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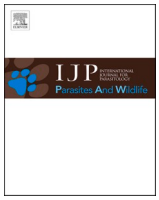

First record and description of actinospore stages (raabeia, triactinomyxon, and aurantiactinomyxon types) of fish parasitic myxozoans from Malaysia

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ABSTRACT

During a 2-month survey in 2023 at Tasik Telabak, Terengganu, Malaysia three distinct actinospore types, namely raabeia, triactinomyxon and aurantiactinomyxon were identified in three invertebrate host species: *Aulodrilus acutus*, *Branchiodrilus* sp., and *Bothrioneurum* sp. utilizing morphometric and molecular analyses. Maximum likelihood of 18S rDNA positioned the raabeia type within the *Myxobolus* clade from fish of the Order Cypriniformes, suggesting a detected actinospore has a potential life cycle development in Cypriniformes and the genus *Myxobolus*. Both triactinomyxon and aurantiactinomyxon types were described solely based on morphology and morphometrics due to preservation error preventing the acquisition of 18S rDNA sequences. The triactinomyxon type in this study exhibited distinct morphology in spore shape and dimensions, characterized by a short style and caudal processes. Conversely, the aurantiactinomyxon type described herein possesses prominent elongated pyriform polar capsules not resembling any previously known aurantiactinomyxon types. These distinctive features, along with host species and geographical location justify their classification as novel types. Histological and microscopic analyses revealed the development of pansporocysts in the intestinal epithelium of the oligochaete host. This study marks the first descriptions of actinospore stages of myxozoans in Malaysia and the initial report of actinospores infecting host species of *Aulodrilus acutus*, *Branchiodrilus* sp. and *Bothrioneurum* sp.

1. Introduction

Myxozoans (Cnidaria, Myxozoa) are microscopic and spore-forming obligate parasites of vertebrates and invertebrates (Lom and Dyková 2006). Their complex life cycles typically involve a myxospore stage that develops in fish and an actinospore stage that develops in annelid worms (Eszterbauer et al., 2015). As the hosts and typical placements of actinospores and myxospores are highly different, the link between myxospore and actinospore stages is not obvious until the traditional life cycle reconstruction and/or molecular analyses, have not connected the two forms in one. Thus, the description of myxozoans is generally based on myxospores collected from fishes, while the annelid-originated free-floating actinospores are classified into morphological collective types until their genetic identity is confirmed. Research on actinosporeans has been relatively limited over decades possibly due to their lack of economic importance of their annelid hosts, despite being the infectious stage to many economically important fish species. Currently, there are approximately 200 identified actinospore types belonging to 20 collective groups, but this number is expected to increase as more than 2600 myxozoan species have been described from both fish and other vertebrate hosts worldwide (Lom and Dyková, 2006; Okamura et al., 2018). In contrast to myxospores, the limited number of descriptions of actinospores is due to under-sampling to collect and examine of oligochaetes (Milanin et al., 2017; Rocha, 2023). Most collective groups are exclusively from freshwater or brackish water, but only four are solely marine. Among 200 actinospore types, 35 were classified as raabeia type (Rocha et al., 2019a), 59 as triactinomyxon (Borkhanuddin et al., 2014a; Székely et al., 2014; Xi et al., 2015; Rangel et al., 2015, 2016) and 64 as aurantiactinomyxon, which, according to the present knowledge, are the morphologically most diverse collective groups (Rocha, 2023; Rocha et al., 2024). The remaining ones belong mainly to types of antonactinomyxon, helioactinomyxon,

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hexactinomyxon, hungactinomyxon, neoactinomyxum, sphaeractinomyxon, seisactinomyxon, and synactinomyxon.

Previous actinospore studies relied solely on morphology and schematic drawings, resulting in initial descriptions lacking adequate comparative data. Subsequent research revealed significant morphological variability among isolates (Hallett et al., 2004; Eszterbauer et al., 2006), and overlap in measurements among different types (Rangel et al., 2016). To address these challenges, recent works increasingly integrate molecular analysis with morphology to prevent misidentification, particularly of actinospores with notable intraspecific morphometric variation (Rosser et al., 2014; Xi et al., 2015, 2017; Rangel et al., 2016; Rocha et al., 2019b). This combined approach enhances understanding of myxozoan biodiversity and evolutionary relationships.

Actinospore fauna have been extensively studied in fish farms and natural waters across Europe (McGeorge et al., 1997; El-Mansy et al., 1998a, b; Xiao and Desser, 1998a, b, c; Hallett et al., 2002; Székely et al., 2000, 2002a, 2005, 2014; Negredo and Mulcahy, 2001; Özer et al., 2002; Oumouna et al., 2003; Rácz et al., 2005; Marcucci et al., 2009; Caffara et al., 2009; Borkhanuddin et al., 2014a; Zhao et al., 2016; Rocha et al., 2019c) and East Asia (Yokoyama et al., 1993, 1995; Székely et al., 2002b, 2003; Xi et al., 2013, 2015; Zhao et al., 2016, 2017). However, to date, no actinospores and myxozoan life cycle have been reported in Malaysia. Although more than 30 species of myxospores were identified in Malaysian fishes in freshwater, estuarine and marine environments (Molnár et al., 2006a, 2006b; Székely et al., 2009a, 2009b, 2012; Bartošová and Fiala, 2011; Borkhanuddin, 2013; Freeman and Kristmundsson, 2015; Fiala et al., 2015; Shahar et al., 2017; Samshuri, 2018; Borkhanuddin et al., 2014b, 2020a, 2020b), their actinosporean stages and oligochaete hosts remained unstudied. In this study, we conducted survey of the actinosporean fauna in freshwater oligochaetes communities of Tasik Telabak, Terengganu. Here we report for the first time the descriptions of three new actinospore types of raabeia, triactinomyxon, and aurantiactinomyxon.

2. Materials and methods

2.1. Study area and sample collection

Tasik Telabak (5°37′58.22″ N, 102°28′44.52″ E) is located in the upstream region of Besut, Terengganu (Fig. 1), and is recognized as a popular recreational area for fishing activities. Recently, the lake has been utilized by local communities for fish farming, including species such as *Oreochromis niloticus* (tilapia) and *Hemibagrus nemurus* (silver catfish). Additionally, various native and invasive fish species can also be found in the lake including *Cichla* sp. (peacock bass), *Barbonymus schwanenfeldii* (tinfoil barb), *Oxyeleotris marmoratus* (marble goby), *Notopterus notopterus* (bronze featherback), *Osteochilus vittatus* (silver shark minnow), *Cyclocheilichthys apogon* (beardless barb), and *Labiobarbus* sp. (common barb). Tasik Telabak was chosen for the study due to the limited information available on fish parasites and the aquaculture activities are newly established, presenting opportunities for economic development and expansion in the region. Moreover, the lake is a crucial economic resource for local communities that depend on its ecosystem for their livelihoods. It serves as an excellent subject for studying biodiversity and the potential for sustainable aquaculture including parasite surveys informing about pathogen-infection risk assessments of economically important cultured species.

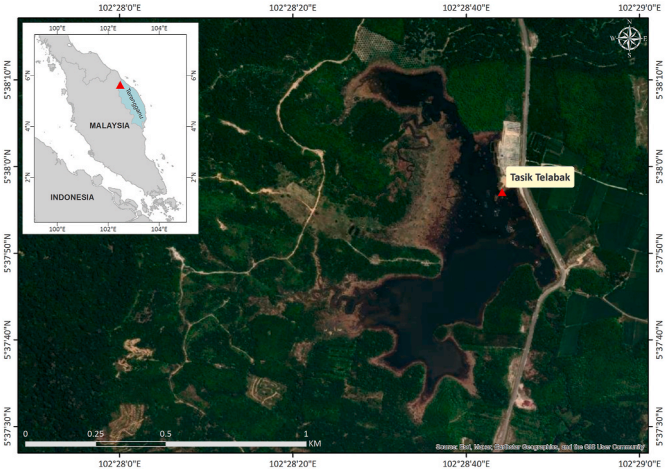

Fig. 1. Map of Peninsular Malaysia showing the studied area. Mud samples with oligochaetes were collected from the site at Tasik Telabak, Hulu Besut, Terengganu (arrowhead).

Mud containing oligochaetes was collected from the shallow parts of the lake on July 19, 2023. The collected sediments were placed in buckets with minimal lake water and transported to the Marine Science Biodiversity Laboratory of the Faculty of Science and Marine Environment at Universiti Malaysia Terengganu. Upon arrival, the mud was maintained in the buckets with an aeration system, regularly supplied with dechlorinated tap water, and kept at an ambient temperature of 33 °C–35 °C. Within 24 h, the oligochaetes were hand-sorted and placed individually into 48-well microtiter plates containing dechlorinated water. Each plate was examined daily for released actinospores using Leica DM IL LED inverted microscope (Leica, Wetzlar, Germany).

