Department of Pathology

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Myxosporean infections of common carp (*Cyprinus carpio*)

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1. Introduction

Myxosporeans are abundant and important parasites in various freshwater and marine fish. As metazoans, they consist of more than one cell and since they are of cnidarian origin, they are related to corals and jellyfish **(Okamura et al., 2015a).** Their complex two-host life cycle involves a great morphological variety of developmental stages. Two infective spore types play a crucial role for the invasion of a vertebrate intermediate host and the final invertebrate host as well as for the scientific identification of the parasite **(Gómez et al., 2014)**. To achieve a complete life cycle under laboratory conditions still proves challenging to researchers all over the world. Decades of controversial debates over taxonomy, nomenclature, origin and interrelations of myxozoan parasites are well documented and lead to confusion as well as an increasing interest within the scientific community. Scientific breakthroughs such as the detailed understanding of *Myxobolus cerebralis*, the causative agent of the economically and ecologically very significant salmonid whirling disease, additionally raised the awareness for myxosporean parasites, also among veterinarians. Some species are monoxenous and therefore known to parasitize only a single host species. However, the majority of myxosporeans is stenoxenous and therefore able to infect a certain range of closely related fish. A characteristical feature of those parasites is their well advanced tissue tropism. Independently from host species or tissue affinity, the parasitic life cycle begins intracellularly and it always involves stages in which further stages develop via a process called internal cleavage **(Molnár, 1994)** or plasmotomy **(Okamura et al., 2015a)**. By far not all myxosporeans are harmful but the more pathogenic species may attack sites such as the skin, gills, fins, muscles, swim bladder, kidney or intestines of their fish host **(Moser & Kent, 1994)**. The presporogonic and sporogonic development of those fish parasites are the main sources of pathological changes. Various degrees of inflammation, reparation and organization accompanied by a loss of tissue function result in problems such as growth retardation, decreased host resistance to other infectious and non-infectious diseases or even death of the affected fish. Grossly recognizable tissue cysts may additionally lead to irritations among consumers and significantly impair the market value of fish **(Roberts, 2012)**. Since farmed common carp production is about 9 % of the total global freshwater aquaculture with a main production and consumption in Asia and Europe **(FAO, 2017)**, the relevance of such infections is clearly given.

2. Aim and Goals

With this thesis, the author would like to give a general introduction on a complex class of fish-pathogenic parasites, with focus on the veterinary perspective on a few myxosporean parasites that are well-known pathogens for the popular food and pet fish species *Cyprinus carpio*. In this context, the main objective is to review and summarize the currently available literature on pathogenic myxosporean infections in common carp, including koi, and to highlight the dynamics and findings relevant for a practicing veterinarian. Over a period of almost two years, the author worked alongside a team of fish pathologists and parasitologists to conduct a series of infection experiments, which aimed to reproduce complete parasitic life cycles under laboratory conditions and to examine the myxosporean fauna present in Hungarian carp. The contents and results of this Diploma thesis may aid a better understanding of myxosporean infections in common carp and help to improve future experimental setups. The practical part of this Diploma thesis was subjected to the following tasks:

1. *Detection of actinospore shedding oligochaetes from Kis-Balaton Lake, Hungary and identification of the released actinospore type.*

2. *Experimental exposure of young common carp to the previously obtained actinospores and post-mortem examination for the presence of myxosporean infection.*

3. Literature Review

3.1. Basic Nomenclature and Taxonomy of pathogenic Myxosporeans

Myxozoans are obligate parasites belonging to the phylum of Cnidaria and the taxon of Metazoa within the animal kingdom, so they consist of several cells and are related to coral polyps, jellyfish, and hydra. Most Myxozoans depend on two different host types to complete their life cycle. Typically invertebrates such as aquatic annelid worms serve as final hosts, while vertebrates such as fish, frogs, birds or shrews act as intermediate hosts **(Moser & Kent, 1994; Okamura et al., 2015a)**. The phylum Myxozoa includes two major taxonomic classes, Malacosporea and Myxosporea **(Lom & Dyková, 2006)**.

Out of those two classes, only the Myxosporea are discussed further in this thesis. As currently known to science, the phylum Myxosporea consists of more than 2200 species. They are assigned to 64 genera and 17 families **(Okamura et al., 2015a)**. So far myxosporean nomenclature and taxonomic classification prove challenging to science. One of the main sources of confusion is the complexity of myxosporean life cycles and the high morphological diversity among the developmental stages, often of even the same species. A look back at history reveals that the first myxosporean species was described by Jurine as early as 1825 and Bütschli introduced the phylum of Myxosporea in 1881. However, it took the scientific community over 100 more years to agree that the actinospore developmental stage and the myxospore developmental stage are both part of the same life cycle rather than belonging to two separate taxonomical classes named Actinosporea and Myxosporea **(Atkinson, 2011; Okamura et al., 2015a)**. This important discovery goes back to the year 1984 when Wolf and Makiw "demonstrated that a myxosporean from salmonids, *Myxobolus cerebralis*, could infect tubificid oligochaetes to produce triactinomyxon actinospores, which could then infect naïve fish and generate new myxospores" **(Atkinson, 2011).** Following this finding, **Kent et al. (1994)** proposed to summarize the former class of Actinosporea in so-called collective groups within the class of Myxosporea. As a result, the actinospore developmental stages can still be grouped based on their complex morphology independently from the myxospore morphology and PCR results. This way, the collective groups help to categorize detected actinospores with or without preexisting information about the exact species they belong. The availability of modern gene sequencing, as well as the classical morphology-based approach, does not necessarily clarify the taxonomic relations. The results of both methods do not always coincide, and the true phylogeny is, therefore, sometimes hard to determine.

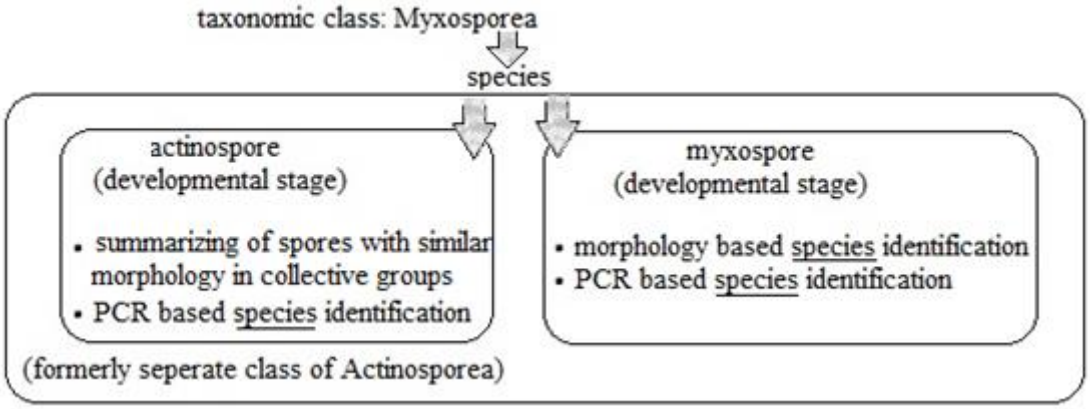


Figure 1: Role of myxosporean actinospores and myxospores in taxonomy

Taxonomic classification based on Morphology

When myxosporeans are taxonomically classified based on their spore morphology, the actinospore developmental stage released by the annelid host and the myxospore stage released by the fish host are evaluated. Actinospores have distinct anatomical features such as the spore size and dimensions, the number of polar capsules, the dimensions of their projections or the length of their style. Nowadays, actinospores which share common morphological features are summarized within a collective group (**Özer et al. 2002; Lom & Dyková, 2006; Yokoyama et al., 2012)**. The myxospore developmental stage has relevant anatomical features such as its shell valves, size, dimensions, surface structure and the coiled polar filaments which are used as a criterion for classification. To determine the species morphologically only the myxospore stage is used nowadays **(Lom & Dyková, 2006).** It is recommended to compare and evaluate a given spore individually on basis of the published literature guidelines available for it **(Borkhanuddin, 2013)**.

Taxonomic classification based on gene sequencing

The taxonomic classification based on molecular genetic markers mainly relies on small subunit (SSU) ribosomal RNA genes, mostly the 18S rRNA gene, in example for *Thelohanellus kitauei* **(Shin et al., 2012)**. Here genetic databases and the availability of PCR results are the key factors for a species identification. The ribosomal small subunit genetic marker has conserved regions which are wide-spread among various eukaryotic species. Therefore it is possible to design suitable PCR-primers that bind to the conserved regions of known and unknown species **(Schmalenberger et al., 2001)**. Variable regions of the SSU rRNA genes are much more unique, and they are often located between conserved regions on the same genome. Those variable regions may only be found in a certain genus or taxon. Therefore the availability of PCR-results for such variable regions is of great practical importance for myxozoan phylogeny and classification **(Fiala, 2006)**. Other molecular markers such as the large subunit (LSU) rRNA provide additional taxonomic information. Regardless of its potential value, the LSU rRNA is less commonly used for PCR because database records are still often lacking **(Bartošová et al., 2009)**.

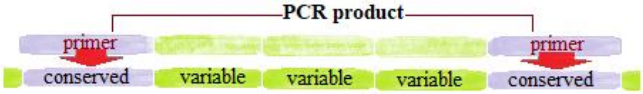


Figure 2: Basic principle of PCR in the identification of new myxosporean species

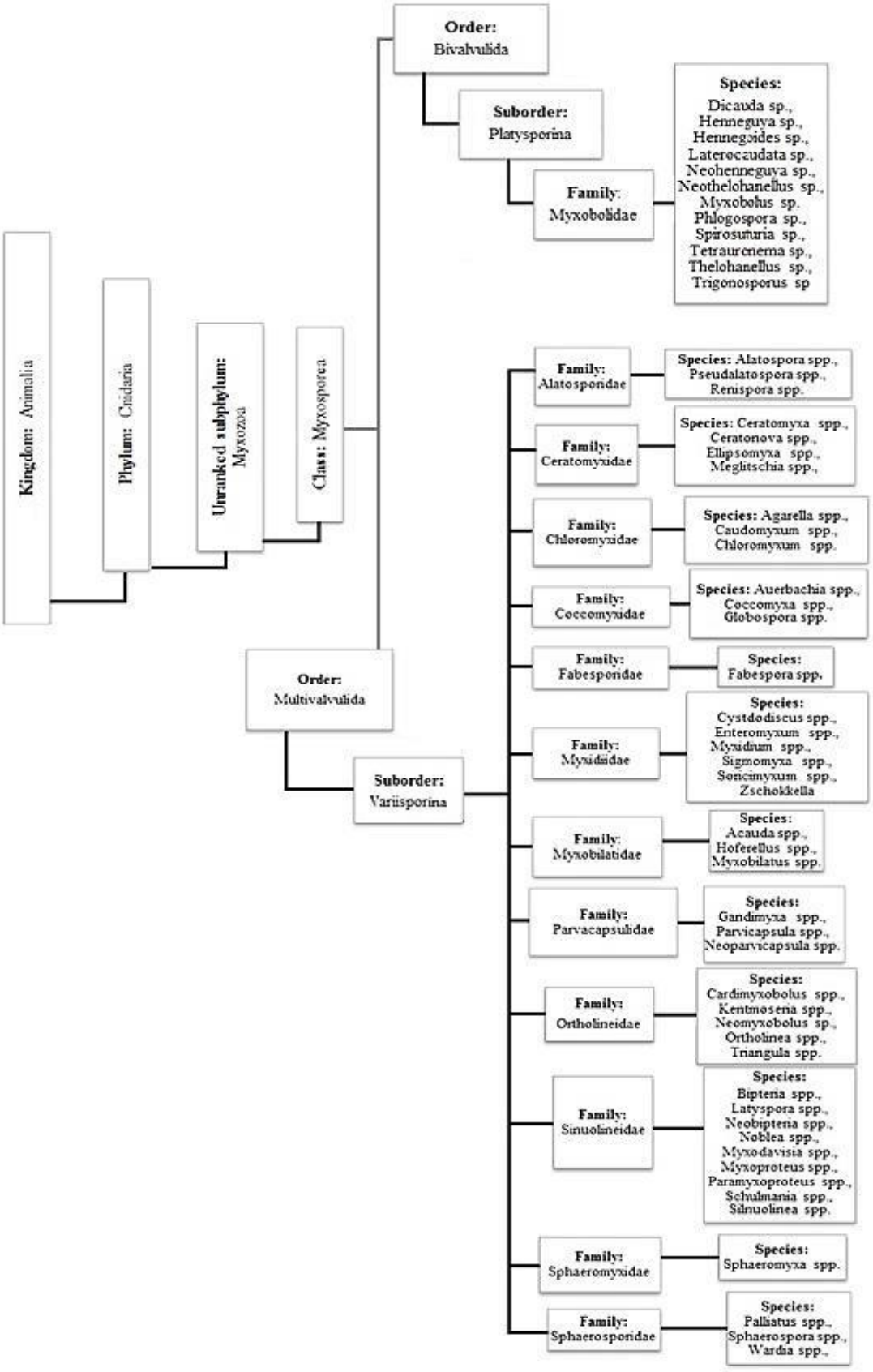


Figure 3: Taxonomic tree of myxosporean parasites, based on **Fiala et al. (2015)**

3.2. Anatomy and Life-cycle of pathogenic Myxosporeans

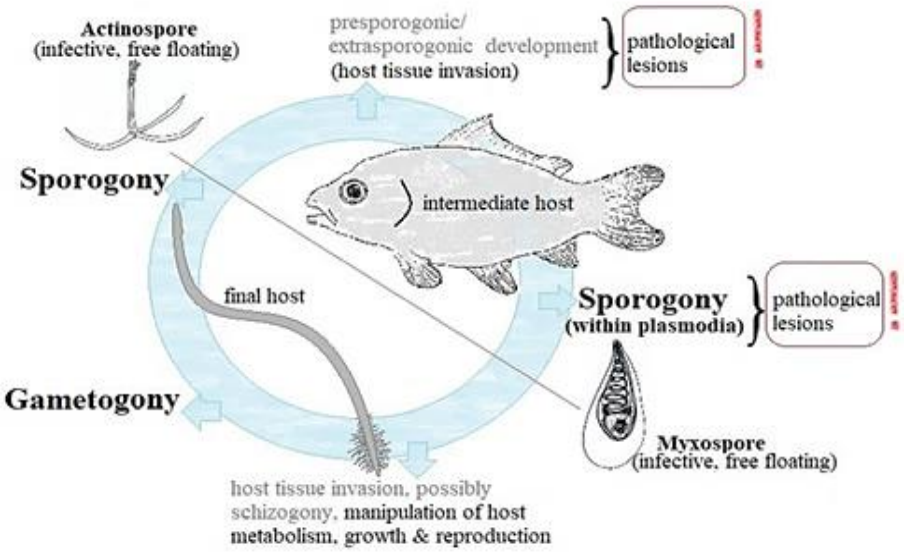


Figure 4: Own schematic illustration of general myxosporean lifecycle

A standard feature of Myxosporeans is their two-host-life-cycle **(Wolf & Makiw, 1984)**, involving a vertebrate intermediate host and a final invertebrate host. Characteristically for myxosporean parasites are so-called "cell within cell stages" **(Lom & Dyková, 2006**; **Morris, 2012)** meaning that a parasitic cell may contain other parasitic cells which again may contain further developmental stages. At certain points of their life, myxosporean stages move actively based on their contractile F-Actin (**Uspenskaya & Raikova 2004)**. This unique feature allows the parasites to invade the host successfully, to spread within tissues and to escape the host immune response **(Feist et al., 2015a)**. Within the vertebrate host, commonly a fish, the parasite proliferates and matures until an infective myxospore is ready to be shed into the surrounding water. The myxospore then enters its final invertebrate host, which is typically an aquatic annelid worm and proceeds with the parasitic development resulting in the formation of another infective spore stage, the actinospore. Once the mature free floating actinospore encounters a fish host, the result is a new parasitic life cycle. During spore formation within both hosts, multicellular stages may form voluminous parasitic aggregates, which elicit damage to the host since they are growing in size, and have a direct mechanical compression effect on the surrounding cells

or tissues. Whether or not the infection is successful and whether it results in an extent of damage that causes significant pathological changes, clinical signs and losses depends on the individual host age, resistance, and status, as well as various environmental factors **(El-Matbouli et al. 1999)**. To date, only a few complete life cycles have been fully documented and best-understood is that of *M. cerebralis* **(Okamura et al. 2015a)**.

3.2.1. Development within the oligochaete host

For all pathologically relevant fish parasites described in this thesis, the final hosts are invertebrates **(Wolf & Markiw, 1984)** belonging to the aquatic annelid worms. Oligochaete worms are mainly bottom dwellers that often get infected with the myxospore stage while they are feeding on debris contaminated with the parasite. A vertical transmission where the body of an infected parent worm spontaneously divides to give rise to a daughter specimen, has also been reported **(Morris & Adams, 2006)**. The parasitic life cycle usually begins in the intestinal tissues of the worm where the next infective spore stage, the actinospore develops and ultimately leaves the worm via defecation. In other cases, the actinospores develop within the oligochaete´s body cavity and are released via pores, or they develop within the integument and are released from there **(Alexander et al., 2015a)**. The actinospore production occurs within pansporocysts, which are growing in size over time as the parasitic stages accumulate. As a result those expanding masses inflict a certain degree of mechanical compression upon the surrounding oligochaete cells and cell organelles **(Székely et al. 1998)**. Nevertheless, myxosporean parasites may persist in their final invertebrate host over a significant time-period, often months, sometimes years without killing the host. There is evidence that the fertility of infected annelid worms may decrease. Other pathophysiological changes such as an “increased growth and respiration” **(Alexander et al., 2015b)** were also observed in affected worms. As a logical consequence of this parasitic manipulation, the larger host with its higher metabolic turnover and reduced energy loss for its reproduction represents a better habitat for the parasite and can yield a greater number of infective actinospores, which eventually infect a vertebrate host to complete the parasitic life cycle **(Alexander et al., 2015b)**. Examples of pathologically important myxosporean species of common carp and their suitable hosts are summarized in the following Table 1.

