et al. 2013
<i>Sphaerospora</i> sp.	<i>Liza ramada</i>	RT	X	JX286626; JX286636; JX286640	Bartošová et al. 2013
<i>Sphaerospora</i> sp.	<i>Mugil curema</i>	GB	X	DQ377695; FJ417076	Fiala 2006
<i>Sphaerospora</i> sp.	<i>Pomoxis nigromaculatus</i>	RT	X	JX286621; JX286630; JX286638	Bartošová et al. 2013
<i>Sphaerospora</i> sp.	<i>Ptychadena anchietae</i>	RT	X	JX286622; JX286631	Bartošová et al. 2013

3.3. Phylogeny of the *Sphaerospora sensu stricto* clade

Figure 7 shows the state of knowledge into phylogeny of the *Sphaerospora s. s.* clade (Bartošová et al. 2013) before the addition of our novel sphaerosporid data (Patra et al. In Prep.: Paper VI). Clustering of its members reflects certain biological features such as habitat type or host group (fish/amphibian) as well as morphological (number of sporoplasms) and sequence features (GC content, introns) (Bartošová et al. 2013).

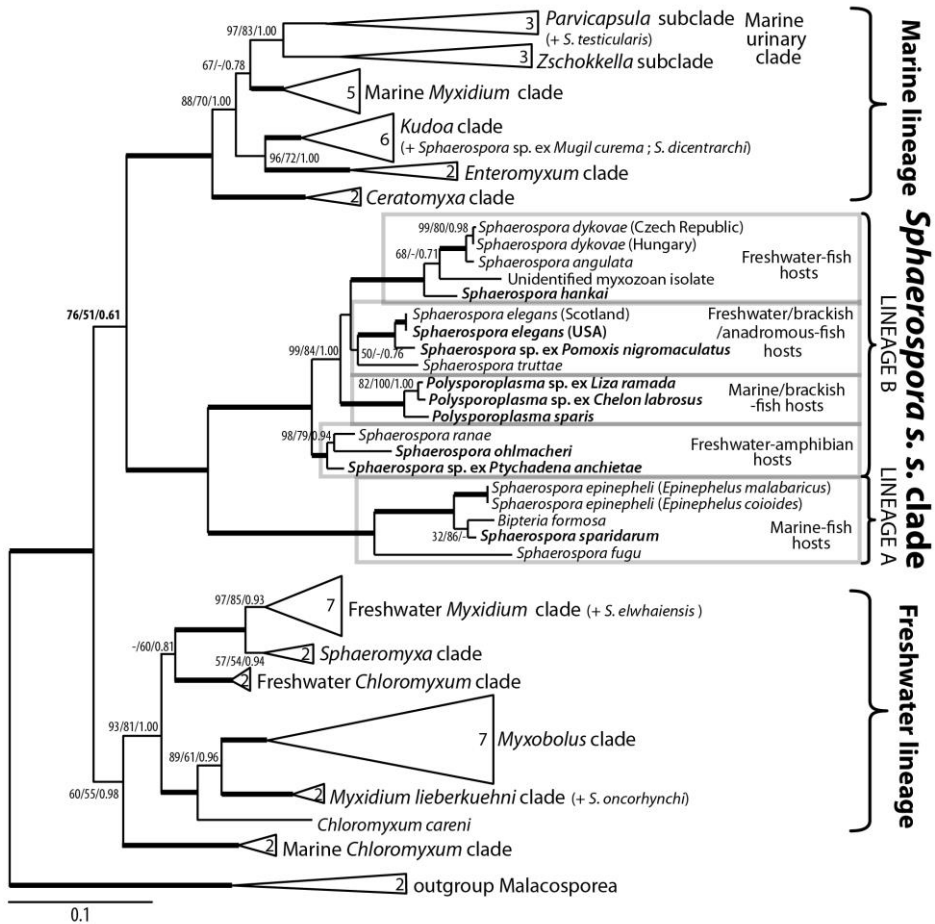


Figure 7: 18S rDNA-based maximum likelihood tree of myxozoan sequences showing *Sphaerospora sensu stricto* clade as a sister clade to marine myxosporeans. Clustering pattern based on different biological characters is also mentioned within the figure. The position of *Sphaerospora sensu lato* species in the tree is indicated within the brackets (Bartošová et al. 2013).

The clade is divided into two lineages: i) lineage A including species only from marine fish that have a single binucleated sporoplasm (except *Sphaerospora epinepheli* Supamattaya, Fischer-Scherl, Hoffmann et Boonyaratpalin, 1991) and shorter AT-rich rDNA inserts (Bartošová et al. 2013; Fiala 2006; Karlsbakk & Kjøie 2009), and ii) lineage B with 2–12 uninucleate sporoplasms and longer, GC-rich rDNA inserts. Lineage B further divides into two clades: 1) species from fish hosts and, 2) species from amphibian hosts. The fish clade of lineage B further splits into three subclades according to host habitat: a) species from strictly freshwater fish, b) species from freshwater/brackish and anadromous fish and, c) polysporoplasmid species from marine/brackish hosts (Bartošová et al. 2013). More recently described species, *S. motemardini* and *Sphaerospora olsoni* Sanders, Jaramillo, Ashford, Feist, Lafferty et Kent, 2015 (Holzer et al. 2013b: Paper I; Sanders et al. 2015) as well as all the species recognized in our current study (Patra et al. In Prep.: Paper VI) cluster according to the abovementioned patterns.

Cophylogeny studies can help to understand host-parasite interactions, coevolution and adaptation (Song et al. 2015; Filipiak et al. 2016). Until recently, cophylogeny of myxozoans and their hosts has not been studied (Holzer et al. In Prep.). Successful myxozoan coadaptation with highly diverged vertebrate and invertebrate hosts may reflect corresponding host-parasite co-evolutionary patterns. Likewise, the wide vertebrate host range of the *Sphaerospora s. s.* dataset presents itself as an optimal candidate to understand the co-evolutionary strategies of these groups.

3.4. Life cycle dilemma

Similar to all other myxozoans, sphaerosporids are believed to have an indirect life cycle, alternating between two hosts (Bartošová et al. 2013; Jirků & Bartošová-Sojtková 2014). In order to reveal the definitive invertebrate host and determine the complete life cycle of *Sphaerospora* species, various experimental trials were performed. Transmission of spores in kidney homogenates of common carp infected with *S. dykova* to *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta) resulted in the production of neoactinomyxum-type spores with 37% prevalence in the invertebrate host (Molnár et al. 1999b). In comparison with natural infection levels in oligochaetes (e.g. Holzer et al. 2004, Rangel et al. 2016, Zhao et al. 2016), this prevalence is extraordinary high and seems to confirm the experimental outcome. However, Eszterbauer et al. (2006) sequenced a neoactinomyxum-type actinospore of identical dimensions from the same oligochaete species, which showed molecular similarity to *Thelohanellus* spp. and not *Sphaerospora s. s.* (~20% 18S rDNA dissimilarity). Morphologically extremely similar actinosporeans may represent distantly related myxozoan genera (Eszterbauer & Székely 2004) or, vice versa (Eszterbauer et al. 2006), hence, molecular proof for the *S. dykova* infection experiment is urgently required to proof/disproof this life cycle. Echinactinomyxon stages from *Lumbriculus variegatus* and *Tubifex tubifex* (Müller, 1774) (Oligochaeta) were believed to represent alternate spore stages of *S. truttae*, as proven by experimental infection (Özer & Wootten 2000). This report was later proven wrong

based on 18S DNA sequence analyses (Holzer et al. 2004). Recently, tetractinomyxon stages from *Capitella* sp. (Polychaete) were found to belong to *S. dicentrarchi* (Rangel et al. 2016). However, *S. dicentrarchi* is not a member of *Sphaerospora* s. s. (see section 3.2) (Kent et al. 2001; Bartošová et al. 2009). Based on the knowledge obtained from molecular data, it is now evident that a definitive invertebrate host for *Sphaerospora* s. s. is yet to be determined.

3.5. Extrasporogonic proliferation in fish blood

Using *in situ* hybridisation, the development within the fish host was extensively studied for *S. truttae* which confirmed the gills as the portals of entry of sphaerosporids into fish (Holzer et al. 2003). Proliferative blood stages were found only three days after infection, while sporogonic stages were found in the kidney tubules 25 days after initial infection and the appearance of mature spores took another 15 days after the detection of early sporogonic stages. In contrast to many myxozoans that are known to use the blood stream for transport to the target organ (Johnson 1980; Kent & Hedrick 1985; Moran et al. 1999a; b; Bjork & Bartholomew 2010; Holzer et al. 2013: Paper I), extrasporogonic stages of various *Sphaerospora* s. s. actually proliferate in the blood being a hallmark of this parasite clade (Baska & Molnár 1988; Lom et al. 1983b; 1991a; Supamattaya et al. 1993, Holzer et al. 2014). In *S. dykova*, small blood stages initially contain only one secondary cell inside the primary cell. Division of the secondary cell ultimately generates eight secondary cells, which later contain one tertiary cell each (Lom & Dyková 1992). Thereafter the primary cell disintegrates and releases all secondary cells, which hence become primary cells again and the proliferative cycle continues. In *S. truttae*, up to 120 secondary cells can be found (Holzer et al. 2003). Blood stages can reach all organs with the blood flow. Within the rete mirabile of the swim bladder of carp, blood stages of *S. dykova* obstruct fine blood vessels, become histozoic and start further proliferation, creating a strong host cell response resulting in SBI. Other areas with small blood vessels such as the kidney and choroidal rete mirabile can accumulate blood stages causing inflammatory responses (Lom et al. 1991; Molnár 1993b). Eventually, after reaching the kidney, sphaerosporids penetrate the epithelial cells of the renal tubules and start further proliferation and sporogony in the intratubular space, with spores being released via the urine (Holzer et al. 2003; Lom et al. 1991; Molnár et al. 1993b).

Motile sphaerosporid blood stages were first described as 'Unidentified Blood Objects' (UBOs) (Csaba 1976) in carp without knowing that they belonged to the Myxozoa. A little later, morphologically similar stages from the intratubular space in the renal tubule (Molnár 1980) and histozoic multicellular 'K-stages' within the swim bladder causing SBI in common carp (Körting 1982; Kovács-Gayer et al. 1982) were described. The link between C-, K- and intratubular stages was made based on their similar morphological characteristics and development, their simultaneous occurrence in infected fish and based on experimental trials focusing on the transmission of C- and K-stages from infected fish to SPF receptor fish. Until recently, these stages were related to *S. dykova*, however, recent molecular

methods have demonstrated that *S. molnari* is the predominant species in carp blood and an important co-factor for *S. dykova*-based SBI (Holzer et al. 2014: Paper III). Mixed infections of these two species are the rule (A. S. Holzer pers. comm.). Successful experimental transmission of sphaerosporid blood stages via intraperitoneal injection into uninfected receptor fish has been reported for several species (McGeorge et al. 1994; Csaba et al. 1984; Molnár & Kovács-Gayer 1986; Körting et al. 1989; Csaba 1976; Patra et al. 2014; Hartigan et al. 2015).

Recently, a comprehensive study was conducted to understand the unique motility of *S. molnari* and potentially other sphaerosporid (Baska & Molnár 1988; Lom et al. 1985) blood stages where the function of this movement was related to evasion from host immune cells (Hartigan et al. 2016b: Paper IV). In *S. molnari*, the primary cell membrane creates and rapidly reabsorbs folds that are used like rudders, moving the parasite around its own axis, making host cell attachment difficult (Hartigan et al. 2016b: Paper IV).

Chapter 4. Objectives of the research

On initiation of the present thesis, the two myxozoan clades of focus in this thesis, i.e. Malacosporea and *Sphaerospora s. s.* belonged to the most species deficient myxozoan lineages, due to the cryptic nature of their representatives and difficulties with obtaining molecular data for their characterisation. Only three malacosporean and 24 sphaerosporid taxa had been characterised molecularly. The main aims of the thesis were to improve the knowledge of these poorly characterized lineages by:

- Exploring their species diversity by morphological and molecular examination of fish hosts
- Determining phylogenetic relationships between newly sequenced members and relating the patterns of clustering to morphological, environmental and biological factors, including host-parasite coevolution
- Identifying valid taxonomic characters for myxozoan species descriptions to avoid misidentifications
- Performing experimental transmissions between vertebrate and invertebrate hosts and establishing life cycles in controlled laboratory systems
- Determining the etiological agents of myxozoan diseases using molecular methods
- Understanding the mechanisms and function of *S. molnari* blood stage motility

5.1. Published paper I

Holzer AS, Pecková H, **Patra S**, Brennan NP,
Yanes-Roca C, Main KL

Severe glomerular disease in juvenile grey snapper *Lutjanus griseus* L. in the Gulf of Mexico caused by the myxozoan *Sphaerospora motemarini* n. sp.

International Journal for Parasitology: Parasites and Wildlife (2013).
2:124–130.

doi: 10.1016/j.ijppaw.2013.03.003

This part is comprised of 7 pages published data, which is present in the original thesis deposited at the Faculty of Science, University of South Bohemia

Abstract

In the eastern Gulf of Mexico, off the coast of Florida, grey snapper, *Lutjanus griseus* was found to be infected with the myxozoan parasite *Sphaerospora motemarini* n. sp., with high prevalence (83%) and intensity of infection occurring in age-0 fish, and with parasite levels decreasing with age (age-1 snapper 40%; age-2 snapper 0%). The morphological, molecular and phylogenetic characterisation of the myxozoan showed that it is a member of the typically marine, polysporoplasmid *Sphaerospora* spp. which form a subclade within the *Sphaerospora sensu stricto* clade of myxozoans, which is characterised by large expansion segments in their SSU rDNA sequences. Presporogonic stages of *S. motemarini* n. sp. were detected in the blood, using PCR. Pseudoplasmodia and spores were found to develop in the renal corpuscles of the host, causing their massive expansion. Macroscopic and histopathological changes were observed in age-0 fish and show that *S. motemarini* n. sp. causes severe glomerulonephritis in *L. griseus* leading to a compromised host condition, which makes it more susceptible to stress (catch-and-release, predators, water quality) and can result in mortalities. These results are discussed in relation to the exploitation of grey snapper populations by commercial and recreational fisheries and with the observed increased mortalities with temperature along the coast of Florida. In the future, we would like to determine prevalence and intensity of infection with *S. motemarini* n. sp. in juvenile *L. griseus* in different areas of the Gulf of Mexico in order to be able to estimate the temperature dependence of *S. motemarini* n. sp. proliferation and to be able to predict its distribution and severity during climatic changes in the Gulf.

5.2. Published paper II

II

Bartošová-Sojková P, Hrabcová M, Pecková H, **Patra S**,
Kodádková A, Jurajda P, Tymi T, Holzer AS

Hidden diversity and evolutionary trends in malacosporean
parasites (Cnidaria: Myxozoa) identified using
molecular phylogenetics.

International Journal for Parasitology (2014). 44:565–577.

doi: 10.1016/j.ijpara.2014.04.005

**This part is comprised of 28 pages published data, which is present in
the original thesis deposited at the Faculty of Science,
University of South Bohemia**

Abstract

Malacosporeans represent a small fraction of myxozoan biodiversity with only two genera and three species described. They cycle between bryozoans and freshwater fish. In this study, we (i) microscopically examine and screen different freshwater/marine fish species from various geographic locations and habitats for the presence of malacosporeans using PCR; (ii) study the morphology, prevalence, host species/habitat preference and distribution of malacosporeans; (iii) perform small subunit/large subunit rDNA and Elongation factor 2 based phylogenetic analyses of newly gathered data, together with all available malacosporean data in GenBank; and (iv) investigate the evolutionary trends of malacosporeans by mapping the morphology of bryozoan-related stages, host species, habitat and geographic data on the small subunit rDNA-based phylogenetic tree. We reveal a high prevalence and diversity of malacosporeans in several fish hosts in European freshwater habitats by adding five new species of *Buddenbrockia* and *Tetracapsuloides* from cyprinid and perciform fishes. Comprehensive phylogenetic analyses revealed that, apart from *Buddenbrockia* and *Tetracapsuloides* clades, a novel malacosporean lineage (likely a new genus) exists. The fish host species spectrum was extended for *Buddenbrockia plumatellae* and *Buddenbrockia* sp. 2. Co-infections of up to three malacosporean species were found in individual fish. The significant increase in malacosporean species richness revealed in the present study points to a hidden biodiversity in this parasite group. This is most probably due to the cryptic nature of malacosporean sporogonic and presporogonic stages and mostly asymptomatic infections in the fish hosts. The potential existence of malacosporean life cycles in the marine environment as well as the evolution of worm- and sac-like morphology is discussed. This study improves the understanding of the biodiversity, prevalence, distribution, habitat and host preference of malacosporeans and unveils their evolutionary trends.

5.3. Published paper III

III

Holzer AS, Hartigan A, **Patra S**,
Pecková H, Eszterbauer E

Molecular fingerprinting of the myxozoan community in
common carp suffering Swim Bladder Inflammation (SBI)
identifies multiple etiological agents.

Parasites & Vectors (2014). 7:398.

doi: 10.1186/1756-3305-7-398

**This part is comprised of 9 pages published data, which is present in
the original thesis deposited at the Faculty of Science,
University of South Bohemia**

Background:

Swim bladder inflammation (SBI) is an important disease of common carp fingerlings in Central Europe. In the 1980s, its etiology was ascribed to multicellular proliferative stages of the myxozoan parasite *Sphaerospora dykova* (formerly *S. renicola*). *S. dykova* was reported to proliferate in the blood and in the swim bladder prior to the invasion of the kidney, where sporogony takes place. Due to the presence of emerging numbers of proliferative myxozoan blood stages at different carp culture sites in recent years we analysed cases of SBI, for the first time, using molecular diagnostics, to identify the myxozoan parasites present in diseased swim bladders.

Methods:

We amplified myxozoan SSU rDNA in a non-specific approach and compared the species composition in swim bladders at culture sites where carp demonstrated 1. No signs of SBI, 2. Minor pathological changes, and 3. Heavy SBI. Based on DNA sequences, we determined the localisation and distribution of the most frequent species by in situ hybridisation, thereby determining which myxozoans are involved in SBI.

Results:

Large multicellular myxozoan swim bladder stages characterised heavy SBI cases and were identified as *S. dykova*, however, blood stages were predominantly represented by *Sphaerospora molnari*, whose numbers were greatly increased in carp with mild and heavy SBI, compared with SBI-free fish. *S. molnari* was found to invade different organs and cause inflammatory changes also in the absence of *S. dykova*. One site with mild SBI cases was characterised by *Buddenbrockia* sp. infection in different organs and a general granulomatous response.