2.2. Microscopic examination

A part of freshly released actinospores from infected oligochaetes were examined on a slide under an Olympus CX33 biological microscope (Olympus Corporation, Japan). The remaining ones were collected in 1.5 mL of Eppendorf tube and preserved in 90% ethanol for molecular studies and transported to Fish Pathology and Parasitology Laboratory of the Veterinary Medical Research Institute at Budapest in Hungary where further examination involved conducting high-magnification examinations and capturing photographs using an Olympus BX53 light microscope equipped with an Olympus DP74 digital camera (Olympus Corporation, Japan). Spore morphology and morphometric parameters were determined from both fresh and fixed spores following the guidelines of Lom et al. (1997). However, comparative studies on spore measurements between fresh and fixed spores were not conducted. Based on findings of Sellyei et al. (2022), where fixed spores in 80% ethanol exhibited no size reduction. Thus, it is hypothesized that spores fixed in 90% ethanol will similarly show no variation in measurements compared to fresh spores. All measurements are given in micrometers (μm) as the range followed by the mean and standard deviation in parentheses.

The infected worms were collected, with the anterior part preserved in 95% ethanol to facilitate species identification through molecular techniques. Consequently, the posterior segment was preserved in 10% neutral buffered formalin, then embedded in paraffin wax, cut in 4–5 μm thick sections and stained with haematoxylin and eosin (H&E) for histological analysis. The histological slides were examined and photographed to determine the site of infection within the oligochaetes.

2.3. Molecular analysis

Preserved actinospores in 95% ethanol were centrifuged at 13,000 rpm for 15 min, and the ethanol was removed by pipetting. The pelleted spores were washed twice with Elution Buffer (10 mM Tris-HCL, pH 8.5) to remove residual ethanol. Genomic DNA extraction was performed from the pellet using Geneaid Tissue Genomic DNA Mini kit (Geneaid Biotech Ltd., Taiwan) following the manufacturer's recommended protocol for animal tissues. Subsequently, a direct PCR was conducted to amplify the 18S rDNA gene of the actinospores using primers Myx1F (Hallett and Diamant, 2001) with ERIB10 (Barta et al., 1997) in 50 μL reaction mixture containing 5 μL of template DNA, 1 × DreamTaq buffer

(10 × ; Thermo Scientific), 200 nM dNTP mix (10 mM; Thermo Scientific), 2.5 U DreamTaq polymerase (5 U; Thermo Scientific), 5 pmol of each primer, and molecular water. The PCR reactions were carried out under the following cycling conditions: initial denaturation step of 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, with a final step of 72 °C for 7 min, and stored at 4 °C. For identification of oligochaete hosts, 16S rRNA gene of mitochondrial DNA (mtDNA) and internal transcribed spacer region (ITS) (internal transcribed spacer 1 - the 5.8S rRNA gene - internal transcribed spacer 2) was amplified using 16sar-L (Palumbi et al., 2002), 16sbr-H (Palumbi et al., 2002), ITS-5 (White et al., 1990) and ITS-4 (White et al., 1990), respectively in a 25 μL reaction mixture containing 1 μL of extracted genomic DNA, 1 × Taq Buffer (10 × ; Thermo Scientific), 0.5 mM dNTP mix (1 mM, MBI Fermentes), 0.5 U Taq Polymerase (2 U; MBI Fermentes), 2.5 pmol of each primer, and molecular water. For the PCR of the oligochaetes, reactions of 16S rRNA and ITS were conducted using the conditions described by Rocha et al. (2020) and Erséus et al. (2017), respectively. PCR products were visualized by agarose gel electrophoresis, purified using DNA Fragment Purification kit (InViTek GmbH, Berlin, Germany), and sequenced bidirectionally using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and read by an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) with primers listed in Table 1.

2.4. Sequence assembly

The partial SSU rDNA sequences obtained from actinospores originating from infected oligochaetes were assembled and checked using Geneious Prime v.11.1 (Kearse et al., 2012). To assess the phylogenetic analysis, 38 published sequences of 18S rDNA were retrieved from GenBank database, and *Chloromyxum cristatum* (AY604198) and *Chloromyxum fluviatile* (GU471265) were selected as outgroups. The nucleotide sequences were aligned by using Clustal W algorithm (Thompson et al., 1994) in MEGA X (Kumar et al., 2018), and poorly aligned, highly variable regions were eliminated using GBlocks v0.91b (Talavera and Castresana, 2007). A phylogenetic tree was obtained using Maximum Likelihood (ML) analysis. ML analysis was calculated using GTR + G + I model according to the model selection by Akaike information criterion (AIC), with bootstrap confidence values calculated with 1000 replications. The resulting consensus tree was visualized in MEGA X and annotated with Inkscape (Free Software Foundation, Inc., MA, USA).

Table 1
List of primers used for DNA amplification and sequencing of actinospores and oligochaete.

Primer	Sequence (5′–3′)	Paired with	Reference
ERIB10	CTTCCGCAGGTTCACCTACGG	Myx1F, ACT3F	Barta et al. (1997)
Myx1F	GTGAGACTGCGGACGGCTCAG	ERIB10, Myxgen4R, ACT1R, MyxospecR	Hallett and Diamant (2001)
ACT3F	CATGGAACGAACAAT	ERIB10, Myxgen4R, ACT1R, MyxospecR	Hallett and Diamant (2001)
Myxgen4R	CTYTGATTTATTCAAGGCAC	Myx1F, ACT3F	Køie et al. (2008)
MyxospecR	CAACAAGTTGATAGGGCAGAA	Myx1F, ACT3F	Fiala (2006)
ACT1R	AATTTCACCTCTCGCTGCCA	Myx1F, ACT3F	Hallett and Diamant (2001)
16sar-L	CGCCTGTTTATCAAAAACAT	16sbr-H	Palumbi et al. (2002)
16sbr-H	CCGGTCTGAACTCAGATCACGT	16sar-L	Palumbi et al. (2002)
ITS-5	TCCTCCGCTTATTGATATGC	ITS-4, 5.8mussR	White et al. (1990)
ITS-4	GGAAGTAAAAGTCGTAACAAGG	ITS-5, 5.8mussF	White et al. (1990)
5.8mussF	CGCAGCCAGCTGCGTGAATTAATGT	ITS-4, 5.8mussR	Källersjö et al. (2005)
5.8mussR	GATGTCGATGTTCAATGTGTCCTGC	ITS-5, 5.8mussF	Källersjö et al. (2005)

3. Results

From July to August 2023, 1312 oligochaetes were collected from Tasik Telabak and screened for myxozoan infection. Nine (0.7%) of them were found to be infected with actinospore stage. The comprehensive survey revealed the presence of eight morphologically distinct species of oligochaetes in the mud samples. However, myxozoan infections were detected only in three genera: *Branchiodrilus*, *Aulodrilus* and *Bothrioneurum.* Worms belonging to *Branchiodrilus* comprised 52% of oligochaetes examined. In total, three actinospore types from three collective groups of raabeia, triactinomyxon and aurantiactinomyxon were identified. Each spore was released from the infected hosts 24 h after mud collection and separation of individual oligochaetes into the cell-well plates.

Host identification was performed using molecular data from the mitochondrial 16S rRNA gene and nuclear ITS region, but two out of three sequences from the ITS region failed. Since the two samples were unsuccessful, we proceeded only with partial 16S rRNA. The ITS sequences obtained from the infected oligochaete revealed a 99.7% similarity to the sequence of *Aulodrilus acutus* (KY637027), while the 16S rRNA sequences revealed 97.5% similarity to *Branchiodrilus semperi* (KY633379) and 83.9% similarity to *Bothrioneurum vejdovskyanum* (KY982552), respectively. It is confirmed that one of the infected worms belongs to *Aulodrilus acutus*, while due to the low similarities of the other two sequences to *Branchiodrilus semperi* and *Bothrioneurum vejdovskyanum*, identification of the oligochaetes was performed only at the genus-level. Each partial 16S rRNA sequence (500 bp) and ITS sequence (1074 bp) of the respective oligochaetes were deposited in GenBank with Accession numbers of PP856691, PP856692, PP855533.

Actinospores have been found to develop in the intestinal epithelium of the nine oligochaetes displaying myxozoan infection. Complete morphological and molecular studies were performed only on the raabeia type, and ones of triactinomyxon and aurantiactinomyxon were compared with morphological and morphometric data from the literature, as attempts to amplify 18S rDNA sequences were unsuccessful. Although the triactinomyxon and aurantiactinomyxon spore types described here were compared solely on morphological and morphometric measurements with known actinospore types, they differed substantially from them in all dimensions. Thus, novel raabeia, triactinomyxon and aurantiactinomyxon types are characterized here for the first time in Malaysia.

3.1. Description of novel actinospore types

3.1.1. Raabeia type nov (Fig. 2A–C)

Description: Spore possesses a spore body and three caudal processes without a style. Spore body elongated oval in side view, 29.7 ± 2.1 (25.4–30.2) μm long and 11.1 ± 0.6 (10.1–10.9) μm wide. Caudal processes equal in length, curved upwards, and tapering to a 'pencil point', 271.2 ± 8.6 (252.0–276.2) μm long and 9.7 ± 0.8 (8.0–10.9) μm wide at the base. Valve cell nuclei located at base of spore body, 3.7 ± 0.6 (2.2–3.8) μm in diameter. Three polar capsules protruding from the anterior end, equally sized and tear-shaped in side view, 5.3 ± 0.6 (4.3–6.4) μm long and 3.6 ± 0.2 (3.2–3.8) μm wide, each containing a

polar tubule exhibiting 4 coils. Polar filament length, 27.8 ± 3.6 (25.6–25.9) μm (n = 3). Number of secondary cells not determined. Measurements were obtained from 20 fresh actinospores.

