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# *Monomyxum ligophori* n. sp. in a ParasiteBlitz: monopisthocotylans as myxozoan hosts in South Carolina and monophyly of a cosmopolitan hyperparasitic clade

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## Abstract

A ParasiteBlitz event offers a brief, intense opportunity to discover diverse parasite species and to reveal life cycles of heteroxenous parasite taxa. In this study, we describe *Monomyxum ligophori* n. sp., a hyperparasitic myxozoan (Monomyxidae) proliferating in two dactylogyrid monopisthocotylan flatworms (*Ligophorus saladensis, Ligophorus mugilinus*) infecting mugilid fishes (*Mugil cephalus, Mugil curema*) on the Atlantic coast of North America. Furthermore, we used DNA barcoding to infer the parasite's complex life cycle, matching its hyperparasitic myxospore stages with actinospore stages infecting the polychaete *Streblospio benedicti* found in the same locality during the ParasiteBlitz and also reported previously from the same region. Thus we report the first life cycle of a myxozoan that most likely does not require a vertebrate host. Hyperparasitic myxozoans are rare with only five species reported worldwide to infect flatworms. This study provides more information on the previously discussed host specificity towards monopisthocotylan hosts of these monomyxid myxozoan hyperparasites. Notably, *Monomyxum ligophori* n. sp. was detected in two out of four gill-infecting parasitic flatworms (being absent in *Ligophorus uruguayensis* and *Metamicrocotyla macracantha*) found infecting the same fish individuals during the ParasiteBlitz. Our molecular data and phylogenetic analysis support the previously suggested common origin of *Monomyxum* species infecting monopisthocotylan flatworms, and contribute to understanding the life cycle and host interactions of this unique hyperparasitic myxozoan lineage.

## Introduction

Hyperparasitism is a type of symbiotic, interspecific relationship involving parasites that act in a host role (Rohde 2002). This phenomenon has been reported only occasionally between two metazoan parasite taxa, with the first comprehensive review concerning mainly mycoparasites dating back to 1964 (Boosalis 1964). In helminths, two-way hyperparasitic relationships have been observed. For example, as a hyperparasite, the polyopisthocotylan flatworm *Cyclocotyla bellones* Otto, 1823, infects the parasitic fish-infecting copepod *Ceratothoa parallela* (Otto, 1828) (Bouguerche et al. 2021). In turn, helminths can be hosts for other parasite groups (Cho et al. 2020; Cort et al. 1960) including myxozoans (Dugarov et al. 2011; Freeman and Shinn 2011).

Myxozoans are a group of microscopic, obligate parasites consisting of over 3000 species, representing about 15% of cnidarian biodiversity (Whipps et al. 2025). They have complex life cycles, with only some 2% demonstrated or inferred from molecular evidence, and these show alternation in spore stages between vertebrate and invertebrate hosts. Myxozoans are most frequently reported as myxospores from their intermediate fish hosts and rarely in other vertebrates including amphibians, reptiles, waterfowl, and mammals (Bartholomew et al. 2008; Dyková et al. 2007; Eiras 2005; Friedrich et al. 2000). Relatively few records exist for myxosporean infections as actinospores in their definitive invertebrate hosts – primarily annelids – where infection prevalence is typically <2% (Rangel et al. 2016). To date, there are at least 14 marine actinospores, of which the myxospore phase remains unknown and the species is not formally described (Atkinson et al. 2019; Yokoyama et al. 2012).

Hyperparasitism involving myxozoan-helminth interactions has been observed haphazardly and rarely. To date, myxozoan hyperparasitism accounts for five myxozoan-helminth combinations including two species of *Fabespora* Naidenova and Zaika, 1969 infecting different digenean flatworm species (Overstreet 1976; Siau et al. 1981); *Myxidium giardi* (Cépède, 1906) infecting the monopisthocotylan *Pseudodactylogyrus bini* (Kikuchi, 1929), a parasite of European eel (*Anguilla anguilla* L.) (Aguilar et al. 2004); and two species of *Monomyxum* Freeman et Shinn, 2011 infecting different monopisthocotylans (Freeman et al. 2009; Freeman and Shinn, 2011).