Table 1: Examples of pathogenic myxosporeans with important lifecycle steps

Disease	Parasite species	Vertebrate host	Invertebrate host	Actinospore type	Source
Swim bladder inflammation of common carp	*Sphaerospora renicola*	*Cyprinus carpio*	*Branchiura sowerbyi*	Neoactinomyxum	**Molnár et al., (1999)**
Intestinal giant cystic disease in common carp	*Thelohanellus kitauei*	*Cyprinus carpio*	*Branchiura sowerbyi*	Aurantiactinomyxon	**Zhao et al., (2016)**
Fin cysts in common carp	*Thelohanellus nikolskii*	*Cyprinus carpio*	*Branchiura sowerbyi*	Aurantiactinomyxon	**Molnár, 1982, Székely et al.1998**
Haemorrhagic thelohanellosis in common carp	*Thelohanellus hovorkai*	*Cyprinus carpio*	*Branchiura sowerbyi*	Aurantiactinomyxon	**Székely et al., 1998, Yokoyama et al., 1998**

Schizogony

After the infectious myxospores left the fish host, they float freely in the water until they are ingested by an oligochaete **(Lom & Dyková, 2006)**. The myxospores reach the worm´s intestinal lumen and decoil their polar filaments, which then anchor to a host cell. In another step, the spore valves open up, and the infective sporoplasm is released into the intercellular space. Then the actual schizogonic phase begins, and the nuclei of the sporoplasm begin to divide, resulting in one big multinucleated cell. During a process called plasmotomy, the cytoplasm of this multinucleated cell divides to form numerous uninucleated cells. Those cells wander between the enterocytes of the annelid host and undergo further schizogonic cycles. As a result, the uninucleated cells give rise to further multinucleated cells, which again divide into cells with a single nucleus **(Lom & Dyková, 2006)**. This way the parasite spreads within its final host and multiplies in numbers. Each uninucleated cell produced during Schizogony either has one α-nucleus or one β-nucleus respectively. To begin the gametogony phase two of the uninucleated cells fuse, resulting in a binucleated cell containing both types of nuclei **(El-Matbouli & Hoffmann 1998)**. Other authors were not able to find evidence for a schizogonic development. Alternatively,

the binucleated cells may derive from the myxospore sporoplasm directly without preliminary schizogony **(Morris & Freeman 2010)**. Further studies are necessary to clarify whether or not and to which extent a schizogony phase is present within the oligochaete host. Nevertheless, the migration of the infective sporoplasm and the development of binucleated cells with an α-nucleus and a β-nucleus are proven.

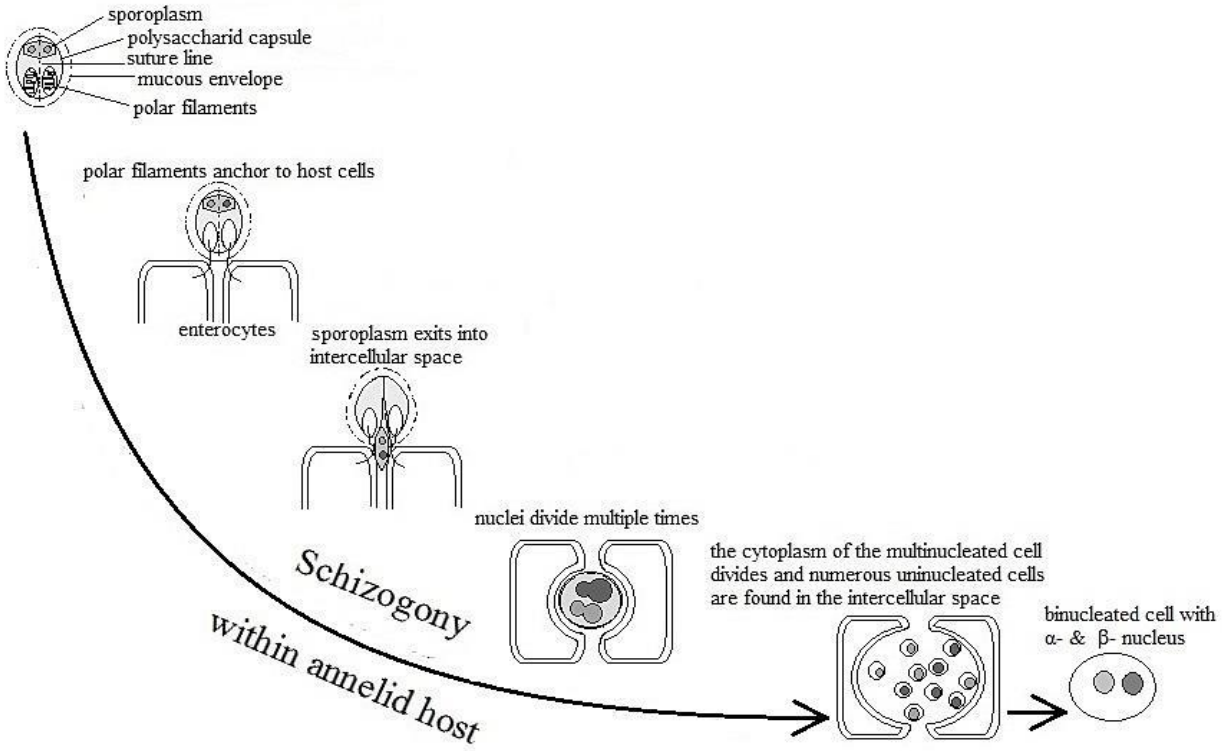


Figure 5: Own schematically illustrated summary of the schizogonic phase within the annelid host, based on **El-Matbouli and Hoffmann (1998); Kent et al. (2001)**

Gametogony

Gametogony is the only sexual reproduction phase during the myxosporean life cycle, and it exclusively occurs within the final host of the parasite. The binucleated cells produced earlier are motile to a certain degree which aids tissue invasion **(Feist et al., 2015a)**. They undergo further nuclear and cellular division, resulting in a developmental stage called pansporocyst. At first, the early pansporocyst consists of two enveloping somatic cells, which are located in the periphery and two generative cells in the center **(Lom & Dyková, 2006)**. One of the generative cells is a smaller α-cell, and one is a larger β-cell **(Janiszewska, 1957)**. The enveloping somatic cells undergo further mitotic divisions,

resulting in several enveloping cells per pansporocyst. In the center of the pansporocyst, the generative cells perform mitotic divisions as well, but here this is followed by meiosis. As a result, haploid gametocytes are produced, half of which are of α-origin, and the other half are of β-origin. When an α-gametocyte fuses with a β-gametocyte, a diploid zygote is formed, and the sexual reproduction is complete **(El-Matbouli & Hoffmann 1998; Lom & Dyková, 2006)**.

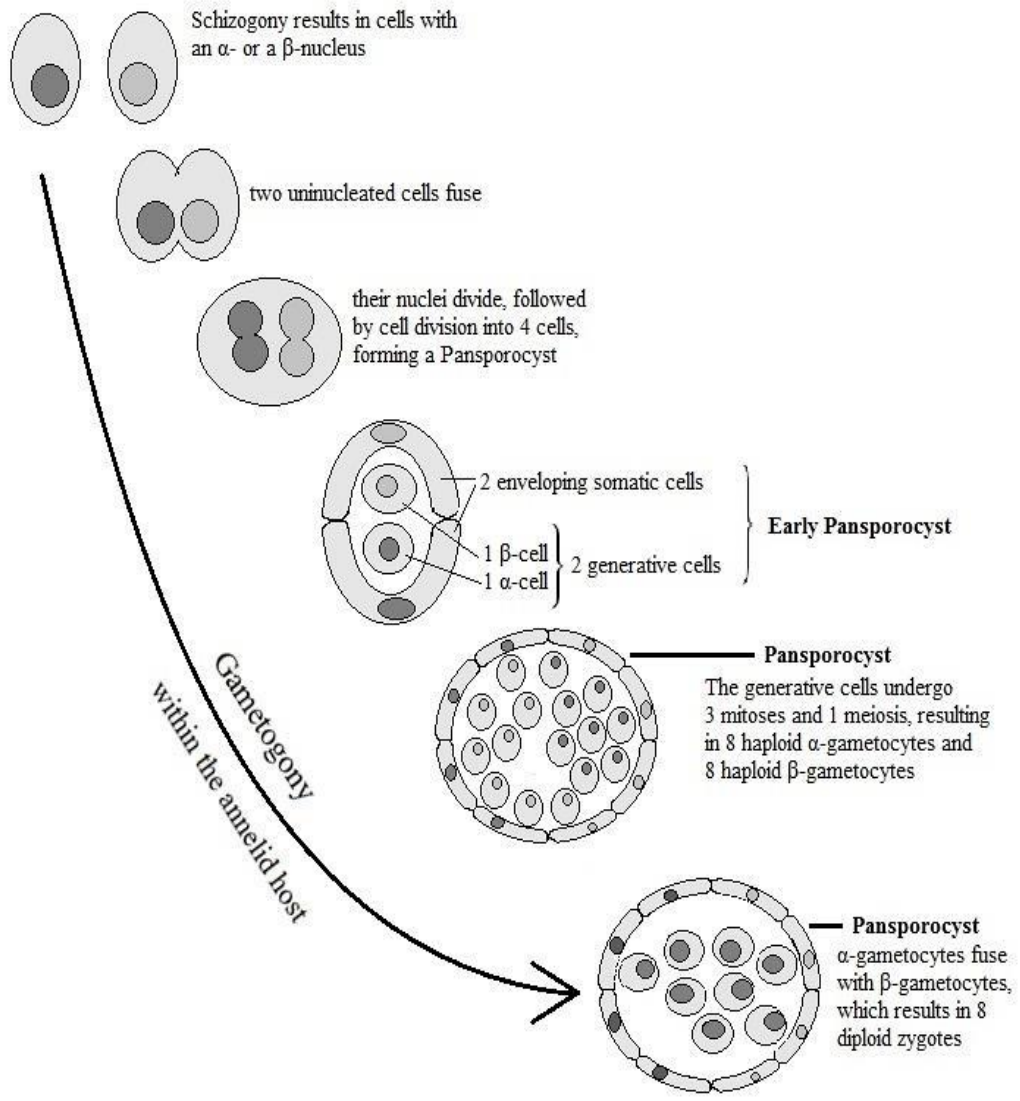


Figure 6: Own schematically illustrated summary of the gametogonic phase within the annelid host, based on **El-Matbouli and Hoffmann (1998); Kent et al. (2001)**

Sporogony

During this asexual developmental phase, the infective actinospore stage is produced within the worm. This requires further mitotic divisions within the pansporocyst and the differentiation of the zygote into three cell types that later become the sporoplasm, valves, and capsule of the actinospore **(Feist et al., 2015c)**. Each zygote eventually becomes one actinospore, usually with a spore body in which the three polar capsules are located and with three shell valves that form the extendible caudal projections of the actinospore. The actual infective internal cell stages of the actinospore are located within the multinucleated sporoplasm **(Lom & Dyková, 2006)**.

3.2.2. Actinospores

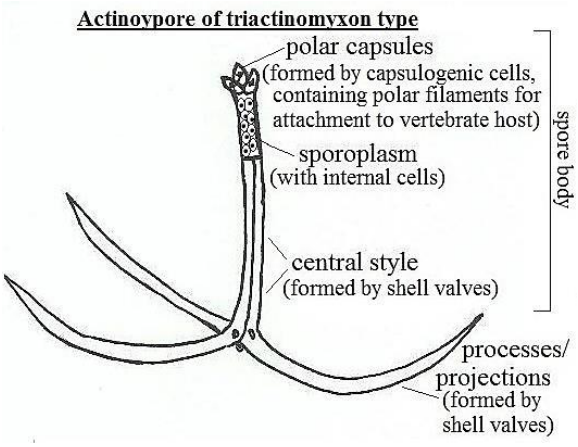


Figure 7: Own schematic drawing of an actinospore stage, triactinomyxon type

"Myxosporea develop in their invertebrate hosts into delicate, tri-radially symmetric actinospores, that are highly variable in size (5-500 μm) and number of infectious cells (2 to >100)" **(Gómez et al., 2014)**. Once it has matured, this infectious stage is released from the pansporocyst and shed into the surrounding water via feces or pores **(Atkinson, 2011)**, where it is infective upon contact with the skin or mucous membranes of the final fish host. Alternatively, the fish may feed on infected oligochaetes, taking up the mature actinospores indirectly per oral. In general an actinospore consist of a spore body and

processes, sometimes also referred to as projections or hooks. When the mature actinospores get in contact with the surrounding water, the processes become inflated via osmotic forces. It is believed that this happens to increase the duration of free-floating and therefore increases the chance for an encounter with a fish host **(Feist et al., 2015c)**. The spore body is located proximally to the processes and consists of three polar capsules **(Lom & Dyková, 2006)**, containing extrudable polar filaments for the attachment to the vertebrate host. A sporoplasm with several infective internal cells is also found at the spore body, as well as the style, which greatly varies in length depending on the actinospore type. Morphologically this developmental stage can be assigned to various collective groups. In an example, the important carp pathogens *Thelohanellus kitauei, T. hovorkai* and *T. nikolskii* have aurantiactinomyxon type actinospores and are therefore assigned to the collective group Aurantiactinomyxon Janiszewska, 1952. Within this collective group, a further grouping into aurantiactinomyxon type 1-5 is possible **(Borkhanuddin, 2013)**. For a morphology-based grouping of actinospores, criteria such as the total actinospore length in micrometers, dimensions of caudal processes, spore body and secondary cell dimensions, the number of secondary cells, length and width of the style and spore body dimensions are taken into consideration **(El-Mansy et al., 1998)**. More examples for different actinospore types among important fish pathogenic myxosporean parasites can be seen in Table 2 and Figure 8.

Table 2: Key features of important actinospore types, based on **Özer et al. (2002)**

Aurantiactinomyxon (Aurantiactinomyxon raabeiiunioris Janiszewska, 1952)	**Triactinomyxon** (Triactinomyxon ignotum Štolc, 1899)	**Neoactinomyxum** (Neoactinomyxum globosum Granata, 1922)	**Raabeia** (Raabeia gorlicensis Janiszewska, 1955)
– no style – 3curved processes of equal length, – 120° angle between projections – single spores, not attached to each other – spherical to ellipsoidal spore body – spherical polar capsules with protruding tips – 32 to 128 internal / secondary cells in sporoplasm – type 3 is the largest, especially long processes – e.g. *Thelohanellus spp., Henneguya spp., Hoferellus spp.*	– with 1 style – 3 processes, curved dorsally with pointed tips – 8-256 internal/ secondary cells – single spores, not attached to each other – long spore body – pear-shaped polar capsules – e.g. *Myxobolus spp*.	– no style – 3 equally short processes – generally rounded triangle- shaped spore – 3 polar capsules with protruding tips – 32 internal/ secondary cells – single spores, not attached to each other – e.g. *Hoferellus carassii*	– no style – 3 long processes, some species with branched tips – Ellipsoidal spore body – Polar capsules with protruding tips – e.g. *Myxobolus dispar, Myxidium truttae*

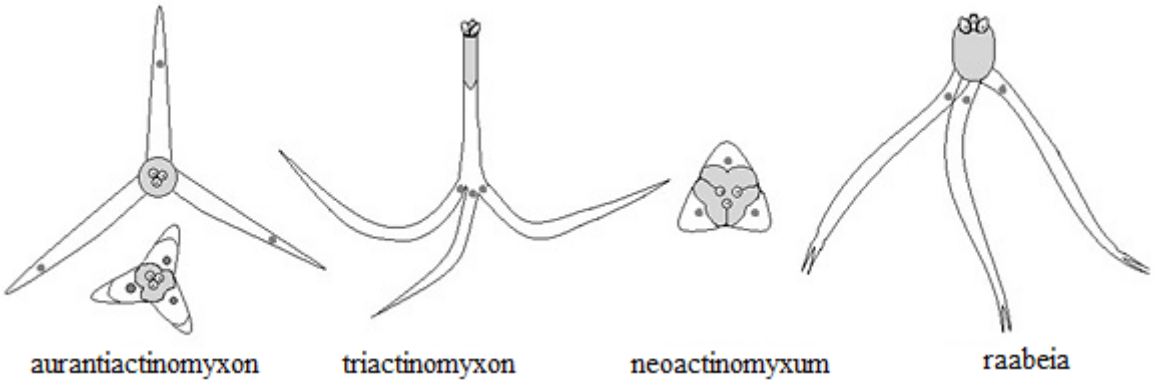


Figure 8: Own schematic drawing of important myxosporean actinospore morphotypes, based on **Özer et al. (2002)**

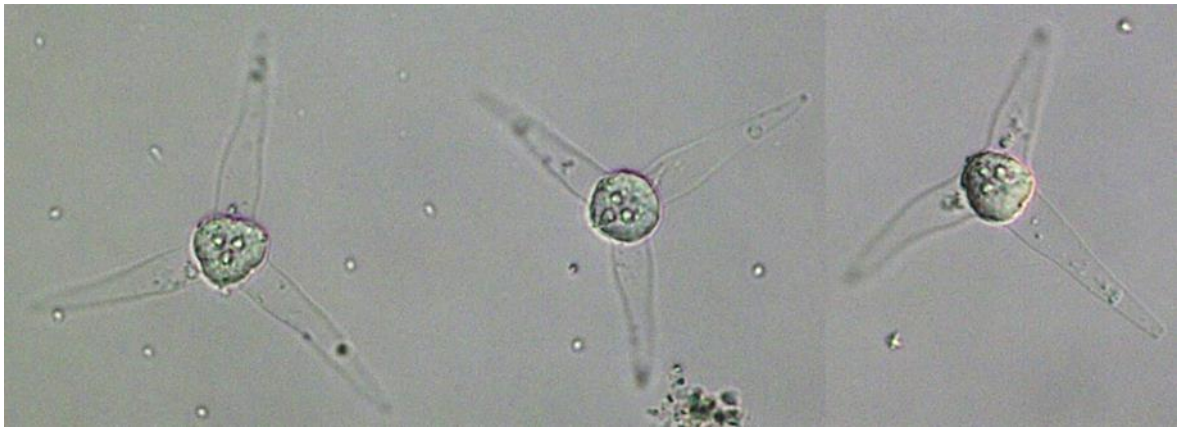


Figure 9: Aurantiactinomyxon type actinospores released by *B. sowerbyi*

3.2.3. Development within the vertebrate host

Once the free-floating actinospore gets in contact with the epithelial surface of the fish host, its polar filaments uncoil and protrude from their polar capsules to pierce into the host cell for attachment **(El-Matbouli & Hoffmann 1998)**. The actinospore targets tissues such as the skin, the epithelium of the buccal cavity **(Lom & Dyková, 2006),** the fins or the gills of the fish **(Behrmann-Godel, 2015)**. The sporoplasm is then released extracellularly and eventually enters the host epithelium **(Kent et al., 2001)**. From there the sporoplasm reaches the intercellular space, where active amoeboid movement aids deeper tissue invasion **(Sarker et al., 2015).**

Horizontal fish to fish transmission is currently not reported for any carp pathogenic myxosporean parasites but certain marine *Enteromyxon spp.* successfully achive per oral transmission even to freshwater fish species **(Gómez et al., 2014)**.