Host: Aulodrilus acutus Ohtaka and Usman (1997).

Site of infection: The intestinal epithelium. Histological analyses have proved that the pansporocysts located in the intestinal epithelium of the infected oligochaetes (Fig. 3A and B). In the thin sections, matured spores can be observed in the pansporocyst (Fig. 3C).

Prevalence: 5.76% (3 infected in 52 oligochaetes examined).

Locality: Tasik Telabak, Hulu Besut, Terengganu.

Type material: Series of phototypes was deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-PAR-20894.

18S rDNA sequence: Two sequences from different individuals with lengths of 1768 bp and 1523 bp were deposited in GenBank under the accession numbers PP856689 and PP856690, respectively.

Remarks: Morphometric measurements of the raabeia type were compared with 35 published descriptions of raabeia (Table 2). Raabeia type of the present study was morphologically similar to raabeia type 1 (Oumouna et al., 2003) and raabeia type (Eszterbauer et al., 2006), but not morphometrically similar. The spore body length of the raabeia type closely resembled raabeia type 4 and raabeia type 6 (Özer et al., 2002), while the width showed the closest resemblance to raabeia type E (Xiao and Desser, 1998a) and raabeia of *Myxobolus lentisuturalis* (Caffara et al., 2009). The length of the caudal processes of the raabeia type differed from those described in the literature, while the width showed the closest resemblance to raabeia type E (Xiao and Desser, 1998a). The length of polar capsules was closest to raabeia type 1 (Oumouna et al., 2003), while their width was similar to raabeia type 2 (Hallett et al., 2004). Thus, the present raabeia type appears to be novel.

Two parallel sequences of 18S rDNA from the raabeia type did not match (≤ 89%) any of the myxozoan sequences available in the GenBank. Distance estimation revealed the highest percentage of similarity (92.4%, 92.1%) with Raabeia sp. (HQ613408, HQ613407), followed by the sequence of *Myxobolus* sp. (KU170935) (90%). The other sequences of Hungactinomyxon sp. (KY784589), Hungactinomyxon type 1 (AY779062) and six *Myxobolus* spp. (AB274267, OK012397, AF378343, OK274150, MF150547, KY784597) indicated a 88.2–89.8% similarity (Table 3). Phylogenetic analysis confirmed that the raabeia type identified in this study was positioned basally in a monophyletic clade together with *Myxobolus cultus*, raabeia of *Myxobolus cultus*, *Myxobolus lentisuturalis*, hungactinomyxon type, *Myxobolus* sp. and *Myxobolus branchiopectin*, with maximum bootstrap support (Fig. 4).

3.1.2. *Triactinomyxon type nov (Fig. 5A–D)*

Description: Spore possesses a spore body, style and three caudal processes. Spore body cylindrical and elongated, 27.9 ± 3.1 (21.7–33.0) μm long and 8.9 ± 0.7 (7.4–10.5) μm wide. Style short, 29.8 ± 3.9

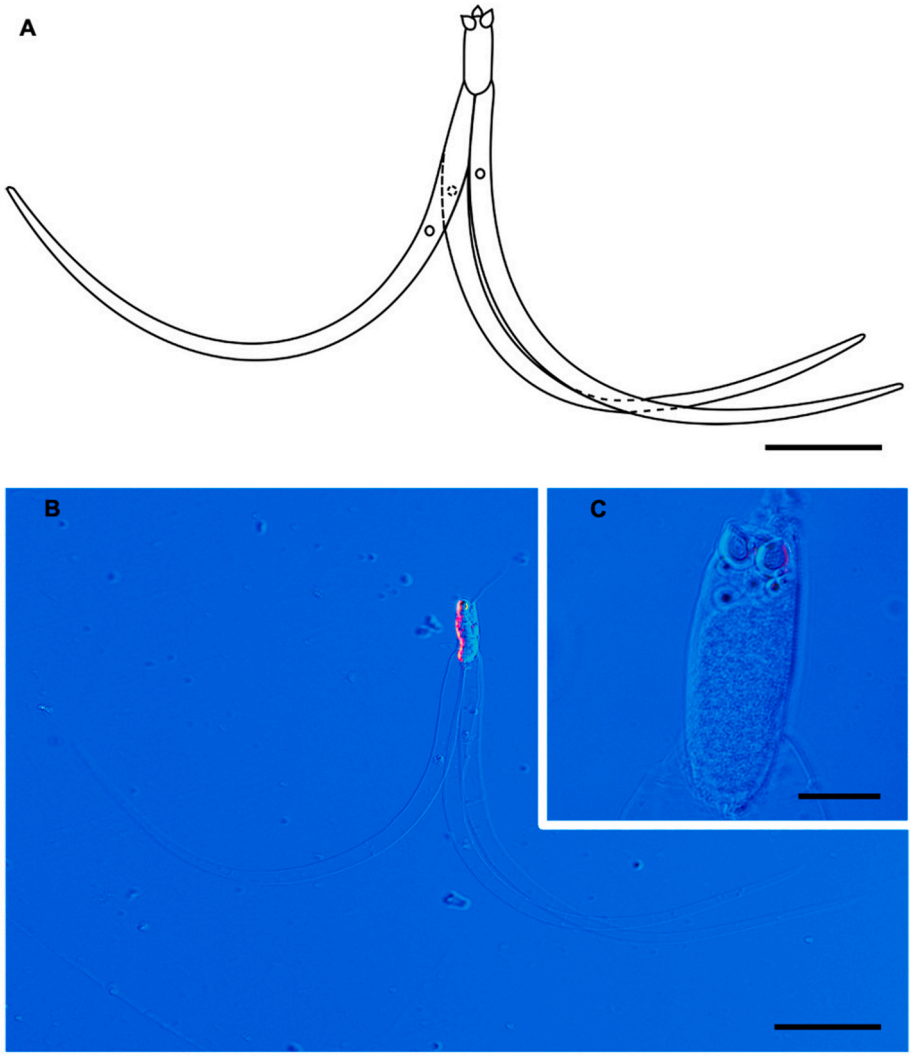

Fig. 2. Raabeia type nov. A) Schematic drawing of mature actinospore. B) Fresh mount of raabeia. C) Higher magnification of spore body showing two of three polar capsules with 4 coils of the polar tubule. Scale bars represent 50 μm except C) 10 μm.

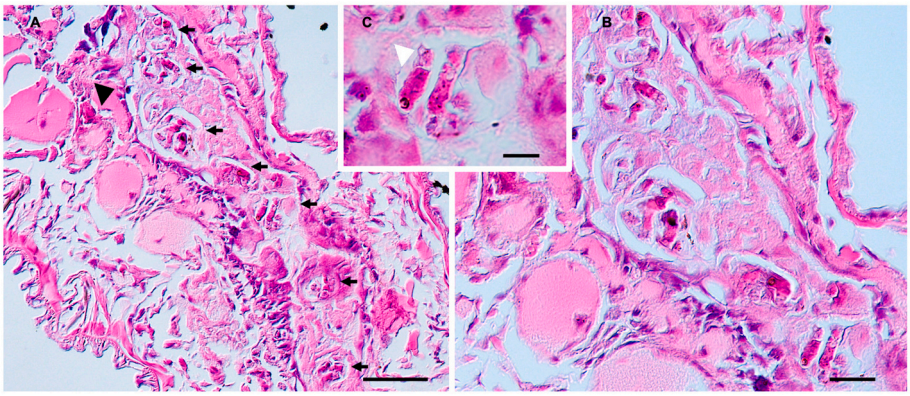

Fig. 3. (A, B) Semithin sections showing multiple pansporocysts (arrow) of the novel raabeia type in the intestinal epithelium (black arrowhead) of the freshwater oligochaete, *Aulodrilus acutus* from Tasik Telabak. C) Enlarged section of the pansporocyst with visible polar capsules (white arrowhead) of mature spores. Stained with H&E. Scale bars represent A) 50 μm; B) 20 μm and C) 10 μm.

Table 2
Comparison of morphometric measurements of Raabeia type spore from the present study and previous literature data. All measurements are in μm and '–' indicates no data. SBL: spore body length, SBW: spore body width, CPL: caudal processes length, CPW: caudal processes width, PCL: polar capsule length, PCW: polar capsule width, SCn: number of secondary cells.

