The Thesis of the PhD Dissertation

NUR NADHIRAH SYAFIQAH BINTI SUHAIMI

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COMPARATIVE STUDIES OF MYXOZOAN PARASITES INFECTING FISHES AND ALTERNATE ANNELID HOSTS IN FRESHWATER ECOSYSTEMS OF HUNGARY AND MALAYSIA

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The PhD School

Name:	Doctoral School of Agricultural and Food Sciences
Discipline:	Animal Science

Head of the Doctoral School:	**Prof. Dr. Melinda Kovács** University Professor, Full Member of the Hungarian Academy of Sciences

Supervisors:	**Honorary Professor Dr. Csaba Székely** Scientific Advisor, Fish Pathology and Parasitology Research Team Leader, HUN-REN Veterinary Medical Research Institute, Budapest, Hungary
	Dr. Boglárka Sellyei Senior Researcher, Fish Pathology and Parasitology Research Team, HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

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Approval of the Supervisors

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1. INTRODUCTION

1.1. Research background

Fish parasites are taxonomically diverse, including monogeneans, trematodes, cestodes, nematodes, crustaceans, protozoans, microsporidians, and myxozoans. One of the most widespread and ecologically important groups among these is the Myxozoa (Grasse, 1970). Myxozoans are microscopic, spore-forming parasites belonging to the phylum Cnidaria. They possess complex two-host life cycles involving a vertebrate host, where myxospores develop, and an invertebrate host, usually annelid worms, where actinospores are produced. Although best known as fish parasites, myxozoans have also been reported from amphibians, reptiles, mammals (Dyková et al., 2007; Prunescu et al., 2007) and birds (Bartholomew et al., 2008).

More than 3,000 myxozoan species have been described worldwide (Whipps et al., 2025a), many of which cause serious diseases in economically important fishes. Several myxozoans are significant pathogens in aquaculture and wild fisheries. *Tetracapsuloides bryosalmonae* causes proliferative kidney disease in salmonids (Hedrick et al., 1993), *Myxobolus cerebralis* causes whirling disease (Hoffman, 1990), *Henneguya ictaluri* induces proliferative gill disease in channel catfish (Pote et al., 2012), and *Kudoa thyrsites* leads to post-mortem muscle degradation in salmon (Whitaker & Kent, 1991). Other species, including *Hofferellus carassii* (Ahmed, 1973), *Sphaerospora renicola* (Holzer et al., 2014), and *Thelohanellus* spp., are associated with organ damage and skeletal deformities in cyprinid fishes (Akhmerov, 1960; Borzák et al., 2021).

Despite extensive research, the continued discovery of new species indicates that myxozoan biodiversity remains underestimated. This dissertation addresses this gap by documenting myxozoan parasites from freshwater ecosystems in Hungary and Malaysia, focusing on fish hosts and

their annelid alternate hosts to improve understanding of their diversity, host associations, and life cycles.

1.2. Problem statement and significance of study

Freshwater ecosystems support high biodiversity and are essential for global aquaculture, yet parasitic infections, particularly myxozoans, are increasingly threatened them. Despite their importance, myxozoan diversity and biology remain poorly understood, especially in Southeast Asia, where aquaculture is rapidly expanding. In contrast, European countries such as Hungary have a longer history of fish parasitological research and well-developed aquaculture systems. More than 100 myxozoan species have been described in Hungary; however, many lakes and fish farms remain unexplored, suggesting that true diversity is still underestimated.

In Malaysia, however, a few dozen parasites have been described so far. Although the country hosts 629 freshwater fish species (Froese & Pauly, 2025), only 23 myxozoan species have been reported from just twelve fish hosts, suggesting that many of its myxozoan fauna remain undocumented (Shariff, 1982; Molnár et al., 2006a,b; Székely et al., 2009a,b, 2014; Borkhanuddin et al., 2013, 2020a). To address this gap, the present study conducted a comprehensive survey of myxozoans from fish farms in Hungary and diverse freshwater habitats in Malaysia, targeting both cultured and wild fish populations to improve knowledge of species diversity, host range, and distribution.

Knowledge of actinosporean stages is also limited due to the labour-intensive nature of oligochaete sampling and screening. To date, 51 actinosporean types have been identified in Hungary, while none have been reported from Malaysia. Due to the lack of data, the life cycles of myxozoan are unknown in Malaysia. Traditionally, life cycle elucidation relied on

experimental infections, but recent advances in molecular techniques now allow a reliable linkage between myxospore and actinospore stages. By integrating morphology, histopathology, and molecular analyses, this study applies an integrative taxonomic approach to resolve species identities and advances knowledge of myxozoan biodiversity in both regions.

1.3. Research aim and objectives

The main aim of this work was to document myxozoan parasites and provide insights into their diversity.

Specifically, the objectives of this study are:

1. To identify and document myxosporean species and actinosporean types infecting fishes and oligochaetes from fish culture ponds in Hungary.

2. To identify and document myxosporean species and actinosporean types infecting wild fishes and oligochaetes in freshwater biotopes of Malaysia.

3. To characterize both myxosporean and actinosporean stages using an integrative approach combining morphological, morphometric, histopathological and molecular analyses.

4. To elucidate life cycles by joining up DNA sequences of corresponding myxosporean and actinosporean stages.

5. To investigate phylogenetic relationships among myxozoans and reveal evolutionary patterns.

2. MATERIALS AND METHODS

2.1. Fish and oligochaete collections in Hungary

Fish were collected using gillnets from ponds at two fish farms in Hungary: Ócsárd near Pécs (45°55'53.544" N, 18°09'14.0472" E) and Dömsöd (47°07'17.33" N, 19°01'34.23" E), between November 2023 and June 2024. The Ócsárd farm consists of 12 ponds covering 38 hectares and primarily produces pikeperch and wels catfish, along with smaller proportions of common carp, grass carp, African catfish, tench, burbot, bream, and gibel carp. Fish for this study were sampled from three ponds.

The Dömsöd farm comprises 13 ponds covering 1.3 hectares and mainly produces common carp, grass carp, wels catfish, and pikeperch, as well as other cyprinids such as silver carp and bream. Fish were sampled from two ponds. In addition, a small number of fish were obtained from a barrage pond in Szigetvár during autumn 2021 and 2022. Details of fish species, sample sizes, and sampling locations are provided in **Table 1**.

Table 1 Fish species, number and size range of specimens examined from each fish farm.

Species	Number of fish specimens examined	Fish size (length)	Locality
Carassius auratus gibelio	112	7–23 cm	Ócsárd, Szigetvár
Cyprinus carpio	27	4–21 cm	Ócsárd, Dömsöd
Rutilus rutilus	5	10–12.5 cm	Ócsárd
Total	**144**		

Sediment containing oligochaetes was collected using a shovel and a 1000 μm mesh net from low-water earthen ponds at Ócsárd, Szigetbecse,

Makád, Dömsöd, and Dinnyés between April 2023 and June 2024. At the Ócsárd fish farm, samples were collected from four ponds. Szigetbecse consists of twelve ponds (1.9 ha), while Makád includes six ponds (87.35 ha). For this study, sediments were sampled from four ponds in Szigetbecse and two ponds in Makád. Although samples were also collected from Dömsöd and Dinnyés, no oligochaetes were detected, and these sites were excluded from further analysis.

All live fish and sediment samples were transported to the Fish Pathology and Parasitology Laboratory at the HUN-REN Veterinary Medical Research Institute (Budapest), and maintained at 23 °C with continuous aeration. Fish were dissected and examined for myxozoan infections using stereo and light microscopy. Oligochaetes were hand-sorted under illumination and individually placed into 48-well microtiter plates with dechlorinated water, while larger species were housed in larger wells of 12 or 24-well microtiter plates. Plates were examined daily for one week to two months for actinospore release using an inverted microscope. Oligochaetes were identified using morphological characteristics (Timm, 1999) and molecular analyses.