Presporogonic phase

In general, the presporogonic development takes place inside the host cells or within the intercellular space between the host cells respectively **(Lom & Dyková, 2006)**. During this phase, the parasite prepares to give rise to plasmodia or pseudoplasmodia which later play a major role in the asexual process of spore formation, sporogony. Location and morphological details of presporogonic stages are often hard to identify, therefore such information is not available for all pathologically relevant myxosporean parasites yet **(Feist et al., 2015b)**. First, the actinospore sporoplasm falls apart and each of its inner cells either enters a host cell to divide intracellularly or in other cases it remains intercellularly to divide there **(Lom & Dyková, 2006)**. Primary mother cells form and undergo further mitoses, so that daughter cells develop within the mother cell. The daughter cells are also referred to as inner secondary cells. In the end, the host cell contains a primary cell, in which several inner secondary cells are formed in which sometimes inner tertiary cells develop **(El-Matbouli et al., 1995; Kent et al., 2001)**. Therefore this is a typical example for myxosporean "cell within cell stages" **(Lom & Dyková, 2006)**. The inner cells then undergo further mitotic divisions and greatly increase in numbers. This growing parasitic aggregate already poses potential harm for the host cell and tissue metabolism due to its compression effect **(El-Matbouli et al., 1995; Kent et al., 2001)**. In certain myxosporean species such as *Myxobolus cerebralis*, an increasing number of accumulating inner cells inevitably lead to the disintegration of the primary cell. The infective inner cells are then found intracellularly and still have to bore through the host cell membrane, or they are directly released into the intercellular space **(Kent et al., 2001), (Feist et al., 2015b)**. From there they begin to invade further and each inner cell turns into a new primary mother cell and produces another bunch of inner cells **(Kent et al., 2001)**. Other myxosporean species keep an intact presporogonic primary cell stage with inner daughter cell stages and proceed with their parasitic tissue invasion **(Feist et al., 2015b).**

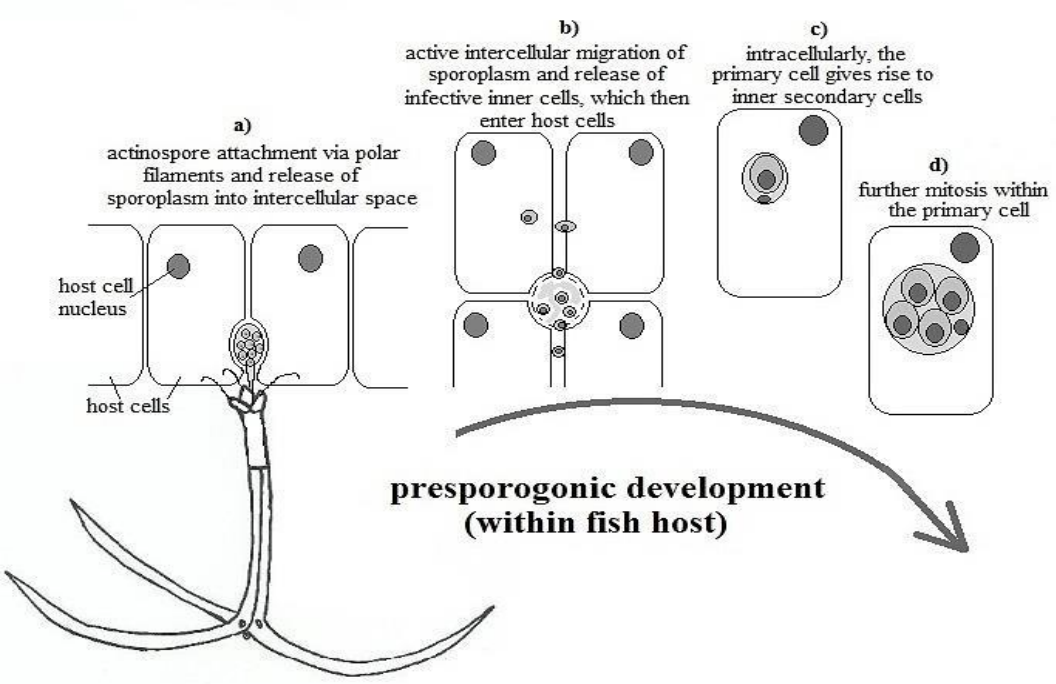


Figure 10: Own schematic illustration of presporogonic development within the fish host, based on **El-Matbouli and Hoffmann (1998); Kent et al. (2001)**

Extrasporogonic phase

Presporogonic phase and sporogony usually take place in the same tissue, but there are exemptions for certain myxosporean species such as *Sphaerospora dykovae* **(Lom & Dyková, 2006)**. Here the presporogonic phase is more accurately termed "extrasporogonic phase" **(Kent et al. 2001, Lom & Dyková, 2006).** In those cases, the presporogonic multiplication continues at a distinct location even though sporogony has already started in its target tissue within the vertebrate host **(Lom & Dykovà 2006)**. Since the presporogonic phase already causes stress to the host cells, this additional extrasporogonic spreading and parasitic proliferation may worsen the destructive effect inflicted by the parasitic infection.

Sporogony

In most myxosporeans, sporogony takes place within a plasmodium in a certain target tissue. The plasmodium consists of a primary cell of presporogonic origin which contains myxospore progenitor stages. Presporogonic primary cells are located inter- or intracellularly within the host tissues **(Lom & Dyková, 2006)**. The aim of the sporogonic phase is the development of the next infective spore stage, the myxospore.

Plasmodia

Characteristical for the sporogonic phase is the formation of a plasmodium, which is a growing parasitic mass, inflicting various degrees of mechanical damage to the host cells surrounding it. A plasmodium can be histozoic, so located "within intercellular spaces of solid tissue" **(Moser & Kent, 1994)** and especially the larger sized specimens may be referred to as "cyst" **(Lom & Dyková, 2006**). More precisely the plasmodia just grossly resemble those and are no true cysts from the pathology point of view **(Molnár, 1994)**. Other plasmodia are coelozoic and therefore situated within the lumen of hollow organs **(Lom & Dyková, 2006; Feist et al., 2015c)**. Generally, histozoic myxosporeans have a higher pathogenic potential compared to coelozoic myxosporeans **(Goméz et al., 2014)**. Plasmodia vary in size and typically contain several vegetative nuclei on which the primary cell metabolism is based. Within the primary or mother cell of presporogonic origin, numerous daughter cells develop and are termed generative cells. Some authors also describe the primary cell as "enveloping cell" **(Kent et al., 2001)**, while its daughter cells may be referred to as "enveloped cells" **(Kent et al., 2001)**. Two types of generative cells exist, the sporogonic cell and the pericyte. When only sporogonic cells are present, they either directly divide further to produce all myxospore components. Or if both types of generative cells are present, the two cell types interact to form the myxospore stage. In the latter case, one cell usually arranges itself peripherally and becomes the pericyte envelope. The second generative cell is a sporogonic cell that remains in the center. As a next step, sporogonic cells and pericytes divide to form a pansporoblast. The term pansporoblast is used when mitotic divisions within a plasmodium lead to several pericytes surrounding a group of sporogonic cells. When the sporogonic cells in the center divide this leads to three more cell types which later differentiate into the myxospore valves, its capsule, and the sporoplasm. At the end of the sporogonic phase, the pansporoblast contains a certain number of mature myxospores **(El-Matbouli & Hoffmann, 1995; Lom & Dyková, 2006).**

Pseudoplasmodia

In contrast to the classical plasmodia, pseudoplasmodia are usually smaller structures with only a single vegetative nucleus and only one type of generative cells, the sporogonic cells. The limited number of sporogonic cells limits the production of myxospores to maximum two per pseudoplasmodium **(Lom & Dyková, 2006).**

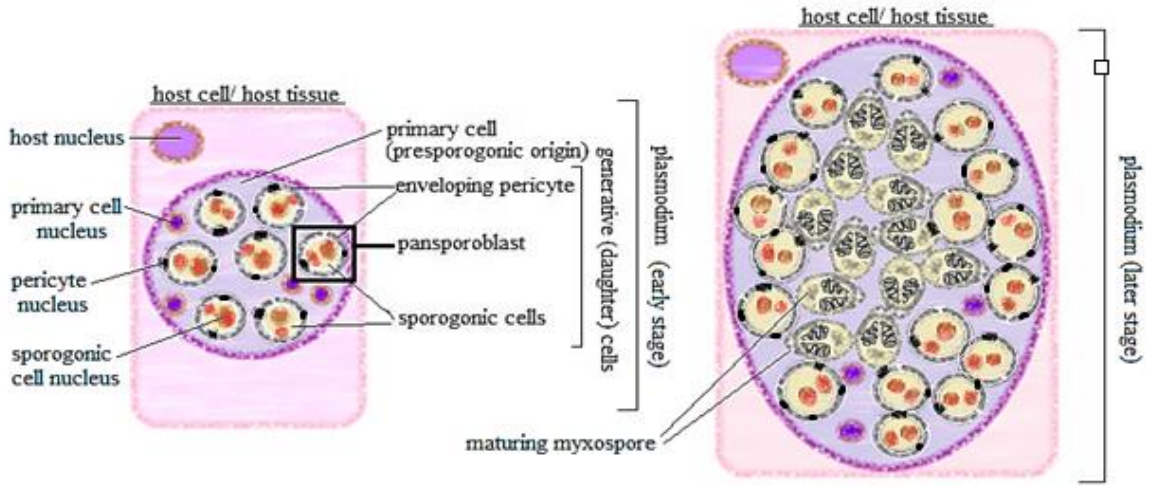


Figure 11: Own schematic illustration of an early and a late plasmodium, developing during schizogony in the fish host, based on **Kent et al. (2001)**

3.2.4. Myxospores

Within their vertebrate host, the myxosporean parasite generally develops "into bilaterally symmetric myxospores with hard shell valves, and are relatively smaller (5-20 μm) with 1 to 2 infectious sporoplasms" **(Goméz et al., 2014)**. At least two but up to shell seven valves meet at a suture line **(Lom & Dyková, 2006)** and the outermost structure of the myxospore is its mucous envelope **(El-Matbouli & Hoffmann, 1998)**. Between one and seven polar capsules are located inside the myxospore, enclosed by the shell valves. Each of the myxospore´s 1-7 polar capsules contains a coiled polar filament **(Lom & Dyková, 1992)** which becomes extruded upon infection and enables the parasitic spore to attach and fasten to the final invertebrate host. Besides those structural elements, there are up to two sporoplasms, filled with one to twelve infective cells **(Sitjà-Bobadilla et al., 1995)**, which initiate the further parasitic development within the final invertebrate host **(Lom & Dyková, 2006)**. Myxospores are infective to the annelid host once they left their pansporocyst and the fish host. They often reach the mud dwelling worms when a tissue cyst ruptures or the fish host dies and sinks to the water bottom where it decays until the myxospores become free **(Atkinson, 2011).** For the morphology based identification of myxospores, criteria such as the general myxospore shape, its length and width in valvular and sutural view, position and number of the polar capsules, the valves as well as the surface structure are taken into consideration **(Borkhanuddin, 2013).**

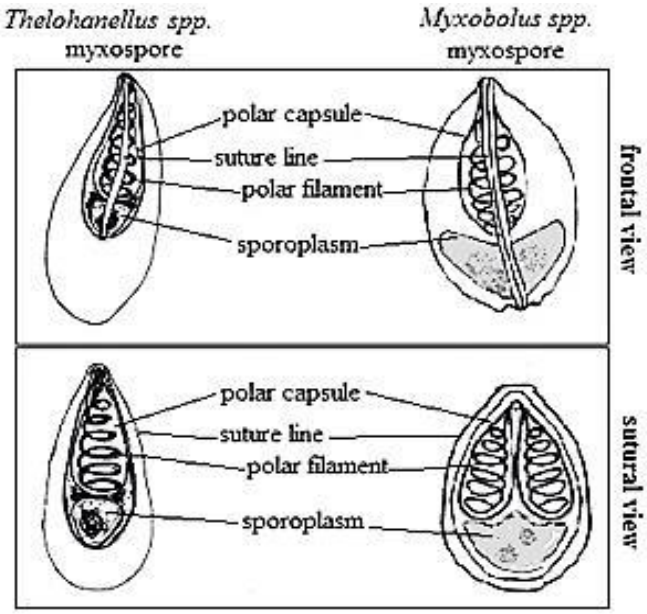


Figure 12: Own schematic illustration of basic myxospore morphology

3.3. Pathological findings caused by Myxosporeans

General Pathogenesis

As the previous general description of a myxosporean life cycle reveals, the veterinary point of view begins with the development within the intermediate vertebrate host. After the myxospore stage has successfully entered its fish host, it proceeds to the presporogonic phase where first histopathological changes may take place. Here the parasitic stages spread intra- and intercellularly with the aim to arrive at their target tissue **(Feist et al., 2015b**). The late presporogonic stages then start to proliferate inter- or intracellularly and often travel with body fluids within the host, while they increase in size. The main pathological changes follow during sporogony, when myxospore-containing plasmodia or pseudoplasmodia grow massively causing various degrees of tissue damage **(Feist et al., 2015c)**. Therefore, clinical and subclinical lesions are usually the consequence of sporogony. Fish fry and fingerlings appear to be most susceptible age group and considerable morbidity and mortality rates may be observed in cultured common carp.

3.3.1. Important diseases in common carp

3.3.1.1. Renal and swim bladder sphaerosporosis *(Sphaerospora dykovae)*

Clinical signs

S. dykovae, may synonymously referred to as *Sphaerospora renicola* **(Gómez et al., 2014)** has a significant pathogenic impact on cultured *Cyprinus carpio* in Europe, Asia, Israel and Australia **(Lom & Dyková, 2006)**. Affected carp usually develop an acute disease accompanied by clinical signs within their first year of life. They may present with balance disorders, odd swimming movements, and dark discoloration. Emaciation, growth retardation **(Körting, 2006)**, as well as exophthalmia, is also commonly seen in affected young carp **(Molnár, 1993).** Chronical cases may present with balance disorders such as upside down, horizontal or vertical swimming positions, fins reaching or exceeding the water surface and grossly visible abdominal enlargement **(Jeney & Jeney, 1995)**.

Pathogenesis

Newly hatched carp fry usually encounter the infective neoactinomyxum type actinospore released by the oligochaetes *Branchiura sowerbyi* or *Tubifex tubifex* **(Molnár et al., 1999)** in late spring or early summer **(Körting, 2006).** Following infection, the coelozoic parasite **(Lom & Dyková, 2006)** propagates in the blood from where it spreads to the swim bladder for further presporogonic development inducing various degrees of inflammation. Thereafter and parallelly, the sporogonic phase of the parasitic life cycle leads to pseudoplasmodia formation and lesions in the kidney tubules. Additional pathological changes may occur when developmental stages obstruct fine capillaries in different organs such as the eye **(Molnár, 1993)**. Acute disease and clinical signs are usually observed during summer in July or August **(Al-Samman et al., 2003)** when the carp are up to three months old **(Molnár, 1993)**. According to **Grupcheva et al. (1985)**, the seasonal pattern of carp sphaerosporosis results in high numbers of blood stages in autumn of the first year of life and during the spring months of the following year, while kidney stages are mainly found soon after hatching and again during the second spring of the young carp´s life. Pathological changes and parasitic K-cells in the swim bladder may be detected as early as two weeks post infection **(Körting, 2006).**

The pathogenicity of *Sphaerospora dykovae* is mainly based on its direct mechanical effect on the host cells, decreased tissue perfusion and the cellular reaction it provokes. Other mechanisms such as antigen challenge and humoral immune response do not play a role in the pathogenesis **(Al-Samman et al., 2003)**.

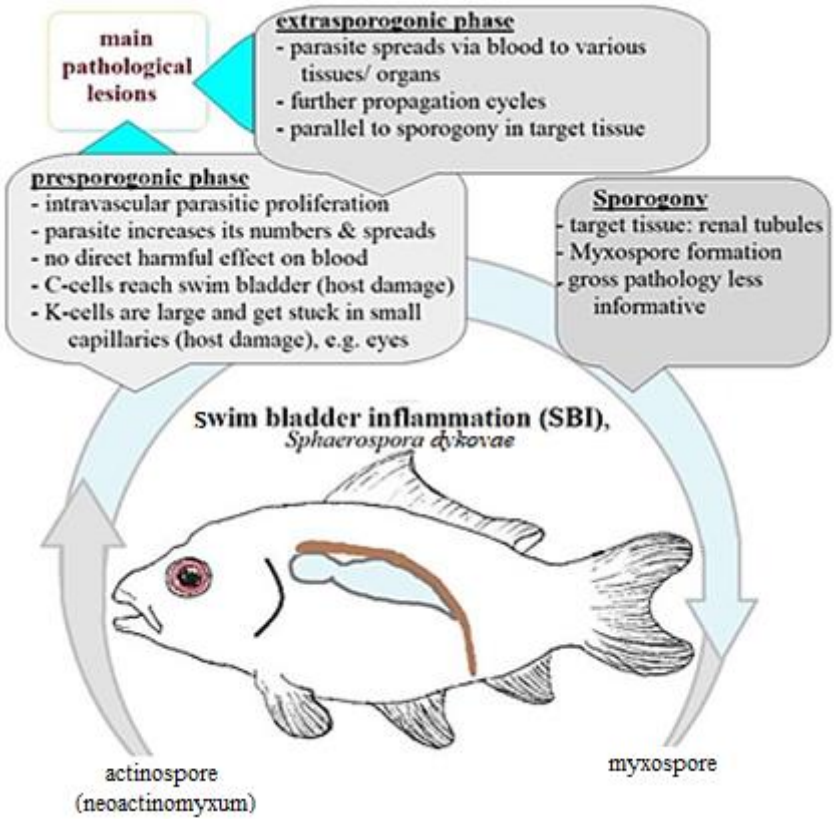


Figure 13: Basic parasitological and pathological features of swim bladder inflammation caused by *Sphaerospora dykovae* (illustrated by Susanne Braun)

Intravascular phase

Post-infection, the presporogonic stages take advantage of the carp´s circulatory system and travel via blood to various organs **(Csaba et al., 1984; Dyková & Lom, 1982)**. Those intravascular stages are referred to as C-cells or Csaba-cells **(Molnár, 1980a)** and are a characteristic feature of *Sphaerospora renicola*. At the beginning of the intravascular phase, the parasite consists of an actively moving primary cell in which several secondary cells containing tertiary cells develop. After a while, the primary cell ruptures and several dozen secondary cells, containing two tertiary cells each, are released into the bloodstream where they spread further **(Baker et al., 1995**; **Csaba et al., 1984)**.