Raabiea type/species	Host	SBL	SBW	CPL	CPW	PCL	PCW	SCn	Reference
Raabeia type	***Aulodrilus acutus***	**29.7**	**11.1**	**271.2**	**9.7**	**5.3**	**3.6**	**–**	**Present study**
Raabeia gorlicensis	*Tubifex tubifex*	35	–	170	–	4	4	32	Janiszewska (1955), 1957
Raabeia magna	*Limnodrilus hoffmeisteri*	51–58	–	–	–	6–7	–	128	Janiszewska (1957)
Raabiea furciligera	*L. hoffmeisteri*	32.8	10.2	125	–	4.4	–	24	Janiszewska and Krztón (1973)
Raabeia noxubeensis	*Amphichaeta* sp.	27.5	11.8	53.9	9.1	–	–	12	Bellerud (1993)
Raabeia type	Unidentified	18.2	12.8	219.3	6.7	7	5	–	McGeorge et al. (1997)
Raabeia type A	*L. hoffmeisteri*	16.0	10.0	145.0	8.0–9.0	4.0	2.0	8	Xiao and Desser (1998a)
Raabeia type B	*L. hoffmeisteri*	25.5	9.0	230.0	14.0	5.5	2.7	16	Xiao and Desser (1998a); Kent et al. (2001)
Raabeia type C	*L. hoffmeisteri*	16.5	9.0	210	10.0–12.0	4.5	2.3	8	Xiao and Desser (1998a)
Raabeia type D	*T. tubifex*	21.5	9.5	290	11.0–12.0	4.5	3.0	16	Xiao and Desser (1998a)
Raabeia type E	*T. tubifex*	24.0	11.0	215	9.0–11.0	4.5	2.6	12	Xiao and Desser (1998a)
Raabeia type F	*L. hoffmeisteri*	16.5	9.0	145	6.0–7.0	4.5	2.5	16	Xiao and Desser (1998a)
Raabeia type 1	*L. hoffmeisteri*	14.1	12.4	202.8	8.2	5.9	4.7	–	El-Mansy et al. (1998a)
Raabeia type 2	*Tubifex* sp.	21.7	7.7	209.4	6.6	5.7	4.0	–	El-Mansy et al. (1998a)
Raabeia type 1	*Branchiura* sp. and *Tubifex* sp.	25.9	11.8	294	9	5.9	3.5	–	El-Mansy et al. (1998b)
Raabeia type 2	*Branchiura sowerbyi*	14.1	12.4	202.8	8.2	5.9	4.7	–	El-Mansy et al. (1998b)
Raabeia type 3	*T. tubifex*	28.2	14.1	183.6	10.6	7.5	5.9	–	El-Mansy et al. (1998b)
Raabeia type 4	Unidentified	21.7	7.7	209.4	6.6	5.7	4.0	–	El-Mansy et al. (1998b)
Raabeia of *Myxobolus dispar*	*T. tubifex*	37.0	–	121.0	10.8	7.5	4.0	32	Molnár et al. (1999)
Raabiea type	Unidentified	15–22	–	160–180	–	–	–	–	Békési et al. (2002)
Raabeia type 1	Unidentified	18.1	15.7	94.5	–	6.0	4.0	–	Özer et al. (2002)
Raabeia type 2	*Lumbriculus variegatus*	18.1	16.1	85.6	–	7.0	6.0	–	Özer et al. (2002)
Raabeia type 3	*T. tubifex*	33.9	12.8	228.3	–	6.5	4.4	16	Özer et al. (2002)
Raabeia type 4	*T. tubifex*	29.6	16.5	142.7	–	8.0	5.0	32	Özer et al. (2002)
Raabeia type 5	*L. variegatus*	23.7	20.2	133.3	–	6.0	5.0	–	Özer et al. (2002)
Raabeia type 6	*T. tubifex*	29.8	17.4	164.8	–	7.8	4.6	–	Özer et al. (2002)
Raabeia type 1	*Tubifex* sp.	35	12	245	–	5.0	3.0	20–28	Oumouna et al. (2003)
Raabeia type 2	Unidentified	18.0	15.0	80.0	–	4.0	3.0	–	Oumouna et al. (2003)
Raabeia type 1	Unidentified	27.2	16.8	213.2	11.2	3.9	3.2	12	Hallett et al. (2004)
Raabeia type 2	Unidentified	22.0	14.2	120.7	7.7	4.2	3.6	8	Hallett et al. (2004)
Raabeia of *Myxobolus cultus*	*Branchiura sowerbyi*	23	10	191	7	4	2.5	16	Eszterbauer et al. (2006)
Raabeia of *Myxobolus lentisuturalis*	*Branchiura sowerbyi*	22.1	10.8	196	–	4.7	2.9	–	Caffara et al. (2009)
Raabeia type 1	*Isochaetides michaelseni*	20	9	126	5	6	3	–	Borkhanuddin et al. (2014a)
Raabeia type 2	*I. michaelseni*	63	5	185	7	5	3	16	Borkhanuddin et al. (2014a)
Raabeia type	*Dero digitata*	28.2	6.44	150.65	7.3	–	–	–	Rosser et al. (2014)
Raabeia type	*Ilyodrilus templetoni*	23.9	12.4	108.0	6.2	5.1	3.6	12	Rocha et al. (2019a)

(22.4–38.6) μm long and 9.5 ± 1.1 (8.0–12.1) μm wide. Total length of spore, 57.6 ± 4.1 (51.2–67.8) μm. Caudal processes short, with blunt tips and equal in length, 29.9 ± 3.2 (24.6–35.1) μm long and 9.7 ± 0.9 (8.2–11.0) μm wide at the base. Largest span of caudal processes, 59.3 ± 3.6 (52.3–66.7) μm. Valve cell nuclei located at the base of caudal processes. Three pyriform polar capsules, equal in size and protruding from the anterior end, 3.4 ± 0.4 (2.8–4.0) μm long and 2.2 ± 0.2 (1.7–2.6) μm wide. Polar filament turns not visible well. Sporoplasm containing 8 secondary cells (Fig. 5D). Measurements were obtained from 25 ethanol-fixed actinospores.

Host: Species of the genus *Branchiodrilus* Michaelsen, 1900.

Site of infection: The intestinal epithelium. The studied worm showed a massive infection, with pansporocysts at various stages of development observed in the intestinal epithelium (Fig. 5E and F).

Table 3
Similarity matrix for 18S rDNA of raabeia type and closely related myxozoan species. The lower triangle region shows genetic *p*-distance, and the upper triangle region shows the percentage (%) of sequence similarities.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Raabeia type PP856689 (present study)		100.0	92.4	92.1	90	89.8	89.6	89.6	89.3	88.3	88.2	82.9	80.7
2	Raabeia type PP856690 (present study)	0.000		92.5	92.2	89.7	90.4	89.1	90.4	89.8	88.8	87.7	82.7	80.2
3	Raabeia sp. HQ613408	0.076	0.075		99.7	98.8	97.6	97.5	99.4	96.8	91.4	92.5	85.2	84.7
4	Raabeia sp. HQ613407	0.079	0.078	0.003		98.4	97.2	97.3	99.1	96.9	91.2	92.5	85.2	84.7
5	*Myxobolus* sp. KU170935	0.100	0.103	0.012	0.016		94.6	94.3	97.9	96.2	89.2	89.3	83.7	80.9
6	Hungactinomyxon sp. KY784589	0.102	0.096	0.024	0.028	0.054		99.4	95.4	95.3	88.9	89.3	83.5	81.4
7	Hungactinomyxon type 1 AY779062	0.104	0.109	0.025	0.027	0.057	0.006		94.2	94.6	88.4	88.9	83.5	81.3
8	*Myxobolus cultus* KY784597	0.104	0.096	0.006	0.009	0.021	0.046	0.058		95.9	89.4	89.4	83.1	81.1
9	*Myxobolus lentisuturalis* MF150547	0.107	0.102	0.032	0.031	0.038	0.047	0.054	0.041		88.9	88.6	83.6	81.7
10	*Myxobolus branchiopectin* OK274150	0.117	0.112	0.086	0.088	0.108	0.111	0.116	0.106	0.111		90.8	82.2	81.6
11	*Myxobolus* sp. AF378343	0.118	0.123	0.074	0.075	0.107	0.107	0.111	0.106	0.114	0.092		81.4	81.2
12	*Myxobolus* sp. OK012397	0.171	0.173	0.148	0.148	0.167	0.163	0.165	0.169	0.164	0.178	0.186		84.1
13	*Myxobolus nagaraensis* AB274267	0.193	0.198	0.153	0.153	0.191	0.186	0.187	0.189	0.183	0.184	0.188	0.159	