2.2 Fish and oligochaete collections in Malaysia

Fish originating from Sungai Tong (Setiu), Sungai Nerus (Kuala Nerus), and small channels around Kuala Terengganu were purchased from a local fish market in Kuala Terengganu, Malaysia, at two-week intervals between July and August 2023 and September and November 2024. Details of sampled species and sample sizes are provided in **Table 2**. Sediment containing oligochaetes was collected from Tasik Telabak in July 2023, September 2024, and October 2024. Tasik Telabak, located in Besut, Terengganu, is a recreational lake recently used for small-scale aquaculture

and inhabited by both native and introduced fish species. The site was selected due to the limited existing data on fish parasites and the growing importance of aquaculture.

Table 2 Fish species, number and size range of specimens collected or examined from each locality in Terengganu.

Species	No. of fish specimens examined	Fish size (length)	Locality
Barbonymus gonionotus	6	15.5–30.0 cm	Sungai Tong
Barbonymus altus	16	9.5–14.5 cm	Sungai Nerus
Barbonymus schwanefeldii	6	13.0–25.0 cm	Sungai Tong
Leptobarbus rubripinna	5	9.5–16.0 cm	Sungai Nerus
Barbodes binotatus	13	8.0–11.5 cm	Sungai Nerus
Labiobarbus leptocheilus	15	7.0–13.0 cm	Sungai Nerus
Osteochilus waandersii	15	7.5–11.0 cm	Sungai Nerus
Trichopodus trichopterus	9	9.0–11.0 cm	Small channels
Trichopodus pectoralis	12	10.0–23.5 cm	Small channels
Channa gachua	12	7.5–15.5 cm	Small channels
Neolissochilus hexagonolepis	5	14.0–15.5 cm	Sungai Tong
Anabas testudineus	3	12.0–13.0 cm	Small channels
Notopterus notopterus	2	22.5–25.0 cm	Sungai Nerus
Total	**119**		

Live fish were transported in oxygenated bags, while sediment samples were transported in buckets to the Marine Science Biodiversity Laboratory, Universiti Malaysia Terengganu. Fish were maintained in tanks and sediments in aerated buckets at ambient temperatures (30–35 °C). Fish were examined for myxosporean infections in external tissues and internal organs using stereo and light microscopy. Oligochaetes were hand-sorted under illumination and placed individually into microtiter plates containing dechlorinated water. Plates were examined daily for actinospore release using

an inverted microscope. Fish were identified morphologically, and oligochaetes were identified using morphological characteristics (Timm, 1999) and molecular analyses.

2.3 Microscopic examination

Fresh myxospores and actinospores collected in Hungary were mounted on glass slides and examined under high magnification using an Olympus BX53 light microscope equipped with a DP74 digital camera. After examination, remaining spores were preserved in 80% ethanol for molecular analyses. For Malaysian samples, fresh myxospores and actinospores were examined using an Olympus CX33 microscope, and the remaining material was preserved in 90% ethanol for molecular studies and in 10% neutral buffered formalin for morphological analysis. All fixed samples were later examined in Budapest (HUN-REN VMRI) using high-magnification microscopy, and Lugol's staining was applied in some cases to enhance sporoplasm visibility.

Myxospore and actinospore measurements were taken following standard guidelines by Lom & Arthur (1989) and Lom et al. (1997), respectively, using ImageJ software, and results are presented as mean, standard deviation, and range. Measurements were compared with published data for species identification. Line drawings were prepared from photomicrographs, and voucher specimens were deposited in the Parasitological Collection of the Hungarian Natural History Museum.

For histological analysis, infected tissues were fixed in neutral buffered formalin or Bouin's solution, embedded in paraffin, sectioned at 3–4 μm, and stained with hematoxylin and eosin. Infected oligochaetes were cut into two parts and preserved for molecular and histological analyses. All slides were examined and photographed using an Olympus DP74 digital camera.

2.4 Molecular analyses

2.4.1. DNA isolation, PCR and Sequencing

DNA was extracted from plasmodia and spores preserved in 80% or 90% ethanol. Prior to extraction, samples were centrifuged, ethanol was removed, and the pellets were washed twice with 10 mM Tris-HCl (pH 8.5). Genomic DNA was isolated using the Geneaid Tissue Genomic DNA Mini Kit following the manufacturer's protocol for animal tissues.

Partial 18S rDNA and 28S rDNA genes were amplified using direct, semi-nested, or nested PCR with various primer sets. For direct and semi-nested PCR, reactions were performed in 25 µL reaction volumes, containing 2 µL of template DNA, 1× DreamTaq buffer (10×; Thermo Scientific), 0.2 mM dNTP mix (10 mM; Thermo Scientific), 1.25 U DreamTaq polymerase (5 U; Thermo Scientific), 12.5 pmol of each primer, and molecular grade water. For nested PCR, reactions were performed in 50 µL volumes, containing 5 µL of template DNA, 1× DreamTaq buffer (10×; Thermo Scientific), 0.2 mM dNTP mix (10 mM; Thermo Scientific), 2.5 U DreamTaq polymerase (5 U; Thermo Scientific), 25 pmol of each primer, and molecular grade water. Different primer combinations and thermal cycling conditions were applied following previously published protocols (Liu et al., 2016a; Úngari et al., 2021; Atkinston & Bartholomew, 2009; Colunga et al., 2024; Bittencourt et al., 2021), with minor modifications where necessary. Semi-nested and nested PCR protocols were used to enhance amplification success, particularly for *Ceratomyxa* species (Zatti et al., 2023).

For nested PCR of the 18S rDNA gene, two successive reactions were performed using ERIB1–ERIB10 primers in the first round (Cech et al., 2015; Eszterbauer et al., 2013) and Myx1F–SphR primers in the second round (Cech et al., 2015). Amplification of 28S rDNA employed several primer pairs following established protocols (Bartošová et al., 2009). Oligochaete samples

were amplified for 16S rRNA and ITS regions using published methods (Rocha et al., 2019a; Erséus et al., 2017).

PCR products were visualized on agarose gels, purified using a DNA Fragment Purification Kit, and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3100 Genetic Analyzer.

2.4.2. Phylogenetic analyses

The 18S rDNA and 28S rDNA sequences obtained in this study were corrected and assembled using Geneious Prime v11.1 (Kearse et al., 2012). Sequence alignments were performed with ClustalW algorithm (Thompson et al., 1994) in MEGA X (Kumar et al., 2018), and poorly aligned regions were removed using GBlocks with less stringent settings (Castresana, 2000; Talavera & Castresana, 2007).

Phylogenetic analyses were conducted using both Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum Likelihood analyses were performed in MEGA X with 1,000 bootstrap replicates, while BI analyses were carried out in MrBayes with Markov Chain Monte Carlo runs of 1,000,000 generations, discarding the first 25% as burn-in (Ronquist et al., 2012). Alignment gaps were treated by partial deletion with a 75% site coverage cut-off. The best-fitting nucleotide substitution models (GTR + G + I or GTR + G) were selected based on Akaike and Bayesian Information Criteria using jModelTest (Darriba et al., 2012). Clades with ML bootstrap values ≥ 70% and Bayesian posterior probabilities ≥ 0.90 were considered well supported.

Phylogenetic trees were visualized using MEGA X and FigTree v. 1.4.4 (Rambaut, 2018), and graphically edited in Inkscape. Genetic distances were calculated using the p-distance model in MEGA X.