By repeating the presporogonic propagation cycle, the parasite manages to increase in numbers and spreads within its host, which additionally challenges the young carp. Towards the end of the presporogonic phase, complex cell-within-cell stages consisting of roughly 30 to 40 primary cells with secondary and tertiary cells develop and are termed K-cells **(Molnár, 1993; Körting, 2006)** after their discoverers Kovács-Gayer and Körting **(Baska & Molnár, 1988)**. Since the K-cells are rather large parasitic aggregates, they easily get stuck within small capillary blood vessels **(Molnár, 1993)** where they cause obstruction possibly resulting in hypoxia, increased vessel permeability, exudation and necrosis in the affected tissue.

Swim bladder inflammation (SBI)

SBI is considered "a common and economically important disease of carp in intensive aquaculture**" (Behrmann-Godel, 2015)**. C-cells invade the swim bladder as they migrate actively from small capillaries into the intercellular space filled with loose fibrous connective tissue **(Csaba et al., 1984)**. The larger K-cells also reach the swim bladder via blood circulation but they rather get passively stuck within small capillaries where they block the blood flow, increase the vascular permeability and cause exudation **(Al-Samman et al., 2003)**. According to **Jeney & Jeney (1995)**, this first stage of SBI is therefore characterized by hyperemia and petechial hemorrhages in the wall of the affected swim bladder. During the second stage of SBI, the hyperemia decreases but the darkly discolored remnants of the previous hemorrhages are still present. Stage one and two of SBI usually remain subclinical **(Jeney & Jeney, 1995)**. When the swim bladder inflammation proceeds to the third stage, the wall already appears thicker than normal and is full with exudate **(Jeney & Jeney, 1995)**. Here the growing number of parasitic stages accumulating in the internal lamina of the swim bladder wall **(Moser & Kent, 1994)** leads to a massive cellular reaction **(Körting, 2006)** mainly by infiltrating lymphocytes **(Moser & Kent, 1994)** , followed by epithelial hyperplasia **(Moser & Kent, 1994).** When the epithelial lamina propria becomes hyperplastic, the rigid wall consequently narrows the lumen and decreases the air content **(Kappe, 2004**). Over time, the macroscopical picture of the swim bladder changes as the wall becomes thick and fibrotic, it no longer has a smooth and shiny appearance due to forming edema, hemorrhages and growing granulation tissue **(Kreier, 1994; Körting, 2006)**. From this stage onwards, affected small carps present with clinical

signs **(Jeney & Jeney, 1995)**. Typical examples for the latter are balance disorders and unusual, eratic swimming patterns **(Körting, 2006)**. The fourth stage of SBI is characterized by a worsening of the situation described for the third stage, may additionally already causing necrosis of the swim bladder wall **(Jeney & Jeney, 1995).** For the fifth and final stage of SBI, **Jeney & Jeney (1995)** describe the formation of cysts. This finding seems to be consistent with the granulomatous tissue proliferation of epithelial or connective tissue origin mentioned by **Körting (2006)**, which may grow towards the lumen of the swim bladder. Furthermore, the fifth stage of SBI is characterized by an accumulation of serous or purulent exudate within the swim bladder lumen **(Jeney & Jeney, 1995).** In cases where the inflammation extends towards the surrounding tissues, peritonitis may develop **(Kreier, 1994)** and adhesions in the surrounding gut, kidney or liver can occur **(Körting, 2006).** On dissection the acutely infected swim bladder is heavier, looks grossly malformed, smaller in size, less transparent and with focal to massive hemorrhages or yellow to brown discoloration on its surface **(Al-Samman et al., 2003; Kappe, 2004; Körting, 2006)**. Surviving carp may later achieve varying degrees of regeneration of the affected swim bladder. A chronic course of swim bladder inflammation is also possible. Here clinical signs are not necessarily typical, the number of pseudoplasmodia in the kidney tubules is low, the pathological changes are of rather mild nature and regeneration is common. Such chronic cases are characterized by capillary dilatation, followed by small hemorrhages which degrade over time so that only traces of haemosiderin remain. The swim bladder wall is just slightly thickened but still opaque **(Al-Samman et al., 2003)**.

Renal sphaerosporosis

The disease is called renal sphaerosporosis and is caused by sporogony within pseudoplasmodia in the convoluted kidney. Histopathology reveals that the main problem is the mechanical damage those growing space-occupying parasitic aggregates inflict on the kidney tubular cells **(Al-Samman et al., 2003)**. As the pseudoplasmodia grow inside their host cells, they displace and compress the host cytoplasm and cell organelles. This way the host cell metabolism is impaired and the affected tubular epithelial cell responds with regressive changes such as atrophy **(Baker, 2008)** swelling and eventual cell-death, followed by compensatory epithelial hyperplasia **(Dyková & Lom, 1982).** Granulation

tissue may also proliferate in the degenerating tubules **(Baker, 2008)**. The accumulation of those parasitic stages leads to tubular dilatation, sometimes to an extent where tubular blockage occurs, resulting in grossly visible renal enlargement and increased kidney weight **(Al-Samman et al., 2003)**. Other gross pathological kidney lesions such as soft, pale and swollen areas are rarely observed but possible **(Körting, 2006)**. Impairment or loss of function and consequently appearing clinical signs are of minor importance in renal sphaerosporosis. Survivors of acute sphaerosporosis and chronically infected carp typically harbor fewer parasitic stages in their kidneys **(Al-Samman et al., 2003).**

Other tissues

An extrasporogonic phase does also occur in *Sphaerospora renicola*. Here the blood stages continue to spread to various organs and increase their numbers even though sporogony within the renal tubules has already begun **(Lom & Dyková, 1982**). The large K-cells sometimes get stuck within the fine capillaries of the rete mirabile in the eye choroid, where they lose their ability to move actively. When K-cells are blocking the blood supply to the surrounding choroidal tissue, hemostasis, leaking blood vessels, hypoxia, and death of the malsupplied cells is the result. Macroscopically the affected fish are often exophthalmic, showing hemorrhages within the orbit and necrosis **(Molnár, 1993)**. Since the C-cells and K-cells spread via blood circulation, they also reach the liver and the spleen where they cause less specific lesions. In relation to the body weight, hepato- and splenomegaly is a common finding in small carp infected with *Sphaerospora renicola* **(Al-Samman et al., 2003)**.

Cause of Death

The loss of tubular function inducing renal failure, abdominal adhesions in the course of inflammatory reactions due to blood vessel blockage and local necrosis, the mentioned organomegaly within an in relation very restricted space, are all possible causes for death itself. Additionally, the navigation difficulties caused by the swim bladder and eye lesions may attract predators and impair the food intake. Nevertheless, a recovery is sometimes possible.

Diagnosis

Fresh or stained blood smears **(Kappe, 2004)** are most likely to yield intravascular stages when they are taken in autumn of the first year or spring of the second year in the young carp´s life **(Grupcheva et al., 1985).** Native kidney samples from freshly dissected carp put on a glass slide and squashed under a coverslip are suitable for microscopical examination and detection of renal stages such as pseudoplasmodia and myxospores **(Al-Samman et al., 2003)**. Fresh, unstained plasmodia are non-motile, quite spherical and about 20 µm in size. Impression smears from renal tissue samples may also be stained with Giemsa to facilitate an examination as the plasmodial cytoplasm displays a bright coloration. Stained plasmodia are typically smaller and may only measure less than half the size of fresh specimens **(Lom & Dyková, 1982**). Renal stages are mainly detectible soon after hatching and peak again during the spring months of the following year **(Grupcheva et al., 1985).** The loose connective tissue of the swim bladder wall **(Csaba et al., 1984)** with its fine capillary rete mirabile can also be used for impression smears. When examined microscopically such smears may reveal the presence of K-cells, especially when the samples are obtained from acutely diseased fish. Impression smears of the eye choroid stained with Giemsa may reveal K-cells of round or amorphous appearance, containing secondary cells which again contain tertiary cells. Native smears may reveal pale, round K-cells resembling a signet-ring **(Molnár, 1993)**. Since each of the parasitic cell-within-cell stages has its own nucleus, this multinucleated parasitic stages can be distinguished from ordinary uninucleated body cells. When mature myxospores are found, they are generally of round shape with one little bump-like appendage on each shell valve **(Lom & Dyková, 2006)**. The two small polar capsules **(Körting, 2006)** are subspherical and the myxospore has two sporoplasms with a single nucleus each **(Lom & Dyková, 2006)**. The myxospores measure about 7 µm in diameter **(Körting, 2006)**. If available additional staining methods such as hematoxylin-eosin (H. E.) or Kossa stain may be applied on fixed and embedded organ samples **(Lom & Dyková, 1982**).

3.3.1.2. Sphaerosporosis in the gills (*Sphaerospora molnari*)

Clinical signs

Sphaerospora molnari is well known to parasitize the gills, skin and nasal pits respectively, significantly challenging the health of young *Cyprinus carpio*. According to **Molnár (1979)**, *S. molnari* targets common carp fry in their first three months of life, while **Lom et al. (1983)** consider carp in their first year of life as the typically susceptible age group. Infected carp may present with macroscopically visible white foci on the gills and dyspnea **(Novakov et al., 2015)**.When the physiologically bright reddish pink gills appear pale and show a rough texture, this can be indicating for *S. molnari* too **(Molnár, 1979)**. Young mirror carp, mainly in their first three months of life, may present with focal epithelial lesions on their anterior body. This cutaneous infection occurs in addition to the classical gill lesions **(Molnár, 1980)**.

Pathogenesis

Since the complete life cycle of *S. molnari* has not been resolved yet, the morphological actinospore type has not yet been identified. After the waterborne actinospore successfully released its sporoplasm into a young carp, presporogonic and extrasporogonic development takes place intravascularly **(Feist et al., 2015b)**. The resulting blood stages are able to move actively and comparatively fast due to two unique contractile actin types **(Hartigan et al., 2016)**. Eventually, the intravascular stages arrive at the gill epithelium, where the histozoic parasite initiates sporogony **(Eszterbauer et al., 2013)**.

Gill Sphaerosporosis

The sporogonic stages settle in the intercellular space along the gill filaments, between the gill lamellae, and to a lesser extent at the gill arch (**Molnár, 1979; Molnár & Eszterbauer, 2015)**. Only the stratified epithelium is parasitized. Sporogony begins in the deep, still undifferentiated stratum germinativum. As the early pansporoblasts differentiate further, they follow the growth direction of their host tissue so that the pseudoplasmodia harboring fully mature myxospores are located most superficially. At some point, the outermost host cells rupture and desquamate so that the infectious myxospores can easily escape into the surrounding water. As **Molnár (1979)** showed during his experiments,

infected fish may shed myxospores over a period of at least four months. The developing pseudoplasmodia stress and compress their host cells, including cytoplasm, nucleus and cell organelles leading to tissue necrosis and epithelial hyperplasia **(Baker, 2008)**. Nevertheless, *Sphaerospora molnari* alone does not parasitize the respiratory epithelium of the gill lamellae, connective tissue, cartilage or blood vessels **(Molnár, 1979)**. Respiratory problems are rather indirect consequences of *S. molnari*, since myxospores and cellular debris are accumulating excessively in between the gill lamellae, where respiratory gas exchange takes place **(Novakov et al., 2015)**.

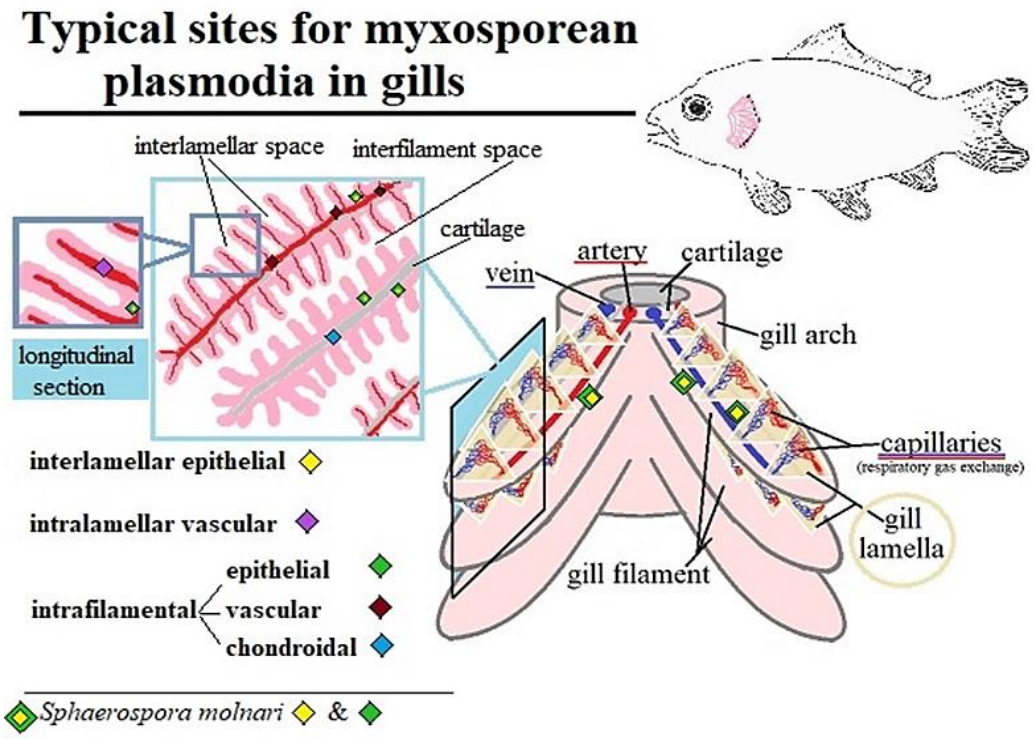


Figure 13: Own schematical illustration of typical locations for *Sphaerospora molnari* plasmodia in the gills of common carp

Cutaneous sphaerosporosis

Additionally to the classical gill lesions, a spread towards the neighboring stratified epithelium of the skin can occur **(Molnár, 1980b; Lom et al., 1983)**. In such cases the gill cavity, skin around the brachial slit and to a lesser degree the skin close to the dorsal and

abdominal fins, the thicker skin areas of the head and focally other cranially located skin areas may be infested with sporogonic stages. The affected cells of the cutaneous stratified epithelium undergo degenerative changes, lose their function and integrity **(Molnár, 1980b)**. This additional cutaneous form of *S. molnari* infection mainly occurs in young mirror carp, a common carp variant with significantly fewer scales **(Molnár, 1980b)**.

Cause of Death

Despite *S. molnari* may cause morbidity rates as high as 100% and infestations to an extent where up to 80% of the gill epithelium harbors *S. molnari* stages do occur, mortalities are rarely caused by *S. molnari* alone **(Molnár, 1979)**. The presence of *S. molnari* acts as a local and general weakening factor for the fish, it is a port of entry for secondary pathogens and co-infections with ciliates or fungi are common findings **(Molnár, 1979)**. In such mixed infections, it is hard to determine the exact origin of the present lesions but some authors see *S. molnari* as the primary causative agent **(Feist & Longshaw, 2006)**.

Diagnosis

A quick method to obtain suitable samples for a parasitological examination is a mucosal scraping from the fish gills. This way, pseudoplasmodia and myxospores **(Molnár, 1979)** can usually be detected. The latter are spherical, measure about 10 µm **(Feist & Longshaw, 2006)** and can be detected even without further staining. Skin scrapings yield suitable samples for the detection of the cutaneous form of sphaerosporosis **(Molnár, 1980b)**. Longitudinal gill sections, stained with haematoxylin-eosin (H.E.), Farkas-Mallory´s stain or Giemsa are recommended for the detection of *Sphaerospora molnari* **(Molnár, 1979)**. Here the filaments with their stratified epithelium as well as the gill lamellae with their respiratory epithelium can be identified and examined. With H.E. staining, the cellular and parasitic details are best seen, while the Farkas-Mallory staining is a metachromatic staining method for maturing myxospores. The young stages start out in light blue then become purple and red until the fully developed myxospores stain yellow **(Molnár, 1979)**. For the veterinary practitioner, the Giemsa staining remains a good choice, since it also stains the myxospores very well **(Molnár, 1979)**. As an infection with *S. molnari* usually results in heavy infestations, microscopic examination often reveals various intracellular sporogonic stages and host cells of an odd

shape with little cytoplasm. The myxospore morphology is typical for *Sphaerosporidae* **(Lom & Dyková, 2006)** and therefore of spherical shape, measuring about 8 – 13 µm **(Molnár, 1979)**. The two polar capsules are of equal dimensions, spherical as well and harbor one polar filament each. *Sphaerospora molnari* myxospores also have two sporoplasm cells with one nucleus each **(Lom et al., 1983)**. Myxospores may accumulate in large numbers within the interlamellar space. Multinucleated pansporoblasts in the deeper epithelial layers readily stain purple on H.E. and Giemsa, a visual effect that becomes intensified by the dark purple nucleus of the occupied host cell **(Molnár, 1979)**.

3.3.1.3. Intestinal giant cystic disease caused by *Thelohanellus kitauei*

Clinical signs

Thelohanellus kitauei is well known to cause clinical disease and tremendous losses among common carp in Asia (**Rhee et al., 1990)**. Genetically the species is also present in European common carp populations **(Zhao et al., 2016)** but clinically apparent cases are exceptionally rare. The exact reason is currently still unknown but this phenomenon is already subject to ongoing research activities. The age-group in which intestinal lesions develop is one to two-year-old carp **(Egusa & Nakajima, 1981)**. *T. kitauei* is also known to attack koi carp *(Cyprinus carpio haematopterus)* **(Shin et al., 2012)** and Israelian carp (*Cyprinus carpio nudus)* **(Rhee et al., 1990).** Affected carp may present with a pale body coloration and pale gills, appear emaciated but with an enlarged abdomen. The anus may be reddened and non-contracted with erosions on it. A mucoid discharge of white or yellow color may also be seen dripping from the anal opening **(Rhee et al., 1990)**. Recently, **Zhao et al. (2016)** described another pathological picture, where macroscopically visible, well circumscribed, pale nodules erupt from the skin, causing a loss of scales in the affected area, local hyperemia and erosions.

Pathogenesis

The pathogenesis begins when the infectious Aurantiactinomyxon type actinospores released by the oligochaete *Branchiura sowerbyi* anchor to a young carp (**Zhao et al., 2016)**. The exact port of entry for the infectious sporoplasm has yet to be demonstrated.