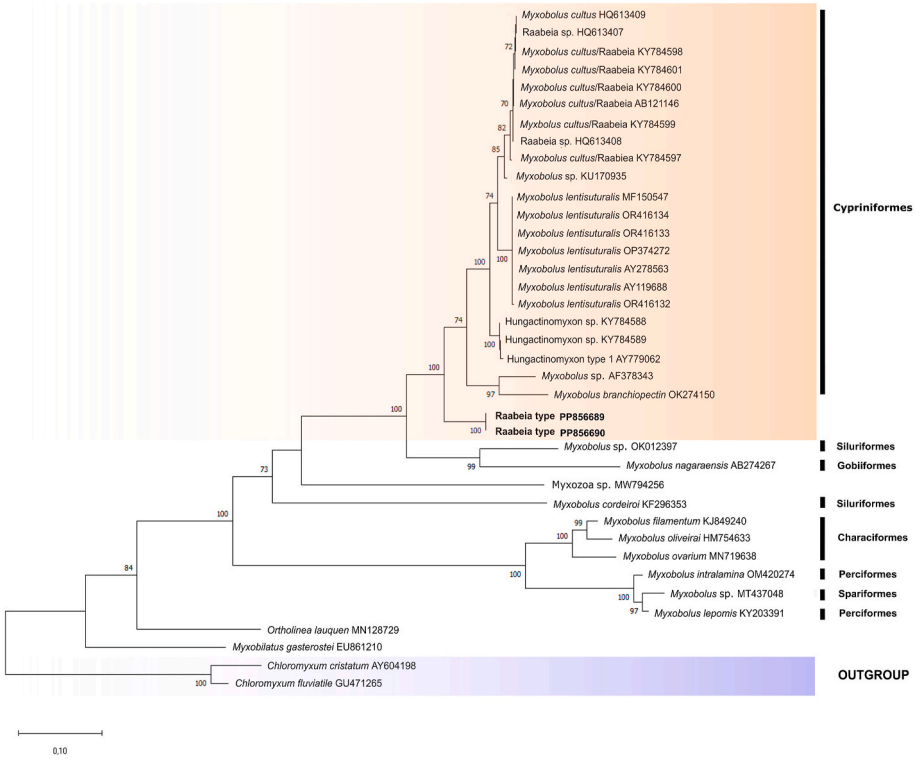

Fig. 4. Maximum likelihood phylogenetic tree based on small subunit 18S ribosomal DNA sequences of raabeia type and related species. The tree is rooted to the *Chloromyxum cristatum* and *Chloromyxum fluviatile* as the outgroup, with bootstrap support ≥70% indicated at nodes. GenBank accession numbers are given in parentheses followed by the name of the parasite species. Actinosporean examined in the present study are in bold. The scale bar indicates the number of expected substitutions per site.

Prevalence: 0.59% (4 infected in 682 oligochaetes examined).

Locality: Tasik Telabak, Hulu Besut, Terengganu.

Type material: Series of phototypes was deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-PAR-20895.

Remarks: Morphometric measurements of the triactinomyxon type were compared with previously published 19 descriptions of triactinomyxon possessing 8 secondary cells (Table 4). The morphology and morphometrics of the present triactinomyxon type are inconsistent with any previously described triactinomyxon types. The spores exhibit short caudal processes and a style length that may be indicative of specific differences from the previously described triactinomyxon types. Thus, the present triactinomyxon type appears to be novel. Throughout this study, several attempts at molecular analyses were conducted; however, we were unable to yield specific band sizes although using several primer combinations. First, primers the same as those used for the raabeia type were tested but did not work with this sample. Subsequently, semi-nested and nested PCRs were performed with different primer combinations, but these attempts were also unsuccessful. This is probably due to errors in sample fixation that hindered the extraction of high-quality genomic DNA.

3.1.3. *Aurantiactinomyxon type nov (Fig. 6A–E)*

Descriptions: Spore body subspherical, 8.8 ± 0.7 (7.6–10.1) μm in diameter. Caudal processes equal in size, elongated, tapering to pointed ends in apical view, and extending in a downward curve from spore body in side view, 36.7 ± 2.7 (30.7–43.8) μm long and 4.9 ± 0.6 (3.9–6.2) μm wide at the base of caudal processes. Valve cell nuclei not visible. Elongated pyriform polar capsules prominently protrude from the apex of spore body in both side view and apical view. Polar capsules

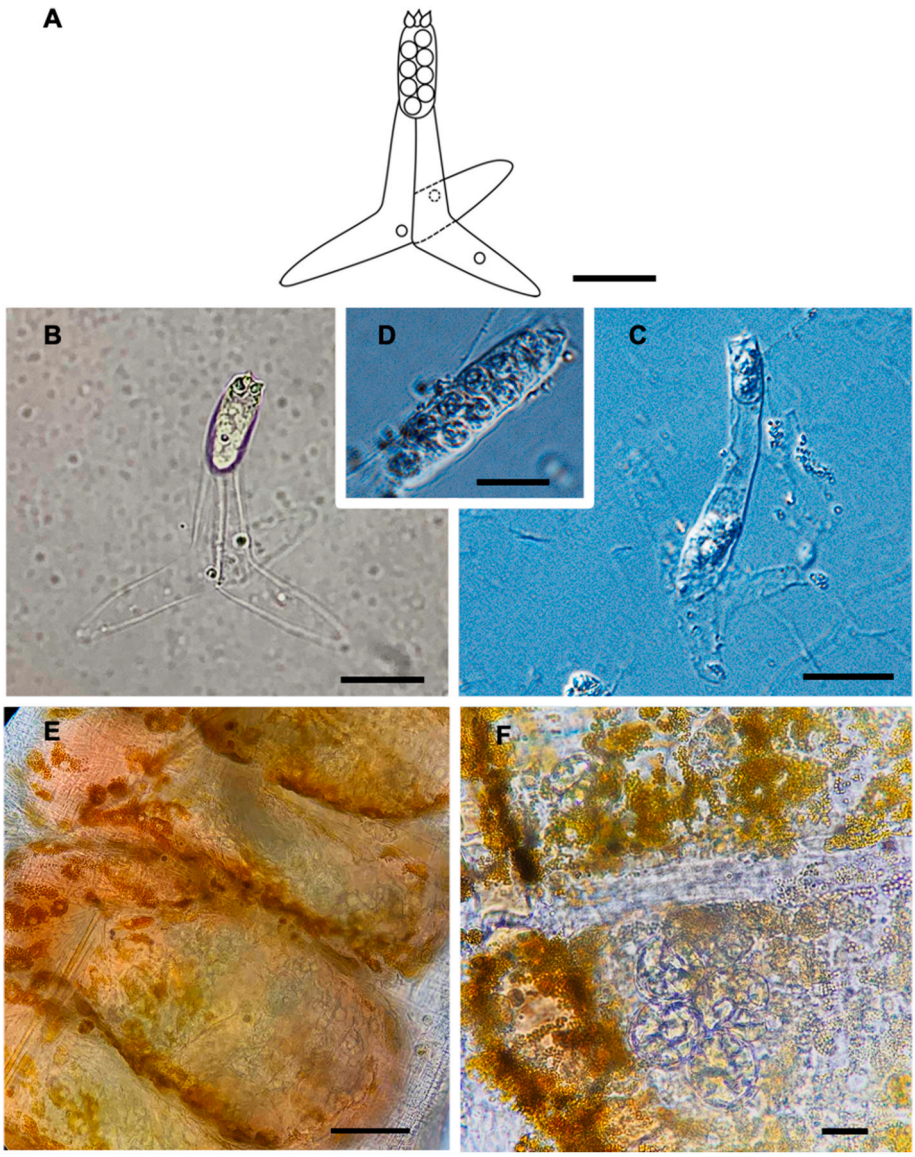

Fig. 5. Triactinomyxon type nov. A) Schematic drawing of mature actinospore. B) Freshly released triactinomyxon from *Branchiodrilus* sp. C) Triactinomyxon spore fixed in 90% ethanol. D) Higher magnification of spore body showing 8 secondary cells. E) Heavily infected *Branchiodrilus* sp. with pansporocysts at various stages of development in the intestinal epithelium. F) Higher magnification showing pansporocysts of triactinomyxon type in the intestinal epithelium. Scale bars represent 20 μm, except D) 10 μm and E) 100 μm.

in side view, 3.6 ± 0.3 (3.0–4.3) μm long and 1.3 ± 0.2 (1.1–1.9) μm wide; polar capsules in apical view, 2.0 ± 0.3 (1.5–2.5) μm in diameter. Polar filament turns not visible. Number of secondary cells not determined. Measurements were obtained from 25 ethanol-fixed actinospores.

Host: Species of the genus *Bothrioneurum* Štolc, 1886.

Site of infection: The intestinal epithelium. The studied worm displayed a severe infection, with pansporocysts at different developmental stages observed in the intestinal epithelium (Fig. 6F). In some parts of the worm, six to seven out of eight actinospores can be seen in the pansporocysts (Fig. 6G).

Prevalence: 2.7% (2 infected in 75 oligochaetes examined).

Locality: Tasik Telabak, Hulu Besut, Terengganu.

Type material: Series of phototypes was deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-PAR-20896.

Remarks: Morphometric measurements of the aurantiactinomyxon type were compared with 64 previously published descriptions of aurantiactinomyxon from freshwater and marine water (Table 5). The morphology and morphometrics of the present aurantiactinomyxon type are inconsistent with any previously described aurantiactinomyxon types. The closest resemblance is found with aurantiactinomyxon type 11 (El-Mansy et al., 1998b) although the present aurantiactinomyxon possess a larger spore body and caudal processes, while type 11 has shorter polar capsules. The spore body of the present aurantiactinomyxon closely resembles that of aurantiactinomyxon type 1 (Milanin et al., 2018), and its polar capsule diameter is similar to aurantiactinomyxon type 1 described by Hallett et al. (1997), while other morphological features do not resemble to any known aurantiactinomyxon types. The spores exhibit prominently elongated pyriform polar capsules, which could be the distinguishing feature of this spore type compared to previously described aurantiactinomyxon types. Therefore, the present aurantiactinomyxon type appears to be novel. During this study, several attempts at molecular analyses were made using various primers to obtain specific band sizes but were unsuccessful. First, primers the same as those used for the raabeia type were tested but did not work with this sample. Subsequently, semi-nested and nested PCR were performed with different primer combinations, but

Table 4
Comparison of morphometric measurements of Triactinomyxon type with 8 secondary cells, from the present study with those from previous literature. All measurements are in μm and '–' indicates no data. SBL: spore body length, SBW: spore body width, SL: style length, SW style width, CPL: caudal processes length, CPW: caudal processes width, PCL: polar capsule length, PCW: polar capsule width, Lo: long, Sh: short.