3. RESULTS AND DISCUSSION

3.1. Myxosporean species in Hungary

A total of 144 specimens representing three fish species were collected from Hungarian fish farms and examined for myxozoan infections. *Rutilus rutilus* showed the highest infection rate (80.0%; 4/5), while *Carassius auratus gibelio* had the lowest rate (51.7%; 58/112). *Cyprinus carpio* showed an intermediate infection rate of 55.6% (15/27). In total, 15 myxosporean species were identified, including eight *Myxobolus* spp., five *Thelohanellus* spp., one *Zschokkella* sp., and one *Sphaerospora* sp. Eight of these species are newly described (*Myxobolus ocsardiensis* n. sp., *Myxobolus pecsensis* n. sp., *Thelohanellus imrei* n. sp., *Zschokkella chezhachei* n. sp., *Thelohanellus serosae* n. sp., *Thelohanellus paranikolskii* n. sp., *Myxobolus* n. sp. 1 and *Myxobolus* n. sp. 2), while the others were previously known (*Myxobolus lentisuturalis, Myxobolus diversus, Sphaerospora molnari, Myxobolus intrachondrealis, Myxobolus basilamellaris, Thelohanellus nikolskii* and *Thelohanellus hovorkai*). *Carassius auratus gibelio* harboured the highest parasite diversity, with seven species (four *Myxobolus*, one *Thelohanellus*, one *Zschokkella*, and one *Sphaerospora*). *Cyprinus carpio* was infected with six species (two *Myxobolus* and four *Thelohanellus*), whereas *R. rutilus* hosted only two *Myxobolus* species (**Table 3**). All examined fish appeared healthy, with no visible lesions, except for individuals infected with *Myxobolus lentisuturalis*.

Myxobolus lentisuturalis is a highly pathogenic species previously reported from China, Italy, Croatia, and the USA, infecting the dorsal muscles of *C. auratus gibelio* and *C. auratus auratus*. The present Hungarian findings are consistent with earlier reports, showing characteristic dorsolateral body deformities caused by muscle infection. Spore morphology closely matched previous descriptions (Dyková et al., 2002; Caffara et al., 2009; Wang et al.,

2019; and Huskanović (2021), with only minor size differences. Similar to reports from China (Caffara et al., 2009), the measured spores were nearly identical in size. *M. lentisuturalis* can be easily diagnosed by visible body deformities. Its detection in Hungary confirms its establishment in Europe and supports the spread of Far Eastern myxozoans into European waters.

Table 3 Myxosporean species detected in the investigated fish species.

Fish species	Myxosporean parasite	Predilection site
Carassius auratus gibelio	*Myxobolus lentisuturalis*	Muscle
	Myxobolus ocsardiensis n. sp.	Kidney, liver
	Myxobolus pecsensis n. sp.	Gill cartilage
	Myxobolus diversus	Fins
	Thelohanellus imrei n. sp.	Connective tissues of gill arch and pharynx
	Zschokkella chezhachei n. sp.	Bile duct
	Sphaerospora molnari	Gill lamellae
Cyprinus carpio	*Myxobolus intrachondrealis*	Gill cartilage
	Myxobolus basilamellaris	Base of gill filaments
	Thelohanellus serosae n. sp.	Serous membrane of kidney
	Thelohanellus paranikolskii n. sp.	Skin
	Thelohanellus nikolskii	Fin rays
	Thelohanellus hovorkai	Serous membrane of intestine
Rutilus rutilus	*Myxobolus* n. sp. 1	Connective tissue of gill arch
	Myxobolus n. sp. 2	Kidney

Myxobolus ocsardiensis n. sp., found in the kidney and liver of *C. auratus gibelio*, showed the greatest morphological and morphometric similarity to *M. hearti*. Phylogenetic analyses placed it within a well-supported clade of kidney-infecting *Myxobolus* species with similar spore shapes and host associations. Comparisons with other kidney-infecting *Myxobolus* species from *Carassius* spp. revealed clear morphological

differences, although molecular data for those species are lacking. Based on its distinct morphology and phylogenetic position, *M. ocsardiensis* n. sp. is recognized as a new species.

Tissue tropism proved to be an important criterion for distinguishing *Thelohanellus* species. Although *T. imrei* n. sp. was morphometrically similar to *T. wangi* and *T. nanhaiensis*, it differed in predilection site and spore features, particularly the straight sutural line. Molecular data further supported its distinction, showing moderate sequence similarity to *T. wangi*. A BLAST search also revealed high similarity between *T. imrei* n. sp. and a previously described Neoactinomyxum type, suggesting a possible life-cycle link that requires further confirmation.

Among the myxosporean species infecting *C. auratus gibelio*, a *Zschokkella* species found in the bile duct showed the highest infection rate. BLAST analysis revealed 100% sequence identity with an undescribed taxon referred to as "*Zschokkella chongqingense*", previously reported from the gallbladder of *C. auratus*. Based on morphological, molecular, and phylogenetic evidence, the present species is described as *Zschokkella chezhachei* n. sp. Moreover, sequence identity with an aurantiactinomyxon type indicated that this actinospore represents the alternate life stage of *Z. chezhachei* n. sp.

Myxobolus pecsensis n. sp. showed close morphological similarity to *M. intrachondrealis*, both infecting cartilage of the gill arches. However, molecular data and morphometric differences confirmed that they are distinct species infecting different hosts (*C. auratus gibelio* and *C. carpio*, respectively). Phylogenetically, *M. pecsensis* n. sp. clustered with several ellipsoidal-spored *Myxobolus* species, including *M. lentisuturalis* and *M. cultus*. Its close relationship with Hungactinomyxon types suggests a possible

actinospore stage, although no definitive link has yet been established. Further oligochaete sampling is therefore needed to clarify its life cycle.

Myxobolus diversus was originally described from the fins of *C. auratus* in China, and was later reported from the same host and predilection site in Hungary. The present spores matched the original description of *M. diversus* from China (Chen & Ma, 1998) but were slightly larger and showed a mucus envelope not previously reported. Histological observations confirmed that plasmodia developed in the intrasegmental spaces between cartilaginous fin-ray segments, consistent with earlier findings. Although no reference sequences are available for the original description, phylogenetic analyses placed *M. diversus* within a clade of gill- and fin-infecting *Myxobolus* species. Based on host, predilection site, and morphology, the parasite found in *C. auratus gibelio* was identified as *M. diversus*.

The spores observed in this study closely matched those of *S. molnari* in morphology and size, with only minor differences. Molecular analysis showed less than 1% divergence from previously published *S. molnari* sequences, confirming conspecificity (Whipps & Kent, 2006; Bartošová & Fiala, 2011; Patra et al., 2017). Although *S. molnari* was originally considered specific to *C. carpio*, it has since been reported from *C. auratus gibelio*. Phylogenetic results from this study demonstrated that the *S. molnari* sequence from *C. auratus gibelio* was identical to those from Hungary and the Czech Republic. These findings confirm that *S. molnari* is not host-specific to common carp and also infects gibel carp.

Six *Myxobolus* species (*M. cyprini, M. cyprinicola, M. dispar, M. encephalicus, M. intrachondrealis,* and *M. basilamellaris*) have been reported from *C. carpio* in Hungary (Molnár et al., 2025). In the present study, *M. intrachondrealis*, a gill cartilage parasite, was detected again, representing the second record of this species from common carp in Hungary and worldwide.

Spore morphology and measurements were consistent with the original description by Molnár (2000), except for differences in the number of polar filament coils, which may reflect limitations of earlier microscopy.

Molecular analysis revealed that *M. intrachondrealis* shared 100% 18S rDNA sequence identity with *Myxobolus cultrati*. Morphometric comparisons showed near-identical spores, with differences limited to host species and reported predilection sites. *Myxobolus cultrati* was described from the retina of *Pelecus cultratus* based only on dispersed spores and molecular data, without confirmed plasmodial localization (Borzák et al., 2016). At the time of its description, sequence data for *M. intrachondrealis* were unavailable, preventing comparison. The newly generated sequences strongly suggest that *M. cultrati* should be regarded as a synonym of *M. intrachondrealis*.

Myxobolus basilamellaris was also detected in *C. carpio* from the Dömsöd fish farm. This species was first described in Europe (Kovacs & Molnár, 1983; Lom & Molnár, 1983; Eszterbauer, 2004) and has since been reported from Syria (Dayoub et al., 2007), China (Wang et al., 2021), and koi carp (Zhang et al., 2023a). Spore morphology and measurements in this study matched recent descriptions. However, the earlier reports noted smaller spores with sutural markings and a mucous envelope, features not observed here or in recent studies.