However, the target location for the pathologically most relevant sporogonic phase is the intestinal mucosa. First, the early sporogonic stages arrive at the submucosal layer, from where they mature and migrate further in a luminal direction to parasitize the lamina proporia mucosae **(Ye et al., 2017)**. The large plasmodia inflict significant pressure onto the surrounding columnar epithelium, leading to a cellular host response in form of infiltrating lymphocytes and granulocytes. Later, the host tries to enclose the noxious parasite by means of a granulomatous capsule. Fibroblasts and other connective tissue components, as well as epithelial cells, proliferate and neoangiogenesis may facilitate a cellular infiltration and nutrient supply. Once the myxospores are mature, they leave the destroyed mucosal layer and enter the intestinal canal from where they are shed into the environment. Following cyst rupture, the remnants are filled up with hyperplastic connective tissue and may atrophy over time **(Rhee et al., 1990)**. Recently, another possible location for sporogony was discovered in the dermal stratum spongiosum and stratum compactum of the skin. Despite plasmodia have not yet been demonstrated, *T. kitauei* myxospores were morphologically and genetically identified. The authors interpret their finding as a “habitat transfer" from the gut epithelium to the skin **(Zhai et al., 2016)**.

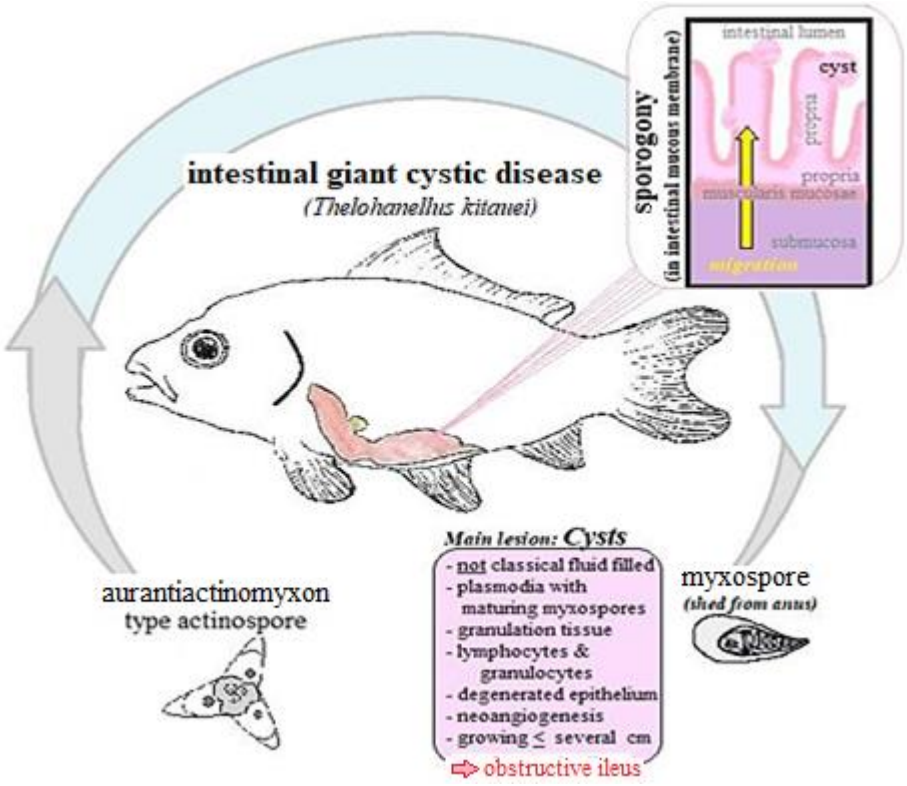


Figure 14: Own schematic illustration of intestinal giant cystic disease *(T. kitauei)*

Cause of Death and Gross Pathology

The actual cause of death is an obstructive ileus, due to large plasmodial tissue cysts originating from the intestinal mucosa, which eventually obstruct the gut lumen partially or totally **(Rhee et al., 1994)** and favor the development of a lethal secondary enteritis **(Wang et al., 2005)**. Single or multiple cysts of variable size impair the transport of ingesta, hinder digestion and nutrient absorption leading to appetite loss and the observed emaciation, as well as yellowish mucoid gut contents **(Egusa & Nakajima, 1981)**. As the cysts grow in size, they increasingly obstruct the gut lumen and begin to distend and overstretch the gut wall locally. The vascularity in the affected area becomes compressed, resulting in hypoxia and accumulation of metabolites in the related tissues. Hyperemia and flaccid, distended intestines, as well as a thin, fragile gut wall, may, therefore, be recognized on dissection **(Rhee et al., 1990)**. Since the carp´s hepatopancreas is physiologically located in close anatomical proximity to the parasitized intestines, the expanding gut parts often cause circulatory disturbances and pressure atrophy to the hepatocytes **(Egusa & Nakajima, 1981).** Over time, the affected fish develop anemia with blood parameters such as erythrocyte count, total protein, albumin and blood triglyceride content below the normal range. Those findings mainly result from the underlining malabsorption **(Wang et al., 2005)** and explain the pale appearance of skin, gills, inner organs, and muscles, as well as the observed ascites **(Rhee et al., 1990)**. Such weakened carps often develop secondary enteritis which can lead to mortalities as well **(Wang et al., 2005)**.

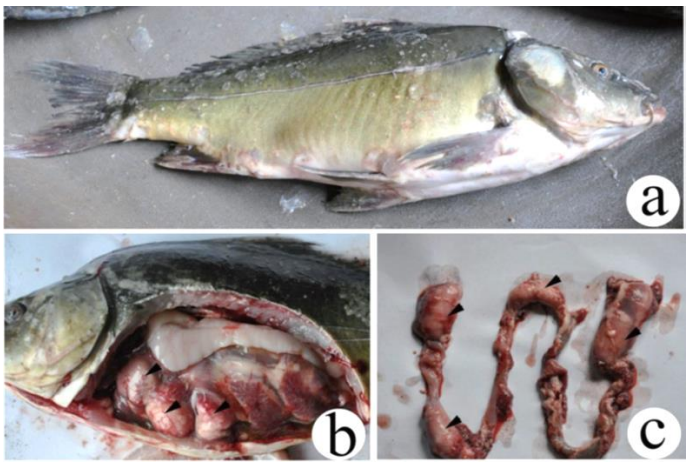


Figure 15: *Thelohanellus kitauei* in common carp. Presentation on gross inspection (a) and at dissection (b), (c). Large cysts (see arrows) are originating from the intestinal wall **(Ye et al., 2017).**

Figure 16: Gross pathological picture of *T. kitauei*. Right pictures (a, b, c) showing skin lesions, left pictures showing classical instestinal lesions **(Zhai et al., 2016).**

Diagnosis

Macroscopically well detectible intestinal cysts are mainly found in Asiatic carp populations. Giemsa or H.E. stained tissue sections from the intestinal lesions are suitable for a histopathological diagnosis of the sporogonic stages and lesions **(Rhee et al., 1990)**. Under field conditions, it may be enough to cut a macroscopic lesion open and examine a native impression smear for myxospores. The pyriform myxospore of *T. kitauei* measures about 25 µm in length, is 8 µm thick and 8 µm wide **(Ye et al., 2017)** with two thin shell valves, a single pyriform polar capsule, and about 9 polar filament turns **(Ye et al., 2017)**. It contains one pyriform sporoplasm with an iodinophilous vacuole, which can additionally be stained with Lugol's solution **(Egusa & Nakajima, 1981)**.

3.3.1.4. Fin cysts in caused by *Thelohanellus nikolskii*

Clinical signs and Gross Pathology

The typical age group developing lesions are fingerlings **(Molnár, 1982)** and rarely two or three-year-old carp may become clinically apparent as well **(Antychowicz et al. 2005)**. Affected carp may present with small, well circumscribed, pale or dark pigmented, protruding, macroscopically visible nodules on the surface of the fins. Those nodules are often of round or elliptical shape, have a firm consistency and are not moveable on palpation. Occasionally, carps with partially or totally missing tail fin can be seen **(Antychowicz et al. 2005)**. Older carps may present with similar plasmodial cysts on the scale tips **(Székely et al. 1998).** The typical season for the aforementioned lesions is during the European summer months **(Antychowicz et al. 2005)**.

Figure 17: Gross pathological picture of *T. nikolskii* lesions on fins (a) and scales (b). (Pictures were provided by the Fish Pathology and Parasitology Research Team, Institute for Veterinary Medical Research, CAR, HAS)

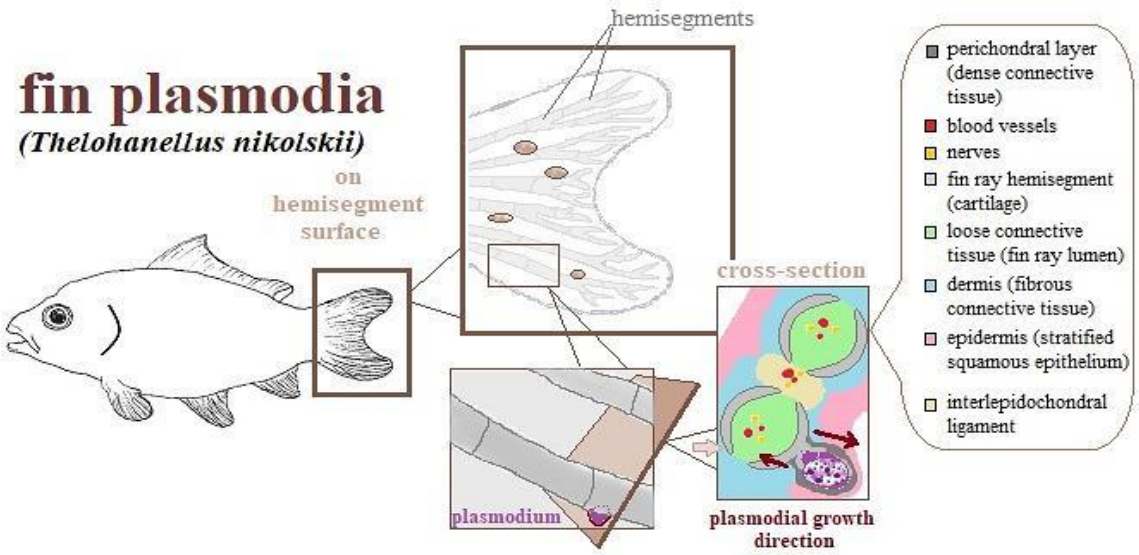


Figure 18: Own schematic illustration of *Thelohanellus nikolskii* development on the fins

Pathogenesis

Following contact with the infectious aurantiactinomyxon type actinospores originating from *Tubifex tubifex* **(Székely et al., 1998)**, the presporogonic phase takes place in currently unidentified tissue locations. The preferred target tissue for sporogonic development is the external surface of the fin ray cartilage where the perichondral cell layer is physiologically located. The latter secretes an extracellular matrix rich in collagen. Over time, the collagen undergoes calcification and a new cartilage is formed from it **(Molnár, 2002b)**. A similar collagenic cartilage is an element of the fish scales, which explains why *T. nikolskii* sometimes forms plasmodia on the scale tips of common carp as well **(Moshu & Molnár, 1997; Székely et al., 1998)**. As the fin plasmodia mature, they

expand and the surrounding perichondral cells respond with hyperplasia and an increased extracellular matrix secretion, later resulting in an increased cartilage synthesis. Consequently, the parasite becomes encapsulated by prominent, dense connective tissue **(Molnár & Eszterbauer, 2015)**. A second tissue, the fibrous connective tissue of the dermis may also respond with proliferation, contributing to the formation of a thick, rigid cyst capsule **(Molnár, 2002b)**. Young plasmodia can histopathologically be identified as cell-in-cell stages with several well-stained nuclei and a capsule with an uneven surface and rich in densely packed collagen fibers **(Desser, 1983)**. Plasmodial growth can also occur towards medially, at the inner side of the cartilaginous fin hemisegment. This often leads to distortion and disruption of the cartilage structure, sometimes even fragmentation of the latter. Such fragments may later become a part of the thick fibrous plasmodial capsule **(Molnár, 2002b)**. Mature plasmodia eventually rupture and myxospores are readily shed into the surrounding water. The rigid, empty nodular remnants persist for a considerable time on the affected fin.

Cause of Death

Death is not a commonly seen consequence of *T. nikolskii* infections but rupturing plasmodia may serve as a port of entry for secondary invading pathogens. Deformed or damaged fins and plasmodial foci may additionally impair the market value of harvested carp intended for human consumption or aquacultural trade.

Situation in koi and other carp subspecies

Thelohanellus nikolskii has been well known to parasitize common carp *(Cyprinus carpio)* in central European aquacultures over the past decades and prevalences up to 100% with sometimes 50 plasmodial nodules per carp are not rare **(Molnár, 2002a)**. According to **Molnár (2002a)**, the European subspecies of common carp *(Cyprinus carpio carpio)* is also susceptible but 15% of the early plasmodial stages detected during his study terminated their life cycle and never matured. Consequently, the European carp appears less susceptible to a clinical manifestation of *T. nikolskii* infection. Considerably fewer young koi (*Cyprinus carpio haematopterus*) from the same ponds and the same age as the examined *Cyprinus carpio* and European carp harbored any plasmodia. When plasmodia were found in koi, those were smaller in size and occurred in smaller numbers compared to common carp (**Molnár, 2002a)**.

Diagnosis

The gross clinical picture is already quite informative and may lead to a tentative diagnosis. A detailed macro- and microscopical examination of living, narcotized carp is one of the diagnostic options to confirm the suspicion. The use of a stereomicroscope may prove useful in this case **(Molnár, 2002a)**. Freshly obtained fin cysts may be examined in the native state or they are cut into sections and stained with H. E. for further histopathological evaluation **(Antychowicz et al. 2005)**. Farkas-Mallory´s stain may also be used when a metachromatic staining of parasitic stages is wished **(Molnár, 2002b)**. The plasmodia usually measure a few millimeters in size and may occur on any fin**.** When mature myxospores are recovered, they are usually pyriform or elliptical, 15 µm long and 11 µm wide. The myxospores contain one but sometimes up to three round polar capsules with one double curled polar filament each **(Antychowicz et al., 2005)**.

3.3.1.5. Haemorrhagic thelohanellosis caused by *Thelohanellus hovorkai*

Clinical signs

T. hovorkai displays a seasonal pattern of actinospore release and parasitoses, with highest prevalences during the summer months **(Liyanage et al., 1998)**. This is an important parasitosis of *Cyprinus carpio*, including ornamental koi and outbreaks can cause high economical losses, especially in Asia **(Yokoyama et al., 1998)**. Infected common carp may present initially with non-specific clinical signs such as anorexia, emaciation, and lethargy. As for various other diseases and conditions, fish infected with *T. hovorkai* may display their malaise as they spend a considerable time every day in surface waters or they rest near the water bottom for a prolonged time **(Yokoyama et al., 1998)**. Clinical signs can develop as early as two weeks post exposure and young koi carp are as susceptible as common carp **(Liyanage et al., 1998)**. At the terminal phase of hemorrhagic thelohanellosis, affected carp often develop diffuse petechial hemorrhages on various parts of the skin, especially on the ventral aspect of the fish body **(Yokoyama et al., 1998)**. Susceptible are *Cyprinus carpio* as early as fingerling-size **(Székely et al., 1998)**, at one year of age **(Liyanage et al., 1998)** but adult carp exceeding this age also acquire the infection **(Yokoyama et al., 1998)**.

Figure 19: Gross pathological picture of haemorrhagic thelohanellosis in carp. (Yokoyama et al., 1998)

Pathogenesis

The final invertebrate host is *Branchiura sowerbyi*, which releases aurantiactinomyxon type actinospores into the water **(Székely et al., 1998)**. Susceptible common carp acquire the infection via ingestion of actinospores or as they swim in water heavily contaminated with the latter **(Liyanage et al., 2003)**. The parasitic host invasion begins when the actinospore releases its infective sporoplasm in close proximity to the gill filaments, which then serve as a port of entry for the migrating parasite. After *T. hovorkai* invaded the gills it travels via a currently unknown route to its target location, the connective tissue, where it initiates sporogony and forms plasmodia **(Liyanage et al., 2003)**. Since the target tissue for *T. hovorkai* is a component of various organs as well as the serosal membranes, *T. hovorkai* has a wide range of suitable locations to develop **(Székely et al., 1998)**. In general, those *T. hovorkai* plasmodia which are developing deeply within a certain host tissue are responsible for more severe pathological lesions, compared to superficially proliferating plasmodia **(Liyanage et al., 1998)**. While maturing plasmodia located close to tissue surfaces simply rupture at some point and release their myxospores into excretory canals, body cavities or the external world, the deeper situated plasmodia liberate their myxospores into the intercellular space, the lymphatic system or the spores enter the blood circulation for further spreading within the fish. Such freely circulating myxospores are prone to get stuck in fine capillaries, where they inflict microscopical traumas to the vessel wall, obstruct the blood supply, cause hemostasis, increased vessel permeability, hemorrhages, edema and local necrosis. The host organism often responds to this noxious stimulus with cellular infiltration and inflammation **(Liyanage et al., 1998)**. Particularly

myxospores, which become trapped within the intermuscular space and the cutaneous intercellular space, seem to attract phagocytes and other inflammatory cells. Macrophages readily phagocytose *T. hovorkai* myxospores and later migrate to melanomacrophage centers, the fish analog to mammalian lymph nodes, for further attempts of degradation and presentation **(Yokoyama et al., 1998)**. Myxospores which remain trapped in the skin cause a local inflammation and may detach from the surrounding epithelium as part of the forming inflammatory debris **(Yokoyama et al., 1998)**. According to **Liyanage et al. (2003)**, *T. hovorkai* may complete its life-cycle within three to five months at temperatures of 20 – 25°C.