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*Monomyxum incomptavermi* (Freeman and Shinn, 2011) infects *Diplectanocotyla gracilis* Yamaguti, 1953, a diplectanid parasite of Indo-Pacific tarpon (*Megalops cyprinoides* [Broussonet, 1782]) and an undescribed *Monomyxum* sp. infects *Haliotrema* sp., a dactylogyrid parasite of flathead (*Platycephalus* sp.) (as reviewed in Freeman and Shinn, 2011). Two families were erected to accommodate lineages of *Myxidium*-like morphotype, which form distinct clades basal to the highly speciose lineage *Kudoa* Meglitsch, 1947, including Monomyxidae with *Monomyxum* as the type genus (Freeman and Kristmundsson 2015).

On the occasion of a ParasiteBlitz (de Buron et al. 2025), taxonomists with specializations across multiple parasite groups provide a comprehensive overview of the parasite diversity in a target ecosystem. As both invertebrates and vertebrates are screened, the likelihood of elucidating complex parasite life cycles increases dramatically. Parasites have been suggested previously as tags for trophic relationships (Lafferty et al. 2006) and ecosystem function. Thus the ParasiteBlitz initiative not only increases the chances of elucidating life cycles in heteroxenous parasites but it indirectly provides insights into trophic relationships in ecosystems.

In the present study, we report the infection of a myxozoan as a hyperparasite of *Ligophorus saladensis* Marcotegui and Martorelli, 2009, and *Ligophorus mugilinus* (Hargis, 1955), monopisthocotylan flatworm intermediate hosts, together with proximal identification of its actinospore stage in a spionid annelid, *Streblospio benedicti* Webster, 1879. The life cycle connection is inferred from small subunit ribosomal DNA sequence identity and supported by physical proximity of both hosts in the tidal creek, thus most likely representing discovery of the first myxozoan life cycle that does not include a vertebrate host. Molecular data enabled us to test the hypothesis of a single evolutionary transition towards a hyperparasitic lifestyle of monopisthocotylan-infecting myxozoans.

## Material and methods

### Sample retrieval

Fish and annelids were collected as part of the ParasiteBlitz event at Stono Preserve, South Carolina, USA (see de Buron et al. 2025). Briefly, fish were collected in a tidal creek by net, and annelids were collected with their mud substrate from intertidal *Spartina* grass flats adjacent to the creek. Fish species of Mugilidae, *Mugil cephalus* Linnaeus, 1758 (n = 1 from impoundment and n = 4 from creek) and *Mugil curema* Valenciennes, 1836 (n = 8 from creek), were dissected and examined for external and internal parasites including squash preparation of different organ tissues. Annelids were sieved from the mud and examined whole in squash preparations. Monopisthocotylan individuals of *L. mugilinus* (n = 47), *L. saladensis* (n = 13), *Ligophorus uruguayensis* Siquier and Ostrowski de Núñez, 2009 (sensu WoRMS 2025) (n = 11) (Monopisthocotyla, Dactylogyridae), and *Metamicrocotyla macracantha* (Alexander, 1954) (n = 1) (Polyopisthocotyla, Microcotylidae) were bisected: with the anterior including the species-level diagnostic copulatory organs mounted in GAP (n = 62) on a slide for morphological evaluation, and the posterior part with attachment organs being stored in absolute ethanol for downstream molecular work (n = 19). Monopisthocotylan species identification was based on Sarabeev and Desdevises (2014) and Marchiori et al. (2015). Comprehensive survey results for major parasite groups will be presented in their respective ParasiteBlitz special collection papers. Monopisthocotylan specimens and annelids (n = 345) were checked for the presence of myxozoan infections using an Olympus BX51 microscope at 400–1000× magnification and digital images captured with an attached Canon DSLR of fresh material (where possible) otherwise from fixed specimens. Myxospores were described following Lom and Arthur (1989) with minor terminological variation noted, and saccimyxon actinospores following Atkinson et al. (2019). To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) (ICZN 2012), details of the species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:DC2B07B9-7F8C-4945-A28E-C818961A61FC

### Molecular characterisation

Molecular characterisation targeted the small subunit ribosomal rDNA allowing comparison with previously described myxozoans. DNA extraction from monopisthocotylans followed the protocol described in Kmentová et al. (2021) with a final elution volume of 30 μL. DNA was extracted from the annelid host with a QIAGEN DNeasy kit using the Animal Tissue protocol, using half volumes of the digestion buffers ATL and AL, and a final elution volume of 60 μL. The 18S rRNA gene was amplified using universal primers ERIB1 (forward; 5′−GTTCCGCAGGTTCACCTACGG−3′) and ERIB10 (reverse; 5′−CTTCCGCAGGTTCACCTACGG−3′) (Barta et al. 1997) then each paired with internal primers ACT1r (reverse; 5′−AATTTCACCTCTCGCTGCCA−3′) (Hallett and Diamant 2001), MYXGEN4F (forward; 5′−GTGCCTTGAATAAATCAGAG−3′) (Diamant et al. 2004) to generate overlapping fragments.