Thelohanellus dogieli was the first *Thelohanellus* species described from Hungary, but was later reclassified as *T. hovorkai* due to strong morphological similarity. Earlier drawings showed elongated pyriform polar capsules, similar to those of *T. serosae* n. sp., but differences in predilection site, spore and polar capsule dimensions confirm they are distinct species. Comparisons are limited because micrographs of *T. dogieli* are unavailable. *T. hovorkai* and *T. serosae* n. sp. share large polar capsules and similar

predilection sites, but differ in mucous envelope shape and plasmodium size. Molecular data clearly separate these taxa, and the actinospore stage of *T. serosae* n. sp. was confirmed, showing 100% sequence identity with aurantiactinomyxon type B2 from *Branchiura sowerbyi.*

Molecular techniques enabled the differentiation of *T. paranikolskii* n. sp. and *T. nikolskii*, two closely related species with overlapping morphology and the same host. *Thelohanellus nikolskii* commonly infects the fins and scales of common carp, while *T. paranikolskii* n. sp. was identified from the skin of the same host. Morphologically and morphometrically, these two species are nearly indistinguishable, differing only slightly in spore thickness, polar tubule coil number, and anterior spore shape. These minor differences alone were insufficient for species separation; however, molecular analyses showed clear genetic divergence, with only 94.5% similarity to *T. nikolskii* and 93.8% to *T. pseudonikolskii*.

To date, 17 *Myxobolus* species have been reported from *R. rutilus*. This study describes two additional novel species infecting the gills and kidneys. *Myxobolus* n. sp. 1 and *Myxobolus* n. sp. 2 are morphologically very similar, differing mainly in sutural markings, but they occupy different predilection sites: the gill connective tissue and the kidney, respectively. *Myxobolus* n. sp. 1 showed the highest similarity to *Myxobolus fundamentalis* and exhibited a ‘muelleri-like’ morphology. Phylogenetically, it clustered within the ‘muelleri-type’ clade but was clearly distinct from related species. *Myxobolus* n. sp. 2 was compared with other kidney-infecting *Myxobolus* species, and clustered genetically with *M. erythrophthalmi*, while remaining distinct from *Myxobolus shaharomae* and *Myxobolus zaikae*. Despite their nearly identical morphology, molecular data and tissue specificity confirm both as new species.

3.2. Actinosporeans in Hungary

A total of 5,976 oligochaetes were collected from sediments at the Ráckeve and Ócsárd fish farms. Myxozoan infections were detected in 71 individuals, giving an overall prevalence of 1.18%. Eight oligochaete species were identified, including *B. sowerbyi*, *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, *Dero* sp., *Stylaria* sp., *Potamothrix* sp., *Branchiodrilus hortensis*, and *Ophidonais serpentina*. Infections were found in four species: *B. sowerbyi*, *T. tubifex*, *L. hoffmeisteri*, and *O. serpentina*. *Branchiura sowerbyi* showed the highest infection rate at 12.1% (38/315), followed by *L. hoffmeisteri* at 1.2% (21/1,721), *O. serpentina* at 0.9% (1/110), and *T. tubifex* at 0.3% (3/986).

In total, ten actinospore types were identified: one triactinomyxon, two raabeia, six aurantiactinomyxon, and one neoactinomyxum type. Among these, one triactinomyxon, five aurantiactinomyxon, and one raabeia type were novel, while the remaining types had been previously described. Molecular analyses linked four actinospore types to their myxosporean stages, including one completing the life cycle of the newly described *Zschokkella chezhachei*, and others associated with *Thelohanellus wangi*, *T. hovorkai*, and *Myxobolus cultus*.

Triactinomyxon type 1 described in this study from *L. hoffmeisteri* differs clearly from previously reported triactinomyxon types. In particular, the length and width of its caudal processes differ from those in the literature. Phylogenetic and genetic distance analyses placed triactinomyxon type 1 within a clade of gill-infecting *Myxobolus* species from cyprinid hosts, showing the highest similarity to *Myxobolus diversicapsularis*, whose life cycle involves triactinomyxon actinospores.

Two raabeia types were identified in this study: one from *L. hoffmeisteri* and the other from *B. sowerbyi*. Raabeia type 1 closely resembled

the previously described “Triactinomyxon ‘F’” (Xiao & Desser, 1998b), as both of them share a cylindrical spore body and 16 secondary cells arranged in two columns; the “Triactinomyxon ‘F’” was previously misclassified as a triactinomyxon type. Based on morphology and supported by phylogenetic analysis, raabeia type 1 was classified within the raabeia group. In contrast, the morphology of raabeia type 2 was consistent with earlier descriptions (Yokoyama et al., 1995; Eszterbauer et al., 2006; Xi et al., 2013) and was confirmed by molecular analysis as the actinospore stage of *M. cultus*. Notably, raabeia type 2 observed in this study possessed short branches at the ends of the caudal processes, a feature not previously reported for raabeia types of *M. cultus*.

All aurantiactinomyxon types identified in this study differed clearly from each other in both morphology and morphometrics. Subsequent molecular analyses confirmed that six of these types are novel. Notably, the 18S rDNA sequence of aurantiactinomyxon type 4 matched that of *Zschokkella chezhachei*, thereby completing its life cycle. In addition, aurantiactinomyxon types 5 and 6 were identified as the actinospore stages of *T. hovorkai*. Although both belong to the same species, they differ morphologically, particularly in the lengths of their caudal processes and polar capsules. This study represents the first record of *O. serpentina* as an annelid host for aurantiactinomyxon actinospore, suggesting greater myxozoan diversity in this species than previously recognized.

Furthermore, neoactinomyxum type 1 observed in this study was consistent with previously described neoactinomyxum type (Xi et al., 2015), but differs by having 32 secondary cells, a feature reported here for the first time. Molecular analyses confirmed that this neoactinomyxum type represented the actinospore stage of *T. wangi*.

3.3 Myxosporeans in Malaysia

A total of 119 fish representing thirteen species were collected from rivers and small channels in Terengganu and examined for myxozoan infections. *Barbonymus gonionotus*, *Osteochilus waandersii*, and *Channa gachua* showed 100% infection rates. *Trichopodus pectoralis* had the next highest prevalence at 66.7% (6/9), followed by *Labiobarbus leptocheilus* at 60.0% (9/15), while the remaining species showed infection rates of 50% or lower (**Table 4**).

Table 4 Infection prevalence rates in the investigated fish species.

Species	Infected fish	Infection rate
Barbonymus gonionotus	6/6	100.0%
Barbonymus altus	7/16	43.7%
Barbonymus schwanefeldii	3/6	50.0%
Leptobarbus rubripinna	2/5	40.0%
Barbodes binotatus	1/13	7.7%
Labiobarbus leptocheilus	9/15	60.0%
Osteochilus waandersii	15/15	100.0%
Trichopodus trichopterus	6/9	66.7%
Trichopodus pectoralis	10/12	83.3%
Channa gachua	12/12	100.0%
Neolissochilus soroides	0/5	0.0%
Anabas testudineus	0/3	0.0%
Notopterus notopterus	0/2	0.0%

Overall, thirty-five myxosporean species were identified, including fifteen *Myxobolus*, eleven *Henneguya*, five *Thelohanellus*, three *Myxidium*, and one *Ceratomyxa* species. Of these, thirty-three were newly described, while two were previously known. The highest parasite diversity was observed in *C. gachua* and *L. leptocheilus*. *Channa gachua* harboured seven species (six *Henneguya* and one *Myxidium*), while *L. leptocheilus* was

infected by seven species comprising five *Myxobolus*, one *Thelohanellus*, and one *Myxidium* (**Table 5**). In addition, *O. waandersii* and *T. pectoralis* each hosted five myxosporean species, whereas *B. gonionotus* was infected by four species. The remaining fish species harboured one or two myxosporean species each (**Table 5**). All examined fish appeared healthy, with no visible lesions.

Table 5 Myxosporean species detected in the investigated fish species.