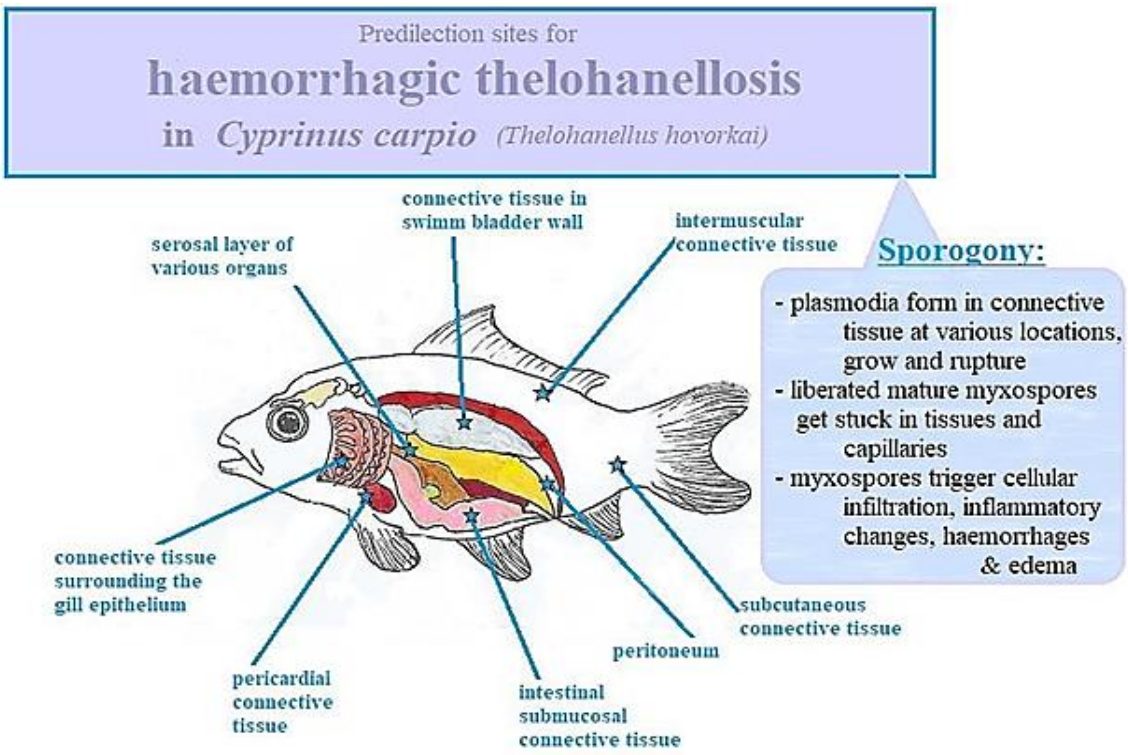


Figure 20: Own schematic illustration of predilection sites for *T. hovorkai* development

Cause of Death

Massive hemorrhages and edema formation within the targeted connective tissue may reach intensities where the common carp eventually succumbs to the parasitic infection **(Yokoyama et al., 2012)**. *Thelohanellus hovorkai* infections usually take a

chronical, progressive course and death may follow several weeks post infection. However, affected carp may recover once they survive the critical summer months, but myxospores may remain in certain tissues and are sometimes found even post recovery **(Yokoyama et al., 1998)**.

Diagnosis

A quick field diagnosis may be carried out via a mucous scraping from the carp´s skin surface. The mucus sample is then placed on a microscope slide and a drop of physiological saline is added before placing a cover slip on it. Such wet mount preparations are suitable for myxospore detection using a conventional light microscope and this non-invasive method poses little harm to living carp **(Yokoyama et al., 1998)**. Tissue samples from various organs with connective tissue components as well as serosal layers and skin lesions are suitable for staining methods such as H.E., Giemsa, and May-Grünwald **(Yokoyama et al., 1998)**. A histopathological diagnosis is then based on further evaluation with a light microscope **(Liyanage et al., 1998)**. Mature *T. hovorkai* myxospores are typically 20 µm long, 10 µm wide, with one 9 µm long and 8 µm wide polar capsule **(Feist & Longshaw, 2006)**.

3.3.1.6. Infection of skeletal muscles with *Myxobolus cyprini*

Clinical signs and Gross Pathology

Myxobolus cyprini is well known to parasitize several fish species of the *Cyprinidae* family in Europe, Asia and North America **(Kent et al., 1996)**. Most susceptible to *M. cyprini* are common carp in their second year of life but carp fry in their first year of life are occasionally affected as well. Despite an ongoing massive infection typically results in pathological changes in various organs, clinically apparent cases are hardly seen. **Molnár & Kovács-Gayer (1985)** mainly detected subclinical and latent courses of infection but also observed clinical signs such as abdominal enlargement, exophthalmos, ascites and reddish discolorated skin due to hemorrhages. An infection with *M. cyprini* may also be referred to as “malignant anemia” **(Yokoyama et al., 2012)**. On post-mortem examination, macroscopically visible plasmodial nodules may be found in the striated musculature.

Pathogenesis

Since the complete life cycle of *M. cyprini* has yet to be revealed, the associated actinospore type and the invertebrate final host have not been described as the present. However, *M. cyprini* is histozoic and undergoes sporogony exclusively within the myocytes of skeletal muscle fibers. It is therefore considered a "specific muscle parasite" (**Molnár & Kovács-Gayer, 1985).** In the course of intracellular parasite proliferation and myxospore maturation, *M. cyprini* develops plasmodia, which can exceed 1 mm in length. Such large parasite aggregations may be synonymously termed "pseudocysts" (**Molnár & Kovács-Gayer, 1985)** and sometimes occupy the entire myofibre, consequently causing local necrosis. As plasmodial growth and the intracellular accumulation of sporogonic stages progresses, the cell organelles, sarcoplasm, and function of the affected myocytes become significantly impaired by massive mechanical compression. Affected myofibrils additionally distend towards the neighboring tissue, exerting further pressure with resulting atrophy of the surrounding myofibrils **(Feist & Longshaw, 2006)**. However, the developing plasmodia do not provoke any cellular host response and the lesions are rarely extensive enough to cause clinically apparent courses of *M. cyprini* infection in common carp (**Molnár & Kovács-Gayer, 1985)**. Once the sporogonic phase has produced a new generation of mature myxospores, the massive intracellular pressure ruptures the affected myocyte and the infective spores can enter the extracellular space for further transportation and shedding. Circumscribed loss of structure and function within the parasitized muscle tissue are therefore histopathologically and occasionally also macroscopically evident in young common carp. Some myxospores never manage to leave their location of origin. Instead, they trigger a cellular host response in form of granuloma formation, leading to an encapsulation. In such cases, epithelioid cells gather around the noxious parasite spore, later followed by connective tissue elements. Other liberated myxospores are phagocytosed by macrophages and transported to the renal, hepatic and splenic melanomacrophage centers (**Molnár & Kovács-Gayer, 1985)**. The latter is the fish equivalent to germinative centers in mammalian lymph nodes and typically harbor macrophages for phagocytosis and antigen presentation to lymphocytes **(Agius & Roberts, 2003)**. Another proportion of myxospores gain access to the lymphatic system and eventually enter the bloodstream from where they circulate to various organs until they eventually get stuck in fine capillaries, where they additionally inflict microscopical traumas (**Molnár & Kovács-Gayer, 1985)**.

Capillary obstruction leads to local hypoxia, increased vessel permeability, hemorrhages, hydropic changes and edema formation as well as localized necrosis within the affected tissues. This may result in the formation of "yellow bodies" (**Molnár & Kovács-Gayer, 1985)**, a type of debris consisting of detached, necrotic host cells arranged envelope-like around myxospores. When the described debris forms in excretory tissues such as the renal tubules, forms near a bile duct lumen or in the intestinal wall, this facilitates a shedding of *M. cyprini* and the parasite may complete its life cycle in an aquatic invertebrate. Those myxospores which are entrapped within the fine skin or gill capillaries, typically exit the fish directly as they induce local hemorrhages, necrosis, and epithelial desquamation. Sporogony within the striated musculature, dissemination of the produced myxospores and their liberation to the environment usually take place parallelly over a period of time within the same common carp (**Molnár & Kovács-Gayer, 1985)**.

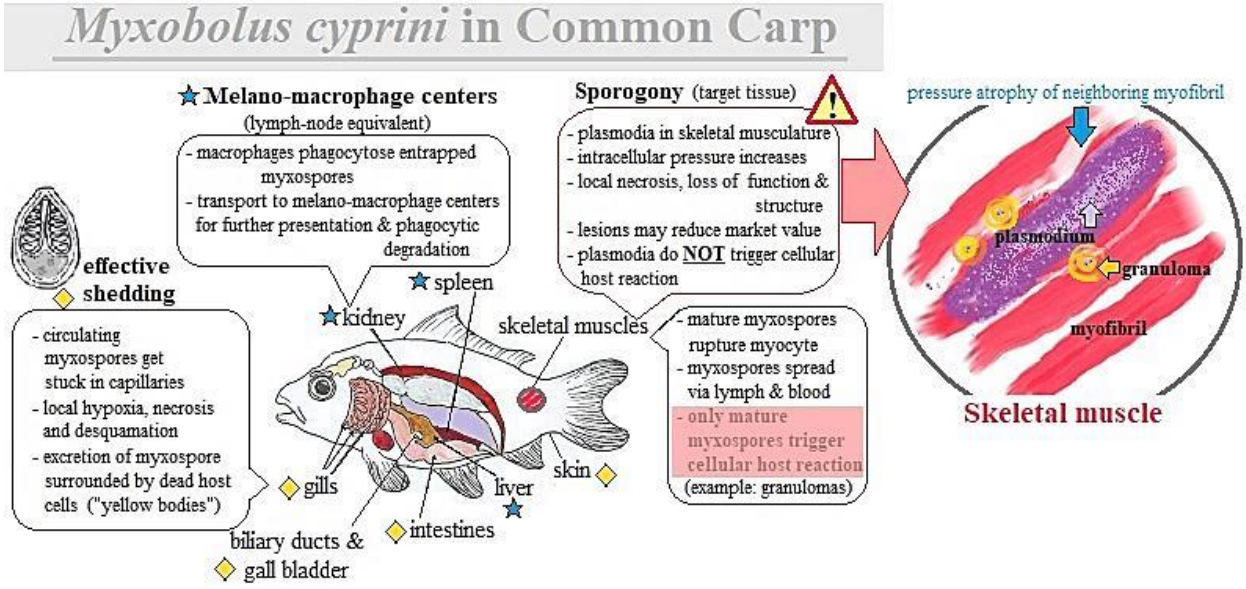


Figure 21: Own schematic illustration of *Myxobolus cyprini* infection in common carp

Cause of Death

Myxobolus cyprini is unique in respect of the most pathogenic life stage. For the other myxosporean parasites described in this thesis, the process of sporogony itself inflicts the most damage to the host and proliferating plasmodia are the first step in an often deadly cascade of events. In contrast, the product of sporogony, the mature myxospores, already

liberated from the plasmodium are the most pathogenic parasitic stage in *Myxobolus cyprini*. Severely infested young carp with a pronounced clinical disease may eventually die from organ failure when an enormous myxospore load induces a massive cellular host reaction, an overwhelming degree of capillary obstruction and the resulting diffuse necrotic changes within the affected organs. Prolonged infection and stress may also increase the likelihood of secondary infections, which further complicate the pathological picture. However, the majority of *M. cyprini* infections do not kill the parasitized young carps (**Molnár & Kovács-Gayer, 1985).** Nevertheless, muscle rarefication and granulomas in the filet may lower market values of cultured common carp and several months of ongoing parasitosis may interfere with weight gain and overall condition of young carp.

Diagnosis

Since infections with *M. cyprini* often remain subclinical or latent, post-mortem inspection, dissection, and histopathological examination are highly recommended for diagnosis. Gross pathology may already give rise to suspicion when focal necrosis and granuloma formation appear in the skeletal musculature. Tissue samples of freshly killed common carp are suitable for native impression smears and further microscopical examination for parasitic stages. Plasmodia are only demonstrable from skeletal muscle samples, while mature myxospores additionally appear in the peritoneal capillaries or fine blood vessels of various viscera such as the pancreas, kidney, gills, skin, and intestines (**Molnár & Kovács-Gayer, 1985)**. The melanomacrophage centers often appear markedly enlarged in histopathological preparations due to increased macrophage activation and myxospore sequestration. Drops of freshly obtained blood put on a microscope slide and placed under a coverslip may reveal the presence of circulating myxospores. Histological sections of the aforementioned tissues can additionally be stained with H.E. or Farkas Mallory´s staining to aid visualization of *M. cyprini*. Fresh, native mucosal scrapings from gills and intestinal mucosa are also suitable for a microscopic examination (**Molnár & Kovács-Gayer, 1985)**. Morphologically, mature myxospores of *M. cyprini* resemble those of other Myxobolus-type myxospores and are of subspherical shape, 10-16 µm long, 8-12 µm wide with two equally sized polar capsules, each containing one coiled polar filament **(Feist & Longshaw, 2006)**. *M. cyprini* myxospores are also demonstrable from feces (**Molnár & Kovács-Gayer, 1985; Kappe, 2004)**.

3.3.1.7. Gill myxobolosis associated with *Myxobolus koi*

Clinical signs

Myxobolus koi usually infects young *Cyprinus carpio*, including ornamental koi **(Camus & Griffin, 2010)** in their first months of life **(Yokoyama et al., 1997)**. An infection with *M. koi* may induce respiratory distress **(Yokoyama et al., 2012),** anorexia and death **(Camus & Griffin, 2010).** Clinically apparent cases typically occur in the summer months and disappear over the colder seasons **(Yokoyama et al., 1997)**. Depending on the time of infection and the type of developing plasmodia, young carp may either present with or without dyspnea. Small whitish foci of less than one millimeter in size, located in the gills or grossly visible plasmodial nodules of at least one millimeter in size may be found on the gills **(Egusa, 1978)**. On gross pathology, the large type plasmodia appear as one to several solitary nodules of pale color and firm consistency.

Pathogenesis

Myxobolus koi is responsible for seasonal waves of parasitoses, often resulting in clinical disease with significant economic losses in common carp culture, especially in Asia **(Egusa, 1978; Yokoyama et al., 1997)**. Again, the life cycle of this myxosporean species has not been resolved yet and the involved actinospore type along with the necessary oligochaete host, has not been identified to date. The exact port of entry, route of spreading within the fish host as well as the morphology of different presporogonic stages has yet to be revealed too. Pathologically most relevant is the sporogonic phase, which is exclusively taking place in the gill epithelium. **Yokoyama et al. (1997)** discovered two distinct pathological pictures as a possible outcome of *Myxobolus koi* infections, a large type of plasmodia with lower prevalence and a small type of plasmodia with a higher prevalence. Especially the large plasmodia are capable of producing high mortality rates in young *Cyprinus carpio* **(Yokoyama et al. 2012)**.

Large Plasmodia

An early plasmodial progenitor begins sporogony within the gill lamellae. Whether the initial sporogonic stages of *M. koi* start their development within the gill epithelium, blood vessels or cartilage is not clear. As the plasmodium with its irregular structure grows in size, harboring more and more it expands and distends the affected lamellae. Later the gill

filament on which the lamella is located becomes involved as well. A capsular layer of slightly eosinophilic, fibrovascular host tissue surrounds the outermost surface of each plasmodium. This indicates that the parasitic proliferation within each plasmodium destroys the intralamellar blood vessels, consequently leaving only a capsule and fibrovascular cords protruding towards the plasmodial center. Eventually, the plasmodium grows to a size where it often reaches the neighboring plasmodium and they commonly fuse as they proceed with their enlargement **(Camus & Griffin, 2010)**. Such plasmodial aggregates can stretch over several gill filaments, sometimes also include the neighboring gill arch. Such large plasmodial aggregates may grow up to several millimeters in size, appearing as solitary or multiple pale or white nodules on the gill surface, where they are well visible at gross pathological inspection **(Yokoyama et al., 1997; Camus & Griffin, 2010)**. Histopathologically, the gill epithelium stressed by the growing plasmodium responds with hypertrophic changes to compensate for the epithelial cells lost due to pressure atrophy. As a result of epithelial hypertrophy, the affected gill filaments may acquire a club-shaped, plump appearance. Since the sporogonic stages proliferate within the gill lamellae, the physiological site for blood-gas exchange, respiration becomes impaired to various degrees. According to **Yokoyama et al. (1997)**, large plasmodia generally develop in small common carp that acquire *M. koi* early in summer, therefore pathological lesions usually peak around July and decline to practically zero cases until October of the same year.

Small Plasmodia

The prevalence of this second possible outcome of *M. koi* infection is highest around August and again no cases are seen during the colder months of the year. Here sporogony leads to plasmodia of a microscopical size of less than 1 mm. Sporogony takes place within the gill lamellae and maximally one small plasmodium develops per lamella. The affected lamella undergoes the typical distension and the increasing mechanical pressure stresses intralamellar capillaries and epithelium. However, the plasmodial growth is limited and neighboring small plasmodia do not fuse with each other **(Yokoyama et al., 1997)**.

Cause of Death

Massive progressive destruction of the respiratory epithelium may lead to pronounced generalized hypoxia and affected carp may eventually suffocate.