Conclusions:

We provide evidence that the etiology of SBI can vary in relation to culture site and disease severity and that emerging numbers of *S. molnari* in the blood represent an important co-factor or precondition for SBI.

5.4. Published paper IV

IV

Hartigan A, Estensoro I, Vancová M, Bílý T, **Patra S**,
Eszterbauer E, Holzer AS

New cell motility model observed in parasitic cnidarian
Sphaerospora molnari (Myxozoa: Myxosporea)
blood stages in fish.

Scientific Reports (2016). 6:39093.

doi: 10.1038/srep39093.

**This part is comprised of 27 pages published data, which is present in
the original thesis deposited at the Faculty of Science,
University of South Bohemia**

Abstract

Cellular motility is essential for microscopic parasites, it is used to reach the host, migrate through tissues, or evade host immune reactions. Many cells employ an evolutionary conserved motor protein- actin, to crawl or glide along a substrate. We describe the peculiar movement of *Sphaerospora molnari*, a myxozoan parasite with proliferating blood stages in its host, common carp. Myxozoa are highly adapted parasitic cnidarians alternately infecting vertebrates and invertebrates. *S. molnari* blood stages (SMBS) have developed a unique "dancing" behaviour, using the external membrane as a motility effector to rotate and move the cell. SMBS movement is exceptionally fast compared to other myxozoans, non-directional and constant. The movement is based on two cytoplasmic actins that are highly divergent from those of other metazoans. We produced a specific polyclonal actin antibody for the staining and immunolabelling of *S. molnari*'s microfilaments since we found that neither commercial antibodies nor phalloidin recognised the protein or microfilaments. We show the *in situ* localization of this actin in the parasite and discuss the importance of this motility for evasion from the cellular host immune response *in vitro*. This new type of motility holds key insights into the evolution of cellular motility and associated proteins.

5.5. Published paper V

V

Patra S, Hartigan A, Morris DJ, Kodádková A, Holzer AS

Description and experimental transmission of *Tetracapsuloides vermiformis* n. sp. (Cnidaria: Myxozoa) and guidelines for describing malacosporean species including reinstatement of *Buddenbrockia bryozoides* n. comb. (syn. *Tetracapsula bryozoides*).

Parasitology (2017). 144:497-511.

DOI: 10.1017/S0031182016001931.

This part is comprised of 35 pages published data, which is present in the original thesis deposited at the Faculty of Science, University of South Bohemia

Abstract

This paper provides the first detailed description of a *Tetracapsuloides* species, *Tetracapsuloides vermiformis* n. sp., with vermiform stages in the bryozoan host, *Fredericella sultana*, and its experimental transmission from *F. sultana* to *Cyprinus carpio*. The suitability of morphological, biological and 18S rDNA sequence data for discrimination between malacosporean species is reviewed and recommendations are given for future descriptions. Presently, malacosporean species cannot be differentiated morphologically due to their cryptic nature and the lack of differential characters of spores and spore-forming stages in both hosts. We examined biological, morphological and molecular characters for the present description and for revising malacosporean taxonomy in general. As a result, *Buddenbrockia plumatellae* was split into two species, with its sac-like stages being ascribed to *Buddenbrockia bryzoides* n. comb. In addition to ribosomal DNA sequences multiple biological features rather than morphological characters are considered essential tools to improve malacosporean taxonomy in the future according to our analysis of the limited traits presently available.

5.6. Unpublished paper VI

VI

**Patra S, Bartošová-Sojková P, Pecková H, Fiala I, Eszterbauer E,
Holzer AS**

Biodiversity and host-parasite cophylogeny of *Sphaerospora*
sensu stricto (Cnidaria: Myxozoa)

(Manuscript in advanced preparation)

**This part is comprised of 40 pages unpublished data, which is present in
the original thesis deposited at the Faculty of Science,
University of South Bohemia**

Abstract

Sphaerospora sensu stricto clade is the most basal myxosporean clade within the Myxozoa (Cnidaria) that groups the parasites of predominantly urinary system of marine and freshwater fishes and amphibians. Sphaerosporids are unique by extremely long insertions in the variable regions of their 18S and 28S rDNA and by formation of motile proliferative stages in hosts' blood. In present study, we microscopically and molecularly examined over 530 fish kidney samples from various geographic locations, mainly in Central Europe, to investigate the sphaerosporid biodiversity. Moreover, we performed the comprehensive phylogenetic analyses of newly gathered and existing 18S rDNA data of *Sphaerospora* spp. to explore the evolutionary trends within the *Sphaerospora sensu stricto* clade. We also implemented different cophylogenetic analyses of sphaerosporids and their vertebrate hosts to understand the host-parasite coevolution strategies. We revealed a high diversity of *Sphaerospora* spp. mainly in freshwater habitats by amplifying thirteen new sphaerosporids 18S rDNA sequences from which one belongs to the presently re-described *Sphaerospora diminuta*. The remaining half of sequences, accompanied by morphological data, represents new *Sphaerospora* spp. Sphaerosporids cluster within their clade according to vertebrate host type, host family and habitat. Cophylogenetic analyses revealed highly significant congruence between the evolutionary history of sphaerosporids and their vertebrate hosts with cyprinids representing a host group of multiple parasite lineages and frequent host switching. This study significantly improved our understanding of the biodiversity, habitat and host preference of sphaerosporids and unveiled interesting aspects of host-parasite co-evolution.

Chapter 6. Summary and general discussion

The most important outcomes from my Ph.D. thesis are:

- 1) Revealing the hidden biodiversity, cryptic species nature and phylogenetic relationships of the poorly characterized but highly diverse myxozoan clades Malacosporea and *Sphaerospora s. s.* in Central Europe
- 2) Cophylogeny of sphaerosporids and their vertebrate hosts in the light of recent evolutionary discoveries
- 3) Revision of taxonomy based on a synthesis of biological and molecular phylogenetic data
- 4) Elucidation of myxozoan life cycles under controlled experimental conditions
- 5) Discovery of the hidden etiology of swim bladder inflammation in common carp using *in situ* hybridisation
- 6) Discovery of a new type of cellular motility in eukaryotes in blood stages of *Sphaerospora molnari* and its contribution to host immune cell evasion

6.1. Revealing the hidden biodiversity, cryptic species nature and phylogenetic relationships of the poorly characterized but highly diverse myxozoan clades Malacosporea and *Sphaerospora s. s.* in Central Europe

At the beginning of my thesis, only three nominal species of the Malacosporea were described along with the additional three further undescribed species. However, malacosporean biodiversity had been predicted much higher (Massard & Geimer 2008), an estimate that was first confirmed when Hartikainen et al. (2014) discovered five new species from bryozoan hosts. Subsequently, we discovered other five new malacosporean species after screening over 500 fish, predominantly from Central Europe (Bartošová-Sojtková et al. 2014: Paper II), by molecular methods. In most cases, infections were not detected by microscopy or cryptic sporogonic or presporogonic stages were detected in an early sporogonic stage (no mature spores). Morphological similarity of pseudoplamodia of malacosporeans and sphaerosporids or species of *Hoferellus* Berg, 1898 and *Ortholinea* Shul'man, 1962 causes difficulties for species identification and discrimination.

In bryozoan hosts, early covert unicellular stages are impossible to identify under the stereomicroscope (Tops et al. 2009; Hartikainen & Okamura 2012). DNA sequencing and comprehensive 18S rDNA-based phylogenetic analyses with all available malacosporean species revealed phylogenetic clustering of the taxa in five distinct clades including *Buddenbrockia*, *Tetracapsuloides* and three novel lineages

(Fiala et al. 2015b; Patra et al. 2017: Paper V). A comparative analysis of morphological features for comparison and identification of malacosporean species in either of their hosts showed that spores are virtually indistinguishable between species or even genera, and that identical plasmodial morphotypes evolved more than once in the Malacosporea. As a consequence, 18S rDNA sequence data are an absolute necessity for the description of new species and only DNA-based methods allow for the elucidation of the true malacosporean diversity (Patra et al. 2017: Paper V).

Another problem had previously impeded phylogenetic characterisation of members of *Sphaerospora s. s.* This phylogenetic clade was relatively recently established, with 18S rDNA sequences of only four members available at that time (Jirků et al. 2007), and it was enriched by studies, mainly from our laboratory, to a total of 32 taxa to date after excluding *sensu lato* members (Bartošová et al. 2013; Eszterbauer et al. 2013; Holzer et al. 2013a; b: Paper I; Sanders et al. 2015; Patra et al. In Prep.: Paper VI). This included an enormous effort regarding amplification of their sequences and subsequent taxonomic reassignments. Sphaerosporid proliferative stages found amongst host blood cells and spore-forming stages are small, mostly disporous, embedded in the renal tubules. Physical isolation of the parasite from hosts' tissue is very difficult (Holzer et al. 2003). This situation is further complicated by difficulties related to molecular isolation. Members of *Sphaerospora s. s.* possess some of the longest insertions in the variable regions of 18S rDNA amongst all eukaryotic organisms (Holzer et al. 2007; Bartošová et al. 2013; Eszterbauer et al. 2013). Amplification of complete 18S rDNA sequences with general eukaryotic or myxozoan-specific 18S rDNA primer combinations generally fail due to unique species-specific insertions and preferential amplification of shorter fragments from DNA of other myxozoans often present in coinfections (Bartošová et al. 2013). To overcome this situation, we progressively designed sets of primers based on newly acquired 18S rDNA of sphaerosporids. Our results characterize *Sphaerospora s. s.* as a monophyletic clade based on 18S rDNA which was supported by 28S rDNA and EF2 sequence data and shine light on the factors affecting clustering of its representatives (Bartošová et al. 2013; Holzer et al. 2013b: Paper I; Patra et al. In Prep.: Paper VI). Apart from phylogenetic and evolutionary studies, new sequences may also aid the future discovery of the still unconfirmed definitive host of this myxozoan clade by possible sequence match(es) of existing *Sphaerospora* sequences with sequence data of actinosporean stages obtained from invertebrate hosts.

Regarding both, malacosporeans and sphaerosporids, Central Europe has proven to be a biodiversity hotspot (Lom et al. 1985; Baska & Molnár 1988; Bartošová-Sojtková et al. 2014: Paper II), where they as they are common in cyprinid vertebrate hosts. However, to be less host biased, future studies could employ a host independent approach, for example screening and analyses of environmental DNA (eDNA) from water and sediment samples, a method with a large potential for an efficient exploration of the biodiversity of aquatic species (Biggs et al. 2015; Bohmann et al. 2014; Deiner & Altermatt 2014; Laramie et al. 2015; Stewart et al. 2017). An initial study in myxozoans showed that screening of environmental

samples and faeces of piscivorous animals is more than promising for estimating the unknown myxozoan biodiversity (Bass et al. 2015; Hartikainen et al. 2016), as water contains all transmitting actinosporean and myxosporean stages, also those of rare and so far undescribed species. However, considering the aberrant 18S rDNA sequences of sphaerosporids, detection assays will have to be carefully designed.

The present thesis has greatly improved our knowledge of taxa belonging to the Malacosporea and *Sphaerospora s. s.* This is of particular importance as Malacosporea is the most basal lineage of the myxozoans, which possesses various unique morphologies (radial symmetry, spore forming motile vermiform stages etc.), recalling towards ancestral cnidarian origin (Jiménez-Guri et al. 2007a). This lineage is considered to be evolutionary older and diverged before the rest of the myxozoan lineages evolved (Anderson et al. 1999; Kodádková et al. 2015). *Sphaerospora s. s.* as a sister clade to either of the two remaining myxosporean lineages or basal to all myxosporeans is also unique due to the development of its members (extrasporogonic stage) and long 18S rDNA inserts reflecting evolutionary patterns that differ from the rest of the myxozoans. Studying these two lineages can enrich our knowledge into the evolution, including origins and divergence of myxozoans from their free-living cnidarian counterparts.

6.2. Cophylogeny of sphaerosporids and their vertebrate hosts in the light of recent evolutionary discoveries

Host-parasite cophylogeny studies have proven of enormous importance for reconstructing the common history of host-parasite taxa and to understand primeval relationships (Baudet et al. 2015). Recently, the evolution and congruence of phylogenetic trees of the Myxozoa and both, their invertebrate and vertebrate hosts was studied for the very first time, including also a comprehensive molecular clock analyses for the timing of events (Holzer et al. In Prep.). Due to the distant relationship of the vertebrate host dataset and the minimal number of taxa available (19 parasites, 19 hosts) in this previous study global fit between the phylogenies of *Sphaerospora s. s.* and their vertebrate hosts was not detected, despite significant congruence estimated by tree-based methods. After combining an additional 13-sphaerosporid 18S rDNA sequences and 11 hosts to the dataset, highly significant congruence was detected by all cophylogenetic methods (Patra et al. In Prep.: Paper VI). Since sphaerosporids are likely evolutionary older than the rest of the annelid-infecting myxozoan lineages, and based on the assumption that myxozoans first used invertebrate hosts only (Holzer et al. In Prep.), archiannelids (polychaetes belonging to the Haplodrilii) or sipunculids present themselves as excellent candidates for the first hosts of sphaerosporids. It would also support the idea of a marine origin of sphaerosporids, whose most basal clade is represented by sequences exclusively from marine teleosts (Bartošová et al. 2013, Patra et al. In Prep.: Paper VI). Ikeda (1912) detected two different morphotypes of actinosporeans in *Petalostoma minutum* (Keferstein, 1862) (= *Nephasoma minuta*) off the UK coast. It would be extremely interesting to obtain 18S rDNA sequences from myxozoans in sipunculids to prove this hypothesis. Additionally, rDNA sequences of sphaerosporids in

evolutionary older, cartilaginous fishes would add another piece to the puzzle of the origins of sphaerosporids. The only described species is *Sphaerospora araii* Arthur et Lom, 1985 from *Raja rhina* Jordan et Gilbert, 1880 but other species may well exist in modern sharks, rays and chimaeras, potentially representing ‘old sphaerosporids’ in ‘old hosts’, similar as the most basal lineages in all other annelid-infecting (polychaete and oligochaete-infecting) myxozoan clades (Gleeson & Adlard 2011; 2012; Kodádková et al. 2015). In future, extensive screening of various ‘old’ vertebrate and invertebrate hosts may be able provide exciting proof for these hypotheses on the early history of sphaerosporid evolution.

6.3. Revision of taxonomy based on a synthesis of biological and molecular phylogenetic data

Since the early discoveries of myxozoans in their fish hosts, spore morphology was the main criterion used for myxozoan descriptions and classification (Kudo 1933; Tripathi 1948; Shul’man 1966). Integration of other basic biological data such as host group/species, habitat, host tissue specificity or characteristics of developmental stages are now additional requirements for taxonomic descriptions (Lom & Arthur 1989).

While morphological descriptions were previously the only means of distinguishing between different myxozoan species, we have now acquired a large database of 18S rDNA GenBank sequences. This helped us to discover ‘true’ relationships and to understand biological features that reflect the patterns of phylogenetic clustering (see details in Chapter 1.2). As these patterns are of special importance for the evolution of the Myxozoa they are hence likely good features for systematics. We have to be aware of the fact that spores are ecological morphotypes, since their shape is likely determined by the optimisation of transmission strategies and dispersion in the environment (Fiala & Bartošová 2010; Fiala et al. 2015b). This may explain why spore morphology contradicts molecular phylogeny to a great degree (Holzer et al. 2004; Fiala 2006; Bartošová & Fiala 2011; Rocha et al. 2013; Karlsbakk et al. 2017) while a number of other characters are important for the design of a new, more ‘accurate’ taxonomic system of the Myxozoa. Only the combination and careful evaluation of morphological and ultrastructural cell development data from both hosts, alongside host characteristics and molecular phylogeny allows for a holistic approach and an accurate design of guidelines for myxozoan descriptions (for malacosporeans see Patra et al. 2017: Paper V).

6.4. Elucidation of myxozoan life cycles under controlled experimental conditions

Myxozoans are known to have indirect two-host life cycles. A total of 53 myxozoan life cycles are described, with about three quarters being molecularly confirmed (section 1.3). Only one complete life cycle is known from malacosporeans (*T. bryosalmonae*) (Feist et al. 2001; Morris & Adams 2006b) but two further are

partially confirmed (Grabner & El-Matbouli 2010a; Patra et al. 2017: Paper V). The definitive invertebrate host group of *Sphaerospora* s. s. remains unconfirmed until alternate spore stages are sequenced. The poor knowledge of life cycles in these two phylogenetic clades prompted us to screen aquatic invertebrates and perform infection experiments (Patra et al. 2013; Patra et al. 2014; Patra et al. 2017: Paper V).

Over the last four years, we PCR screened over 576 aquatic invertebrates from different *S. molnari* and *S. dykova*e enzooic ponds in the Czech Republic and Hungary, but did not find any infected hosts. Water samples from a number of ponds confirmed the presence of *S. molnari* infective spores in the water column, at several sites, but PCR detection was limited to the month of May and June. The narrow window of release may explain why *Sphaerospora* s. s.-infected invertebrates are so difficult to find. Finally, we decided to perform a less targeted but more controlled laboratory experiment by exposing invertebrate-rich sediments to SPF carp, in isolated aquaria. Oligochaete-rich muddy sediments did not result in infection of fish with malacosporeans or sphaerosporids but cohabitation of fish with invertebrate fauna on twigs and stones resulted in infection with *S. molnari* (100% prevalence, Patra et al. 2013; 2014) and *T. vermiformis* (Patra et al. 2017: Paper V), hence narrowing down the microhabitat of invertebrate hosts. We later found vermiform stages of *T. vermiformis* in the bryozoan host *F. sultana*, resulting in successful transmission of the fourth malacosporean species to date (Patra et al. 2017: Paper V).

Mixed infections in invertebrate and vertebrate hosts are a major problem when aiming to provide experimental proof of myxozoan life cycles. These issues have led to confusion and misidentifications in the past, that were only discovered after DNA sequencing of these wrongly ascribed life cycle stages (e.g. Holzer et al. 2004; Eszterbauer et al. 2006). Controlled experimental conditions in isolated aquaria systems and the use of SPF hosts (vertebrate and invertebrate cultures) prevent contamination and are of great advantage for studying the developmental cycles and pathological changes induced by individual species in the absence of other myxozoans and other aquatic parasites (e.g. common ectoparasites). It is essential to elucidate more myxozoan life cycles, especially from phylogenetic groups where little such information is available. It is furthermore important to go beyond relating a morphotype from a vertebrate host to one from the invertebrate host and to investigate the biological differences in myxozoan development between members of different evolutionary clades or between pathological vs. non-pathological species. As in our study on *T. vermiformis*, it is of importance to collect a multitude of data, from light microscopy to ultrastructure and molecular information, hence providing more comprehensive data on different parasite stages as these are required for a more holistic understanding of myxozoan life cycles and development.