Fish species	Myxosporean parasite	Predilection site
Barbonymus gonionotus	*Myxobolus gonionoti* n. sp.	Gill filaments
	Myxobolus barbonymi n. sp.	Muscle
	Thelohanellus gonionoti n. sp.	Caudal fin
	Thelohanellus zahrahae	Gill filaments
Barbonymus altus	*Myxobolus faizahae* n. sp.	Muscle
	Thelohanellus barbonymi n. sp.	Gill arch
Barbonymus schwanefeldii	*Myxobolus dykovae*	Gill lamellae
	Ceratomyxa schwanefeldii n. sp.	Gallbladder
Leptobarbus rubripinna	*Myxobolus* n. sp. 3	Muscle
Barbodes binotatus	*Myxobolus* n. sp. 4	Ovaries
Labiobarbus leptocheilus	*Myxobolus* n. sp. 5	Connective tissue of gill arch
	Myxobolus n. sp. 6	Gill filaments
	Myxobolus n. sp. 7	Fin rays
	Myxobolus n. sp. 8	Ovaries
	Myxobolus n. sp. 9	Muscle
	Thelohanellus n. sp. 1	Skin
	Myxidium n. sp. 1	Gallbladder

Osteochilus waandersii	*Myxobolus* n. sp. 10	Cartilage of gill arch, bulbus arteriosus of heart
	Myxobolus n. sp. 11	Gill cartilage
	Myxobolus n. sp. 12	Muscle
	Myxobolus n. sp. 13	Muscle
	Thelohanellus n. sp. 2	Fin rays
Trichopodus trichopterus	*Henneguya* n. sp. 1	Gill lamellae
Trichopodus pectoralis	*Henneguya* n. sp. 2	Gill lamellae
	Henneguya n. sp. 3	Cartilaginous tissue of gill arch
	Henneguya n. sp. 4	Gill arch
	Henneguya n. sp. 5	Pharynx
	Myxidium n. sp. 2	Gallbladder
Channa gachua	*Henneguya* n. sp. 6	Serous membrane of internal organs
	Henneguya n. sp. 7	Ovaries
	Henneguya n. sp. 8	Vertebral column
	Henneguya n. sp. 9	Gills
	Henneguya n. sp. 10	Muscle
	Henneguya n. sp. 11	Muscle near caudal peduncle
	Myxidium n. sp. 3	Gallbladder

The genus *Barbonymus* includes ten species widely distributed in Southeast Asia, with *B. schwanefeldii*, *B. gonionotus*, and *B. altus* being common and commercially important in Malaysia. Examination of these fishes revealed six novel myxozoan species (three *Myxobolus*, two *Thelohanellus,* and one *Ceratomyxa*) and two previously known species (*M. dykovae* and *T. zahrahae*), based on morphology and 18S rDNA analyses. Two muscle-infecting species identified here, *Myxobolus barbonymi* n. sp. and *Myxobolus faizahae* n. sp., displayed 'pseudodispar-type' morphology, with intracellular and intramuscular plasmodia, respectively. Molecular and

phylogenetic analyses confirmed both as new species and showed that muscle-infecting *Myxobolus* species tend to cluster together phylogenetically.

In this study, two new gill-infecting species (*Myxobolus gonionoti* n. sp. and *Thelohanellus barbonymi* n. sp.) were identified along with two known species (*Myxobolus dykovae* and *Thelohanellus zahrahae*). *Myxobolus gonionoti* n. sp. showed the closest morphological similarity to *M. dykovae* but differed in some features, supporting its novelty. Although it could be confused with *Myxobolus macrocapsularis* previously reported from *B. gonionotus* (Ky & Te, 2007; Chinh et al., 2023), BLAST analysis showed only 77% sequence similarity, confirming it as a distinct species. Earlier reports of *M. macrocapsularis* were based solely on morphology, suggesting that those spores may have been misidentified and could represent *M. gonionoti* n. sp.

Plasmodia of *T. barbonymi* n. sp. were found in the gill arches, an unusual site for *Thelohanellus* species, which typically infect gill lamellae or filaments. Only a few species, such as *Thelohanellus valeti*, have been reported from gill arches, supporting the novelty of *T. barbonymi* n. sp. Despite morphological similarity to *T. zahrahae*, genetic data and host differences confirm *T. barbonymi* n. sp. as a distinct species.

This study also identified two previously described gill-infecting species, *T. zahrahae* and *M. dykovae*. Their morphology matched earlier descriptions, and molecular analyses of 18S rDNA confirmed their identities. *Thelohanellus zahrahae* differed from other *Thelohanellus* species from *B. gonionotus*, such as *Thelohanellus catlae*, mainly in plasmodium shape, further supporting its correct identification.

The morphometrics of *Thelohanellus gonionoti* n. sp. were distinct from previously described *Thelohanellus* spp., including those with truncated anterior ends. 18S rDNA and pairwise distance analyses confirmed *T. gonionoti* n. sp. as a valid new species. Phylogenetically, it clusters within the

gill-infecting *Thelohanellus* clade alongside T. *barbonymi* n. sp., *T. zahrahae*, and related species.

To date, nineteen *Ceratomyxa* species have been reported in gall bladders of freshwater fish from South America, Europe, and Asia, with eleven exhibiting motile, worm-like plasmodia, primarily in South American species (Zatti et al., 2017; Zatti et al., 2018a; da Silva et al., 2020; Adriano & Okamura, 2021; Araújo et al., 2022; Bittencourt et al., 2022; Franzolin et al., 2022; Zatti et al., 2023). Plasmodia of *Ceratomyxa schwanefeldii* n. sp. also showed this motility, indicating it is not exclusive to South America. Morphologically, *C. schwanefeldii* n. sp. resembled *Ceratomyxa* sp. 7 in elongated shape and blunt poles (Adriano et al., 2021), but its undulatory movement is slower, similar to *Ceratomyxa ranunculiformis*. Phylogenetic analysis of 18S rDNA placed *C. schwanefeldii* n. sp. in a distinct freshwater lineage closely related to *Unicapsulocaudum mugilum*, sharing 92.8% sequence similarity. Furthermore, 28S rDNA analysis positioned *C. schwanefeldii* n. sp. near marine *Ceratomyxa* species, though limited sequences restrict resolution. This study provides the second 28S rDNA sequence for a freshwater-host *Ceratomyxa*.

Previous studies reported *M. leptobarbi* from the muscle of *L. hoevenii* in Malaysia (Székely et al., 2009a), and *M. koi* from the gallbladder and kidneys of *L. rubripinna* in Thailand (Thumvittayakul et al., 2018). In this study, *Myxobolus* n. sp. 3 was described from the muscle of *L. rubripinna*. Morphologically, it resembled *M. leptobarbi* except for the number of polar filament coils and a mucus envelope, and showed similarities to *M. koi* with differences in spore length. Molecular analyses confirmed *Myxobolus* n. sp. 3 as a distinct species, suggesting host specificity. Comparison with the available *M. koi* sequence from *C. carpio* further supported its distinctness.

Spores reported by Thumvittayakul et al. (2018) from *L. rubripinna* may have been misidentified and likely correspond to *Myxobolus* n. sp. 3.

Myxobolus n. sp. 4 is the first myxozoan reported from female *B. binotatus* and the first from ovarian tissue in Malaysia. Morphologically, it resembled *M. csabai* and *M. nekrasovae*, and its morphometrics were similar to *Myxobolus* n. sp. 12 from *O. waandersi*, differing only in sutural markings. Molecular analysis confirmed it as distinct, forming a basal branch within the gill-infecting *Myxobolus–Thelohanellus* clade with unequal polar capsules.

The genus *Labiobarbus* is widespread in Southeast Asia, with five species reported in Peninsular Malaysia. Only *L. leptocheilus* was collected in this study, from which seven novel *Myxobolus* species were described. *Myxobolus* n. sp. 5 was found in the gill-arch connective tissue and had morphology that resembled *M. tribolodonus* and *M. paludinosus*. Molecular analysis grouped it with newly described species (*Myxobolus* n. sp. 9, *Myxobolus* n. sp. 13) and similar spore morphotypes (*Myxobolus* n. sp. 9, *Myxobolus* n. sp. 13, *Myxobolus csabai*, *Myxobolus tasikkenyirensis*).