Diagnosis

In order to diagnose *M. koi*, it is possible to anesthetize or euthanize the fish and to excise a sample of gill tissue for further examination **(Yokoyama et al., 1997)**. A stereomicroscope is the equipment of choice for detecting plasmodia right on the spot without further staining **(Yokoyama et al., 1997)**. Once a plasmodium is identified, it is placed on a microscope slide and squashed with another slide to aid a better detection of present myxospores. In situations where an immediate microscopical examination is impossible, a wet mount preparation may prove useful. For the latter, a suspected plasmodium is preserved in 70% ethanol first. Later, it is put onto a microscope slide with physiological NaCl solution, placed under a cover-slip and squashed to liberate myxospores, followed by a light microscopical evaluation **(Camus & Griffin, 2010)**. When further histopathological methods are available, gill samples may be stained with H.E., May-Grünwald-Giemsa **(Yokoyama et al., 1997)** or Lugol's iodine solution **(Camus & Griffin, 2010)**. With the latter staining, no iodinophorous vacuole is detectible in *M. koi* myxospores **(Yokoyama et al., 1997)**. Typically, *M. koi* myxospores are pyriform, about 14 µm long, 8 µm wide, have two polar capsules, each measuring 9 µm in length and 2 µm in width, with polar filaments coiling 8 times **(Crawshaw & Sweeting, 1986; Yokoyama et al., 1997; Camus & Griffin, 2010)**. A serological method, the indirect fluorescent antibody technique (IFAT) is also available to test for *M. koi* (**Yokoyama et al. 1997).**

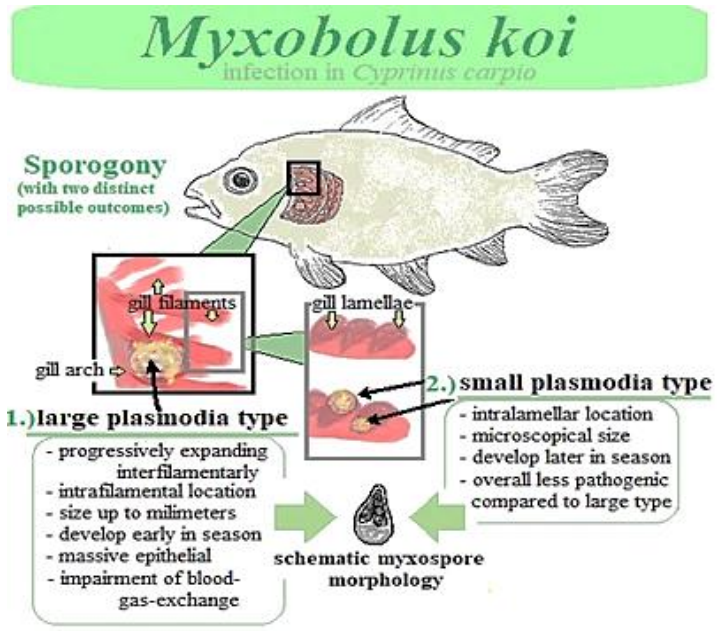


Figure 22: Schematic illustration of Myxobolus koi infection (by Susanne Braun)

3.4. Possible impact on Aquaculture and Hobby, treatment and preventive options

Current situation and possible impact on Aquaculture

As the oldest domesticated fish species **(Balon, 2004)**, the common carp *(Cyprinus carpio)* was first cultivated by the Romans about 2000 years ago. Originally endemic in the Danube river, this omnivorous fish was introduced worldwide **(Balon, 2004),** where it continues to thrive in various water conditions and a wide temperature range of 3 to 35 °C **(Eaton et al., 1995)**. Currently, about 9 % of the total global freshwater aquaculture is common carp and the production tends to increase together with the growing international aquacultural production **(FAO, 2017)**. Asia and Europe are traditionally the main producing and consuming regions, while international shipping and trade are low. Cultivated common carp are often reared in extensive or semi-intensive systems and are therefore produced rather environmentally friendly in ponds. Alternatively, intensive carp cultures with high stocking densities exist as well, especially in Asia **(Hartmann et al., 2015)**. It is common practice to either hatch the carp eggs in separate outdoor ponds or they are hatched in special containers and then transferred to ponds a few days later **(FAO, 2017)**. This means the most susceptible age group is usually kept in an environment where a contamination with myxosporeans and other pathogens is a plausible scenario. Since common carp feed on zooplankton throughout their life and since they are considered a bottom-dwelling species with a strong instinct to dig in muddy sediment for food **(Garcia-Berthou, 2001)**, they are naturally prone to encounter potentially infected oligochaetes as well as freshly released actinospores. Myxosporean parasites may arrive at aquacultural facilities, laboratories, and hobby ponds via various routes. The infective spore stages may travel over significant distances in contaminated water, when the latter is relocated via human activity, water currents, draining systems and so on. Hobbyists may introduce the parasites via wild caught fish, aquatic plants or live food **(Baker, 2008)**. Free-floating myxospores may remain infective for about three months **(Nehring et al., 2015)** and waterborne actinospores usually sustain their viability for a few days **(Özer & Wootten, 2002; Kallert & El-Matbouli, 2008)**, but their ability to successfully invade a host decreases as they age. The introduction of infected invertebrates is another risk factor for aquaculture and hobby keepers. Mud dwelling annelid worms are a fix component of natural aquatic ecosystems all over the world. They gain access to extensive and intensive aquaculture systems via sediment, waterfowl and other wildlife, water drains and various

other ways. Farmers and hobbyists may also catch or buy oligochaetes to supply their fish with life food. According to **Hallett et al. (2006)** and the authors own unpublished observation, even commercially packed tubificid worms purchased in large pet shops may harbor and release actinospores. Water, mud, wildlife or fomites may also serve as mechanical vectors for the infectious spore stages of myxosporean parasites.

Prevention and treatment

Prolonged feeding of the antiparasitic drug Fumagillin in medicated feedstuffs is an effective measure for prevention and treatment of several early myxosporean infections. **Rhee et al. (1993)** fed carp already clinically infected with *T. kitauei* with 10.6 mg Fumagillin for a period of four weeks. Despite the original lesions did not resolve none of the almost market-sized carp died from the infection. The same researchers exposed subclinically infected subadult common carp weighing around 500g to half of the original Fumagillin dosis and achieved a preventive effect. No side effects of the drug were detected and **Rhee et al. (1993)** concluded that "cumulative mortalities of fish and the extrusion rates of the polar filaments of the spores were significantly decreased in a dose-independent fashion". **Molnár (1987)** also obtained positive results, when he experimentally used Fumagillin against *Sphaerospora renicola*. Treating water contaminated with actinospores with UV-radiation has been demonstrated to successfully prevent *Myxobolus cerebralis* infections **(Hedrick et al., 2000**). Another relevant parameter is the water-flow-through in aquacultural systems. **Hallett & Bartholomew (2008)** found that a low water flow favors high oligochaete densities and high parasite proliferation rates, potentially increasing the infectious spore load. Whether and to what degree the latter findings apply to carp pathogenic myxosporeans has yet to be revealed. The effective prevention and management of secondary pathogens and parasitic co-infections is of major importance **(Jeney & Jeney, 1995)**. Supplementing fish food with substances known for their stimulating effect on the immune system, such as prebiotics, probiotics, vitamins and herbal compounds may enhance the host resistance to various pathogens, possibly including myxosporeans as well. Nevertheless exact scientific data for the effectiveness of such additives on myxosporean infections is lacking until now **(Sitjá-Bobadilla et al., 2015)**. However some authors consider a high-quality nutrition with high vitamin content as a suitable supportive measure **(Körting, 2006)**. Prevention may also

begin at the oligochaete level. Experiments conducted by **El-Matbouli & Hoffmann (1991)** revealed, that *M. cerebralis* myxospores are extremely resistant to freezing temperatures. The actinospores were exposed to – 20°C and temporarily entered an inactive, dormant state but remained infective to their oligochaete host *Tubifex tubifex* for at least 3 months. Therefore, treating objects, water or ponds with extreme cold or allowing an exposure to cold winter temperatures does not necessarily eliminate the parasite from an establishment. **El-Matbouli & Hoffmann (1991)** also demonstrated that neither ingestion, nor digestion by northern pike and mallard duck destroyed the infective stages of *M. cerebralis*, which indicates, species other than the typical hosts of the parasite are hardly preventable risk factors in the spreading and transmission of infective stages. Comparable experimental data for carp pathogenic myxosporean parasites is not available so far. Carp of the most susceptible age may be reared in water kept free from oligochaetes, which is difficult to achieve in practice, especially in extensive or semi-intensive carp cultures or hobby ponds. Hatchery-tanks or hobby aquaria may still be suitable for fine filtration systems, maintaining the circulating water free from myxosporean stages. One option to minimize the risk of actinospore transmission, while still ensuring the carp have a suitable soil to display their natural digging behavior is, to actively choose a certain soil type, which is an unsuitable habitat for the oligochaete species in question. **Liyanage et al. (2003)** demonstrated that *Branchiura sowerbyi*, the final host of several pathogenic myxosporeans, is not able to populate sandy pond bottoms since it prefers the softer mud soil. The same study revealed that other non-vector oligochaete species readily thrive in a sandy substrate. Completely foregoing any substrate on the water bottom can serve as an option for very young fry or in the case of laboratory setups. If live oligochaetes are intended as a food source, they can first be stored at temperatures well outside the parasite´s optimal temperature range. Experiments indicate that certain oligochaetes infected with myxosporeans, temporarily stop releasing actinospores at 4 °C **(Rácz, 2004)**. Under field conditions, the oligochaetes may escape the hungry carps and resume actinospore production in warmer water or mature intraoligochaete stages may eventually infect the fish post ingestion. **Rácz (2004)** also proved that certain zooplankton, such as *Cyclops spp*. are natural predators of

free-floating myxosporean stages. A major limitation of this method for spore reduction is the aquatic food chain, meaning that among others, the pond fish themselves readily feed on *Cyclops spp*. and other zooplankton. **Liu et al. (2011)** discovered that the bacteria species *Aeromonas veronii* can produce a unique chitinase enzyme which lyses the shell valves of the infective myxospores. The researchers isolated the responsible gene and transferred it to *E. coli*, which then began to produce the biologically active enzyme. As a fish food additive, this bacterial enzyme may help to reduce the shedding of infectious myxospores in the future. **Yang et al. (2014)** found evidence that *Thelohanellus kitauei* significantly relies on LDL-receptor based endocytosis to supply itself with host-derived fat. *T. kitauei* also uses certain proteolytic enzymes and protease inhibitors to interfere with the host immune system and aid parasitic invasion. Furthermore, the parasite is unable to perform de novo synthesis of glucose and is highly dependent on such host resources. According to the researchers, those and several other detected mechanisms may serve as potential targets for future pharmacological agents against myxosporeans. After **Tun et al. (2000)** observed two microsporidian parasites within intestinal plasmodia in clinically diseased Tiger Puffer (*Takifugu rubripes*), **Morris & Freeman (2010)** discovered that the parasitic microsporidian *Flabelliforma magnivora* not just parasitizes oligochaetes, it also parasitizes myxosporeans present within the oligochaetes and therefore significantly hampers actinospore formation and release. Using microsporidian parasites against myxosporean parasites might, therefore, become an option for future prevention or treatment of myxosporean infections. Developing suitable vaccines against myxosporeans is already subject of ongoing research. Since immunological processes in fish require further detailed further studies and the various myxosporean developmental stages may express different antigens, the development of fast and effective vaccines is a complex task. However, certain fish species were already shown to aquire immunity against their myxosporean parasites once they survived an initial exposure. Selecting genetically more resistant host strains for aquaculture may also become more and more relevant in the future **(Sitjà-Bobadilla et al., 2015)**.

Figure 23: Triactinomyxon type actinospores detected in commercial live food

4. Material and Methods

4.1. Source of oligochaetes

All oligochaetes used during the laboratory experiments were collected from Kis-Balaton Water Reservoir in Western Hungary. From decades of previous research, the water body is well known to harbor various species of oligochaetes, as well as fishes and myxosporeans. Zones with water levels not exceeding 120 cm in depth and located near the water edge where semiaquatic vegetation was growing, were chosen as sites for oligochaete collection. A net of 1 000 µm mesh size was used to recover portions of soil, rich in organic matter and oligochaetes inhabiting the latter. Most of the debris was carefully removed and oligochaetes were transported to the laboratory. There, the oligochaetes were spread on a tray with a few millimeters water level for better visualization and identification. *Branchiura sowerbyi, Tubifex tubifex and Limnodrilus spp.* were found to be present but only *Branchiura sowerbyi* were selected for the experiments. Each *B. sowerbyi* specimen was transferred into an individual 6-24 ml cell-well plate and provided with dechlorinated tap water according the regime introduced by **Yokoyama et al. (1991)**. They were kept in the cell-well plates for 3 to 7 days at room temperature and each plate was checked for released actinospores regularly, using a Zeiss Treval 3 inverted microscope. After that, the oligochaetes were individually transferred to small plastic cups with a volume of 100 ml each. The cups were stocked with a layer of 2cm sterilized mud, enriched with small pieces of chicken feces for a higher organic matter content and they were aerated continuously. As a food source for the *Branchiura sowerbyi*, commercial granulated fish food was given twice a week and evaporating water was filled up with fresh tap water. Each cup was stocked with a single *B. sowerbyi* specimen and for the duration of the experiments, a temperature between 18 and 24°C was maintained. The infected oligochaetes were kept in the laboratory even after the fish had been exposed to the released actinospores and random checks for further actinospore liberation were performed.

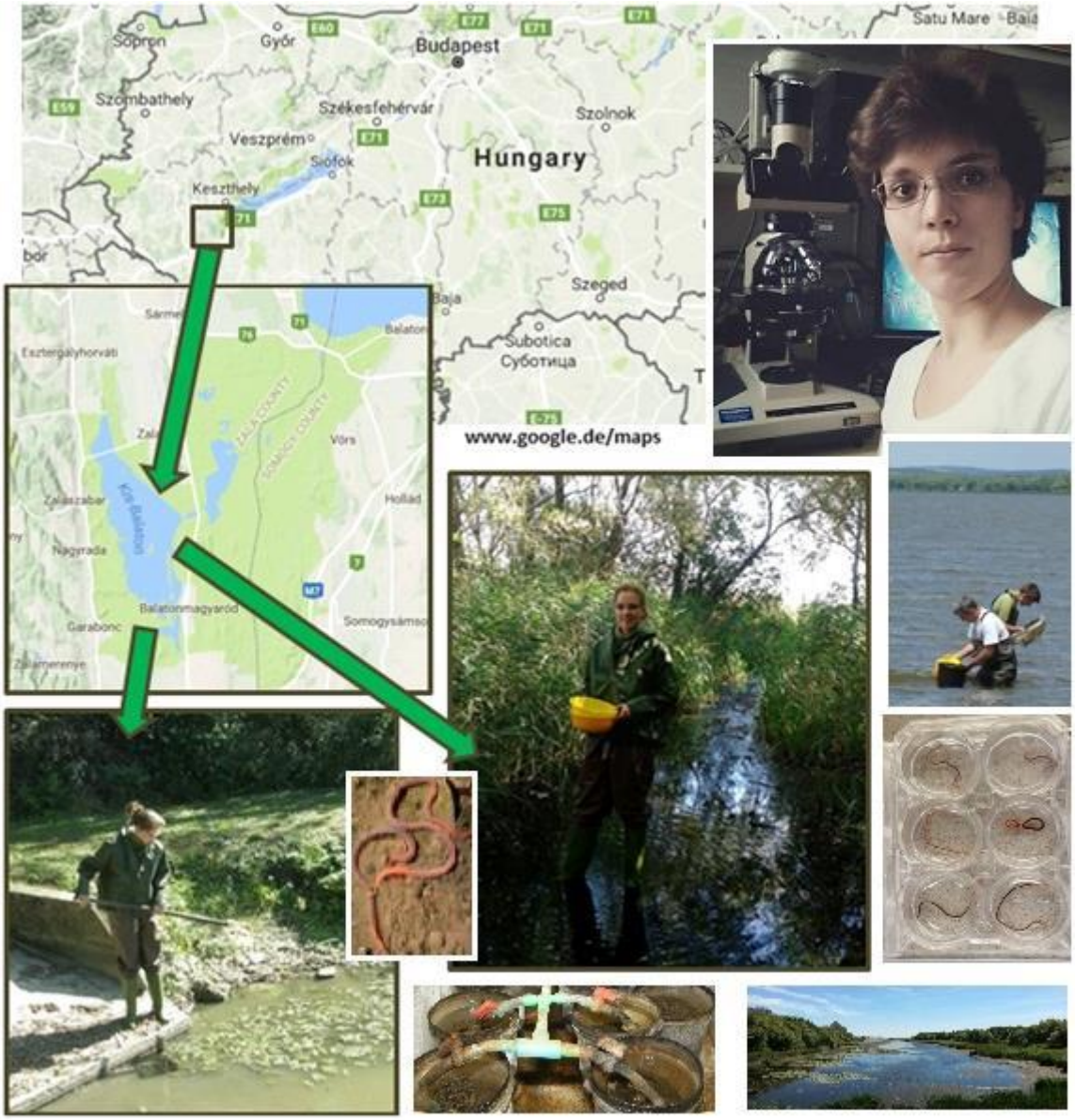


Figure 24: Oligochaete collection at Kis Balaton, 7th October 2015

4.2. Actinospore extraction

In cases where free floating actinospores were detected in the cell-well plate, they were removed with a plastic pipette and placed on a microscope slide under a coverslip right away. For actinospore extraction from the small cups, the water was filtrated through a 10 µm mesh and the last few drops within the mesh were collected with a pipette, placed on microscope slides and checked immediately. An Olympus BH-2 microscope was used for a detailed bright and phase-contrast field examination and photos of the actinospores were taken, using a DP-20 digital camera connected to the microscope.

Meanwhile the *Branchiura sowerbyi*, from which the actinospores were harvested, remained in their individual cell-wells or small cups. All containers were checked for further actinospore release on a regular basis. When the oligochaetes were found to be shedding spores, the infection experiments were prepared. The morphological identification of the harvested actinospores, carried out by the fish research group, were based on the keys provided by **Özer et al. (2002)**, **Lom & Dyková (2006)**, **Yokoyama et al. (2012)** and actinospore dimensions were measured in micrometers. The exact spore dimensions, as well as a micrometer bar on each photo will be provided in the scientific papers, which are in progress but have not been published yet. Actinospores from each infected oligochaete experimental group were preserved in 80% ethanol for PCR analysis, which was later performed by colleagues from the Fish Pathology and Parasitology Research Team.

Figure 25: Aerated plastic buckets for individual oligochaete keeping

4.3. Experimental infection of small carp

The animal experimentations conducted in this research project are approved by the Laboratory Animal Research Board (Munkahelyi Állatkísérleti Bizottság, MAB). The related license number is PEI/001/1002-13/2015.

In total, a number of 180 special pathogen free *Cyprinus carpio* were used for the experimental infections and all of them were in their first year of life. For each of the 14 experimental groups plus one control group, 12 small carp were placed in a small, permanently aerated glass

aquarium containing a volume of 15 *l* water each. No substrate and no filtration pump were added and the carp were fed once a day with commercial fish food flakes. The fish were exposed to water contaminated with actinospores obtained from the previously separated *B. sowerbyi* and kept at room temperature for 7 – 23 months, depending on the experimental group. Dissections were carried out one by one in monthly intervals. Table 3 summarizes the experimental setups in greater detail.