6.5. Discovery of the hidden etiology of swim bladder inflammation in common carp using *in situ* hybridisation

In Central Europe, SBI causes up to 100% mortality in fingerlings of common carp (Waluga & Budzynska 1980). In the 1980s, *S. dykova*e was believed to be the

causative agent of the disease due to the morphological similarity of parasite developmental stages in the blood, the swim bladder and the renal tubules (Molnár & Kovács-Gayer 1986). Based on our recent finding of emerging numbers of sphaerosporid blood stages in European carp ponds (Holzer et al. 2014: Paper III) and their molecular identification as *S. molnari* (Eszterbauer et al. 2013), we decided to reinvestigate the myxozoan species involved in SBI, using molecular methods (PCR and *in situ* hybridisation). Large multicellular swim bladder stages, which characterise heavy SBI cases, were identified as *S. dykova*. However, blood stages were predominantly represented by *S. molnari*, whose numbers were greatly increased in carp with mild and heavy SBI, compared with SBI-free fish (Holzer et al. 2014: Paper III). *S. molnari* was found to invade different organs and cause inflammatory changes also in the absence of *S. dykova*. One site with mild SBI cases was characterised by unknown *Buddenbrockia* sp. infection in different organs and a general granulomatous response. We provided evidence that the etiology of SBI can vary in relation to culture site and disease severity and that emerging numbers of *S. molnari* in the blood represent an important co-factor or precondition for SBI (Holzer et al. 2014: Paper III). Future studies should aim at determining changes in the number of blood stages in relation to temperature. Water temperatures in European freshwater habitats have increased by 1–3°C over the last century (EEA 2012). Proliferation at high temperatures is expected to happen faster, hence potentially linking the observation of higher blood stage numbers in carp to climate change in Central Europe. Design of a quantitative PCR assay and monitoring of infected fish over time could provide substantial data for estimating changes along with future temperature predictions. The same molecular tools could elucidate these changes also for commonly occurring mixed *S. molnari*/*S. dykova* infections.

6.6. Discovery of a new type of cellular motility in eukaryotes in blood stages of *Sphaerospora molnari* and its contribution to host immune cell evasion

Motility is one of the most common features of living organisms. Different types of motility have been reported in the Myxozoa and while mechanisms differ considerably, they are all based on adaptations of the same basic tool: the actomyosin machinery (Hartigan et al. 2016b: Paper IV). The evolutionary older malacosporeans develop vermiform stages inside bryozoan hosts that exhibit true obliquely orientated tetra-radial muscle blocks (Jiménez-Guri 2007a). These cause active, random, twisting movements or rhythmic sinusoidal or spiralling movements within the bryozoan body cavity, aiming at release of the myxoworms from the host body (Okamura et al. 2002; Canning et al. 2002; 2008; Jiménez-Guri et al. 2007b; Gruhl & Okamura 2012; Patra et al. 2017: Paper V). All other myxozoans lost true muscle cells during the evolution but adopted other types of cellular motility. Amoeboid movement has commonly been described in myxozoans, during active host invasion (El-Matbouli et al. 1995a; Eszterbauer et al. 2009), in myxozoans living in body liquids such as the bile, e.g. *Ceratomyxa puntazzi* Alama-Bermejo, Raga et al. Holzer, 2011, that need to stay afloat (Alama-Bermejo et al. 2012) and, in kidney tubules, e.g. pseudoplasmodia of *Sphaerospora diminuta* Li et al. Dessler, 1985 (Li &

Desser 1985; Lom et al. 1989b). Moreover, a sinusoid movement was observed in the spore valve cells of *Fabespora vermicola* Overstreet, 1976 most probably to release from host tissue (Weidner and Overstreet 1979). A different type of motility is the nematode-like movement of the worm-shaped plasmodia of *Ceratomyxa vermiformis* Adriano et Okamura, 2017 in the bile of *Colossoma macropomum* (Cuvier, 1816) (Adriano & Okamura 2017) which likely promotes exit of the whole plasmodium (rather than liberated spores) into the gut via the common bile duct.

While all these types of motilities find similarities in that of other unicellular or multicellular organisms, the fast, continuous and non-directional twitching and “dancing” motility of *S. molnari* blood stages in common carp blood is unique amongst eukaryotes (Hartigan et al. 2016b: Paper IV). The motility, promoted by a rapid formation and reabsorption of membrane folds of the primary cell that propel the parasites around their axis, was coined Membrane Fold Induced Tumbling (MFIT) (Hartigan et al. 2016b: Paper IV). Interestingly, not only the relevant motor protein (highly derived actin in *S. molnari*) but also the function of MFIT differs from other organisms by being non-directional and evasive to host-cell attachment. This may cause limited initial recognition of the parasite and subsequently limited activation of other host defense mechanisms that are necessary for innate or adaptive immune responses. For a better understanding of the interaction of *S. molnari* with the host’s immune cells, further immune assays, such as with serum peroxidases, degranulation assays, respiratory burst activity, measuring of reactive oxygen species and nitric oxide production, as well as gene expression analysis of immune-related genes in carp via RNA-seq should be performed. Apart from these innate immune parameters, it would be interesting to study IgM and IgT expression in infected carp to determine acquired specific immunity to *S. molnari*, providing some protection to re-infection as observed in other myxozoans (Gorgolione et al. 2013; Bailey et al. 2017; Alvarez-Pellitero 2008; Alvarez-Pellitero et al. 2008).

References:

- Abd-Elfattah A, Fontes I, Kumar G, Soliman H, Hartikainen H, Okamura B, El-Matbouli M (2014) Vertical transmission of *Tetracapsuloides bryosalmonae* (Myxozoa), the causative agent of salmonid proliferative kidney disease. *Parasitology* 141:482–490
- Adriano EA, Okamura B (2017) Motility, morphology and phylogeny of the plasmodial worm, *Ceratomyxa vermiformis* n. sp (Cnidaria: Myxozoa: Myxosporea). *Parasitology* 144:158–168
- Aguar JC, Adriano EA, Mathews PD (2017) Morphology and molecular phylogeny of a new *Myxidium* species (Cnidaria: Myxosporea) infecting the farmed turtle *Podocnemis expansa* (Testudines: Podocnemididae) in the Brazilian Amazon. *Parasitol Int* 66:825–830
- Alama-Bermejo G, Raga JA, Holzer AS (2011) Host-parasite relationship of *Ceratomyxa puntazzi* n. sp (Myxozoa: Myxosporea) and sharpnose seabream *Diplodus puntazzo* (Walbaum, 1792) from the Mediterranean with first data on ceratomyxid host specificity in sparids. *Vet Parasitol* 182:181–192
- Alama-Bermejo G, Bron JE, Antonio Raga J, Holzer AS (2012) 3D morphology, ultrastructure and development of *Ceratomyxa puntazzi* stages: first insights into the mechanisms of motility and budding in the Myxozoa. *PLoS One* 7
- Al-Samman A, Molnár K, Székely C, Reiczigel J (2003) Reno-, hepato- and splenomegaly of common carp fingerlings (*Cyprinus carpio* L.) diseased in swimbladder inflammation caused by *Sphaerospora renicola* Dyková, et Lom, 1982. *Acta Vet Hung* 51:321–329

- Alvarez-Pellitero P (2008) Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. *Vet Immunol Immunopathol* 126:171–198
- Alvarez-Pellitero P, Palenzuela O, Sitjà-Bobadilla A (2008) Histopathology and cellular response in *Enteromyxum leei* (Myxozoa) infections of *Diplodus puntazzo* (Teleostei). *Parasitol Int* 57:110–120
- Amandi A, Holt RA, Fryer JL (1985) Observations on *Myxobolus insidiosus* (Myxozoa: Myxosporea) a parasite of salmonid fishes. *Fish Pathology* 20:287–304
- Anderson CL, Canning EU, Okamura B (1998) A triploblast origin for Myxozoa? *Nature* 392:346–347
- Anderson CL, Canning EU, Okamura B (1999) Molecular data implicate bryozoans as hosts for PKX (Phylum Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. *Parasitology* 119:555–561
- Anderson CL, Canning EU, Schafer SM, Yokoyama H, Okamura B (2000) Molecular confirmation of the life cycle of *Thelohanellus hovorkai* Achmerov, 1960 (Myxozoa: Myxosporea). *Bull Eur Assoc Fish Pathol* 20:111–115
- Andree KB, El-Matbouli M, Hoffman RW, Hedrick RP (1999) Comparison of 18S and ITS-1 rDNA sequences of selected geographic isolates of *Myxobolus cerebralis*. *Int J Parasitol* 29:771–775
- Arthur JR, Lom J (1985) *Sphaerospora araii* n. sp. (Myxosporea: Sphaerosporidae) from the kidney of a longnose skate (*Raja rhina* Jordan and Gilbert) from the Pacific Ocean off Canada. *Can J Zool* 63:2902–2906
- Atkinson SD, Hallett SL, Bartholomew JL (2007) The life cycle of *Chloromyxum auratum* (Myxozoa) from goldfish, *Carassius auratus* (L.), involves an antonactinomyxon actinospore. *J Fish Dis* 30:149–156
- Atkinson SD, Bartholomew JL (2009) Alternate spore stages of *Myxobilatus gasterostei*, a myxosporean parasite of three-spined sticklebacks (*Gasterosteus aculeatus*) and oligochaetes (*Nais communis*). *Parasitol Res* 104:1173–1181
- Atkinson SD, Bartošová-Sojtková P, Whipps CM, Bartholomew JL (2015) Approaches for Characterising Myxozoan Species. In: Okamura B, Gruhl A, Bartholomew JL (eds) *Myxozoan Evolution, Ecology and Development*. Springer International Publishing, Cham, p 111–123
- Azevedo C, Corral L, Matos E (1997) Light and ultrastructural data on *Henneguya testicularis* n. sp. (Myxozoa, Myxobolidae), a parasite from the testis of the amazonian fish *Moenkhausia oligolepis*. *Syst Parasitol* 37:111–114
- Azizi R, Rangel LF, Castro R, Santos MJ, Bahri S (2016) Morphology, seasonality and phylogeny of *Zschokkella trachini* n. sp (Myxozoa, Myxosporea) infecting the gallbladder of greater weever *Trachinus draco* (L.) from Tunisian waters. *Parasitol Res* 115:4129–4138
- Baerwald MR (2013) Temporal expression patterns of rainbow trout immune-related genes in response to *Myxobolus cerebralis* exposure. *Fish Shellfish Immun* 35:965–971
- Bailey C, Segner H, Wahli T (2017) What goes around comes around: an investigation of resistance to proliferative kidney disease in rainbow trout *Oncorhynchus mykiss* (Walbaum) following experimental re-exposure. *J Fish Dis* (ahead of print)
- Barta JR, Martin DS, Liberator PA, Dashkevich M, Anderson JW, Feighner SD, Elbrecht A, Perkins-Barrow A, Jenkins MC, Danforth HD, Ruff MD, Profous-Juchelka H (1997) Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. *J Parasitol* 83:262–271
- Bartholomew JL, Whipple MJ, Stevens DG, Fryer JL (1997) The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *J Parasitol* 83:859–868
- Bartholomew JL, Whipple MJ, Campton D (2001) Inheritance of resistance to *Ceratomyxa shasta* in progeny from crosses between high- and low-susceptible strains of rainbow trout (*Oncorhynchus mykiss*). *Bull Natl Res Inst Aquacult, Suppl* 5:71–75
- Bartholomew JL, Atkinson SD, Hallett SL (2006) Involvement of *Manayunkia speciosa* (Annelida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a myxozoan parasite of Pacific salmon. *J Parasitol* 92:742–748
- Bartholomew JL, Atkinson SD, Hallett SL, Lowenstine LJ, Garner MM, Gardiner CH, Rideout BA, Keel MK, Brown JD (2008) Myxozoan parasitism in waterfowl. *Int J Parasitol* 38:1199–1207
- Bartošová P, Fiala I, Hypša V (2009) Concatenated SSU and LSU rDNA data confirm the main evolutionary trends within myxosporeans (Myxozoa: Myxosporea) and provide an effective tool for their molecular phylogenetics. *Mol Phylogenet Evol* 53:81–93
- Bartošová P, Fiala I (2011) Molecular evidence for the existence of cryptic species assemblages of several myxosporeans (Myxozoa). *Parasitol Res* 108:573–583
- Bartošová P, Freeman MA, Yokoyama H, Caffara M, Fiala I (2011) Phylogenetic position of *Sphaerospora testicularis* and *Latyspora scomberomori* n. gen. n. sp (Myxozoa) within the marine urinary clade. *Parasitology* 138:381–393
- Bartošová P, Fiala I, Jirků M, Cinková M, Caffara M, Fioravanti ML, Atkinson SD, Bartholomew JL, Holzer AS (2013) *Sphaerospora sensu stricto*: Taxonomy, diversity and evolution of a unique lineage of myxosporeans (Myxozoa). *Mol Phylogenet Evol* 68:93–105

- Bartošová-Sojková P, Hrabcová M, Pecková H, Patra S, Kodádková A, Jurajda P, Tymi T, Holzer AS (2014) Hidden diversity and evolutionary trends in malacosporean parasites (Cnidaria: Myxozoa) identified using molecular phylogenetics. *Int J Parasitol* 44:565–577
- Baska F, Molnár K (1988) Blood stages of *Sphaerospora* spp. (Myxosporea) in cyprinid fishes. *Dis Aquat Organ* 5:23–28
- Baska F (1990) *Chloromyxum inexpectatum* n. sp. and *Sphaerospora colomani* n. sp. (Myxozoa: Myxosporea), parasites of the urinary system of the sterlet, *Acipenser ruthenus* L. *Syst Parasitol* 16:185–193
- Bass D, Stentiford GD, Littlewood DTJ, Hartikainen H (2015) Diverse applications of environmental DNA methods in Parasitology. *Trends Parasitol* 31:499–513
- Baudet C, Donati B, Sinimeri B, Crescenzi P, Gautier C, Matias C, Sagot MF (2015) Cophylogeny Reconstruction via an Approximate Bayesian Computation. *Syst Biol* 64:416–431
- Benajiba MH, Marqués A (1993) The alternation of actinomyxidian and myxosporidian sporadic forms in the development of *Myxidium giardi* (parasite of *Anguilla anguilla*) through oligochaetes. *Bull Eur Assoc Fish Pathol* 13:100–103
- Bermúdez R, Vigliano F, Marcaccini A, Sitjà-Bobadilla A, Quiroga MI, Nieto JM (2006) Response of Ig-positive cells to *Enteromyxum scophthalmi* (Myxozoa) experimental infection in turbot, *Scophthalmus maximus* (L.): A histopathological and immunohistochemical study. *Fish Shellfish Immun* 21:501–512
- Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson JW, Arnell A, Brotherton P, Williams P, Dunn F (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol Conserv* 183:19–28
- Bjork SJ, Bartholomew JL (2010) Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. *Int J Parasitol* 40:1087–1095
- Bjork SJ, Zhang YA, Hurst CN, Alonso-Naveiro ME, Alexander JD, Sunyer JO, Bartholomew JL (2014) Defenses of susceptible and resistant Chinook salmon (*Onchorhynchus tshawytscha*) against the myxozoan parasite *Ceratomyxa shasta*. *Fish Shellfish Immun* 37:87–95
- Bocharova TA, Donetz ZS (1974) New species of myxosporidians (myxosporidia, cnidosporidia) from fishes of the Wasjugan Basin. *Parasitology* 8:74–76
- Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, Knapp M, Douglas WY, Bruyn M (2014) Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol Evol* 29
- Bond FF (1938) Cnidosporidia from *Fundulus heteroclitus* Lin. *Trans Am Microsc Soc* 52:107–122
- Borkhanuddin M, Cech G, Molnár K, Székely C (2013) The life cycle of three *Myxobolus* spp. and two *Thelohanellus* spp. (Myxozoa) from fishes of Lake Balaton and Kis Balaton reservoir. In: 16th EAFP international conference on diseases of fish and shellfish. Tampere, Finland, 2–6 September 2013
- Braem F (1911) Beiträge zur Kenntnis der Fauna Turkestans. VII. Bryozoen und deren Parasiten. *Trav. Soc. Imp. Natur. St. Petersburg* 42, 1–56. (In Russian)
- Brickle P, Kalavati C, MacKenzie K (2001) Two new species of myxozoan parasites (Myxosporea, Bivalvulida) from toothfish *Dissostichus eleginoides* Smitt, 1898 (Pisces, Nototheniidae). *Acta Parasitol* 46:250–253
- Burton PM (2008) Insights from diploblasts; the evolution of mesoderm and muscle. *J Exp Zool Part B-Molecular Dev Evol* 310B:5–14
- Bütchli O (1882) Myxosporidia. In: Winter CF (ed) *Bronn's Klassen und Ordnungen des Tierreichs 1 Protozoa*, Leipzig, 590–603
- Caffara M, Raimondi E, Florio D, Marcer F, Quaglio F, Fioravanti ML (2009) The life cycle of *Myxobolus lentisuturalis* (Myxozoa: Myxobolidae), from goldfish (*Carassius auratus auratus*), involves a Raabeia-type actinospore. *Folia Parasitol* 56:6–12
- Canning EU, Okamura B, Curry A (1996) Development of a myxozoan parasite *Tetracapsula bryozoides* gen. n. et sp. n. in *Cristatella mucedo* (Bryozoa: Phylactolaemata). *Folia Parasitol* 43:249–261
- Canning EU, Curry A, Feist SW, Longshaw M, Okamura B (1999) *Tetracapsula bryosalmonae* n. sp. for PKX organism, the cause of PKD in salmonid fish. *Bull Eur Assoc Fish Pathol* 19:203–206
- Canning EU, Curry A, Feist SW, Longshaw M, Okamura B (2000) A new class and order of myxozoans to accommodate parasites of bryozoans with ultrastructural observations on *Tetracapsula bryosalmonae* (PKX organism). *J Eukaryot Microbiol* 47:456–468
- Canning EU, Tops S, Curry A, Wood TS, Okamura B (2002) Ecology, development and pathogenicity of *Buddenbrockia plumatellae* Schröder, 1910 (Myxozoa, Malacosporea) (syn. *Tetracapsula bryozoides*) and establishment of *Tetracapsuloides* n. gen. for *Tetracapsula bryosalmonae*. *J Eukaryot Microbiol* 49:280–295
- Canning EU, Okamura B (2004) Biodiversity and evolution of the myxozoa. *Adv Parasitol* Vol 56 56:43–131
- Canning EU, Curry A, Hill SLL, Okamura B (2007) Ultrastructure of *Buddenbrockia allmani* n. sp. (Myxozoa, Malacosporea), a parasite of *Lophopus crystallinus* (Bryozoa, Phylactolaemata). *J Eukaryot Microbiol* 54:247–262