Additionally, *Myxobolus* n. sp. 6, *Myxobolus* n. sp. 7, and *Myxobolus* n. sp. 8 were found in the gill arches, filaments, fins, and ovarian tissue, respectively, sharing pyriform spores. Only *Myxobolus* n. sp. 8 had equal polar capsules, while *Myxobolus* n. sp. 6 and *Myxobolus* n. sp. 7 had unequal capsules. Uniquely, *Myxobolus* n. sp. 7 displayed a 'toyamai-like' structure with one prominent and one rudimentary capsule extending over three-quarters of the spore, unlike similar species reported from gills. Its occurrence in fins may explain its basal phylogenetic position, suggesting undiscovered fin-infecting 'toyamai-like' species in Malaysia.

Previous studies reported *M. tasikkenyirensis* from *O. vittatus* and *M. osteochili* from *O. hasselti* (Székely et al., 2009a) that are morphologically similar to *Myxobolus* n. sp. 9 and *Myxobolus* n. sp. 13. Phylogenetic analyses

showed that *Myxobolus* n. sp. 9 and *Myxobolus* n. sp. 13 formed a sister group with *M. tasikkenyirensis* and *M. csabai*, likely reflecting tissue tropism, as all occur in muscle except *M. csabai* (kidneys); the *M. osteochili* was excluded from the analysis due to low sequence similarity (<88%). Despite similar morphotypes and predilection sites, differences in host species (*L. leptocheilus* vs *O. waandersii*) and 18S rDNA confirmed *Myxobolus* n. sp. 9 and *Myxobolus* n. sp. 13 as distinct species.

In addition, two novel gill-cartilage species (*Myxobolus* n. sp. 10 and *Myxobolus* n. sp. 11) were identified from *O. waandersii* in Malaysia. Spores of *Myxobolus* n. sp. 11 were small with thick shell valves, and *Myxobolus* n. sp. 10 had large spores and developed in an unusual predilection site, under gill filament bases and bulbus arteriosus. Phylogenetic analysis placed *Myxobolus* n. sp. 11 with other gill-cartilage species and raabeia-type actinospores, whereas *Myxobolus* n. sp. 10 formed a basal branch, confirming both as distinct, novel species.

Two novel *Myxobolus* species, *Myxobolus* n. sp. 12 and *Myxobolus* n. sp. 13, were identified from the muscle of *O. waandersii*. *Myxobolus* n. sp. 12 had unequal polar capsules and larger oval plasmodia developing intracellularly within muscle fibres. The *Myxobolus* n. sp. 13 had equal polar capsules with smaller elongate-oval plasmodia and developed intercellularly. Molecular, morphological, and histological evidence confirmed their distinction.

Most *Thelohanellus* species infect gills, but this study described two additional novel species: *Thelohanellus* n. sp. 1 from the skin of L. *leptocheilus* and *Thelohanellus* n. sp. 2 from connective tissue between fin rays of *O. waandersii*. *Thelohanellus* n. sp. 1 formed large, whitish, oval plasmodia resembling *Thelohanellus leshanensis* and *Thelohanellus liaohoensis*, with phylogenetic proximity to *Myxobolus* n. sp. 12, representing

the first skin-infecting *Thelohanellus* in Malaysia. *Thelohanellus* n. sp. 2 had small, oval spores with polar capsules leaning to the spore side, resembling *Thelohanellus goldi*, an Indian species, but differing in polar-tubule coils and host, and formed a basal lineage among myxozoans from the same host, confirming its novelty.

BLASTn analysis showed that the *Myxidium* sample from the gallbladder of *L. leptocheilus* shared 99.4% sequence similarity with *Zschokkella* sp. (KM401441) from *Labeo rohita*, for which, however, no morphological data are available. The spores of *Myxidium* n. sp. 1 exhibited fusiform or sigmoid with pointed ends and pyriform polar capsules, which differed clearly from known *Zschokkella* spp., supporting its status as a new species.

The genus *Trichopodus* includes six valid species, four of which occur in Malaysia, namely *T. trichopterus, T. pectoralis, T. leerii,* and *T. cantoris* (Froese & Pauly, 2025), and are valued both as ornamental and bait fish. Previous studies reported *Henneguya daoudi* (Székely et al., 2009a) and *Henneguya schizura* (Ky & Te, 2007) from *T. trichopterus*. In this study, *Henneguya* n. sp. 1 was found in gill filaments of *T. trichopterus*, morphologically resembling *H. daoudi* but differing in caudal appendage length. Molecular analysis showed 98.7% similarity to *H. daoudi*, and phylogenetically, both formed a sister group to *Henneguya* n. sp. 2 from *T. pectoralis*. The lack of sequence data for *H. schizura* and shorter sequence of *H. daoudi* limits comprehensive comparisons, emphasizing the need for further molecular studies.

Trichopodus pectoralis is widely distributed across Southeast Asia and was introduced to Malaysia for subsistence fisheries without becoming invasive. This study represents the first survey of myxozoan parasites in *T. pectoralis*, identifying five novel species. *Myxidium* n. sp. 2 was found in the

gallbladder, morphologically resembling *Myxidium djolonensis* (fusiform with truncated ends) but differing in spore dimensions, host, and geographical distribution. Phylogenetic analyses placed it within the *Myxidium* clade, closely related to *Zschokkella* sp. (MT840090) from *Etroplus suratensis* and Myxosporea (KP030767) from *Synodontis* sp., confirming its genus assignment.

In addition to *Myxidium* n. sp. 2, four novel *Henneguya* species were identified from different tissues of *T. pectoralis*, such as *Henneguya* n. sp. 2 from gill lamellae, *Henneguya* n. sp. 3 from gill cartilage, *Henneguya* n. sp. 4 from gill arches, and *Henneguya* n. sp. 5 from the pharynx. Each species showed distinct morphology, with *Henneguya* n. sp. 2 and *Henneguya* n. sp. 3 having ellipsoidal spores, *Henneguya* n. sp. 4 and *Henneguya* n. sp. 5 having fusiform spores, and *Henneguya* n. sp. 2 possessed the longest caudal appendages. Notably, *Henneguya* n. sp. 3 exhibited a unique, previously unreported polar capsule arrangement with unequal capsules deviating from the median plane, supporting its novelty. Phylogenetic analyses confirmed all four *Henneguya* as distinct species.

Studies on myxozoan parasites of fish in the genus *Channa* are limited, with most records originating from India and China. *Channa gachua* was previously known to host only a single myxozoan species. However, the present study revealed seven novel myxozoan species, including one *Myxidium* sp. and six *Henneguya* spp., from several organs and tissues.

Plasmodia and free spores of *Myxidium* n. sp. 3 were detected in the gallbladder. This species is morphologically distinct, possessing a concave spore body with slightly truncated ends, a feature not previously reported in genus *Myxidium*. Molecular analyses showed the highest similarity (91.0%) to *M. chuatsi* and phylogenetic analysis placed *Myxidium* n. sp. 3 as a basal

lineage within the *Myxidium* clade. This study represents the first record of a *Myxidium* species from *C. gachua.*

Previous studies have reported twelve *Henneguya* species from five *Channa* species: *C. argus*, *C. maculatus*, *C. striata*, *C. punctatus* and *C. marulius* (Sarkar et al., 1985; Chen & Ma, 1998; Chaudhary et al., 2017), but none had been described from *C. gachua* prior to this study. Here, six novel *Henneguya* species, namely *Henneguya* n. sp. 6, *Henneguya* n. sp. 7, *Henneguya* n. sp. 8, *Henneguya* n. sp. 9, *Henneguya* n. sp. 10 and *Henneguya* n. sp. 11 were identified from several predilection sites, including serous membranes, ovaries, vertebral column, gills, and muscle tissues. *Henneguya* n. sp. 6 and *Henneguya* n. sp. 10 showed oval spores resembling *H. caquetaia*, *H. guanduensis*, *H. disparis*, and *H. ovaliformis*. The former two species are larger, and detailed morphometric data are lacking for the latter two. Despite their similar morphology, *Henneguya* n. sp. 6 and *Henneguya* n. sp. 10 differed in caudal appendages and polar capsule lengths, supporting their recognition as distinct species.