Table 3: Overview on experimental groups with young *Cyprinus carpio* exposed to actinospores from previously collected and individually kept *B. sowerbyi*

actinospore experimental group code	**fish infection experimental setup**	**duration of experiment** (date of infection - date of last dissection)
KB0	12 parasite free 1st year old common carp specimen exposed to contaminated water	25th June 2015 – 10th November 2017
KB1	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 1st July 2016
KB2	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 1st March 2016
KB3	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 22th November 2016
KB4	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 17th November 2016
KB6	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 22th November 2016
KB7	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 29th March 2017
KB10	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 22th November 2016
KB11	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 29th March 2017
KB12	12 parasite free 1st year old common carp specimen exposed to contaminated water	30th July 2015 – 28th February 2017
KBR1	12 parasite free 1st year old common carp specimen exposed to contaminated water	7th October 2015 – 28th February 2017

KBA-2016-1	12 parasite free 1st year old common carp specimen exposed to contaminated water	7th September 2016 – 17th August 2017
KBA-2016-2	12 parasite free 1st year old common carp specimen exposed to contaminated water	7th September 2016 – 17th August 2017
KBA-2016-3	12 parasite free 1st year old common carp specimen exposed to contaminated water	7th September 2016 – 17th August 2017
KBA-2016-4	12 parasite free 1st year old common carp specimen exposed to contaminated water	7th September 2016 – 17th August 2017

Figure 26: Experimental setup for *Cyprinus carpio* infection experiments

4.4. Dissection of small carps

Following exposure to water, contaminated with actinospores from the oligochaete experimental groups, the small *Cyprinus carpio* were given at least 6 weeks of time to develop detectible signs of a myxosporean infection. After those 6 weeks the fish were euthanized one by one, in monthly intervals to allow dissection and microscopical examination of potential myxosporean target tissues. Freshly euthanized carp were placed on a petri dish and dissected under a stereomicroscope to ensure precise inspection and excision of tissue samples from fins, skeletal muscle, gills, buccal mucosa, skin, eye, brain, blood, swimbladder and serosa. The liver, kidney, spleen and intestines were removed entirely and examined throughout. All aforementioned tissues were freshly placed on microscope slides and squashed under coverslips. An Olympus BH-2 microscope was used to screen the native preparations for myxosporean developmental stages such as plasmodia and myxospores. Findings were documented photomicroscopically and when an infection was suspected, the whole fish carcass was transferred into a plastic tube filled with 2 m*l* of

80% ethanol-solution. The tubes were then stored so that colleges from the fish research group could perform PCR analyses later on.

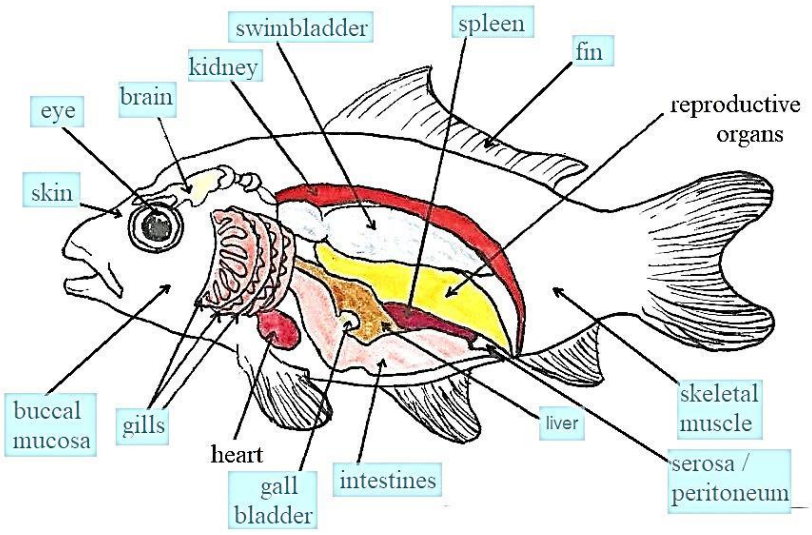


Figure 27: Own schematic illustration of *Cyprinus carpio* anatomy. Routinely excised and examined tissues are highlighted in blue.

5. **Results**

Results based on the 1st oligochaete collection in 2015

The research team obtained 128 *B. sowerbyi* specimens during the first sampling on 21st of July 2015. Until the 30th of July 2015, a total of ten infected *B. sowerbyi* started to shed actinospores and photos were taken of each actinospore sample. Each of the ten infected worms became the basis for one experimental group, namely KB0, KB1, KB2, KB3, KB4, KB6, KB7, KB10, KB11 and KB12. The oligochaete in KB1 was the only specimen to release neoactinomyxum type actinospores and three out of the 12 small common carp, exposed to those spores consequently harbored myxospore stages. The three fish were all dissected 11 months post infection and the few detected myxospores in their abdominal cavity were identified as belonging to a *Thelohanellus* species (Figure 28). According to the PCR carried out by Réka Borzák, a colleague of the research team, the sequenced *Thelohanellus* species is new to science. The other nine experimental groups were all releasing aurantiactinomyxon type actinospores but the fish infection experiments, which were subsequently performed, failed to produce any myxospore stages. Nevertheless,

actinospores from each experimental group were preserved for PCR sequencing. The colleague in charge so far identified close genetic relations to some *Thelohanellus spp.* using the actinospores collected by us. More detailed results of the molecular findings will be published later on. For more detailed information about the experimental results, see Table 4.

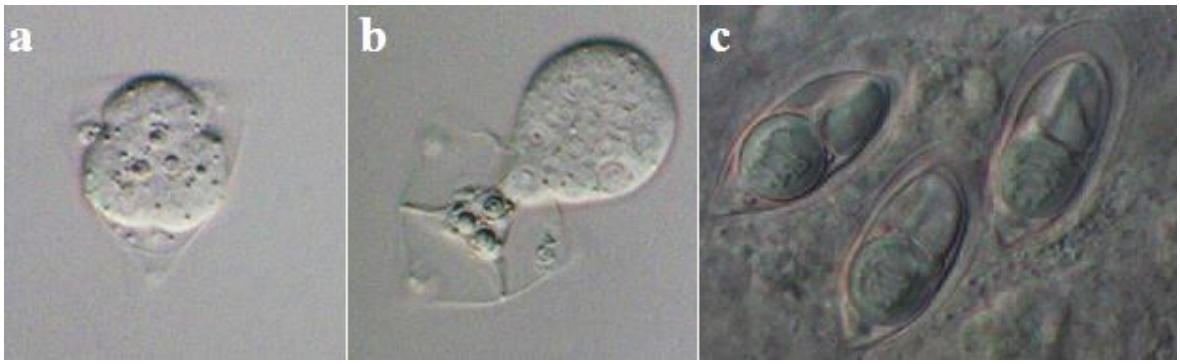


Figure 28: Results from KB1 revealed; a new *Thelohanellus spp.*. (a) unstained neoactinomyxon type actinospore, (b) release of infective sporoplasm, (c) myxospores from small carp

Results based on the 2nd oligochaete collection in 2015

The author joined the second collection on 7th October 2015 and we brought a total number of 112 *B. sowerbyi* to the laboratory. Two months post collection, during a routine screening of our small oligochaete containers and I was able to demonstrate raabeia type actinospores from the single *B. sowerbyi* specimen in KBR1 (Figure 29). Using photomicrographs, I documented the finding. Again a sample was preserved for PCR analysis and the actinospore contaminated water from the small oligochaete container was transferred to an aquarium with 12 SPF *Cyprinus carpio* in their first year of life. The carps were dissected in monthly intervals over a period of 16 months and three weeks but neither me nor my colleges identified any plasmodia or myxospore stages within the fish. Table 4 summarizes further details about the experimental results.

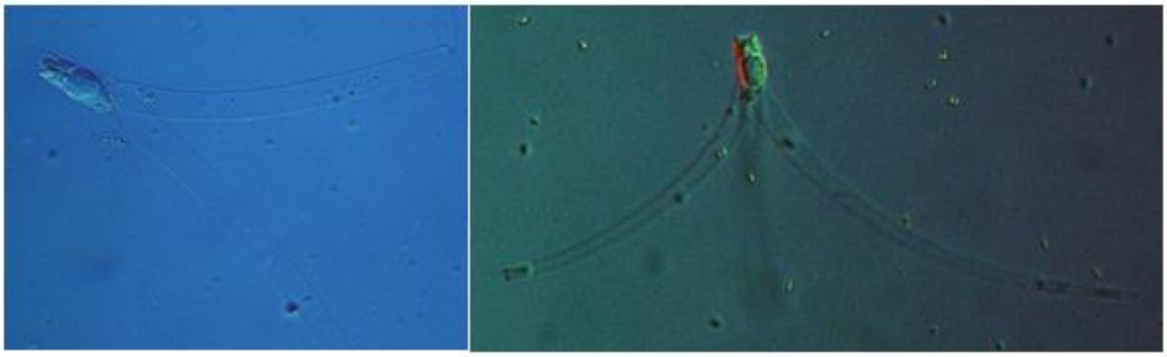
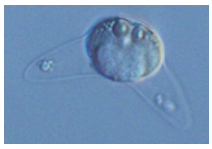
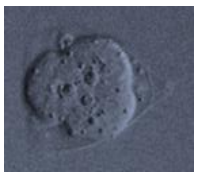
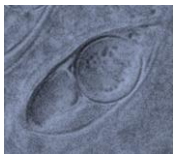
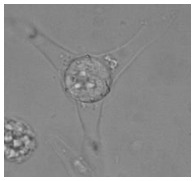
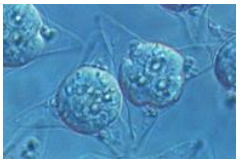
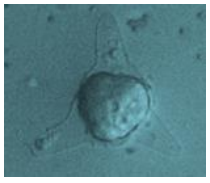
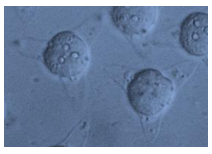
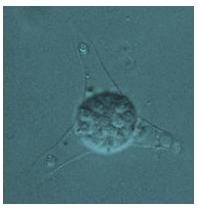


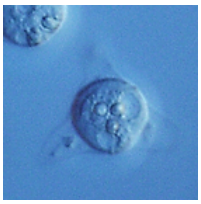
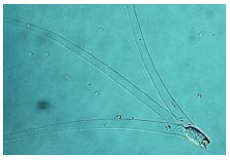
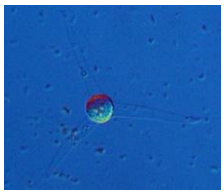
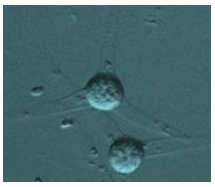
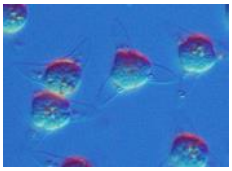
Figure 29: Raabeia type actinospores obtained from *B. sowerbyi* in sample cup KBR1.

Results based on the 1st, 2nd and 3rd collection in 2016

From three more collection trips on 5th July 2016, 3rd August 2016 and 1st September 2016, a total number of 143, 110 and 105 *B. sowerbyi* respectively was recovered. While the oligochaetes from July and August failed to release actinospores, the trip in September yielded three shedding *B. sowerbyi* specimen. The latter became the basis of our experimental groups KBA-2016-1, KBA-2016-2 and KBA-2016-3. Each of the three oligochaetes liberated aurantiactinomyxon type actinospores, which were again examined microscopically and documented photographically. KBA-2016-2 and KBA-2016-3 released a sufficient number of actinospores to keep samples for later PCR sequencing and to perform infection experiments with young carp as well. Réka Borzák, the colleague responsible for PCR analyses so far managed to match the KBA-2016-3 actinospores with *Thelohanellus kitauei*. KBA-2016-1 however, only produced enough actinospores to try an infection experiment with young *Cyprinus carpio*, instead of keeping the sample only for PCR analyisis. Over a period of 11 months and ten days, the exposed small carp were dissected in regular intervals every month but neither me, nor my colleges detected the presence of any plasmodia or myxospore stages in any of the examined fish. More detailed information about the experimental results, are summarized in Table 4.

Table 4: Detailed summary of experimental results

Actinospore experimental group code	**actinospore type/ PCR data of the actinospore**	**result** (infection prevalence in exposed fish)	**actinospore microscopic picture**	**myxospore microscopic picture**
KB0	aurantiactinomyxon/ no PCR data	0/12		no
KB1	neoactinomyxum/ PCR data exists	3/12 *(*PCR: **putative new** *Thelohanellus sp.)*		
KB2	aurantiactinomyxon/ PCR data exists	0/12		no
KB3	aurantiactinomyxon/ PCR data exists	0/12		no
KB4	aurantiactinomyxon/ PCR data exists	0/12 *(*PCR: **putative new** *Thelohanellus sp.)*		no
KB6	aurantiactinomyxon/ PCR data exists	0/12		no
KB7	aurantiactinomyxon/ PCR data exists	0/12		no
KB10	aurantiactinomyxon/ PCR data exists	0/12		no

KB11	aurantiactinomyxon/ no PCR data	0/12		no
KB12	aurantiactinomyxon/ PCR data exists	0/12		no
KBR1	raabeia/ PCR data exists	0/12		no
KBA-2016-1	aurantiactinomyxon/ no PCR data	0/12		no
KBA-2016-2	aurantiactinomyxon/ PCR data exists	0/12		no
KBA-2016-3	aurantiactinomyxon/ PCR data exists	0/12 (PCR match: ***T. kitauei***)		no

6. Discussion

According to Hungarian and international scientific publications, amongst others, the myxosporean parasitoses summarized in this Diploma thesis are responsible for severe pathological changes and economical losses in cultured common carp. Previous studies, conducted by the Fish Pathology and Parasitology Research Team prove the presence of all myxosporean parasites mentioned in this thesis in Hungary. Nevertheless, the experimental reproduction of complex myxosporean life cycles under laboratory conditions is well-

known to be a rather time consuming, difficult task, seemingly especially for *Thelohanellus* species in Hungary. The Hungarian research team has a rather good position in this respect but achieved most of their breakthroughs with *Myxobolus* species. Another dilemma is the fact that clinically diseased carp, as well as macroscopically and microscopically visible pathological lesions due to infections with *Thelohenallus spp., (*except for *T. nikolskii)* seem to occur exceptionally rare in Europe, compared to Asia, where the same pathogens cause massive lesions. The exact underlining reasons and responsible parameters are still subject to ongoing research. Although the main invertebrate host of carp-pathogenic myxosporean parasites, *Branchiura sowerbyi*, has stable populations of sufficient quantities in the Kis-Balaton Reservoir and the oligochaete collections took place during the typical season for myxosporean development, the prevalence of actively shedding specimen was found to be considerably lower than the author expected. Out of the 598 *B. sowerbyi* collected in 2015 and 2016, only 14 specimens released actinospores in the laboratory. However, the experimental setup provided a suitable environment for the collected oligochaetes so that they remained alive over the course of the experiments and the routinely performed microscopical inspection of all experimental containers yielded in most cases enough actinospores to examine, photograph and measure a few specimens, preserve their DNA for later PCR analysis and to continue with the experimental infection of special pathogen free common carp fingerlings. Retrospectively, it could have been a good decision to check the oligochaete in question even more frequently for further actinospore release. Anyways, it is a common practice in the laboratory to keep formerly shedding oligochaetes for several years, as long as there is space available, and to randomly check their containers for further actinospores. Therefore it is still not impossible to obtain more data later on. The 14 groups of SPF common carp fingerlings, were less than one year old and therefore potentially most susceptible to various myxosporean parasitoses while still small enough to allow the screening of entire organs rather than just small tissue samples. The known predilection sites for myxosporean sporogony were dissected with greatest care and native squash preparations were examined methodically, using 200 and 400 times maginification. Therefore, the author expected to detect more than one successful infection, yielding myxospore stages from the dissected fish hosts. In retrospect, it is hard to determine

whether histological staining methods such as Giemsa or H.E. would have increased the chance to visualize early sporogonic stages and developing plasmodia. On the other hand, in all cases where the author doubted her negative finding or found a possibly suspicious structure in a preparation, a second opinion was obtained by asking Dr. Székely or Dr. Molnár and samples were preserved for future PCR analysis. Not all of these samples have been sequenced to date and possible further results will be published later on. Therefore the collected and fixed samples may provide a final answer to the question whether or not more than one experimental infection was actually successful.

7. Conclusion

Despite the results gained during previous two years of intensive research did not meet the author´s expectations, the time spent in the laboratory was definitely worth it. Working together with a group of experts in their fields and having the chance to interactively gain rare knowledge about various fish parasites was a unique and advantageous opportunity in many respects. After all, the complexity of myxosporean parasitic life cycles and their often unpredictable reactions to a laboratory environment is well-known among researchers. Continuing the optimisation of experimental setups and examination schedules is crucial in order to improve our understanding of myxosporean life cycles and parasitoses, far beyond *Thelohanellus spp.* in Hungary. Since infected oligochaetes often shed intermittently in variable quantities and the time periods where spores are liberated is not steady or predictable either, timing and patience are crucial. In order to increase the chance of success in future myxosporean infection experiments, it is advised to invest even more time into regular oligochaete collection trips and shorter spore-screening-intervals for infected worms and fish. The experimental carp may be exposed to higher actinospore concentrations per experimental setup and random tissue samples, obtained from experimentally exposed carp may be stained for additional histopathological examinations. A further chance for future experiments is feeding infected *Branchiura sowerbyi* or pieces of their body (intestine) to experimental fish as a trial to increase infection rates. Nevertheless, the newly obtained practical experiences lead the author to conclude, that a detection of mature infective myxosporean stages is a realistic task, also for a veterinarian.

7. Abstract

Myxosporean infections in common carp *(Cyprinus carpio)* are responsible for a wide range of pathological findings and are well-known to cause heavy economical losses in the main carp producing countries in Europe and Asia. The complex two-host life cycle of myxosporean parasites obligatory relies on an invertebrate final host, typically an aquatic annelid worm and a vertebrate intermediate host, usually a fish. Each host type sheds a rather resistant infectious spore with a unique morphology. From a veterinary point of view, the intrapiscine development is most relevant. Actively or passively migrating presporogonic stages may obstruct fine capillaries in various organs and sporogony typically leads to large intra- or intercellular parasitic proliferation, the so-called plasmodia, which inflict a massive mechanical pressure before they eventually rupture, causing further tissue damage. The released myxospores may then trigger local or distant cellular host reactions, while others are shed to the environment. In this Diploma thesis, the author reviews the available literature to give an introduction to the pathological and parasitological dynamics of myxosporean infections in common carp. Furthermore, this thesis draws attention to seven clinicopathologically relevant myxosporean infections, their recognition, diagnosis, as well as prevention and treatment options. As an active member of the Fish Pathology and Parasitology research team, the author additionally worked on a series of field- and laboratory experiments aiming to identify and propagate myxosporeans obtained from a natural water body in Hungary. The author collected suitable invertebrate hosts from Kis-Balaton Resevoir, kept and checked them for released infective actinospore stages, identified the latter through a microscope, documented her findings photographically, obtained specimens for fish experimental infections, dissected the exposed *Cyprinus carpio* regularly and preserved samples for further PCR analysis. As a result, the neoactinomyxum type actinospores obtained from one oligochaete *(B. sowerbyi)* specimen successfully infected three SPF common carp fingerlings and the few consequently detected myxospores served as evidence for a successful intrapiscine development. They were identified as *Thelohanellus sp*. and later on, molecular data confirmed a putative new species.

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