- Canning EU, Curry A, Okamura B (2008) Early development of the myxozoan *Buddenbrockia plumatellae* in the bryozoans *Hyalinella punctata* and *Plumatella fungosa*, with comments on taxonomy and systematics of the Myxozoa. *Folia Parasitologica* 55:241–255
- Carriero MM, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2013) Molecular Phylogeny of the *Myxobolus* and *Henneguya* Genera with Several New South American Species. *PLoS One* 8
- Casal G, Matos E, Matos P, Azevedo C (2008) Ultrastructural description of a new myxosporean parasite *Kudoa aequidens* sp. n. (Myxozoa, Myxosporea), found in the sub-opercular musculature of *Aequidens plagiozonatus* (Teleostei) from the Amazon River. *Acta Protozoo* 47:135–141
- Chang ES, Neuhofer M, Rubinstein ND, Diamant A, Philippe H, Huchon D, Cartwright P (2015) Genomic insights into the evolutionary origin of Myxozoa within Cnidaria. *Proc Natl Acad Sci U S A* 112:14912–14917
- Ching H, Munday D (1984). Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. *Can J Zool* 62:1075–1080.
- Chen C, Hsieh S (1984) A new genus and two new species of family Myxobolidae from freshwater fishes of China (Myxosporidia: Myxobolidae). *Acta Zootaxa Sin* 9:113–117
- Chen C, Ma C (1998) Myxozoa, Myxosporea. Fauna Sinica. In. Science Press, Beijing, China p. 993
- Cho JB, Kim KH (2001) Light and electron microscopic observations of *Leptotheca koreana* n. sp (Myxosporea) in the kidney of cultured rockfish *Sebastes schlegeli*. *Dis Aquat Organ* 46:189–195
- Clifton-hadley RS, Bucke D, Richards RH (1987) A study of the sequential clinical and pathological changes during proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *J Fish Dis* 10:335–352
- Cohn L (1902) Zur Kenntniss der Myxosporidien. *Zentralbl Bakteriol* 1:628–632
- Collins AG (2009) Recent Insights into Cnidarian Phylogeny. *Smithson Contrib Mar Sci*:139–149
- Csaba G (1976) An unidentifiable extracellular sporozoan parasite from the blood of the carp. *Parasitol Hung* 9:21–24
- Csaba G, Kovács-gayer E, Békési L, Bucsek M, Szokolczai J, Molnár K (1984) Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry. *J Fish Dis* 7:39–56
- Cuesta A, Salinas I, Rodríguez A, Muñoz P, Sitjà-Bobadilla A, Alvarez-Pellitero P, Meseguer J, Esteban MA (2006a) Cell-mediated cytotoxicity is the main innate immune mechanism involved in the cellular defence of gilthead seabream (Teleostei : Sparidae) against *Enteromyxum leei* (Myxozoa). *Parasite Immunol* 28:657–665
- Cuesta A, Muñoz P, Rodríguez A, Salinas I, Sitjà-Bobadilla A, Alvarez-Pellitero P, Esteban MA, Meseguer J (2006b) Gilthead seabream (*Sparus aurata* L.) innate defence against the parasite *Enteromyxum leei* (Myxozoa). *Parasitology* 132:95–104
- Davey A, Caldach-Giner JA, Houeix B, Talbot A, Sitjà-Bobadilla A, Prunet P, Pérez-Sánchez J, Cairns MT (2011) Molecular profiling of the gilthead sea bream (*Sparus aurata* L.) response to chronic exposure to the myxosporean parasite *Enteromyxum leei*. *Mol Immunol* 48:2102–2112
- Davis HS (1917) Myxosporidia of Beaufort region. A schematic and biological study. In, Book 35. Wash. Bull. U. S. Bur. Fish 199–239
- Deiner K, Altermatt F (2014) Transport distance of invertebrate environmental DNA in a natural river. *PLoS One* 9
- Desser SS, Lom J, Dyková I (1986) Developmental stages of *Sphaerospora ohlmacheri* (Whinery, 1893) n. comb. (Myxozoa:Myxosporea) in the renal tubules of bullfrog tadpoles, *Rana catesbeiana*, from Lake of Two Rivers, Algonquin Park, Ontario. *Can J Zool* 64:2213–2217
- Diamant A (1992) A new pathogenic histozoic *Myxidium* (Myxosporea) in cultured gilt-head sea bream *Sparus aurata* L. *Bull Eur Assoc Fish Pathol* 12:64–66
- Diamant A (1997) Fish-to-fish transmission of a marine myxosporean. *Dis Aquat Organ* 30:99–105
- Diamant A, Ucko M, Paperna I, Colorni A, Lipshitz A (2005) *Kudoa iwatai* (Myxosporea: Multivalvulida) in wild and cultured fish in the Red Sea: Redescription and molecular phylogeny. *J Parasitol* 91:1175–1189
- Diamant A, Ram S, Paperna I (2006) Experimental transmission of *Enteromyxum leei* to freshwater fish. *Dis Aquat Organ* 72:171–178
- Dogiel VA (1948) Parasitic protozoa of fishes from Peter the Great Bay. *Trans. All-Union Sci. Inst. Lake River Fish. Leningrad* 27, 17–37.
- Dogiel VA (1965) *General Parasitology*, Vol. Oxford University Press, UK
- Dörfler C, El-Matbouli M (2007) Isolation of a subtilisin-like serine protease gene (MyxSubtSP) from spores of *Myxobolus cerebralis*, the causative agent of whirling disease. *Dis Aquat Organ* 73:245–251
- Dumortier BC, van Beneden PJ (1850) Histoire naturelle des polyps composés d'eau douce. *Nouveaux Mémoires de l'Académie Royale des Sciences et Belle/Lettres de Bruxelles* 16:33–96
- Dyková I, Lom J (1982) *Sphaerospora renicola* n. sp., a myxosporean from carp kidney, and its pathogenicity. *Z Parasitenkd-Parasitology Res* 68:259–268

- Dyková I, Lom J (1997) Light and electron microscope observations on *Sphaerospora ojiroveci* n. sp. (Myxozoa) from the kidney of *Pangasius sutchi* (Teleostei). *Eur J Parasitol* 33:444–451
- Dyková I, Lom J, (2007) Histopathology of Protistan and Myxozoan infection in fishes. An Atlas. Academia, Praha, 219
- Dyková I, Pecková H, Kostka M (2008) Introduction of *Mayorella gemmifera* Schaeffer, 1926 into phylogenetic studies of Amoebozoa. *Acta Protozool.* 47:205–210
- Dyková I, Tymi T, Kostka M (2011) Xenoma-like formations induced by *Soricimyxum fegati* (Myxosporea) in three species of shrews (Soricomorpha: Soricidae), including records of new hosts. *Folia Parasitol* 58:249–256
- European Environment Agency (EEA) (2012): Climate change, impacts and vulnerability in Europe 2012. An indicator-based report. <http://www.eea.europa.eu/media/publications/climate-impacts-andvulnerability>, 2012.
- Eiras JC (2005) An overview on the myxosporean parasites in amphibians and reptiles. *Acta Parasitol* 50:267–275
- El-Mansy A, Molnár K (1997a) Extrapiscine development of *Myxobolus drjagini* Akhmerov, 1954 (Myxosporea : Myxobolidae) in oligochaete alternative hosts. *Acta Vet Hung* 45:427–438
- El-Mansy A, Molnár K (1997b) Development of *Myxobolus hungaricus* (Myxosporea : Myxobolidae) in oligochaete alternate hosts. *Dis Aquat Organ* 31:227–232
- El-Mansy A, Molnár K, Székely C (1998) Development of *Myxobolus portucalensis* Saraiva & Molnár, 1990 (Myxosporea : Myxobolidae) in the oligochaete *Tubifex tubifex* (Muller). *Syst Parasitol* 41:95–103
- El-Matbouli M, Hoffmann RW (1989) Experimental transmission of two *Myxobolus* spp. developing bisporogeny via tubificid worms. *Parasitol Res* 75:461–464
- El-Matbouli M, Hoffmann RW (1992) *Sphaerospora scardinii* n. sp. (Myxosporea, Sphaerosporidae) observed in the kidney of rudd *Scardinius erythrophthalmus*. *Dis Aquat Organ* 14:23–29
- El-Matbouli M, Fischer-Scherl T, Hoffmann RW (1992) Transmission of *Hoferellus carassi carassii* Achmerov, 1960 to goldfish *Carassius auratus* via an aquatic oligochaete. *Bull Eur Assoc Fish Pathol* 12:54–56
- El-Matbouli M, Hoffmann RW (1993) *Myxobolus carassii* Klokaceva, 1914 also requires an aquatic oligochaete, *Tubifex tubifex* as an intermediate host in its life cycle. *Bull Eur Assoc Fish Pathol* 13:189–192
- El-Matbouli M, Hoffmann RW, Mandok C (1995a) Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout cartilage. *J Fish Biol* 46:919–935
- El-Matbouli M, Hoffmann R, Kern R (1995b) *Sphaerospora bramae* (Myxosporea: Sphaerosporidae) in the kidney of common bream (*Abramis brama*). *Bull Eur Assoc Fish Pathol* 6:197–199
- El-Matbouli M, Hoffmann R, Kern R (1996) *Sphaerospora coregoni* n. sp. (Myxosporea: Sphaerosporidae) observed in the kidney of whitefish (*Coregonus lavaretus*). *Eur J Protistol* 32:389–398
- El-Matbouli M, Hoffmann RW (1998) Light and electron microscopic studies on the chronological development of *Myxobolus cerebralis* to the actinosporean stage in *Tubifex tubifex*. *Int J Parasitol* 28:195–217
- El-Matbouli M, Hoffmann RW, Schoel H, McDowell TS, Hedrick RP (1999) Whirling disease: host specificity and interaction between the actinosporean stage of *Myxobolus cerebralis* and rainbow trout *Oncorhynchus mykiss*. *Dis Aquat Organ* 35:1–12
- Estensoro I, Jose Redondo M, Alvarez-Pellitero P, Sitjà-Bobadilla A (2010) Novel horizontal transmission route for *Enteromyxum leei* (Myxozoa) by anal intubation of gilthead sea bream *Sparus aurata*. *Dis Aquat Organ* 92:51–58
- Estensoro I, Redondo MJ, Salesa B, Kaushik S, Pérez-Sánchez J, Sitjà-Bobadilla A (2012) Effect of nutrition and *Enteromyxum leei* infection on gilthead sea bream *Sparus aurata* intestinal carbohydrate distribution. *Dis Aquat Organ* 48:29–42
- Estensoro I, Alvarez-Pellitero P, Sitjà-Bobadilla A (2013a) Antigenic characterization of *Enteromyxum leei* (Myxozoa: Myxosporea). *Dis Aquat Organ* 106:149–162
- Estensoro I, Jung-Schroers V, Alvarez-Pellitero P, Steinhagen D, Sitjà-Bobadilla A (2013b) Effects of *Enteromyxum leei* (Myxozoa) infection on gilthead sea bream (*Sparus aurata*) (Teleostei) intestinal mucus: glycoprotein profile and bacterial adhesion. *Parasitol Res* 112:567–576
- Estensoro I, Mulero I, Redondo MJ, Alvarez-Pellitero P, Mulero V, Sitjà-Bobadilla A (2014a) Modulation of leukocytic populations of gilthead sea bream (*Sparus aurata*) by the intestinal parasite *Enteromyxum leei* (Myxozoa: Myxosporea). *Parasitology* 141:425–440
- Estensoro I, Redondo MJ, Alvarez-Pellitero P, Sitjà-Bobadilla A (2014b) Immunohistochemical characterization of polyclonal antibodies against *Enteromyxum leei* and *Enteromyxum scopthalmi* (Myxozoa: Myxosporea), intestinal parasites of fish. *J Fish Dis* 37:785–796
- Eszterbauer E, Székely C, Molnár K, Baska F (2000) Development of *Myxobolus bramae* (Myxosporea : Myxobolidae) in an oligochaete alternate host, *Tubifex tubifex*. *J Fish Dis* 23:19–25

- Eszterbauer E, Székely C (2004) Molecular phylogeny of the kidney-parasitic *Sphaerospora renicola* from common carp (*Cyprinus carpio*) and *Sphaerospora* sp. from goldfish (*Carassius auratus auratus*). *Acta Vet Hung* 52:469–478
- Eszterbauer E, Marton S, Rácz OZ, Letenyi M, Molnár K (2006) Morphological and genetic differences among actinosporean stages of fish-parasitic myxosporeans (Myxozoa): difficulties of species identification. *Sys Parasitol* 65:97–114
- Eszterbauer E, Kallert DM, Grabner D, El-Matbouli M (2009) Differentially expressed parasite genes involved in host recognition and invasion of the triactinomyxon stage of *Myxobolus cerebralis* (Myxozoa). *Parasitology* 136:367–377
- Eszterbauer E (2011) Erratum to 'Molecular phylogeny of the kidney-parasitic *Sphaerospora renicola* from common carp (*Cyprinus carpio*) and *Sphaerospora* sp. from goldfish (*Carassius auratus auratus*)'. *Acta Vet Hung* 59:409–409
- Eszterbauer E, Sipos D, Forró B, Bartošová P, Holzer A (2013) Molecular characterization of *Sphaerospora molnari* (Myxozoa), the agent of gill sphaerosporosis in common carp *Cyprinus carpio*. *Dis Aquat Organ* 104:59–67
- Eszterbauer E, Atkinson S, Diamant A, Morris D, El-Matbouli M, Hartikainen H (2015) Myxozoan Life Cycles: Practical Approaches and Insights. In: Okamura B, Gruhl A, Bartholomew JL (eds) *Myxozoan Evolution, Ecology and Development*. Springer International Publishing, Cham, p 175–198
- Evans NM, Holder MT, Barbeitos MS, Okamura B, Cartwright P (2010) The phylogenetic position of Myxozoa: exploring conflicting signals in phylogenomic and ribosomal data sets. *Mol Biol Evol* 27:2733–2746
- Evlanov IA (1981) New species of Myxosporidians from fishes of the Kalingrad Region. *Parazitologiya* 15:168–170
- Fantham HB, Porter A, Richardson LR (1939) Some Myxosporidia found in certain freshwater fishes in Quebec Province, Canada. *Parasitology* 31:1–77
- Fantham HB, Porter A (1943) On a myxosporid (*Sphaerospora periphthalmi* n. sp.) found in African and Indian mudskippers (Pisces) and its possible significance. *Proc Zool Soc Lond* 112:113–119
- Feist SW, Bucke D (1987) Ultrastructural aspects of PKX, the causative agent of proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *J Fish Dis* 10:323–327
- Feist SW, Longshaw M, Canning EU, Okamura B (2001) Induction of proliferative kidney disease (PKD) in rainbow trout *Oncorhynchus mykiss* via the bryozoan *Fredericella sultana* infected with *Tetracapsula bryosalmonae*. *Dis Aquat Organ* 45:61–68
- Feist SW, Morris DJ, Alama-Bermejo G, Holzer AS (2015) Cellular Processes in Myxozoans. In: Okamura B, Gruhl A, Bartholomew JL (eds) *Myxozoan Evolution, Ecology and Development*. Springer International Publishing, Cham, p 139–154
- Feng JM, Xiong J, Zhang JY, Yang YL, Yao B, Zhou ZG, Miao W (2014) New phylogenomic and comparative analyses provide corroborating evidence that Myxozoa is Cnidaria. *Mol Phylogenet Evol* 81:10–18
- Ferguson HW, Needham EA (1978) Proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *J Fish Dis* 1:91–108
- Ferguson JA, Atkinson SD, Whipps CM, Kent ML (2008) Molecular and morphological analysis of *Myxobolus* spp. of salmonid fishes with the description of a new *Myxobolus* species. *J Parasitol* 94:1322–1334
- Fiala I (2006) The phylogeny of *Myxosporea* (Myxozoa) based on small subunit ribosomal RNA gene analysis. *Int J Parasitol* 36:1521–1534
- Fiala I, Bartošová P (2010) History of myxozoan character evolution on the basis of rDNA and EF-2 data. *Bmc Evol Biol* 10:13
- Fiala I, Bartošová-Sojková P, Whipps CM (2015a) Classification and Phylogenetics of Myxozoa. In: Okamura B, Gruhl A, Bartholomew JL (eds) *Myxozoan Evolution, Ecology and Development*. Springer International Publishing, Cham, p 85–110
- Fiala I, Bartošová-Sojková P, Okamura B, Hartikainen H (2015b) Adaptive Radiation and Evolution Within the Myxozoa. In: Okamura B, Gruhl A, Bartholomew JL (eds) *Myxozoan Evolution, Ecology and Development*. Springer International Publishing, Cham, p 69–84
- Filipiak A, Zajac K, Kübler D, Kramarz P (2016) Coevolution of host-parasite associations and methods for studying their cophylogeny. *Invert Surviv J* 13:56–65
- Fischer-Scherl T, El-Matbouli M, Hoffmann R (1986) A new *Sphaerospora* sp. in brown trout (*Salmo trutta m. fario*) in Germany. *Bull Eur Assoc Fish Pathol* 6:16–19
- Fleurance R, Sauvegrain C, Marques A, Le Breton A, Guereaud C, Cherel Y, Wyers M (2008) Histopathological changes caused by *Enteromyxum leei* infection in farmed sea bream *Sparus aurata*. *Dis Aquat Organ* 79:219–228
- Fomena A, Marques A, Bouix G (1993) Myxosporidea (Myxozoa) of *Oreochromis niloticus* (Linnaeus 1757) (teleost Cichlidae) in fish-farming pools at Melen (Yaounde, Cameroon, central Africa). *J Afr Zool* 107:45–56