The PCR for the amplification of myxozoan DNA sequences used 2xMangoMix (12.5 μL), 0.5 μL of the forward and reverse primers (0.2 μM), and 9.5 μL ddH$_2$O with 2 μL of DNA extract, for a total of 25 μL per reaction. PCR cycling conditions: initial denaturation 2 min at 94°C, 30 cycles of 20 sec at 94°C, 30 sec at 53°C, and 1 min 30 sec at 72°C, final elongation of 7 min at 72°C, and cooling to 4°C. The amplified products were purified using the GeneJet Gel extraction kit and sequenced using the same primers as for the PCR reaction by Macrogen Europe. PCR for the myxozoan infection in the annelid sample was performed according to Atkinson et al. (2019), with PCR products sequenced at Oregon State University's Center for Quantitative Life Sciences.

### Phylogenetic reconstruction

We augmented the 18S rDNA alignment of myxozoan sequences from Freeman and Kristmundsson (2015) and included our novel sequence data on *Monomyxum* and the saccimyxon data (Atkinson et al. 2019) (see Table 1). Sequences were aligned using Muscle v5.1 under the Parallel Perturbed Probcons algorithm and with four threads (Edgar 2004) in Geneious v2025.0.2. Poorly aligned positions and divergent regions were removed with trimAl v.1.3 using the automated 1 option. The final alignment constituted of 1561 bp including gaps. The optimal substitution model was selected according to the Bayesian information criterion as implemented in ModelFinder in IQ-Tree (Kalyaanamoorthy et al. 2017). Tree topologies were estimated through Bayesian Inference (BI) and Maximum Likelihood (ML) methods using, respectively, MrBayes v3.2.6 (Ronquist et al. 2012) on the CIPRES Science Gateway online server (Miller et al. 2010) and IQ-Tree v1.6.12 (Nguyen et al. 2015). *Ceratonova shasta* (GQ358729) and *Myxidium gadi* (GQ890673) were selected as outgroups due to their documented close relationship with histozoic marine myxozoans (Fiala and Bartošová 2010). BI used two parallel runs and four chains of Metropolis-coupled

**Table 1.** GenBank accession numbers of 18S rDNA sequences used for the phylogenetic reconstruction in the present study

| Myxozoan species | GenBank accession number | Reference |
|---|---|---|
| *Ceratonova shasta* | AF001579 | Bartholomew et al. (1997) |
| *Enteromyxum leei* | AY520574 | Yanagida et al. (2004) |
| *Enteromyxum fugu* | AY520573 | Yanagida et al. (2004) |
| *Enteromyxum scophthalmi* | AF411335 | Palenzuela et al. (2002) |
| *Gastromyxum rafii* | KT002406 | Freeman and Kristmundsson (2015) |
| *Gastromyxum bulani* | KT002405 | Freeman and Kristmundsson (2015) |
| *Kudoa eugerres* | MH581487 | Casal et al. (2019) |
| *Kudoa cookii* | JX090294 | Heiniger et al. (2013) |
| *Kudoa carcharhini* | GU324970 | Gleeson et al. (2010) |
| *Kudoa hemiscylli* | GU324958 | Gleeson et al. (2010) |
| *Kudoa islandica* | KJ451388 | Kristmundsson and Freeman (2014) |
| *Kudoa neurophila* | AY172511 | Grossel et al. (2003) |
| *Kudoa trachuri* | AB693043 | Li et al. (2013) |
| *Monomyxum incomptavermi* | GQ368246 | Freeman and Kristmundsson (2015) |
| *Monomyxum* sp. | GQ368245 | Freeman and Kristmundsson (2015) |
| *Monomyxum ligophori* n. sp. myxospore stage | PX612002 | Current study |
| *Monomyxum ligophori* n. sp. actinospore stage | PX612003 | Current study |
| Saccimyxon type actinospore | MH791159 | Atkinson et al. (2019) |
| *Myxidium gadi* | GQ890673.1 | Mackenzie et al. (2010) |
| *Unicapsula andersenae* | AY302725 | Whipps et al. (2004) |
| *Unicapsula pflugfelderi* | AM931470 | Alama-Bermejo et al. (2009) |
| *Unicapsula seriolae* | AB971677 | Tomochi et al. (2014) |

Markov chain Monte Carlo iterations, run for 100 million generations with a burnin fraction of 0.25, sampling trees every 1000th generation. Convergence was assessed by the average standard deviation of split frequencies (<0.01 in all datasets) and the effective sample size (>200) using Tracer v1.7 for BI analyses (Rambaut et al. 2018). Branch support values for the ML analysis were estimated using ultrafast bootstrap approximation (Hoang et al. 2018) and Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT) (Guindon et al. 2010) with 10,000 replicates (as recommended in the IQ-Tree manual). The resulting tree topologies were visualised in FigTree v1.4.4 (Rambaut 2018).