Triactinomyxon type/species	Host	SBL	SBW	SL	SW	CPL	CPW	PCL	PCW	Reference
Triactinomyxon type	***Branchiodrilus hortensis***	**27.9**	**8.9**	**29.8**	**9.5**	**29.9**	**9.7**	**3.4**	**2.2**	**Present study**
Triactinomyxon ignotum	*Tubifex tubifex*	40	14	175	26	193	22	5	3	Štolc, 1899; Marques (1984)
Triactinomyxon ohridensis	*T. ohridensis*	20–30	–	120–140	–	–	–	–	–	Georgevitch (1940); Marques (1984)
Triactinomyxon petri	*Lumbriculus* sp.	–	–	–	–	–	–	–	–	Georgevitch (1940); Marques (1984)
Triactinomyxon type 4	*Limnodrilus hoffmeisteri*	45	12.9	149	23.5	281.7	20.8	8.0	5.9	El-Mansy et al., 1998a
Triactinomyxon type 3	*Nais elinguis, T. Tubifex, L. hoffmeisteri*	47.1	10.6	102	9.4	128	10.6	7.0	3.5	El-Mansy et al., 1998a,b
Triactinomyxon type C	*L. hoffmeisteri*	18	10–13	195	32	290	25	5	3	Xiao and Desser (1998a)
Triactinomyxon of *Myxobolus pseudodispar*	*T. Tubifex, L. hoffmeisteri*	50.4	15.8	157.3	15.8	Lo: 196.6 Sh: 127	Lo: 13.6 Sh:	5.1	3	Székely et al. (1999), 2001
Triactinomyxon type	*T. tubifex*	–	–	170	–	160	–	4	3	Oumouna et al. (2003)
Triactinomyxon type	*T. tubifex*	37.6	12.2	134.3	16.5	Lo: 200.6 Sh: 154.4	Lo: 15 Sh: 14.9	5.3	3	Rácz and Timm (2002)
Triactinomyxon type 2	Tubificid	–	–	162	–	188	–	4	6	Lowers and Bartholomew (2003)
Triactinomyxon type 3	Tubificid	–	–	192	–	270	–	5	6	Lowers and Bartholomew (2003)
Triactinomyxon type 4	Tubificid	–	–	103	–	221	–	5	5	Lowers and Bartholomew (2003)
Triactinomyxon type 5	Tubificid	–	–	94	11	123	–	2	3	Lowers and Bartholomew (2003)
Triactinomyxon type	*T. tubifex*	25.6	10	112	19.2	Lo: 193.2 Sh: 115.4	Lo: 11.4 Sh: 12.7	4.3	2.5	Hallett et al. (2004)
Triactinomyxon type A	*T. newaensis*	37	12	241	11	150	13	5.5	2.7	Eszterbauer et al. (2006)
Triactinomyxon type B	*T. tubifex*	31	9	205	8	140	14	5	2.5	Eszterbauer et al. (2006)
Triactinomyxon type C1	*T. tubifex*	44	9	113	9	169	10	4	3	Eszterbauer et al. (2006)
Triactinomyxon type C2	*T. tubifex*	–	–	–	7.7	173	13.7	–	–	Eszterbauer et al. (2006)
Triactinomyxon type	*Psammoryctides albicola*	30	13	130	17	120	–	3	2	Székely et al. (2007)

these attempts were also unsuccessful. This failure is probably due to errors in sample fixation that impeded the extraction of high-quality genomic DNA.

4. Discussion

During our study, we described three actinospore types from three collective groups namely raabeia, triactinomyxon, and aurantiactinomyxon from 1312 examined oligochaetes collected from Tasik Telabak. In the past, several attempts at actinospore surveys in Malaysia were conducted in various locations such as Tasik Kenyir, fish farms in Terengganu and Kelantan, but these efforts were unsuccessful in detecting actinospores (Székely C., personal communication). Nevertheless, about 26 myxospores were described found in fishes from those areas (Molnár et al., 2006a, 2006b; Székely et al., 2009a, 2009b, 2012; Borkhanuddin et al., 2014b). Therefore, this study is the first successful report describing actinospore stages of myxozoans in Malaysia.

The raabeia type described in this study showed minimal morphometric differences from other raabeia types in the literature, with only some overlapping features (Table 2). These spores were morphologically similar to raabeia type 1 (Oumouna et al., 2003) and raabeia type of Eszterbauer et al. (2006), but they possessed at least one different characteristic. A comparison of 18S rDNA sequences indicated that the raabeia type in our study did not resemble any myxozoan sequences in GenBank although more than 30 myxozoan species were identified in Malaysia. Notably, despite the presence of the same fish species in Malaysia and neighbouring countries like Vietnam and Thailand, the current sequences of actinospores also did not match any of the myxozoan species identified in those adjacent countries (Thumvittayakul et al., 2018; Chinh et al., 2023). Genetic distances among the raabeia type and other closely related species are no greater than 92% (Table 3), indicating that the raabeia type in this study is genetically different from them. This distinction may be attributed to the geographical isolation among Malaysia, China, Hungary, the USA, and Japan. The phylogenetic tree revealed that our raabeia type was grouped in a clade consisting of *Myxobolus* sp. from fish belonging to Order Cypriniformes (Fig. 4). This analysis suggested that their corresponding myxospore stages may develop in Cypriniformes and close affinities to the genus *Myxobolus*. Future work should aimed at collecting more cyprinids to elucidate the life cycle by finding the potential myxospore stage of raabeia type.

According to the presently available data, the second most diverse actinospore collective group after aurantiactinomyxon is triactinomyxon, with 59 types described types in the literature (Borkhanuddin et al., 2014a; Székely et al., 2014; Xi et al., 2015; Rangel et al., 2015, 2016). This actinospore type is the most common type representing the actinospore stage from the genus *Myxobolus*. The triactinomyxon identified in this study differed from all known triactinomyxon types possessing 8 secondary cells in terms of their spore shape and dimensions. Caudal processes, spore body, and style are essential morphological traits for the identification of triactinomyxons (Xiao and Desser, 1998a; Özer et al., 2002; Hallett et al., 2004, 2005; Xi et al., 2017). The weirdest features distinguishing our spores from known triactinomyxon types are the style and caudal process which in the present spores are much shorter compared to the others. The ratio of caudal processes to spore body reaches 1.07 (29.9/27.9), whereas for most triactinomyxon types the ratio ranges between 2.72 and 16 (Table 4). Moreover, our spores lack the typical anchor-shaped caudal process characteristics of triactinomyxons, further supporting their