- Fomena A, Bouix G (1994) New Myxosporidea species (Myxozoa) from freshwater teleosts in southern Cameroon (Central Africa). *J Afr Zool* 108:481–491
- Foott JS, Hedrick RP (1987) Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *J Fish Biol* 30:477–483
- Freeman MA, Shinn AP (2011) Myxosporean hyperparasites of gill monogeneans are basal to the Multivalvulida. *Parasit Vectors* 4
- Freeman MA, Kristmundsson A (2015) Histozoic myxosporeans infecting the stomach wall of elopiform fishes represent a novel lineage, the Gastromyxidae. *Parasit Vectors* 8
- Fujita T (1912) Notes on New Sporozoan Parasites of Fishes. *Zool Anz Leipzig* 39: 17–20
- Funk VA, Olafson RW, Raap M, Smith D, Aitken L, Haddow JD, Wang D, Dawson-Coates JA, Burke RD, Miller KM (2008) Identification, characterization and deduced amino acid sequence of the dominant protease from *Kudoa paniformis* and *K. thyrstites*: A unique cytoplasmic cysteine protease. *Comp Biochem Phys B* 149:477–489
- Gleeson R, Adlard R (2011) Morphological and genetic analysis of three new species of *Ceratomyxa* Thélohan, 1892 (Myxozoa: Myxosporidia) from carcharhinid sharks off Australia. *Syst Parasitol* 80:117–124
- Gleeson RJ, Adlard RD (2012) Phylogenetic relationships amongst *Chloromyxum* Mingazzini, 1890 (Myxozoa: Myxosporidia), and the description of six novel species from Australian elasmobranchs. *Parasitol Int* 61:267–274
- Gonzalez SF, Buchmann K, Nielsen ME (2007) Real-time gene expression analysis in carp (*Cyprinus carpio* L.) skin: Inflammatory responses caused by the ectoparasite *Ichthyophthirius multifiliis*. *Fish Shellfish Immun* 22:641–650
- Gordon DP (1999) Bryozoan diversity in New Zealand and Australia. Other 99%: the Conservation and Biodiversity of Invertebrates. eds. W. Ponder and D. Lunney, p 199–204
- Gorgoglione B, Wang T, Secombes CJ, Holland JW (2013) Immune gene expression profiling of Proliferative Kidney Disease in rainbow trout *Oncorhynchus mykiss* reveals a dominance of anti-inflammatory, antibody and T helper cell-like activities. *Vet Res* 44:55
- Grabner DS, El-Matbouli M (2008) Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa : Malacosporidia) to *Fredericella sultana* (Bryozoa : Phylactolaemata) by various fish species. *Dis Aquat Organ* 79:133–139
- Grabner DS, El-Matbouli M (2010a) Experimental transmission of malacosporidian parasites from bryozoans to common carp (*Cyprinus carpio*) and minnow (*Phoxinus phoxinus*). *Parasitology* 137:629–639
- Grabner DS, El-Matbouli M (2010b) *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporidia) portal of entry into the fish host. *Dis Aquat Organ* 90:197–206
- Grassé PP, Lavette A (1978) La myxosporidie *Sphaeromyxa sarazei* et le novel embranchement de Myxosporidia (Myxozoa). *Recherches sur l'état pluricellulaire primitive et considerations phylogénétiques*. *Annls Sci nat (Paris)* 2: 193–285.
- Grossheider G, Körting W (1992) First evidence that *Hofereilus cyprini* (Doflein, 1898) is transmitted by *Nais* sp. *Bull Eur Assoc Fish Pathol* 12:17–20
- Gruhl A, Okamura B (2012) Development and myogenesis of the vermiform *Buddenbrockia* (Myxozoa) and implications for cnidarian body plan evolution. *Evodevo* 3:10
- Gunter NL, Adlard RD (2009) Seven new species of *Ceratomyxa* Thélohan, 1892 (Myxozoa) from the gall-bladders of serranid fishes from the Great Barrier Reef, Australia. *Syst Parasitol* 73:1–11
- Gunter NL, Whipps CM, Adlard RD (2009) *Ceratomyxa* (Myxozoa: Bivalvulida): Robust taxon or genus of convenience? *Int J Parasitol* 39:1395–1405
- Gunter N, Adlard R (2010) The demise of *Leptotheca* Thélohan, 1895 (Myxozoa: Myxosporidia: Ceratomyxidae) and assignment of its species to *Ceratomyxa* Thélohan, 1892 (Myxosporidia: Ceratomyxidae), *Ellipsomyxa* Køie, 2003 (Myxosporidia: Ceratomyxidae), *Myxobolus* Bütschli, 1882 and *Sphaerospora* Thélohan, 1892 (Myxosporidia: Sphaerosporidae). *Syst Parasitol* 75:81–104
- Gurley RR (1894) The Myxosporidia, or Psorosperms of fishes, and the epidemics produced by them. *Rep U.S. Fish Com* 26:65–304
- Hallett SL, Lester RJ (1999) Actinosporidia (Myxozoa) with four developing spores within a pansporocyst: *Tetraspora discoidea* n.g. n.sp. and *Tetraspora rotundum* n.sp. *Int J Parasitol* 29:419–427
- Hallett SL, Atkinson SD, Holt RA, Banner CR, Bartholomew JL (2006) A new myxozoan from feral goldfish (*Carassius auratus*). *J Parasitol* 92:357–363
- Hallett SL, Bartholomew JL (2012) *Myxobolus cerebralis* and *Ceratomyxa shasta*. In: Woo PTK, Buchmann K (eds) Fish parasites: pathobiology and protection. CABI, Oxfordshire, p 131–162
- Hallett S, Atkinson S, Bartholomew J, Székely C (2015) Myxozoans Exploiting Homeotherms. In: Okamura B, Gruhl A, Bartholomew JL (eds) Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, p 125–135

- Hartigan A, Fiala I, Dyková I, Jirků M, Okimoto B, Rose K, Phalen DN, Slapeta J (2011) A suspected parasite spill-back of two novel *Myxidium* spp. (Myxosporea) causing disease in Australian endemic frogs found in the invasive cane toad. PLoS One 6
- Hartigan A, Patra S, Holzer AS (2015) Getting myxozoan parasites hot under the collar: a real-time PCR assay to quantify proliferative blood stages of *Sphaerospora molnari* in *Cyprinus carpio* in a range of temperatures. In: 9th International Symposium on Fish Parasites. Valencia, Spain
- Hartigan A, Wilkinson M, Gower DJ, Streicher JW, Holzer AS, Okamura B (2016a) Myxozoan infections of caecilians demonstrate broad host specificity and indicate a link with human activity. Int J Parasitol 46:375–381
- Hartigan A, Estensoro I, Vancová M, Bílý T, Patra S, Eszterbauer E, Holzer AS (2016b) New cell motility model observed in parasitic cnidarian *Sphaerospora molnari* (Myxozoa: Myxosporea) blood stages in fish. Sci Rep 6:1–12
- Hartikainen H, Okamura B (2012) Castrating parasites and colonial hosts. Parasitology 139:547–556
- Hartikainen H, Fontes I, Okamura B (2013) Parasitism and phenotypic change in colonial hosts. Parasitology 140:1403–1412
- Hartikainen H, Gruhl A, Okamura B (2014) Diversification and repeated morphological transitions in endoparasitic cnidarians (Myxozoa: Malacosporea). Mol Phylogenet Evol 76:261–269
- Hartikainen H, Bass D, Briscoe AG, Knipe H, Green AJ, Okamura B (2016) Assessing myxozoan presence and diversity using environmental DNA. Int J Parasitol 46:781–792
- Hastings AB (1943) Note on the Vermiform bodies found in some Polyzoa. Discovery rep 22:301–510
- Hedrick RP, McDowell T, Groff JM (1990) *Sphaerospora ictaluri* n. sp. (Myxosporea, Sphaerosporridae) observed in the kidney of channel catfish *Ictalurus punctatus* Rafinesque. J Protozool 37:107–112
- Hedrick RP, Wishkovsky A, Modin JC, Toth RJ (1991) Three myxosporeans found in the cranial and branchial tissues of rainbow trout in California. J Aquat Anim Health 3:55–62
- Hedrick RP, MacConnell E, Kinkelin PD (1993) Proliferative kidney disease of salmonid fish. Annu Rev Fish Dis 3:277–290
- Hedrick RP, El-Matbouli M, Adkison MA, MacConnell E (1998) Whirling disease: re-emergence among wild trout. Immunol Rev 166:365–376
- Hedrick RP, Baxa D V, Kinkelin PD, Okamura B (2004) Malacosporean-like spores in urine of rainbow trout react with antibody and DNA probes to *Tetracapsuloides bryosalmonae*. Parasitol Res 92:81–88
- Henderson M, Okamura B (2004) The phylogeography of salmonid proliferative kidney disease in Europe and North America. Proc R Soc B-Biological Sci 271:1729–1736
- Hill SLL, Okamura B (2007) Endoparasitism in colonial hosts: patterns and processes. Parasitology 134:841–852
- Holland JW, Okamura B, Hartikainen H, Secombes CJ (2011) A novel minicollagen gene links cnidarians and myxozoans. Proc R Soc B-Biological Sci 278:546–553
- Holzer AS, Sommerville C, Wootten R (2003) Tracing the route of *Sphaerospora truttae* from the entry locus to the target organ of the host, *Salmo salar* L., using an optimized and specific in situ hybridization technique. J Fish Dis 26:647–655
- Holzer AS, Sommerville C, Wootten R (2004) Molecular relationships and phylogeny in a community of myxosporeans and actinosporeans based on their 18S rDNA sequences. Int J Parasitol 34:1099–1111
- Holzer AS, Sommerville C, Wootten R (2006a) Molecular identity, phylogeny and life cycle of *Chloromyxum schurovi* Shul'man & Ieshko 2003. Parasitol Res 99:90–96
- Holzer AS, Sommerville C, Wootten R (2006b) Molecular studies on the seasonal occurrence and development of five myxozoans in farmed *Salmo trutta* L. Parasitology 132:193–205
- Holzer AS, Wootten R, Sommerville C (2007) The secondary structure of the unusually long 18S ribosomal RNA of the myxozoan *Sphaerospora truttae* and structural evolutionary trends in the Myxozoa. Int J Parasitol 37:1281–1295
- Holzer AS, Stewart S, Tildesley A, Wootten R, Sommerville C (2010) Infection dynamics of two renal myxozoans in hatchery reared fry and juvenile Atlantic cod *Gadus morhua* L. Parasitology 137:1501–1513
- Holzer AS, Bartošová P, Pecková H, Tým I, Atkinson S, Bartholomew J, Sipos D, Eszterbauer E, Dyková I (2013a) Who's who' in renal sphaerosporids (Bivalvulida: Myxozoa) from common carp, Prussian carp and goldfish - molecular identification of cryptic species, blood stages and new members of *Sphaerospora sensu stricto*. Parasitology 140:46–60
- Holzer AS, Pecková H, Patra S, Brennan NP, Yanes-Roca C, Main KL (2013b) Severe glomerular disease in juvenile grey snapper *Lutjanus griseus* L. in the Gulf of Mexico caused by the myxozoan *Sphaerospora motemarinii* n. sp. Int J Parasitol Parasites Wildl 2:124–130
- Holzer AS, Hartigan A, Patra S, Pecková H, Eszterbauer E (2014) Molecular fingerprinting of the myxozoan community in common carp suffering Swim Bladder Inflammation (SBI) identifies multiple etiological agents. Parasit Vectors 7

- Holzer AS, Bartosova-Sojkova P, Born-Torrijos A, Kodadkova A, Hartigan A, Fiala I Joint evolution of myxozoans and their hosts. Manuscript in advanced preparation
- Ikeda I (1912) Studies on some Sporozoon parasites of Sipunculoids. I. The Life-History of a New Actinomyxidian, *Tetractinomyxon intermedium* g. et sp. nov. Arch für Protist 25:240–272
- Jacob E, Bremen HB (1953) Eine bislang unbekannte Sphaerosporose des Flussaals, hervorgerufen durch *Sphaerospora reichenowi* nova species, mit eigenartigen Sitz im Darm. Berl Munch Tierarztl 66:326–328
- Jiménez-Guri E, Philippe H, Okamura B, Holland PWH (2007a) *Buddenbrockia* is a Cnidarian worm. Science 317:116–118
- Jiménez-Guri E, Okamura B, Holland PWH (2007b) Origin and evolution of a myxozoan worm. Integr Comp Biol 47:752–758
- Jirků M, Fiala I, Modrý D (2007) Tracing the genus *Sphaerospora*: rediscovery, redescription and phylogeny of the *Sphaerospora ranae* (Morelle, 1929) n. comb. (Myxosporea, Sphaerosporidae), with emendation of the genus *Sphaerospora*. Parasitology 134:1727–1739
- Jirků M, Bartošová P, Kodádková A, Mutschmann F (2011) Another chloromyxid lineage: molecular phylogeny and redescription of *Chloromyxum careni* from the asian horned frog *Megophrys nasuta*. J Eukaryot Microbiol 58:50–59
- Jirků M, Bartošová-Sojková P (2014) Ultrastructure and localisation of late-sporogonic developmental stages of *Sphaerospora ranae* (Myxosporea: Sphaerosporidae). Folia Parasitol 61:311–321
- Johnson KA (1980) Host susceptibility, histopathologic and transmission studies on *Ceratomyxa shasta*, a myxosporidan parasite of salmonid fish. Fish Pathol 14:183–184
- Jones S, Fiala I, Prosperi-Porta G, House M, Mumford S (2011) *Sphaerospora elwhaiensis* sp. n. (Myxosporea: Sphaerosporidae) from landlocked sockeye salmon *Oncorhynchus nerka* (Salmoniformes: Salmonidae) in Washington State, USA. Folia Parasitol 58:87–94
- Jurine L (1825) Histoire des poissons du Lac Léman. Mem la Société Phys d'histoire Nat Genève 3:133–235
- Kabata Z, Whitaker DJ (1989) *Kudoa thyrsites* Gilchrist, 1924 (Myxozoa) in the cardiac muscle of pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*Salmo gairdneri*). Can J Zool 67:341–342
- Kalavati C, Rao NB (2005) *Leptotheca mackenzii* n. sp. (Myxozoa: Myxosporea) infecting the urinary bladder of the frog, *Lemnectes limnonectes limnocharis*. J Environ Sociobiol 2:25–32
- Kallert DM, El-Matbouli M, Haas W (2005a) Polar filament discharge of *Myxobolus cerebralis* actinospores is triggered by combined non-specific mechanical and chemical cues. Parasitology 131:609–616
- Kallert DM, Eszterbauer E, El-Matbouli M, Erseus C, Haas W (2005b) The life cycle of *Henneguya nuesslini* Schuberg & Schröder, 1905 (Myxozoa) involves a triactinomyxon-type actinospore. J Fish Dis 28:71–79
- Kallert DM, Eszterbauer E, Erseus C, El-Matbouli M, Haas W (2005c) Life cycle studies of *Myxobolus parviformis* sp. n. (Myxozoa : Myxobolidae) from bream. Dis Aquat Organ 66:233–243
- Kallert DM, Bauer W, Haas W, El-Matbouli M (2011) No shot in the dark: Myxozoans chemically detect fresh fish. Int J Parasitol 41:271–276
- Karlsbakk E, Sæther PA, Høstlund C, Fjellsøy KR, Nylund A (2002) *Parvicapsula pseudobranchicola* n. sp. (Myxozoa), a myxosporidian infecting the pseudobranch of cultured Atlantic salmon (*Salmo salar*) in Norway. Bull Eur Assoc Fish Pathol 22:381–387
- Karlsbakk E, Kjøie M (2009) *Bipteria formosa* (Kovalova et Gaevskaya, 1979) comb. n. (Myxozoa: Myxosporea) in whiting *Merlangius merlangus* (Teleostei: Gadidae) from Denmark. Folia Parasitol 56:86–90
- Karlsbakk E, Kjøie M (2012) The marine myxosporean *Sigmomyxa sphaerica* (Thélohan, 1895) gen. n., comb. n. (syn. *Myxidium sphaericum*) from garfish (*Belone belone* (L.)) uses the polychaete *Nereis pelagica* L. as invertebrate host. Parasitol Res 110:211–218
- Karlsbakk E, Kristmundsson A, Albano M, Brown P, Freeman MA (2017) Redescription and phylogenetic position of *Myxobolus aeglefini* and *Myxobolus platessae* n. comb. (Myxosporea), parasites in the cartilage of some North Atlantic marine fishes, with notes on the phylogeny and classification of the Platysporina. Parasitol Int 66:952–959
- Kaschkovskiy VV, Razmashkim DA, Skripchenko GE (1974) Infections and parasites of fish in fish breedings of Siberia and the urals. Sredn Knizhn izd, Sverdlovsko 15–26
- Katayama T, Wada H, Furuya H, Satoh N, Yamamoto M (1995) Phylogenetic position of the dicyemid mesozoa inferred from 18S rDNA sequences. Biol Bull 189:81–90
- Kawai T, Sekizuka T, Yahata Y, Kuroda M, Kumeda Y, Iijima Y, Kamata Y, Sugita-Konishi Y, Ohnishi T (2012) Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw fish. Clin Infect Dis 54:1046–1052
- Kelley GO, Adkison MA, Leutenegger CM, Hedrick RP (2003) *Myxobolus cerebralis*: identification of a cathepsin Z-like protease gene (MyxCP-1) expressed during parasite development in rainbow trout, *Oncorhynchus mykiss*. Exp Parasitol 105:201–210