Although both *Henneguya* n. sp. 10 and *Henneguya* n. sp. 11 were found in skeletal muscle, their plasmodia developed in different locations. Histological sections showed that *Henneguya* n. sp. 10 occurred in the muscle of the trunk, whereas *Henneguya* n. sp. 11 developed in the muscle of the caudal peduncle. The two species also differed morphologically, with *Henneguya* n. sp. 10 having oval spores and *Henneguya* n. sp. 11 exhibiting ellipsoidal spores. The closest similarity was observed between *Henneguya* n. sp. 11 and *Henneguya* n. sp. 8 from the vertebral column, which also has ellipsoidal spores but differs slightly in size. Likewise, *Henneguya* n. sp. 7 and *Henneguya* n. sp. 9 showed similar elongated to lanceolate spores but differed in predilection sites, infecting ovarian tissue and gills, respectively. Considering the strong organ and tissue specificity of myxozoans, the six

Henneguya species described here are recognized as novel taxa based on their distinct predilection sites and morphological features.

3.4 Actinosporeans in Malaysia

A total of 2,666 oligochaetes were isolated from sediments at Tasik Telabak, representing eight morphologically distinct species, with *Branchiodrilus* and *Aulophorus* being the most abundant genera. Myxozoan infections were detected in 24 individuals, giving an overall prevalence of 0.90%. Infections occurred in four oligochaete species: *Bothrioneurum* sp., *Branchiodrilus* sp., *Aulodrilus acutus*, and *Aulophorus* sp. The highest prevalence was recorded in *A. acutus* (3.37%), followed by *Aulophorus* sp. (1.90%), *Bothrioneurum* sp. (1.30%), and *Branchiodrilus* sp. (0.37%). Six novel actinospore types were identified, comprising one triactinomyxon, one raabeia, and four aurantiactinomyxon types. None of the obtained sequences matched known myxosporean sequences in GenBank, so corresponding life cycles could not be determined.

Raabeia type 1 showed minor morphometric overlaps with previously described raabeia types but differed by at least one diagnostic character. Phylogenetic analysis placed raabeia type 1 within a Cypriniformes-infecting *Myxobolus* clade, closely related to a newly described species from *O. waandersii*, suggesting its myxospore stage likely develops in cyprinid hosts.

The aurantiactinomyxon collective group is one of the most diverse, with about 64 known types (Rocha, 2023; Rocha et al., 2024). In this study, four novel aurantiactinomyxon types were identified from two oligochaete hosts, none of which matched previously described types morphologically or morphometrically. All four types differed clearly from one another, particularly in caudal process length. Three types were found in the same host (*Aulophorus* sp.) with distinct caudal process size. Aurantiactinomyxon type

2 having exceptionally long processes, while aurantiactinomyxon type 1 had the shortest and was overall smaller. Aurantiactinomyxon type 4, collected from a different host, was distinguished by its prominent, elongated pyriform polar capsules, a feature not reported in any other aurantiactinomyxon type. Molecular analyses confirmed that the examined aurantiactinomyxon types represent distinct species, except for aurantiactinomyxon type 4, for which genetic data could not be obtained due to preservation problems.

Based on current knowledge, triactinomyxon is the second most diverse actinospore collective group after aurantiactinomyxon, with 59 types described (Borkhanuddin et al., 2014a; Székely et al., 2014; Xi et al., 2015; Rangel et al., 2015, 2016b), and it represents the most common actinospore type for the genus *Myxobolus*. The triactinomyxon type 1 identified in this study differed from all known triactinomyxon types in having eight secondary cells, as well as in both spore shape and dimensions. Its most distinctive features were the exceptionally short style and caudal processes, with a caudal process to spore body ratio of 1.07, the lowest range reported for other triactinomyxon types. In addition, the absence of the typical anchor-shaped caudal processes further supported its recognition as a novel type, although molecular data could not be obtained due to preservation issues.

4. CONCLUSION AND RECOMMENDATIONS

The large number of myxosporean species and actinosporean stages identified highlights the still largely unexplored diversity of myxozoans in Malaysian and Hungarian freshwater ecosystems. In Hungary, surveys of three fish farms revealed fifteen myxosporean species and ten actinosporean types, including eight new myxosporean species and seven new actinosporean types. Although Hungary has fewer fish species than Malaysia due to its landlocked geography, aquaculture plays an important role in fish production. Myxozoan studies in Hungarian fish farms have been limited, and this survey expanded coverage to three additional farms. Further investigations across more fish farms and host species are needed, as only a small fraction of Hungarian fishes are currently known to host myxozoans.

Most of the fifteen species described in this study were found in *C. auratus gibelio*, an invasive species in Hungary that has received limited research attention. Previously, only three myxosporean species had been recorded from *C. auratus gibelio* in Hungary. This study identified seven species, including four new ones, and documented *M. lentisuturalis* for the first time in Hungary. These findings show that invasive fish can host distinct myxozoan assemblages in new environments. Further surveys of other invasive fish species in Hungary are therefore recommended.

Several new *Thelohanellus* species were identified, including *T. imrei* n. sp. from the gill arch and pharyngeal connective tissues, *T. serosae* n. sp. from the serous membranes of the kidney and intestine, and *T. paranikolskii* n. sp. from the skin. Although *T. serosae* n. sp. and *T. paranikolskii* n. sp. were morphologically similar to the known species *T. hovorkai* and *T. nikolskii*, respectively, molecular analyses clearly distinguished them, highlighting the importance of molecular tools in resolving cryptic diversity.

Furthermore, 35 myxosporean species were recorded from two rivers and small channels across Kuala Terengganu, together with six actinosporean types from Tasik Telabak, increasing the number of known Malaysian species from 41 to 76. This study also provided the first successful descriptions of actinosporean stages in Malaysia; however, none could be linked to known myxospore stages, likely due to short sampling periods and low oligochaete abundance. Therefore, broader and long-term surveys across diverse aquatic habitats are essential.

The fish examined in this study included both native and introduced species, but endemic species were not investigated. Malaysia is known to host 49 endemic fish species, primarily distributed in Sabah and Sarawak (Froese & Pauly, 2025). Given that 35 myxosporean species were identified from native and introduced hosts, it is likely that endemic Malaysian fishes also harbour substantial undiscovered myxozoan diversity. Accordingly, future studies should expand myxozoan surveys in these regions to include a broader range of endemic fish species.

Two Malaysian actinospore types, aurantiactinomyxon type 4 and triactinomyxon type 1, were described based solely on morphology because molecular data could not be obtained, underscoring the need for improved preservation and future genetic analyses to clarify their phylogenetic relationships and identify their corresponding myxosporean stages and fish hosts.

Finally, myxo- and actinospore stages of three myxozoan life cycles were successfully linked using molecular data, confirming the effectiveness of genetic approaches, although experimental infections remain essential for validation. While 18S rDNA was primarily used in this study, future research should incorporate 28S rDNA to improve phylogenetic resolution and strengthen evolutionary and taxonomic inferences.