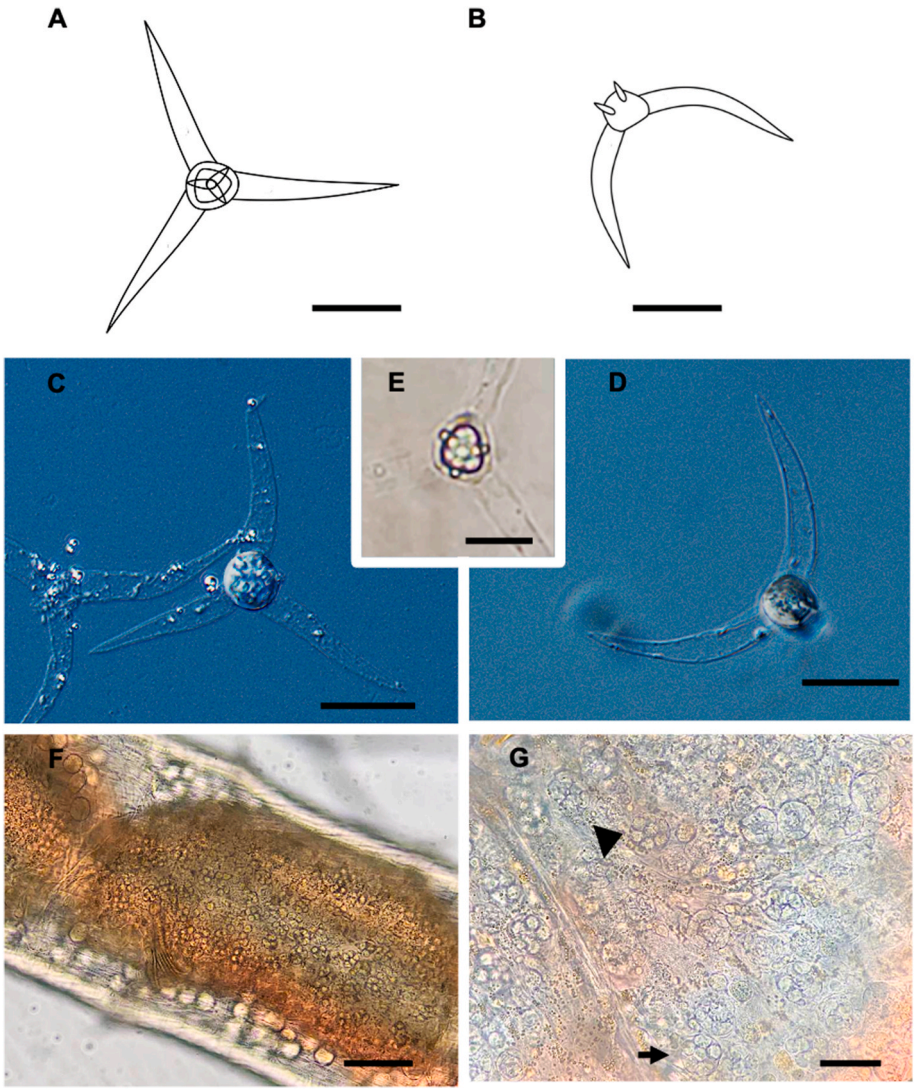

Fig. 6. Aurantiactinomyxon type nov. A) Schematic drawing of apical view. B) The side view of mature actinospores. C) Fixed (90% ethanol) aurantiactinomyxon spore in the apical view. D) Fixed (90% ethanol) aurantiactinomyxon spore in the side view. E) Higher magnification of fresh spore body showing three elongated polar capsules. F) Heavily infected *Bothrioneurum* sp. with pansporocysts at various stages of development in the intestinal epithelium. G) Pansporocyst showing six to seven of eight actinospores (arrowhead). In some spores, polar capsules can be seen (arrow). Scale bars represent 20 μm except E) 10 μm and F) 100 μm.

classification as a novel type.

The aurantiactinomyxon type of the present study also does not show morphological and morphometric resemblance to any previously described aurantiactinomyxon types (Table 5). The most similar is with aurantiactinomyxon type 11 (El-Mansy et al., 1998b) but noticeable differences can be observed in caudal processes length, width and polar capsule diameter. However, the present Aurantiactinomyxon exhibited a unique feature, having prominent, elongated pyriform polar capsules protruding from the anterior of the spore body, distinguishing it from any known aurantiactinomyxon types (Fig. 6A–E). Therefore, based on these distinctive morphological and morphometric characteristics, we believe that the aurantiactinomyxon type described herein represents a novel type.

Traditionally, spore morphology was used as the sole criterion for myxozoan identification before the advent of molecular techniques. Later, many researchers suggested that morphological descriptions of actinospores be completed with 18S rDNA gene sequence data, particularly in cases with overlapping or similar morphological features. For instance, studies by Hallett et al. (2002), Eszterbauer et al. (2006), Zhao et al. (2016) and Rocha et al. (2019b) have reported instances where genetically identical aurantiactinomyxon type, neoactinomyxum type, and sphaeractinomyxon type actinospores exhibited phenotypic variations in spore shape. In this study, due to preservation error and the low probability of resampling, we were unable to obtain 18S rDNA sequences for our triactinomyxon type and aurantiatinomyxon type. Therefore, we relied solely on morphological and morphometric data for comparison. Despite this limitation, we are confident in classifying them as novel types based on their unique features, host species and geographical location. However, we recommend the collection of further samples from the same sampling site for molecular analysis to confirm their status as novel types and to identify their myxosporean stage and fish host.

Most raabeia, triactinomyxon and aurantiactinomyxon actinospore types have been reported to infect oligochaetes from the families Naididae (Rosser et al., 2014; Rocha et al., 2019a; Rocha, 2023). To date, no other myxosporean infections have been reported from populations of *Aulodrilus acutus*, *Branchiodrilus* sp., and *Bothrioneurum* sp. worldwide, suggesting a new record of alternate annelid hosts within the family Naididae in freshwater environments. This new host record broaden the range of annelid species known to host myxozoans. Moreover, *A. acutus*, *Branchiodrilus* sp., and *Bothrioneurum* sp. are widely distributed in East and/or Southeast Asia (Ohtaka, 2018; Brinkhurst and Jamieson 1971),

Table 5
Comparison of morphometric measurements of Aurantiactinomyxon type spore in the present study with 64 previous published ones in the literatures. All measurements are in μm and '–' indicates no data. SBD: spore body diameter, CPL: caudal processes length, CPW: caudal processes width, PCL: polar capsule length, PCW: polar capsule width, SCn: number of secondary cells, D: diameter.

Aurantiactinomyxon type/species	Host	SBD	CPL	CPW	PCL	PCW	SC	Reference
Aurantiactinomyxon type	***Bothrioneurum vejdovskyanum***	**8.8**	**36.7**	**4.9**	**3.6 D: 1.9**	**1.3**	–	**Present study**
Aurantiactinomyxon raabei junioris	*Limnodrilus hoffmeisteri, Tubifex* sp.	17	25–30	–	–		16	Janiszewska (1957); Marques (1984)
Aurantiactinomyxon pavinsis of *Chloromyxum truttae*	*Stylodrylus heringianus, Tubifex* sp.	8–12	10–20	6–8	3.0	2.0	12–16	Ormières (1968); Marques (1984); Oumouna et al. (2003); Holzer et al. (2004); Marcucci et al., 2009
Aurantiactinomyxon stellans	Unidentified	15–20	70–90	15–20	D: 8–10	–	<16	Marques (1984)
Aurantiactinomyxon trifolium	Unidentified	20–25	40–50	17–20	–	–	32	Marques (1984)
Aurantiactinomyxon minor	*Dero digitata, L. hoffmeisteri*	13–16	26–36	9.1–13.0	D: 2.7	–	~12	Styer et al. (1992); Negredo and Mulcahy (2001)
Aurantiactinomyxon of *Hoferellus carassii*	*T. tubifex, Lophochaeta ignota, L. hoffmeisteri, Nais elinguis*	23.5	48.8	11.7	–	–	22	El-Matbouli et al. (1992); Trouillier et al. (1996)
Aurantiactinomyxon of *Hoferellus cyprini*	*Nais* spp.	~12.7	~31.1	~6.9	–	–	–	Grossheider and Körting, 1992
Aurantiactinomyxon mississippiensis of *Henneguya mississippiensis*	*Dero digitata*	L: 14.2 W: 13.6	32.4	7.3	–	–	30	Bellerud (1993); Hanson et al. (2001)
Aurantiactinomyxon type	*Dero digitata*	~23.0	40	8	–	–	–	Pote and Waterstrat (1993)
Aurantiactinomyxon type 1	*Branchiura sowerbyi*	11	16	–	–	–	8	Yokoyama et al. (1993); Yokoyama (1997)
Aurantiactinomyxon of *Thelohanellus hovorkai*	*B. sowerbyi*	18–22	25–33	–	D: 2	–	32	Yokoyama et al. (1993); Yokoyama (1997); Anderson et al. (2000)
		18.6	29.0	9.2	3.42	3.36	32	Székely et al. (1998)
Aurantiactinomyxon type 1	*Pacifidrilus vanus*	L: 10.1 W: 10.7	~3.0	~3.0	D: 1.9	–	–	Hallett et al. (1997)
Aurantiactinomyxon type 2	*Pacifidrilus darvelli, Limnodriloides toloensis*	L: 9.4–12.5 W: 11.6–14.0	–	–	D: 2.5–3.0	–	–	Hallett et al. (1997)
Aurantiactinomyxon type 3	*Pacifidrilus vanus*	L: 9.4–10.6 W: 6.9–10.9	–	–	–	–	–	Hallett et al. (1997)
Aurantiactinomyxon type	Unidentified, *Lumbriculus variegatus*	13.7	25.6	12.0	D: 2.7	–	–	McGeorge et al. (1997); Özer and Wootten (2001)
Aurantiactinomyxon type 1	*T. tubifex*	18.3	17.5	9.9	D: 2.0	–	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 2	*B. sowerbyi*	22.8	65.7	10.5	4.0	1.7	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 3	*B. sowerbyi*	22.8	70.3	8.0	D: 2.9	–	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 4	*B. sowerbyi*	19.4	55.7	11.2	D: 2.9	–	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 3 and Aurantiactinomyxon type 5	*B. sowerbyi*	9.9	17.2	3.9	D: 1.4	–	–	El-Mansy et al. (1998a), 1998b
Aurantiactinomyxon type 6	*Limnodrilus* sp.	19.7	24.2	11.2	D: 2.8	–	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 7	Actinospores collected from water	18.9	24.4	9.5	2.8	2.5	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 8	*Limnodrilus* sp.	22.6	12.2	9.0	1.4	–	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 1 and Aurantiactinomyxon type 9	*B. sowerbyi*	18.8	51.3	9.5	2.3	–	–	El-Mansy et al. (1998a), 1998b
Aurantiactinomyxon type 10	*B. sowerbyi*	15.5	16.7	8.8	1.7	–	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 11	Actinospores collected from water	8.5	31.9	3.7	3.4	2.0	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 12	*B. sowerbyi*	12.1	26.5	8.7	2.8	3.1	–	El-Mansy et al. (1998b)
Aurantiactinomyxon type 2	*Limnodrilus* sp.	21.1	22.6	11.7	2.8	2.0	–	El-Mansy et al. (1998a)
Aurantiactinomyxon of *Thelohanellus nikolskii*	*T. tubifex*	21.1	13.4	9.0	D: 2.1	–	16	Székely et al. (1998)
	Nais sp.	10.3	14.6	6.5	3.3	2.6	8	Borkhanuddin (2013); Borzák et al. (2021)
Aurantiactinomyxon type	*L. hoffmeisteri*	L: 12.0 W: 11.0	24.0	13.0–16.0	3.0	1.5	64–128	Xiao and Desser (1998b)
Aurantiactinomyxon type	*B. sowerbyi*	8.1	6.1	5.6	1.6	1.1	64	Székely et al. (2000)
Aurantiactinomyxon type	*L. hoffmeisteri*	–	–	–	–	–	–	Kent et al. (2001)
Aurantiactinomyxon type A1	*L. ignota*	14.4	21.1	16.1	D: 3.0	–	10	Negredo and Mulcahy (2001); Negredo et al. (2003)
Aurantiactinomyxon type A3	*L. ignota*	~9.1	20.8	10.4	–	–	~10	Negredo and Mulcahy (2001)
Aurantiactinomyxon type	*T. tubifex*	19.4	37.3	15.7	D: 3.1	–	30	Hallett et al. (2002)
		19.7	87.7	13.1				
Aurantiactinomyxon type 1	*T. tubifex*	14.4	32.0	14.8	D: 2.7	–	64–128	Özer et al. (2002)
		14.2	33.0		D: 2.5			Holzer et al. (2004)
Aurantiactinomyxon type 2	*T. tubifex*	14.9	24.8	15.3	D: 2.5	–	64	Özer et al. (2002)
Aurantiactinomyxon type 3	*T. tubifex*	L: 24.0	114.5	–	4.0	3.2	32	Özer et al. (2002)
		W: 21.8 L: 21.1	114.0	–	4.0	3.5	–	Holzer et al. (2004)