- Kelley GO, Zagmutt-Vergara FJ, Leutenegger CM, Adkison MA, Baxa DV, Hedrick RP (2004) Identification of a serine protease gene expressed by *Myxobolus cerebralis* during development in rainbow trout *Oncorhynchus mykiss*. *Dis Aquat Organ* 59:235–248
- Kent ML, Hedrick RP (1985) PKX, the causative agent of Proliferative Kidney Disease (PKD) in pacific salmonid fishes and its affinities with the Myxozoa. *J Protozool* 32:254–260
- Kent ML, Hedrick RP (1986) Development of the PKX myxosporean in rainbow trout *Salmo gairdneri*. *Dis Aquat Organ* 1:169–182
- Kent ML, Whitaker DJ, Margolis L (1993a) Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, a myxosporean parasite of pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (lumbriculidae). *Can J Zool Can Zool* 71:1207–1211
- Kent ML, Whitaker DJ, Margolis L (1993b) *Sphaerospora oncorhynchi* n.sp. (Myxosporidia: Sphaerosporidae) from the kidney of sockeye salmon (*Oncorhynchus nerka*) in British Columbia and its possible relationship to the myxosporean causing proliferative kidney disease in salmonid fishes. *Can J Zool* 71:2425–2430
- Kent ML, Margolis L, Corliss JO (1994) The demise of a class of protists - taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grassé, 1970. *Can J Zool Can Zool* 72:932–937
- Kent ML, Khattra J, Hedrick RP, Devlin RH (2000) *Tetracapsula renicola* n. sp (Myxozoa : Saccosporidae); The PKX myxozoan - The cause of proliferative kidney disease of salmonid fishes. *J Parasitol* 86:103–111
- Kent ML, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallett SL, Lester RJG, Longshaw M, Palenzuela O, Siddall ME, Xiao CX (2001) Recent advances in our knowledge of the Myxozoa. *J Eukaryot Microbiol* 48:395–413
- Kinkelin PD, Gay M, Forman S (2002) The persistence of infectivity of *Tetracapsula bryosalmonae*-infected water for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis* 25:477–482
- Kodádková A, Bartošová -Sojková P, Holzer AS, Fiala I (2015) *Bipteria vetusta* n. sp. - an old parasite in an old host: tracing the origin of myxosporean parasitism in vertebrates. *Int J Parasitol* 45:269–276
- Koehler A, Romans P, Desser S, Ringuette M (2004) Encapsulation of *Myxobolus pendula* (Myxosporidia) by epithelioid cells of its cyprinid host *Semotilus atromaculatus*. *J Parasitol* 90:1401–1405
- Kjøie M, Whipps CM, Kent ML (2004) *Ellipsomyxa gobii* (Myxozoa : Ceratomyxidae) in the common goby *Pomatoschistus microps* (Teleostei : Gobiidae) uses *Nereis* spp. (Annelida : Polychaeta) as invertebrate hosts. *Folia Parasitol* 51:14–18
- Kjøie M, Karlsbakk E, Nylund A (2007) A new genus *Gadimyxa* with three new species (Myxozoa, Parvicapsulidae) parasitic in marine fish (Gadidae) and the two-host life cycle of *Gadimyxa atlantica* n. sp. *J Parasitol* 93:1459–1467
- Kjøie M, Karlsbakk E, Nylund A (2008) The marine herring myxozoan *Ceratomyxa auerbachii* (Myxozoa : Ceratomyxidae) uses *Chone infundibuliformis* (Annelida : Polychaeta : Sabellidae) as invertebrate host. *Folia Parasitol* 55:100–104
- Konovalov SM (1967) Особенности паразитофауны Щуки р. Пенжины (On certain peculiarities of parasites fauna of pike from the Penzhina River). *Parasitology* 6:539–546
- Körting W (1982) Protozoan parasites associated with swimbladder inflammation (SBI) in young carp. *Bull Eur Assoc Fish Pathol* 2:25–28
- Körting W, Kruse P, Steinhagen D (1989) Development of "Csaba cells" in experimentally infected *Cyprinus carpio*. *Angew Parasitol* 30:185–188
- Kovács-Gayer E, Csaba G, Békési L, Bucsek M, Szokolczai J, Molnár K (1982) Studies on the protozoan etiology of swim bladder inflammation in common carp fry. *Bull Eur Assoc Fish Pathol* 2:22–24
- Kovács-Gayer E (1983) Histopathological studies on protozoan swim bladder inflammation of common carp fry. *Parasitol Hung* 16:39–46
- Kovalova AA, Zubchenko AV (1984) New information on the North Atlantic Myxosporidae fauna. *Parazitologiya* 18:484–487
- Kpacha TK, Diebakate C, Toguebaye BS (1996) Myxosporidies (Myxozoa, Myxosporidia) of the genera *Sphaeromyxa* Thélohan, 1892 *Myxidium* Butschli, 1882, *Zschokkella* Auerbach, 1910, *Bipteria* Kovalova, Zubchenko and Krasin, 1983 and *Leptotheca* Thélohan, 1895 parasites of fish from the coast of Senegal (West Africa). *J Afr Zool* 110:309–317
- Kudo R (1919) Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Illinois biological monographs 5
- Kudo R (1933) A taxonomic consideration of Myxosporidia. *Trans Am Microsc* 52:195–216
- Kulemina IV (1969) New species of endoparasitic protozoa from the fry of the lake Seliger. *J Zool* 48:1295–1298
- Kumar G, Abd-Elfattah A, El-Matbouli M (2014) Differential modulation of host genes in the kidney of brown trout *Salmo trutta* during sporogenesis of *Tetracapsuloides bryosalmonae* (Myxozoa). *Vet Res* 45:101

- Kyslík J (2016) Phylogeny of Myxozoa based on cnidarian specific genes. Master thesis. University of South Bohemia, Czech Republic
- Landsberg JH (1987) Myxosporean parasites of the catfish, *Clarias lazera* (Valenciennes). *Syst Parasitol* 9:73–81
- Laramie MB, Pilliod DS, Goldberg CS (2015) Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biol Conserv* 183:29–37
- Leis EM, Rosser TG, Baumgartner WA, Griffin MJ (2017) *Henneguya laseae* n. sp from flathead catfish (*Pylodictis olivaris*) in the upper Mississippi River. *Parasitol Res* 116:81–89
- Li LX, Desser SS (1985) The protozoan parasites of fish from two lakes in Algonquin Park, Ontario. *Can J Zool* 63:1846–1858
- Lin DJ, Hanson LA, Pote LM (1999) Small subunit ribosomal RNA sequence of *Henneguya exilis* (Class Myxosporea) identifies the actinosporean stage from an oligochaete host. *J Eukaryot Microbiol* 46:66–68
- Liu XH, Batueva MD, Zhao YL, Zhang JY, Zhang QQ, Li TT, Li AH (2016) Morphological and molecular characterisation of *Myxobolus pronini* n. sp (Myxozoa: Myxobolidae) from the abdominal cavity and visceral serous membranes of the gibel carp *Carassius auratus gibelio* (Bloch) in Russia and China. *Parasit Vectors* 9
- Lom J, Dyková I, Pavlásková M, Grupcheva G (1983a) *Sphaerospora molnari* sp. n. (Myxozoa, Myxosporea), an agent of gill, skin and blood sphaerosporosis of common carp in Europe. *Parasitology* 86:529–535
- Lom J, Dyková I, Pavlásková M (1983b) “Unidentified” mobile protozoans from the blood of carp and some unsolved problems of myxosporean life cycles. *J Protozool* 30:497–508
- Lom J, Noble ER (1984) Revised classification of the class Myxosporea Bütschli, 1881. *Folia Parasitol* 31:193–205
- Lom J, Pavlaskova M, Dyková I (1985) Notes on kidney-infecting species of the genus *Sphaerospora* Thélohan (Myxosporea), including a new species *S. gobionis* sp.nov., and on myxosporean life cycle stages in the blood of some freshwater fish. *J Fish Dis* 8:221–232
- Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. *J Fish Dis* 12:151–156
- Lom J, Dyková I, Feist S (1989a) Myxosporea-induced xenoma formation in pike (*Esox lucius* L.) renal corpuscles associated with *Myxidium lieberkuehni* infection. *Eur J Parasitol* 24:271–280
- Lom J, Desser SS, Dyková I (1989b) Some little-known and new protozoan parasites of fish from Lake Sasajewun, Algonquin Park, Ontario. *Can J Zool* 67:1372–1379
- Lom J, Pike AW, Feist SW (1991) Myxosporean vegetative stages in the choroidal rete mirabile of *Gasterosteus aculeatus* infected with *Myxobilatus gasterostei* and *Sphaerospora elegans*. *Dis Aquat Organ* 11:67–72
- Lom J, Dyková I (1992) Chapter 7 Myxosporidia (Phylum Myxozoa). In: Lom J, Dyková I (eds) *Protozoan parasites of fish. Developments in Aquaculture and Fisheries Science*, Elsevier, Amsterdam, 26:159–235
- Lom J, Dyková I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol* 53:1–36
- Longshaw M, Le Deuff RM, Harris AF, Feist SW (2002) Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to *Tetracapsula bryosalmonae* infected bryozoans. *J Fish Dis* 25:443–449
- Longshaw M (2004) Studies of myxozoan parasites of freshwater fish and invertebrate hosts. Doctoral dissertation. University of Plymouth, England
- Marcus E (1941) Sobre Bryozoa do Brasil. In: Book 5. Boletim da Faculdade de Filosofia Ciências e letras, Universidade de Sao Paulo, Zoologica 5:2–208
- Marton S, Eszterbauer E (2011) The development of *Myxobolus pavlovskii* (Myxozoa: Myxobolidae) includes an echinactinomyxon-type actinospore. *Folia Parasitol* 58:157–163
- Marton S, Eszterbauer E (2012) The susceptibility of diverse species of cultured oligochaetes to the fish parasite *Myxobolus pseudodispar* Gorbunova (Myxozoa). *J Fish Dis* 35:303–314
- Martone CB, Spivak E, Busconi L, Folco EJE, Sánchez JJ (1999) A cysteine protease from myxosporean degrades host myofibrils in vitro. *Comp Biochem Phys B* 123:267–272
- Massard JA, Geimer G (2008) Global diversity of bryozoans (Bryozoa or Ectoprocta) in freshwater: an update. *Bull Soc Nat Luxemb* 109:139–148
- McGeorge J, Sommerville C, Wootten R (1994) Light and electron microscope observations on extrasporogonic and sporogonic stages of a myxosporean parasite of the genus *Sphaerospora* Thélohan, 1892 from Atlantic salmon, *Salmo solar* L., in Scotland. *J Fish Dis* 17:227–238
- McGeorge J, Sommerville C, Wootten R (1997) Studies of actinosporean myxozoan stages parasitic in oligochaetes from the sediments of a hatchery where Atlantic salmon harbour *Sphaerospora truttae* infection. *Dis Aquat Organ* 30:107–119

- McGurk C, Morris DJ, Bron JE, Adams A (2005) The morphology of *Tetracapsuloides bryosalmonae* (Myxozoa : Malacosporea) spores released from *Fredericella sultana* (Bryozoa : Phylactolaemata). *J Fish Dis* 28:307–312
- McGurk C, Morris DJ, Auchinachie NA, Adams A (2006a) Development of *Tetracapsuloides bryosalmonae* (Myxozoa : Malacosporea) in bryozoan hosts (as examined by light microscopy) and quantitation of infective dose to rainbow trout (*Oncorhynchus mykiss*). *Vet Parasitol* 135:249–257
- McGurk C, Morris DJ, Adams A (2006b) Sequential development of *Buddenbrockia plumatellae* (Myxozoa : Malacosporea) within *Plumatella repens* (Bryozoa : Phylactolaemata). *Dis Aquat Organ* 73:159–169
- Molnár K (1980) Renal sphaerosporosis in the common carp *Cyprinus carpio* L. *J Fish Dis* 3:11–19
- Molnár K, Kovács-Gayer E (1986) Experimental induction of *Sphaerospora renicola* (Myxosporea) infection in the common carp (*Cyprinus carpio*) by transmission of SB-protzoans. *J Appl Ichthyol* 2:86–94
- Molnár K (1991) *Sphaerospora danubialis* sp. n. (Myxosporea: Sphaerosporidae) from the kidney of freshwater percid fishes. *Parasitol Hung* 24:53–58
- Molnár K (1993a) *Sphaerospora siluri* n. sp. (Myxosporea: Sphaerosporidae) in the kidney of the sheatfish (*Silurus glanis*). *Acta Vet Hung* 41:341–347
- Molnár K (1993b) The occurrence of *Sphaerospora renicola* K-stages in the choroidal rete mirabile of the common carp. *Folia Parasitol* 40:175–180
- Molnár K (1994) Comments on the host, organ and tissue specificity of fish myxosporeans and on the types of their intrapiscine development. *Parasitol Hung* 27:5–20
- Molnár K, El-Mansy A, Székely C, Baska F (1999a) Development of *Myxobolus dispar* (Myxosporea : Myxobolidae) in an oligochaete alternate host, *Tubifex tubifex*. *Folia Parasitol* 46:15–21
- Molnár K, El-Mansy A, Székely C, Baska F (1999b) Experimental identification of the actinosporean stage of *Sphaerospora renicola* Dyková & Lom 1982 (Myxosporea : Sphaerosporidae) in oligochaete alternate hosts. *J Fish Dis* 22:143–153
- Molnár K, Marton S, Székely C, Eszterbauer E (2010) Differentiation of *Myxobolus* spp. (Myxozoa: Myxobolidae) infecting roach (*Rutilus rutilus*) in Hungary. *Parasitol Res* 107:1137–1150
- Monteiro AS, Okamura B, Holland PWH (2002) Orphan worm finds a home: *Buddenbrockia* is a myxozoan. *Mol Biol Evol* 19:968–971
- Moran JDW, Margolis L, Webster JM, Kent ML (1999a) Development of *Kudoa thyrsites* (Myxozoa : Myxosporea) in netpen-reared Atlantic salmon determined by light microscopy and a polymerase chain reaction test. *Dis Aquat Organ* 37:185–193
- Moran JDW, Whitaker DJ, Kent ML (1999b) Natural and laboratory transmission of the marine myxozoan parasite *Kudoa thyrsites* to Atlantic salmon. *J Aquat Anim Health* 11:110–115
- Morris DJ, Adams A, Richards RH (2000) In situ hybridisation identifies the gill as a portal of entry for PKX (Phylum Myxozoa), the causative agent of proliferative kidney disease in salmonids. *Parasitol Res* 86:950–956
- Morris DJ, Adams A (2006a) Transmission of freshwater myxozoans during the asexual propagation of invertebrate hosts. *Int J Parasitol* 36:371–377
- Morris DJ, Adams A (2006b) Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa : Malacosporea), the causative organism of salmonid proliferative kidney disease, to the freshwater bryozoan *Fredericella sultana*. *Parasitology* 133:701–709
- Morris DJ, Adams A (2007a) Sacculogenesis and sporogony of *Tetracapsuloides bryosalmonae* (Myxozoa : Malacosporea) within the bryozoan host *Fredericella sultana* (Bryozoa : Phylactolaemata). *Parasitol Res* 100:983–992
- Morris DJ, Adams A (2007b) Sacculogenesis of *Buddenbrockia plumatellae* (Myxozoa) within the invertebrate host *Plumatella repens* (Bryozoa) with comments on the evolutionary relationships of the Myxozoa. *Int J Parasitol* 37:1163–1171
- Morris DJ, Adams A (2008) Sporogony of *Tetracapsuloides bryosalmonae* in the brown trout *Salmo trutta* and the role of the tertiary cell during the vertebrate phase of myxozoan life cycles. *Parasitology* 135:1075–1092
- Morris DJ (2010) Cell formation by myxozoan species is not explained by dogma. *Proc R Soc B-Biological Sci* 277:2565–2570
- Morris DJ (2012) A new model for myxosporean (Myxozoa) development explains the endogenous budding phenomenon, the nature of cell within cell life stages and evolution of parasitism from a cnidarian ancestor. *Int J Parasitol* 42:829–840
- Moser M, Kent ML, Dennis D (1989) Gall-Bladder Myxosporea in coral-reef fishes from Heron Island, Australia. *Aust J Zool* 37:1–13
- Moshu AJ (1992) Description of *Sphaerospora luciopercae*, sp. n. (Protista: Myxosporea) - Parasite of the European Pikeperch *Stizostedion Lucioperca* (L.). *Bul. Akad. Stiinte Republicii Moldova*. 2:54–56

- Moshu AJ, Trombitsky ID (2008) *Sphaerospora zingeli* sp. n. (Myxozoa: Myxosporea), kidney parasite of *Zingel zingel* (Linnaeus, 1766) (Percidae) from the Dniester River. In: International Conference Transboundary Dniester river basin management and the EU water Framework Directive. Chisinau, Republic of Moldova. p 204–208
- Moshu AJ, Trombitsky ID (2007) Two new cnidosporean species (Cnidosporea: Sphaerosporidae, Myxobolidae), parasites of the european mudminnow (*Umbra krameri*) from lower Dniester River. Bull Acad Sci Mol Life Sci Nr. 2. p 81–89.
- Muñoz P, Cuesta A, Athanassopoulou F, Golomazou H, Crespo S, Padros F, Sitjà-Bobadilla A, Albinana G, Esteban MA, Alvarez-Pellitero P, Meseguer J (2007) Sharpnose sea bream (*Diplodus puntazzo*) humoral immune response against the parasite *Enteromyxum leei* (Myxozoa). Fish Shellfish Immun 23:636–645
- Naldoni J, Zatti JA, Capodifoglio KR, Milanin T, Maia AA, Silva MR, Adriano EA (2015) Host-parasite and phylogenetic relationships of *Myxobolus filamentum* sp. n. (Myxozoa: Myxosporea), a parasite of *Brycon orthotaenia* (Characiformes: Bryconidae) in Brazil. Folia Parasitol 62:14
- Nesnidal MP, Helmkamp M, Bruchhaus I, El-Matbouli M, Hausdorf B (2013) Agent of Whirling Disease meets orphan worm: phylogenomic analyses firmly place Myxozoa in Cnidaria. PLoS One 8
- Nemeczek A (1926) Beiträge zur Kenntnis der Myxosporidien fauna Brasiliens. In Archiv für Protistenkunde 54:137–149
- Noble ER (1939) Myxosporidia from tide pool fishes of California. J Parasitol 25:359–363
- Ohnishi T, Kikuchi Y, Furusawa H, Kamata Y, Sugita-Konishi Y (2013) *Kudoa septempunctata* invasion increases the permeability of human intestinal epithelial monolayer. Foodborne Pathog Dis 10:137–142
- Okamura B (1996) Occurrence, prevalence, and effects of the myxozoan *Tetracapsula bryozoides* parasitic in the freshwater bryozoan *Cristatella mucedo* (Bryozoa: Phylactolaemata). Folia Parasitol 43:262–266
- Okamura B, Curry A, Wood TS, Canning EU (2002) Ultrastructure of *Buddenbrockia* identifies it as a myxozoan and verifies the bilaterian origin of the Myxozoa. Parasitology 124:215–223
- Okamura B, Canning EU (2003) Orphan worms and, homeless parasites enhance bilaterian diversity. Trends Ecol Evol 18:633–639
- Okamura B, Gruhl A (2015) Myxozoan Affinities and Route to Endoparasitism. In: Okamura B, Gruhl A, Bartholomew JL (eds) Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, p 23–44
- Okamura B, Gruhl A, Reft AJ (2015) Cnidarian Origins of the Myxozoa. In: Okamura B, Gruhl A, Bartholomew JL (eds) Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, p 45–68
- Okamura B, Gruhl A (2016) Myxozoa plus *Polypodium*: A Common Route to Endoparasitism. Trends Parasitol 32:268–271
- Özer A, Wootton R (2000) The life cycle of *Sphaerospora truttae* (Myxozoa : Myxosporea) and some features of the biology of both the actinosporean and myxosporean stages. Dis Aquat Organ 40:33–39
- Palenzuela O, Alvarez-Pellitero P, Sitjà-Bobadilla A (1999) Glomerular disease associated with *Polysporoplasma sparis* (Myxozoa) infections in cultured gilthead sea bream, *Sparus aurata* L. (Pisces : Teleostei). Parasitology 118:245–256
- Paperna I, Cave DD (2001) Kidney sphaerosporosis with extrasporogonic stages in the blood in *Baryancistrus* sp. (Loricaridae) from Amazonian Brazil. Parasite 8:71–74
- Patra S, Hartigan A, Holzer AS (2013) An approach to solve the puzzles created by the members of the *Sphaerospora sensu stricto* clade (myxozoa). In: 2nd annual meeting of European Centre of Ichthyoparasitology. Hradec nad Moravici, Czech Republic
- Patra S, Hartigan A, Holzer AS (2014) Preliminary attempts to reveal the life cycle of *Sphaerospora molnari* (Myxozoa). In: 7th International symposium on aquatic animal health, Oregon, USA
- Patra S, Hartigan A, Morris DJ, Kodádková A, Holzer AS (2017) Description and experimental transmission of *Tetracapsuloides vermiformis* n. sp. (Cnidaria: Myxozoa) and guidelines for describing malacosporean species including reinstatement of *Buddenbrockia bryozoides* n. comb. (syn. *Tetracapsula bryozoides*). Parasitology 144:497–511
- Patra S, Bartošová-Sojtková, P Pecková H, Fiala I, Eszterbauer E, Holzer AS Biodiversity and host-parasite cophylogeny of *Sphaerospora sensu stricto* (Cnidaria: Myxozoa). Manuscript in advanced preparation
- Plehn M (1925) Eine neue Schleienkrankheit. In, Book 28. Allgem. Fischerei-ZTG. (Neudamm) 28:299–300
- Polyanskii YI (1955) Materialy po parazitologii ryb severnykh morei SSSR. Parazity ryb Barentseva morya (Data on fish parasitology of the northern seas of USSR. Fish parasites of Barents Sea). In: Tr. Zool. Inst. Akad. Nauk SSSR, Vol. 19. (Israel Program for Scientific Translations, Jerusalem 1966).
- Pote LM, Hanson LA, Shivaji R (2000) Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp (Myxozoa : Myxosporea). J Aquat Anim Health 12:230–240