5. NEW SCIENTIFIC RESULTS

1. Documentation of eight novel myxosporean species from three fish hosts collected from three fish farms in Hungary. These records were supported by morphological, morphometric, histological, and molecular analyses. The newly identified species belonged to three genera: *Myxobolu*s, *Thelohanellus*, and *Zschokkella.* Additionally, *Myxobolus diversus* and *Myxobolus intrachondrealis* were provided with molecular data for the first time. Of these, 6 species were scientifically described.
2. Description of seven novel actinosporean types from two fish farms in Hungary. These actinosporeans belonged to three collective groups: Raabeia, Aurantiactinomyxon, and Triactinomyxon.
3. Documentation of 33 novel myxosporean species from native and introduced fish species collected from two rivers and small channels across Kuala Terengganu, Terengganu, Malaysia. These records were supported by morphological, morphometric, histological, and molecular analyses. The newly identified species belonged to five genera: *Myxobolus*, *Thelohanellus*, *Henneguya*, *Myxidium* and *Ceratomyxa*. Of these, 6 species were scientifically described.
4. First report and description of actinosporean stages in Malaysia, specifically from Tasik Telabak, Terengganu. Six novel actinosporean types were identified belonging to three collective groups: Raabeia, Aurantiactinomyxon, and Triactinomyxon.
5. Elucidation of three new myxosporean life cycle from fish farms in Hungary, as listed below:
    - *Zschokkella chezhachei* n. sp.
    - *Thelohanellus imrei* n. sp.
    - *Thelohanellus serosae* n. sp.

6. LIST OF PUBLICATIONS

6.1. Peer-reviewed journal articles

1. **Suhaimi, N.S.**, Colunga-Ramírez, G., Sellyei, B., Cech, G., Molnár, K., & Székely, C. (2023). The first detection of *Myxobolus lentisuturalis* Dyková, Fiala et Nie, 2002, a highly pathogenic muscle-infecting parasite of gibel carp (*Carassius auratus gibelio* Berg, 1932) in Hungary. *Journal Fish Diseases*, 46 (12), 1367–1376. https://doi.org/10.1111/jfd.13855.
2. Colunga-Ramírez, G., **Suhaimi, N.S.**, Cech, G., Molnár, K., Székely, C., & Sellyei, B. (2024). Morphological and molecular characterisation of two closely related species: *Myxobolus tihanyensis* n. sp. and *Myxobolus sandrae* Reuss, 1906. *International Journal of Parasitolology: Parasites and Wildlife,* 23, 100909. https://doi.org/10.1016/j.ijppaw.2024.100909.
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6. **Suhaimi, N.S.**, Cech, G., Molnár, K., Székely, C., & Sellyei, B. (2025). Infection of the gibel carp (*Carassius auratus gibelio* Berg, 1932) with myxozoan parasites in a pond farm of Hungary. *Aquaculture Reports*, 42, 102833. https://doi.org/10.1016/j.aqrep.2025.102833.
7. **Suhaimi, N.S.**, Székely, C., Sellyei, B., & Cech, G. (2025). Actinosporean diversity in a Hungarian fish farm and description of the life cycle of

Zschokkella chezhachei. *International Journal of Parasitolology: Parasites and Wildlife*, 101124. https://doi.org/10.1016/j.ijppaw.2025.101124.

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9. **Suhaimi, N.S.**, Sellyei, B., Mosonyi-Borzák, R., Cech, G., Molnár, K., & Székely, C. (2026). *Thelohanellus* species in common carp (*Cyprinus carpio*) and their life cycles from fish farms and Lake Balaton in Hungary. *Aquaculture Reports*. 46, 103380. https://doi.org/10.1016/j.aqrep.2026.103380.

6.2. Publications in progress

1. **Suhaimi, N.S.**, Székely, C., Cech, G., Sellyei, B., & Borkhanuddin, M.H. (2026). Description of three new types of aurantiactinomyxon actinospore (Myxozoa: Myxosporea) from the oligochaete *Aulophorus* sp. in Tasik Telabak, Malaysia. *Pasitology Research*. (Submitted)
2. **Suhaimi, N.S.**, Székely, C., Cech, G., Sellyei, B., & Borkhanuddin, M.H. (2026). Three novel *Myxidium* species (Myxozoa: Myxosporea) from fishes in river and small channels across Terengganu, Malaysia. *Acta Tropica*.

6.3. Conference abstracts/ papers/ oral and poster presentations

1. **Suhaimi N.S.,** Colunga-Ramírez, G., Sellyei B., Cech G., Molnár K. & Székely C. (2023). The first occurrence of *Myxobolus lentisuturalis* Dyková, Fiala et Nie, 2002, a pathogenic muscle-infecting parasite of gibel carp (*Carassius auratus gibelio* Berg, 1932) in Hungary. [Oral presentation]. In: Proceeding of Academical Days/ Parasitology-Zoology-Fish Pathology Session, Budapest, Hungary, January 20–23, 2023.
2. **Suhaimi N.S.,** Colunga-Ramírez, G., Sellyei B., Cech G., Molnár K. & Székely C. (2023). The first occurrence of *Myxobolus lentisuturalis* Dyková, Fiala et Nie, 2002, a pathogenic muscle-infecting parasite of gibel carp (*Carassius auratus gibelio* Berg, 1932) in Hungary. [Oral

presentation]. In: Proceedings of the 47th Hungarian Scientific Conference on Fisheries & Aquaculture (A XLVII. Halászati Tudományos Tanácskozás), Szarvas, Hungary, June 7–8, 2023.

3. **Suhaimi N.S.,** Colunga-Ramírez*, G.*, Sellyei B., Cech G., Molnár K. *&* Székely C. (2023). The first diagnosis of *Myxobolus lentisuturalis* a highly pathogenic muscle-infecting parasite of gibel carp (*Carassius auratus gibelio*) in Hungary [Poster presentation]. In: Proceedings of the 21st International EAFP Conference on Diseases of Fish and Shellfish, Aberdeen, Scotland, September 11–14, 2023.
4. **Suhaimi N.S.,** Borkhanuddin, M.H., Sellyei, B., Cech, G., & Székely, C. (2024). Exploration of actinospores and myxospores (myxozoa) in the freshwater ecosystems of Terengganu, Malaysia: a preliminary study [Oral presentation]. In: Proceeding of Academical Days / Parasitology-Zoology-Fish Pathology Session, Budapest, Hungary, January 30–31, 2024.
5. **Suhaimi N.S.** (2024). A journey from marine biologist to fish parasitologist [Oral presentation]. The Society of Hungarian Parasitologists/ The Situation of Domestic Fish Parasitology, Budapest, Hungary, April, 10, 2024.
6. **Suhaimi N.S.,** Borkhanuddin, M.H., Sellyei, B., Cech, G., & Székely, C. (2024). Survey on actinospores and myxospores (Myxozoa) in the freshwater ecosystems of Terengganu, Malaysia: A preliminary study. [Oral presentation]. In: Proceedings of the 48th Hungarian Scientific Conference on Fisheries & Aquaculture (A XLVII. Halászati Tudományos Tanácskozás), Szarvas, Hungary, June 5–6, 2024.
7. Colunga-Ramírez, G., **Suhaimi, N.S.**, Cech, G., Molnár, K., Székely, C., & Sellyei, B. (2024). Differentiation of the closely related *Myxobolus tihanyensis* n. sp. and *Myxobolus sandrae* Reuss, 1906 [Poster presentation]. 1st Joint Meeting of the Central and Eastern European Branches of the European Association of Fish Pathologists (EAFP), Brno, Czech Republic, December 4–6, 2024.
8. **Suhaimi, N.S.**, Cech, G., Molnár, K., Székely, C., & Sellyei, B. (2025). Infection of the gibel carp (*carassius auratus gibelio* berg, 1932) with myxozoan parasites in a pond farm of Hungary [Oral presentation]. In: Proceedings of the 49th Hungarian Scientific Conference on Fisheries & Aquaculture (A XLVII. Halászati Tudományos Tanácskozás), Szarvas, Hungary, June 3–4, 2025.

9. **Suhaimi, N.S.**, Cech, G., Molnár, K., Székely, C., & Sellyei, B. (2025). Infection of the gibel carp (*carassius auratus gibelio* berg, 1932) with myxozoan parasites in a pond farm of Hungary [Poster presentation]. In: Proceedings of the 22st International EAFP Conference on Diseases of Fish and Shellfish, Heraklion, Greece, September 1–4, 2025.