(*continued on next page*)

Table 5 (*continued*)

Aurantiactinomyxon type/species	Host	SBD	CPL	CPW	PCL	PCW	SC	Reference
		W: 19.3						
Aurantiactinomyxon type 4	*T. tubifex*	11.9	28.3	11.9	D: 2.5	–	32	Özer et al. (2002)
Aurantiactinomyxon type 1	Unidentified	16.1	76.0	–	5.0	4.0	–	Oumouna et al. (2003)
Aurantiactinomyxon type 1	*T. tubifex*	13.5	12.4	13.5	2.0	1.0	8	Székely et al. (2003)
Aurantiactinomyxon type	*B. sowerbyi*	19.6	10.5	15.2	D: 2.7	–	64	Székely et al. (2004)
Aurantiactinomyxon type A	*B. sowerbyi*	20	47	10	D: 3.0	–	32	Eszterbauer et al. (2006)
Aurantiactinomyxon type B	*B. sowerbyi*	18.0	24.0	9.8	2.5	2.6	–	Eszterbauer et al. (2006)
		19.0	16.0	8.4				
Aurantiactinomyxon of *Myxobolus intimus*	*L. hoffmeisteri*	13.8	20.1	10.4	D: 3.1	–	16	Hallett et al. (2006)
Aurantiactinomyxon type 1	Unidentified	12	26.6	10.1	D: 2.3	–	16	Hallett et al. (2006)
Aurantiactinomyxon type	*T. tubifex*	11.0	23.0	11.0	–	–	~16	Morris and Freeman (2010)
Aurantiactinomyxon type	*B. sowerbyi*	19.7	170.8	12.9	3.1	1.7	64	Xi et al. (2013)
Aurantiactinomyxon of *Henneguya ictaluri*	*Dero digitata*	20.9	27.7	10.0	–	–	–	Rosser et al. (2014)
Aurantiactinomyxon of *Henneguya exilis*	*Dero digitata*	11.7	42.5	6.5	–	–	–	Rosser et al. (2014)
Aurantiactinomyxon type JD	*B. sowerbyi*	L: 15.6	21.7	14.0	D: 2.3	–	>30	Xi et al. (2015)
		W: 21.2						
Aurantiactinomyxon of *Thelohanellus kitauei*	*B. sowerbyi*	19.7	20.4	8.9	3.4	2.8	>28	Zhao et al. (2016)
		20.9	19.7	11.6	3.0	2.4	32	
Aurantiactinomyxon of *Thelohanellus testudineus*	*B. sowerbyi*	15.5	13.2	7.4	2.5	2.0	32	Zhao et al. (2017)
Aurantiactinomyxon type	*Pristina americana*	10.9	18.6	9.0	D: 2.1	–	–	Milanin et al. (2017)
Aurantiactinomyxon type	*L. variegatus*	10.4	15.4	8.5	–	–	–	Freeman and Kristmundsson (2018)
Aurantiactinomyxon type 1	*Pristina synclites*	8.7	14.6	6.9	D: 1.3	–	–	Milanin et al. (2018)
Aurantiactinomyxon type 2	*Pristina synclites*	11.2	30.4	7.0	D: 1.5	–	–	Milanin et al. (2018)
Aurantiactinomyxon of *Paramyxidium giardi*	*Tubifex* spp., *Tubificoides pseudogaster*	L: 14.4	22.4	15.5	D: 2.6	–	–	Rocha et al. (2019c)
		W: 12.7						
Aurantiactinomyxon type 1	*Ilyodrilus templetoni*	L: 13.5	30.5	9.3	3.5	2.5	–	Rocha et al. (2024)
		W: 13.0						
Aurantiactinomyxon type 2	*Ilyodrilus templetoni*	13.7	23.2	11.6	3.1	2.3	–	Rocha et al. (2024)
Aurantiactinomyxon type 3	*Ilyodrilus templetoni*	L: 16.7	41.5	10.2	3.7	3.0	–	Rocha et al. (2024)
		W: 14.3						

and are not common in Europe (Timm, 2009). However, *Branchiodrilus* sp. and *Bothrioneurum* sp. have recently been reported also from Europe (Šporka and Mláka, 2008; Odabaşı et al., 2017; Atanacković et al., 2021). Their invasion may disrupt native ecosystems by outcompeting with native species for resources and occupying the habitats previously colonized by the native residents. In this case, they possibly serve as recent hosts for myxozoans in the new environments. This study represents the first documentation of actinosporean infection in these host species. In the future, it is recommended to point to a detailed study of these host species as they seem susceptible to myxozoan infections.

The prevalence rate of infected worms in this study was low (0.7%), consistent with previous studies indicating a typically low percentage of infection in wild environments (Xiao and Desser, 1998a, 1998b; Rosser et al., 2014; Xi et al., 2013, 2015; Zhao et al., 2016; Milanin et al., 2017; Rocha et al., 2019a, 2019c, 2024). Conversely, El-Mansy et al. (1998a) and Székely et al. (2003) reported a high prevalence of actinospores found in wild oligochaete populations of Lake Balaton and Japan. Our results could be explained by limited sampling time where oligochaete collection was conducted once (over a 2-month period) rather than year-round and seasonally (McGeorge et al., 1997; Oumouna et al., 2003; Eszterbauer et al., 2006). In addition, some worms died after one week of separation in the cell-well plates without releasing actinospores during the observation period. Moreover, sampling of oligochaetes was performed in July to August, which coincides with the dry season in Malaysia. According to Patra (2023), a high abundance of oligochaetes is recorded in the rainy/monsoon season, as due to relatively high rainfall favours dense macrophyte growth due to increased food resources, shelter and suitable breeding areas. In addition, higher fish populations can occur in rivers during the rainy/monsoon season from November to March (Radhi et al., 2017) due to increased water flow and nutrient availability (Saifullah et al., 2014), which often coincides with the spawning season. Some fish species also use the increased water levels to migrate to breeding grounds. The higher presence of fish and oligochaetes during the monsoon season provides optimal conditions for actinospores proliferation as more potential hosts are available, leading to higher infection rates. Therefore, seasonal sampling during pre-monsoon (July–October), monsoon (November–March) and post-monsoon (March–June) periods is crucial to acquire more accurate data on the occurrence of actinospores in Malaysia.

In conclusion, one actinospore type was described through both morphological and molecular analyses, and two other actinospore types were described solely based on morphological features, all found from three oligochaete host species. Further studies involving molecular analyses of triactinomyxon and aurantiactinomyxon are needed to confirm their status as novel types. Additionally, comprehensive surveys of fish populations particularly belonging to Cypriniformes in this geographic location are required as they may serve as potential hosts for the raabeia type described here. Remarkably, this study represents the first documentation of actinospore stages of myxozoans in Malaysia.

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CRediT authorship contribution statement

Nadhirah Syafiqah Suhaimi: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Boglárka Sellyei:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Gábor Cech:** Writing – review & editing, Validation, Supervision, Software, Methodology, Data curation, Conceptualization. **Csaba Székely:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Muhammad Hafiz Borkhanuddin:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation,

Data curation, Conceptualization.

Declaration of competing interest

None.

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