- Prunescu C-C, Prunescu P, Pucek Z, Lom J (2007) The first finding of myxosporean development from plasmodia to spores in terrestrial mammals: *Soricimyxum fegati* gen. et sp. n. (Myxozoa) from *Sorex araneus* (Soricomorpha). *Folia Parasitol* 54:159–164
- Rácz OZ, Székely C, Molnár K (2004) Intraoligochaete development of *Myxobolus intimus* (Myxosporea: Myxobolidae), a gill myxosporean of the roach (*Rutilus rutilus*). *Folia Parasitol* 51:199–207
- Rangel LF, Santos MJ, Cech G, Székely C (2009) Morphology, molecular data, and development of *Zschokkella mugilis* (Myxosporea, Bivalvulida) in a polychaete alternate host, *Nereis diversicolor*. *J Parasitol* 95:561–569
- Rangel LF, Cech G, Székely C, Santos MJ (2011) A new actinospore type Unicapsulactinomaxon (Myxozoa), infecting the marine polychaete, *Diopatra neapolitana* (Polychaeta: Onuphidae) in the Aveiro Estuary (Portugal). *Parasitology* 138:698–712
- Rangel LF, Rocha S, Castro R, Severino R, Casal G, Azevedo C, Cavaleiro F, Santos MJ (2015) The life cycle of *Ortholinea auratae* (Myxozoa: Ortholineidae) involves an actinospore of the triactinomaxon morphotype infecting a marine oligochaete. *Parasitol Res* 114:2671–2678
- Rangel LF, Castro R, Rocha S, Severino R, Casal G, Azevedo C, Cavaleiro F, Santos MJ (2016) *Tetractinomaxon* stages genetically consistent with *Sphaerospora dicentrarchi* (Myxozoa: Sphaerosporidae) found in *Capitella* sp. (Polychaeta: Capitellidae) suggest potential role of marine polychaetes in parasite's life cycle. *Parasitology* 143:1067–1073
- Ray RA, Holt RA, Bartholomew JL (2012) Relationship between temperature and *Ceratomyxa shasta*-induced mortality in Klamath River salmonids. *J Parasitol* 98:520–526
- Redondo MJ, Palenzuela O, Riaza A, Macias A, Alvarez-Pellitero P (2002) Experimental transmission of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. *J Parasitol* 88:482–488
- Redondo MJ, Palenzuela O, Alvarez-Pellitero P (2004) Studies on transmission and life cycle of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. *Folia Parasitol* 51:188–198
- Robledo D, Ronza P, Harrison PW, Losada AP, Bermúdez R, Pardo BG, Redondo MJ, Sitjà-Bobadilla A, Quiroga MI, Martínez P (2014) RNA-seq analysis reveals significant transcriptome changes in turbot (*Scophthalmus maximus*) suffering severe enteromyxosis. *Bmc Genomics* 15:1149
- Rocha S, Casal G, Rangel L, Severino R, Castro R, Azevedo C, Santos MJ (2013) Ultrastructural and phylogenetic description of *Zschokkella auratis* sp. nov. (Myxozoa), a parasite of the gilthead seabream *Sparus aurata*. *Dis Aquat Organ* 107:19–30
- Rocha S, Casal G, Rangel L, Castro R, Severino R, Azevedo C, Santos MJ (2015) Ultrastructure and phylogeny of *Ceratomyxa auratae* n. sp. (Myxosporea: Ceratomyxidae), a parasite infecting the gilthead seabream *Sparus aurata* (Teleostei: Sparidae). *Parasitol Int* 64:305–313
- Ronza P, Robledo D, Bermúdez R, Losada AP, Pardo BG, Sitjà-Bobadilla A, Quiroga MI, Martínez P (2016) RNA-seq analysis of early enteromyxosis in turbot (*Scophthalmus maximus*): new insights into parasite invasion and immune evasion strategies. *Int J Parasitol* 46:507–517
- Rosser TG, Griffin MJ, Quiniou SMA, Khoo LH, Greenway TE, Wise DJ, Pote LM (2015) Small subunit ribosomal RNA sequence links the myxospore stage of *Henneguya mississippiensis* n. sp. from channel catfish *Ictalurus punctatus* to an actinospore released by the benthic oligochaete *Dero digitata*. *Parasitol Res* 114:1595–1602
- Ruidisch S, Elmatbouli M, Hoffmann RW (1991) The role of tubificid worms as an intermediate host in the life-cycle of *Myxobolus pavlovskii* (Akhmerov, 1954). *Parasitol Res* 77:663–667
- Rumyantsev EA (1997) *Leptotheca schulmani* sp. n. (Myxosporidia: Ceratomyxidae)-a parasite of coregonid fishes (Coregonidae). *Parazitologiya* 5:466
- Ryce EKN (2003) Factors affecting the resistance of juvenile rainbow trout to whirling disease. Doctoral dissertation. Montana State University, Bozeman
- Ryland JS (2005) Bryozoa: an introductory overview. *Denisia* 16:9–20
- Sanders JL, Jaramillo AG, Ashford JE, Feist SW, Lafferty KD, Kent ML (2015) Two myxozoans from the urinary tract of topmelt, *Atherinops affinis*. *J Parasitol* 101:577–586
- Sarkar NK, Ghosh S (1991) Two new coelozoic Myxosporida (Myxozoa: Myxosporea) from estuarine teleost fishes (Mugilidae) of West Bengal, India. *Zool Soc* 44:131–135
- Sarkar NK (2010) Seven new Myxosporea (Myxozoa: Bivalvulida) from marine fishes of the Bay of Bengal off West Bengal coast, India. *J Environ Sociobiol* 7:7–16
- Saulnier D, Brémont M, Kinkelin PD (1996) Cloning, sequencing and expression of a cDNA encoding an antigen from the myxosporean parasite causing the proliferative kidney disease of salmonid fish. *Mol Biochem Parasit* 83:153–161

- Schlegel M, Lom J, Stechmann A, Bernhard D, Leipe D, Dyková I, Sogin ML (1996) Phylogenetic analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: Evidence that Myxozoa are Metazoa and related to the Bilateria. Arch Für Protistenkd 147:1–9
- Schmidt-Posthaus H, Bettge K, Forster U, Segner H, Wahli T (2012) Kidney pathology and parasite intensity in rainbow trout *Oncorhynchus mykiss* surviving proliferative kidney disease: time course and influence of temperature. Dis Aquat Organ 97:207–218
- Schröder O (1910) *Buddenbrockia plumatella*, eine neue Mesozoenart aus *Plumatella repens* L. und *Plumatella fungosa* Pall. Z Wiss Zool 96:525–537
- Schröder O (1912) Zur Kenntnis der *Buddenbrockia plumatellae* Ol. Schröder. Zeitschrift für wissenschaftliche Zoologie 102:79–91
- Schuermans-Stekhoven JJH (1920) Ueber einige Myxosporidien des Stichling. In, Arch Für Protistenkd 41:321–329
- Seagrave C, Bucke D, Alderman D (1980) The causative agent of Proliferative Kidney Disease may be a member of the Haplosporidia. In: Ahne W (ed) Fish Disease Third CORPAQ-Session Verlag, Berlin p 174–181
- Seipel K, Schmid V (2006) Mesodermal anatomies in cnidarian polyps and medusae. Int J Dev Biol 50:589–599
- Shin SP, Nguyen VG, Jeong JM, Jun JW, Kim JH, Han JE, Baek GW, Park SC (2014) The phylogenetic study on *Thelohanellus* species (Myxosporea) in relation to host specificity and infection site tropism. Mol Phylogenet Evol 72:31–34
- Shinn AP, Pratoomyot J, Bron JE, Paladini G, Brooker EE, Brooker AJ (2015) Economic costs of protistan and metazoan parasites to global mariculture. Parasitology 142:196–270
- Shpirer E, Chang ES, Diamant A, Rubinstein N, Cartwright P, Huchon D (2014) Diversity and evolution of myxozoan minicollagens and nematogalectins. BMC Evol Biol 14: 205
- Shul'man SS (1966) Myxosporidia of the USSR, Nauka, Moscow-Leningrad Translated version published for the United States Department of the Interior and National Science Foundation, Amerind Publishing Co. Pvt. Ltd., New Delhi, India 1988, p 631
- Siddall ME, Martin DS, Bridge D, Desser SS, Cone DK (1995) The demise of a phylum of protists: Phylogeny of Myxozoa and other parasitic Cnidaria. J Parasitol 81:961–967
- Sitjà-Bobadilla A, Alvarez-Pellitero P (1990) *Sphaerospora testicularis* sp. nov. (Myxosporea: Sphaerosporidae) in wild and cultured sea bass, *Dicentrarchus labrax* (L.), from the Spanish Mediterranean area. J Fish Dis 13:193–203
- Sitjà-Bobadilla A, Alvarez-Pellitero P (1992) Light and Electron Microscopic description of *Sphaerospora dicentrarchi* n. sp. (Myxosporea: Sphaerosporidae) from wild and cultured sea bass, *Dicentrarchus labrax* L. J Protozool 39:273–281
- Sitjà-Bobadilla A, Alvarez-Pellitero P (1993) Pathologic effects of *Sphaerospora dicentrarchi* Sitjà-Bobadilla and Alvarez-Pellitero, 1992 and *S. testicularis* Sitjà-Bobadilla and Alvarez-Pellitero, 1990 (Myxosporea: Bivalvulida) parasitic in the Mediterranean sea bass *Dicentrarchus labrax* L. (Teleostei: Serranidae) and the cell-mediated immune reaction: A light and electron microscopy study. Parasitol Res 79:119–129
- Sitjà-Bobadilla A, Alvarez-Pellitero P (1994) Revised classification and key species of the genus *Sphaerospora* Davies, 1917 (Protozoa: Myxosporea). Res Rev Parasitol 54:67–80
- Sitjà-bobadilla A, Alvarez-Pellitero P (1995) Light and electron microscopic description of *Polysporoplasma* n. g. (Myxosporea: Bivalvulida), *Polysporoplasma sparis* n. sp. from *Sparus aurata* (L.), and *Polysporoplasma mugilis* n. sp. from *Liza aurata* L. Eur J Protistol 31:77–89
- Sitjà-Bobadilla A, Alvarez-Pellitero P (2001) *Leptotheca sparidarum* n. sp. (Myxosporea : Bivalvulida), a parasite from cultured common dentex (*Dentex dentex* L.) and Gilthead sea bream (*Sparus aurata* L.) (Teleostei : Sparidae). J Eukaryot Microbiol 48:627–639
- Sitjà-Bobadilla A, Redondo MJ, Bermúdez R, Palenzuela O, Ferreira I, Rianza A, Quiroga I, Nieto JM, Alvarez-Pellitero P (2006) Innate and adaptive immune responses of turbot, *Scophthalmus maximus* (L.), following experimental infection with *Enteromyxum scophthalmi* (Myxosporea : Myxozoa). Fish Shellfish Immunol 21:485–500
- Sitjà-Bobadilla A, Palenzuela O, Rianza A, Macias MA, Alvarez-Pellitero P (2007) Protective Acquired Immunity to *Enteromyxum scophthalmi* (Myxozoa) is Related to Specific Antibodies in *Psetta maxima* (L.) (Teleostei). Scand J Immunol 66:26–34
- Sitja-Bobadilla A, Calduch-Giner J, Saera-Vila A, Palenzuela O, Alvarez-Pellitero P, Perez-Sanchez J (2008) Chronic exposure to the parasite *Enteromyxum leei* (Myxozoa : Myxosporea) modulates the immune response and the expression of growth, redox and immune relevant genes in gilthead sea bream, *Sparus aurata* L. Fish Shellfish Immunol 24:610–619
- Smothers JF, Vondohlen CD, Smith LH, Spall RD (1994) Molecular evidence that the myxozoan protists are metazoans. Science 265:1719–1721

- Song Y, Gokhale CS, Papkou A, Schulenburg H, Traulsen A (2015) Host-parasite coevolution in populations of constant and variable size. *Bmc Evol Biol* 15: 212
- Sterud E, Simolin P, Kvellestad A (2003) Infection by *Parvicapsula* sp. (Myxozoa) is associated with mortality in sea-caged Atlantic salmon *Salmo salar* in northern Norway. *Dis Aquat Organ* 54:259–263
- Sterud E, Forseth T, Ugedal O, Poppe TT, Jorgensen A, Bruheim T, Fjeldstad HP, Mo TA (2007) Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* (Myxozoa). *Dis Aquat Organ* 77:191–198
- Stewart K, Ma H, Zheng J, Zhao J (2017) Using environmental DNA to assess population-wide spatiotemporal reserve use. *Conserv Biol* (In Press)
- Štolc A (1899) Actinomyxidia, eine neue Gruppe der Mesozoa, der Myxosporidien verwandt. 8:1–12
- Su XQ, White RWG (1994) New myxosporeans (Myxozoa, Myxosporidia) from marine fishes of Tasmania, Australia. *Acta Protozool* 33:251–259
- Supamattaya K, Fischerscherl T, Hoffmann RW, Boonyaratpalin S (1991) *Sphaerospora epinepheli* n. sp. (Myxosporidia: Sphaerosporidae) observed in grouper (*Epinephelus malabaricus*). *J Protozool* 38:448–454
- Supamattaya K, Fischerscherl T, Hoffmann RW, Boonyaratpalin S (1993) Light and Electron Microscope Observations on presporogonic and sporogonic stages of *Sphaerospora Epinepheli* (Myxosporidia) in grouper (*Epinephelus Malabaricus*) *J Eukaryot Microbiol* 40:71–80
- Swearer SE, Robertson DR (1999) Life history, pathology, and description of *Kudoa ovivora* n. sp (Myxozoa, Myxosporidia): an ovarian parasite of Caribbean labroid fishes. *J Parasitol* 85:337–353
- Székely C, El-Mansy A, Molnár K, Baska F (1998) Development of *Thelohanellus hovorkai* and *Thelohanellus nikolskii* (Myxosporidia : Myxozoa) in oligochaete alternate hosts. *Fish Pathol* 33:107–114
- Székely C, Molnár K, Eszterbauer E, Baska F (1999) Experimental detection of the actinospores of *Myxobolus pseudodispar* (Myxosporidia : Myxobolidae) in oligochaete alternate hosts. *Dis Aquat Organ* 38:219–224
- Székely C, Rác O, Molnár K, Eszterbauer E (2002) Development of *Myxobolus macrocapsularis* (Myxosporidia : Myxobolidae) in an oligochaete alternate host, *Tubifex tubifex*. *Dis Aquat Organ* 48:117–123
- Székely C, Hallett SL, Atkinson SD, Molnár K (2009) Complete life cycle of *Myxobolus rotundus* (Myxosporidia: Myxobolidae), a gill myxozoan of common bream *Abramis brama*. *Dis Aquat Organ* 85:147–155
- Székely C, Borkhanuddin MH, Cech G, Kelemen O, Molnár K (2014) Life cycles of three *Myxobolus* spp. from cyprinid fishes of Lake Balaton, Hungary involve triactinomyxon-type actinospores. *Parasitol Res* 113:2817–2825
- Székely C, Cech G, Atkinson SD, Molnár K, Egyed L, Gubányi A (2015) A novel myxozoan parasite of terrestrial mammals: description of *Soricimyxum minuti* sp. n.(Myxosporidia) in pygmy shrew *Sorex minutus* from Hungary. *Folia Parasitol* 62:045
- Tajdari J, Matos E, Mendonca I, Azevedo C (2005) Ultrastructural morphology of *Myxobolus testicularis* sp. n., parasite of the testis of *Hemiodopsis microlepis* (Teleostei : Hemiodontidae) from the NE of Brazil. *Acta Protozool* 44:377–384
- Takizawa F, Gómez D, Parra D, Zhang YA, Bjork S, Bartholomew J, Wiens GD, Sunyer JO (2013) Identification of regulatory B and T cell subsets in teleost fish. In: 1st international conference of fish and shellfish immunology, Vigo, Spain
- Taticchi MI, Gustinelli A, Fioravanti ML, Caffara M, Pieroni G, Prearo M (2004) Is the worm-like organism found in the statoblasts of *Plumatella fungosa* (Bryozoa, Phylactolaemata) the vermiform phase of *Tetracapsuloides bryosalmonae* (Myxozoa, Malacosporidia)? *Ital J Zool* 71:143–146
- Technau U, Scholz CB (2003) Origin and evolution of endoderm and mesoderm. *Int J Dev Biol* 47:531–539
- Thélohan P (1892) Observations sur les Myxosporidies et essai de classification de ces organismes. Présidence de M Franchet 4:165–178
- Thélohan P (1895) Recherches sur les Myxosporidies. *Bull Biol Fr Bel* 26:100–394
- Toledo-Guedes K, Sanchez-Jerez P, Mora-Vidal J, Girard D, Brito A (2012) Escaped introduced sea bass (*Dicentrarchus labrax*) infected by *Sphaerospora testicularis* (Myxozoa) reach maturity in coastal habitats off Canary Islands. *Mar Ecol-Evol Persp* 33:26–31
- Tops S, Hartikainen H, Okamura B (2009) The effects of infection by *Tetracapsuloides bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). *Int J Parasitol* 39:1003–1010
- Tripathi YR (1948) Some new myxosporidia from Plymouth with a proposed new classification of the order. 39:110–119
- Trouillier A, ElMatbouli M, Hoffmann W (1996) A new look at the life-cycle of *Hoferellus carassii* in the goldfish (*Carassius auratus auratus*) and its relation to kidney enlargement disease (KED). *Folia Parasitol* 43:173–187
- Tun T, Yokoyama H, Ogawa K, Wakabayashi H (2000) Myxosporeans and their hyperparasitic microsporeans in the intestine of emaciated tiger puffer. *Fish Pathol* 35:145–156

- U-taynapun K, Chirapongsatankul N, Maneesaay P, Itami T, Tantikitti C (2012) A new host record of *Sphaerospora epinepheli* (Myxosporea: Bivalvulida) occurring on orange-spotted grouper *Epinephelus coioides* from Thailand: Epidemiology, histopathology and phylogenetic position. *Vet Parasitol* 188:215–224
- Urawa S (1994) Life cycle of *Myxobolus arcticus*, a myxosporean parasite of salmonid fishes. *Int Symp Aquat Anim Heal*, Seattle, 4–8 September 1994, Book of Abstracts, P-W-10.3
- Urawa S, Freeman MA, Johnson SC, Jones SRM, Yokoyama H (2011) Geographical variation in spore morphology, gene sequences, and host specificity of *Myxobolus arcticus* (Myxozoa) infecting salmonid nerve tissues. *Dis Aquat Organ* 96:229–37
- Uspenskaya AV (1995) Alternation of actinosporean and myxosporean phases in the life-cycle of *Zschokkella nova* (myxozoa). *J Eukaryot Microbiol* 42:665–668
- Wales JH, Wolf H (1955) Three protozoan diseases of trout in California. *California Fish Game* 41: 183–187
- Waluga D, Budzynska H (1980) *Sphaerospora* invasion as the cause of a massive mortality in carp populations. *Gospodarka rybna* 7:5–7
- Weidner E, Overstreet RM (1979) Sporogenesis of a myxosporidan with motile spores. *Cell Tissue Res* 201:331–342
- Weill R (1938) L'interprétation des cnidosporidies et la valeur taxonomique de leur cnidome. Leur cycle comparé a la phase larvaire des narcoméduses cuninides. *Travaux de la Station Zoologique de Wimereaux* 13: 727–744
- Whipps CM, Adlard RD, Bryant MS, Lester RJG, Findlay V, Kent ML (2003) First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* n. sp. from sweeper (*Pempheris ypsilychnus*). *J Eukaryot Microbiol* 50:215–219
- Whipps CM, El-Matbouli M, Hedrick RP, Blazer V, Kent ML (2004a) *Myxobolus cerebralis* internal transcribed spacer 1 (ITS-1) sequences support recent spread of the parasite to North America and within Europe. *Dis Aquat Organ* 60:105–108
- Whipps CM, Gossel G, Adlard RD, Yokoyama H, Bryant MS, Munday BL, Kent ML (2004b) Phylogeny of the multivalvulidae (Myxozoa : Myxosporea) based on comparative ribosomal DNA sequence analysis. *J Parasitol* 90:618–622
- Whipps CM, Kent ML (2006) Phylogeography of the cosmopolitan marine parasite *Kudoa thyrsites* (Myxozoa: Myxosporea). *J Eukaryot Microbiol* 53:364–373
- Wolf K, Markiw ME (1984) Biology contravenes taxonomy in the Myxozoa - new discoveries show alternation of invertebrate and vertebrate hosts. *Science* 225:1449–1452
- Wolf K, Markiw ME, Hiltunen JK (1986) Salmonid whirling disease: *Tubifex tubifex* (Müller) identified as the essential oligochaete in the protozoan life cycle. *J Fish Dis* 9:79–81
- Wood TS (2001) Bryozoans. In: Thorp J, Covich A (eds) *Ecology and classification of North American freshwater invertebrates*, p 505–525
- Xi BW, Zhang JY, Xie J, Pan LK, Xu P, Ge XP (2013) Three actinosporean types (Myxozoa) from the oligochaete *Branchiura sowerbyi* in China. *Parasitol Res* 112:1575–1582
- Xi BW, Zhou ZG, Xie J, Pan LK, Yang YL, Ge XP (2015) Morphological and molecular characterization of actinosporeans infecting oligochaete *Branchiura sowerbyi* from Chinese carp ponds. *Dis Aquat Organ* 114:217–228
- Xiao C, Desser SS (1997) *Sphaerospora ovophila* n. sp. and *Myxobolus algonquinensis* n sp (Myxozoa, Myxosporea), ovarian parasites of fish from Algonquin Park, Ontario, Canada. *J Eukaryot Microbiol* 44:157–161
- Xiao C, Feng S (1997) Description of new species of Myxosporidia from freshwater fishes of Wuling Mountains area, China. In: Song, DX (ed) *Invertebrates of Wuling Mountains area, Southwestern China*, p 168–196
- Yahata Y, Sugita-Konishi Y, Ohnishi T, Toyokawa T, Nakamura N, Taniguchi K, Okabe N (2015) *Kudoa septempunctata* - Induced gastroenteritis in humans after Flounder Consumption in Japan: a case - controlled study. *Jpn J Infect Dis* 68:119–123
- Yang CZ, Zhou Y, Zhao YJ, Huang W, Huang C (2017) Erection of *Unicapsulocaudum mugilum* gen. et sp nov (Myxozoa: Ceratomyxidae) based on its morphological and molecular data. *J Nat Hist* 51:457–467
- Yasuda H, Ooyama T, Nakamura A, Iwata K, Palenzuela O, Yokoyama H (2005) Occurrence of the myxosporean emaciation disease caused by *Enteromyxum leei* in cultured Japanese flounder *Paralichthys olivaceus*. *Fish Pathol* 40:175–180
- Yokoyama H, Ogawa K, Wakabayashi H (1993) Involvement of *Branchiura sowerbyi* (Oligochaeta, Annelida) in the transmission of *Hofereilus carassii* (Myxosporea, Myxozoa), the causative agent of Kidney Enlargement Disease (KED) of goldfish *Carassius auratus*. *Fish Pathol* 28:135–139

- Yokoyama H, Ogawa K, Wakabayashi H (1995) *Myxobolus cultus* n. sp. (Myxosporea, Myxobolidae) in the goldfish *Carassius auratus* transformed from the actinosporean stage in the oligochaete *Branchiura sowerbyi*. J Parasitol 81:446–451
- Yokoyama H (1997) Transmission of *Thelohanellus hovorkai* Achmerov, 1960 (Myxosporea: Myxozoa) to common carp *Cyprinus carpio* through the alternate oligochaete host. Syst Parasitol 36:79–84
- Yokoyama H, Masuda K (2001) *Kudoa* sp. (Myxozoa) causing a post-mortem myoliquefaction of North-Pacific giant octopus *Paroctopus dofleini* (Cephalopoda : Octopodidae). Bull Eur Assoc Fish Pathol 21:266–268
- Yoshino TP, Moser M (1974) Myxosporidia (Protozoa) in macrourid fishes (*Coryphaenoides* spp.) of northeastern Pacific. J Parasitol 60:655–659
- Yurakhno VM, Maltsev VN (2002) New data on myxosporeans of mullets in the Atlantic Ocean basin. Ekol Morya 61:39–44
- Zhang Y, Salinas I, Li J, Parra D, Bjork S, Xu Z, La Patra SE, Bartholomew J, Sunyer JO (2010) IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nat immunol 11: 827–835
- Zhang ZQ (2011) Animal biodiversity: An outline of higher-level classification and taxonomic richness. Zootaxa 3148:1–237
- Zhao YJ, Song WB (2009) Myxozoan parasites of *Leptopthea Thélohan*, 1895 (Myxosporea, Bivalvulida) from marine fishes of Qingdao coastal waters off the Yellow Sea in China, with description of a new species. Acta Hydrobiol Sin 33:61–66
- Zhao YJ, Al-Farraj SA, Al-Rasheid KAS, Song WB (2015) Data on ten new myxosporean parasites (Myxozoa, Myxosporea, Bivalvulida) from the Yellow Sea, China. Acta Protozool 54:305–323
- Zhao DD, Borkhanuddin MH, Wang WM, Liu Y, Cech G, Zhai YH, Székely C (2016) The life cycle of *Thelohanellus kitauei* (Myxozoa: Myxosporea) infecting common carp (*Cyprinus carpio*) involves aurantiactinomycxon in *Branchiura sowerbyi*. Parasitol Res 115:4317–4325

Curriculum Vitae

SNEHA PATRA, M.Sc.

Laboratory of Fish Protistology
Institute of Parasitology; Biology Centre CAS & Faculty of Science
University of South Bohemia
Branisovska 31, 37005 Ceske Budejovice, Czech Republic
Email: snehampatra@gmail.com, patra@paru.cas.cz

Education

2012 May- present: Ph.D. in Parasitology, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Faculty of Science, University of South Bohemia, Czech Republic. Topic- Malacosporea and *Sphaerospora sensu stricto*: Myxozoan clades with unique biology and evolution.

2006-2008: Masters in Science (Subject Zoology with special paper Cytogenetics) from Dinabandhu Andrews College (affiliated to Calcutta University), India

2003-2006: Bachelors in Science (Subject Zoology) from Chandernagore College, University of Burdwan, India

Awards & Achievements

2009: Qualified for Research Eligibility Test, Visva Bharati University, India

2011-2012: Research visit (self-financed; 2 months) under the guidance of Astrid S. Holzer, Institute of Parasitology, Biology Centre CAS, Czech Republic

2014: Paper featured on cover: International Journal for Parasitology, July-2014 issue, Bartošová-Sojtková P., Hrabcováb M., Pecková H., **Patra S.**, Kodádková A., Jurajdac P., Tylm T., Holzer A.S. (2014) Hidden diversity and evolutionary trends in malacosporean parasites (Cnidaria: Myxozoa) identified using molecular phylogenetics. International Journal for Parasitology. 44, 565–577. doi: 10.1016/j.ijpara.2014.04.005

2014 and 2015: Scholarship for the foreign internship from the Faculty of Science, University of South Bohemia

2015: Young scientist travel grant, 9th International Symposium on Fish Parasites, Valencia, Spain

2015: 3rd best oral presentation, 9th International Symposium on Fish Parasites, Valencia, Spain

Professional experience

2010-2011: Contractual teacher (Subject- Biology and Life Science) in Tollygung Girl's High School, Kolkata, India

2014 and 2015: Teaching assistant of the practical course "Field parasitology" (Faculty of Science, University of South Bohemia, České Budějovice)

Scientific Workshops

2010: 9th Refresher courses on "Collection and preservation techniques of zoological specimens" organised by Training & extension division, Zoological survey of India, Kolkata, India

2013: Workshop on "Histology of fish, focusing on toxicological studies in trout and carp" organised by Faculty of fisheries and protection of waters, Vodňany, Czech Republic

Bench Skills

- **Molecular techniques:** DNA, RNA extraction; cDNA preparation; PCR; real time PCR; gel electrophoresis (Agarose, PAGE); nucleotide sequence alignment; primer designing; Phylogenetic analysis (RAxML, PAUP*, Mr. Bayes); handling Geneious v8.0.5, , EditSeq, DNASTar, BioEdit; *In-situ* hybridisation with Biotin and Digoxigenin (DIG) labelling; Western Blotting
- **Microscopy:** Light microscopy, Scanning Electron microscopy and Transmission Electron microscopy; usage of Image J software
- **Histology:** Gram staining (basic fuchsin) procedure in bacteria; Microtome and tissue processing and staining; Haematoxylin-Eosin staining, Giemsa staining
- ***Drosophila*: molecular techniques:** Polytene chromosome staining; imaginal disc preparation; study in genetic crosses; *Drosophila* chromosome mapping; P lac z experiment; X-Gal staining
- **Biochemistry:** Protein estimation; handling Colorimeter
- **Physiology:** Human karyotyping; handling experience *Drosophila* flies and larva
- **Haematology:** Experience in practical tests on Erythrocyte Sedimentation Rate (E.S.R.), ABO blood grouping, Coagulation Time, Bleeding Time, Hb concentration, Total Count, Differential Count, Haemin crystal in human blood
- **Statistical Analysis:** Chi square; Student T test; Correlation; Regression; Anova
- **Ecology:** Percentage saturation of dissolved oxygen; Spatial variations of oxygen concentration in water bodies; estimation of total hardness and alkalinity of water samples; estimation of chemical toxicity chemical; qualitative and quantitative estimation of zooplankton community, primary productivity and assessment of nutrient status aquatic body
- **Field study experience:** Study of biodiversity of an ecosystem; quadrat study; species diversity, abundance dominance calculation; Shannon-weaver index;

zooplanktons collection and preservation; Ethology of *ex-situ* conserved animal (giraffe)

- **Fish rearing:** Handling and rearing fish (common carp); *Artemia* culture

Conferences Presentations

As a presenter:

Patra S., Holzer A.S., Pecková H., Brennan N.P., Yanes-Roca C., Main K.L. (2013) *Sphaerospora motemari* n. sp. causes glomerular disease in juvenile grey snapper *Lutjanus griseus* L: A reason for host population declines in the Gulf of Mexico? (poster). 43rd Jirovec's Protzooloogical Days, Nový Dvůr, Czech Republic

Patra S., Hartigan A., Holzer A.S. (2013) An approach to solve the puzzles created by the members of the *Sphaerospora sensu stricto* clade (myxozoa) (talk) 2nd annual meeting of European Centre of Ichtyoparasitology, Hradec nad Moravicí, Czech Republic

Patra S., Hartigan A. and Holzer A.S. (2014) Preliminary attempts to reveal the life cycle of *Sphaerospora molnari* (Myxozoa) (talk) 7th International symposium on aquatic animal health, Oregon, USA

Patra S., Hartigan A., Morris D.J., Kodádková A., Holzer A.S. (2015) Description of a new *Tetracapsuloides* species and its life cycle – what are the features that best define a malacosporean species? (talk) 9th International Symposium on Fish Parasites, Valencia, Spain

Patra S., Fiala I., Bartošová-Sojtková P., Pecková H., Holzer A.S. (2016) Molecular diversity of new members of the *Sphaerospora sensu stricto* clade (Cnidaria: Myxozoa) (talk) 5th annual meeting of European Centre of Ichtyoparasitology, Prušánky, Czech Republic

As a co-author:

Holzer A.S., Bartošová P., Hartigan A., Alama-Bermejo G., **Patra S.**, Pecková H., Fiala I. (2012) Myxozoan parasite biodiversity, evolution, life cycle stages and host-parasite interaction, 1st annual meeting of European Centre of Ichtyoparasitology, Šatov, Czech Republic.

Hartigan A., Bartošová P., **Patra S.**, Pecková H., Eszterbauer E., Dyková I., Holzer A.S. (2012) A second look at swim bladder inflammation of common carp in Central Europe, 1st annual meeting of European Centre of Ichtyoparasitology, Šatov, Czech Republic

Bartošová P., Loudová M., Pecková H., **Patra S.**, Kodádková A., Holzer A.S. (2013) Hidden biodiversity and evolution trends in the malacosporean parasites (Cnidaria: Myxozoa), 43rd Jirovec's Protzooloogical Days, Nový Dvůr, Czech Republic

Bartošová P., Loudová M., Pecková H., **Patra S.**, Kodádková A., Holzer A.S. (2013) Revealing cryptic diversity of malacosporean parasites (Myxozoa: Cnidaria)

using molecular phylogenetics, European Association of Fish Pathologists, Tampere, Finland

- Hartigan A., **Patra S.**, Kodádková A., Fiala I., Holzer A.S. (2013) Laboratory of fish Protistology: biodiversity, strange biology and complex host-parasite relationships of Myxozoa, 2nd annual meeting of European Centre of Ichtyoparasitology, Hradec nad Moravicí, Czech Republic
- Holzer A.S., Hartigan A., **Patra S.**, Eszterbauer E. (2014) Emerging numbers of motile myxozoan blood stages in common carp – A closer look at *Sphaerospora molnari*, a parasite on the rise, 7th International symposium on aquatic animal health, Oregon, USA
- Hartigan A., **Patra S.**, Holzer A.S. (2015) Getting myxozoan parasites hot under the collar: a real-time PCR assay to quantify proliferative blood stages of *Sphaerospora molnari* in *Cyprinus carpio* in a range of temperatures, 9th International Symposium on Fish Parasites, Valencia, Spain

Publications

- Holzer AS, Pecková H, **Patra S**, Brennan NP, Yanes-Roca C, Main KL (2013) Severe glomerular disease in juvenile grey snapper *Lutjanus griseus* L. in the Gulf of Mexico caused by the myxozoan *Sphaerospora motemari* n. sp. International Journal for Parasitology: Parasites and Wildlife 2: 124–130. doi: 10.1016/j.ijppaw.2013.03.003
- Bartošová-Sojtková P, Hrabcová M, Pecková H, **Patra S**, Kodádková A, Jurajda P, Tým T, Holzer AS (2014) Hidden diversity and evolutionary trends in malacosporan parasites (Cnidaria: Myxozoa) identified using molecular phylogenetics. International Journal for Parasitology 44: 565–577. doi: 10.1016/j.ijpara.2014.04.005
- Holzer AS, Hartigan A, **Patra S**, Pecková H, Eszterbauer E (2014) Molecular fingerprinting of the myxozoan community in common carp suffering Swim Bladder Inflammation (SBI) identifies multiple etiological agents. Parasites & Vectors 7: 398. doi: 10.1186/1756-3305-7-398
- Hartigan A, Estensoro I, Vancová M, Bílý T, **Patra S**, Eszterbauer E, Holzer AS (2016) New cell motility model observed in parasitic cnidarian *Sphaerospora molnari* (Myxozoa:Myxosporae) blood stages in fish. Scientific Reports 6: 39093. doi: 10.1038/srep39093
- Patra S***, Hartigan A, Morris DJ, Kodádková A, Holzer AS (2017) Description and experimental transmission of *Tetracapsuloides vermiformis* n. sp. (Cnidaria: Myxozoa) and guidelines for describing malacosporan species including reinstatement of *Buddenbrockia bryozoides* n. comb. (syn. *Tetracapsula bryozoides*). Parasitology 144: 497-511. doi: 10.1017/S0031182016001931
- * Corresponding Author**

Publication (In Prep.)

Patra S, Bartošová-Sojková P, Pecková H, Fiala I, Eszterbauer E, Holzer AS. Biodiversity and host-parasite cophylogeny of *Sphaerospora sensu stricto* (Cnidaria: Myxozoa). Manuscript in advanced preparation.