## Results

### Infection parameters

Four species of parasitic flatworms were found on the gills of mugilid fishes: monopisthocotylan individuals of *Ligophorus mugilinus*, *L. saladensis*, *L. uruguayensis* (Monopisthocotyla, Dactylogyridae), and a polyopisthocotylan, *Metamicrocotyla macracantha* (Alexander, 1954) (Polyopisthocotyla, Microcotylidae). Morphological examination revealed the presence of myxozoan hyperparasitism in two monopisthocotylan species: mature myxospores were observed in three monogenean-host species combinations, being *L. mugilinus* ex *M. cephalus* with a prevalence of 2.8%, *L. saladensis* ex *M. cephalus* with a prevalence of 14.3%, and *L. saladensis* ex *M. curema* with a prevalence of 16.7%, respectively, all recorded from a single locality. At the infrapopulation level of the monopisthocotylan host (per infected fish host individual), the prevalence of *Monomyxum ligophori* n. sp. was 7.7% from *L. mugilinus* ex *M. cephalus*, 50.0% from *L. saladensis* ex *M. cephalus*, and 50.0% from *L. saladensis* ex *M. curema*. The prevalence of hyperparasitic infection was 12.5% ex *M. curema* and 40.0% ex *M. cephalus* across sampling sites. The overall prevalence of the myxozoan hyperparasite across different fish host species and sampling sites was 23.1%. The infection parameters of the monopisthocotylan hosts are summarized in Table 2. Additional visually negative specimens of *Ligophorus* spp., for which a DNA sample is available (n = 16), were *Monomyxum*-negative by PCR. All specimens of *L. uruguayensis* (n = 11) and *M. macracantha* (n = 1) were negative for myxozoan infection visually and by PCR (n = 5). No myxospores of *Monomyxum* were observed in any of the fish hosts or invertebrates by visual screening, in the broader ParasiteBlitz (de Buron et al. 2025). The myxozoan infection consisted of myxospores found throughout the body of all three infected specimens of *Ligophorus* spp. (Figure 1); while some paired spores were evident, no earlier developmental stages were observed. Measurements of myxospores of *Monomyxum ligophori* n. sp. from each infected monopisthocotylan individual and from *Monomyxum incomptavermi*, the only other species of *Monomyxum* for which measurements are available, are presented in Table 3.

We examined 345 annelids (128 *Streblospio benedicti*, 103 *Manayunkia aestuarina*, and 214 of undetermined species) and found only one overt myxozoan infection, which presented as saccimyxon actinospores and pansporocyst developmental stages in the tegument of *S. benedicti* (Figure 2). Measurements of fresh actinospores (in microns: average ± standard deviation, range, number measured): spore length 8.7 ± 0.5, 8.0–9.6, n = 10; spore width/thickness 5.8 ± 0.4, 5.1–6.7, n = 10; and polar capsules (nematocysts) spherical, diameter 2.1 ± 0.2, 1.8–2.4, n = 29. Morphology, morphometrics, annelid host, and DNA sequence data (see below) were consistent with the saccimyxon type of Atkinson et al. (2019).

**Table 2.** Infection parameters of the monopisthocotylan species across different fish host species including number of screened/infected host individuals, intensity of infection, and range from creek locality (a single specimen of *Mugil cephalus* screened from the impoundment was not infected by monopisthocotylans). More information on the sampled localities are available in de Buron et al. (2025)

| | *Ligophorus mugilinus* | *Ligophorus saladensis* | *Ligophorus uruguayensis* |
|---|---|---|---|
| *Mugil cephalus* | 4/4 (100%), 9 (1–16) | 3/4 (75.0%), 2.3 (0–4) | 2/4 (50.0%), 2.5 (0–4) |
| *Mugil curema* | 5/8 (62.5%), 2.2 (0–4) | 3/8 (37.5%), 2 (0–3) | 3/8 (37.5%), 2 (0–4) |

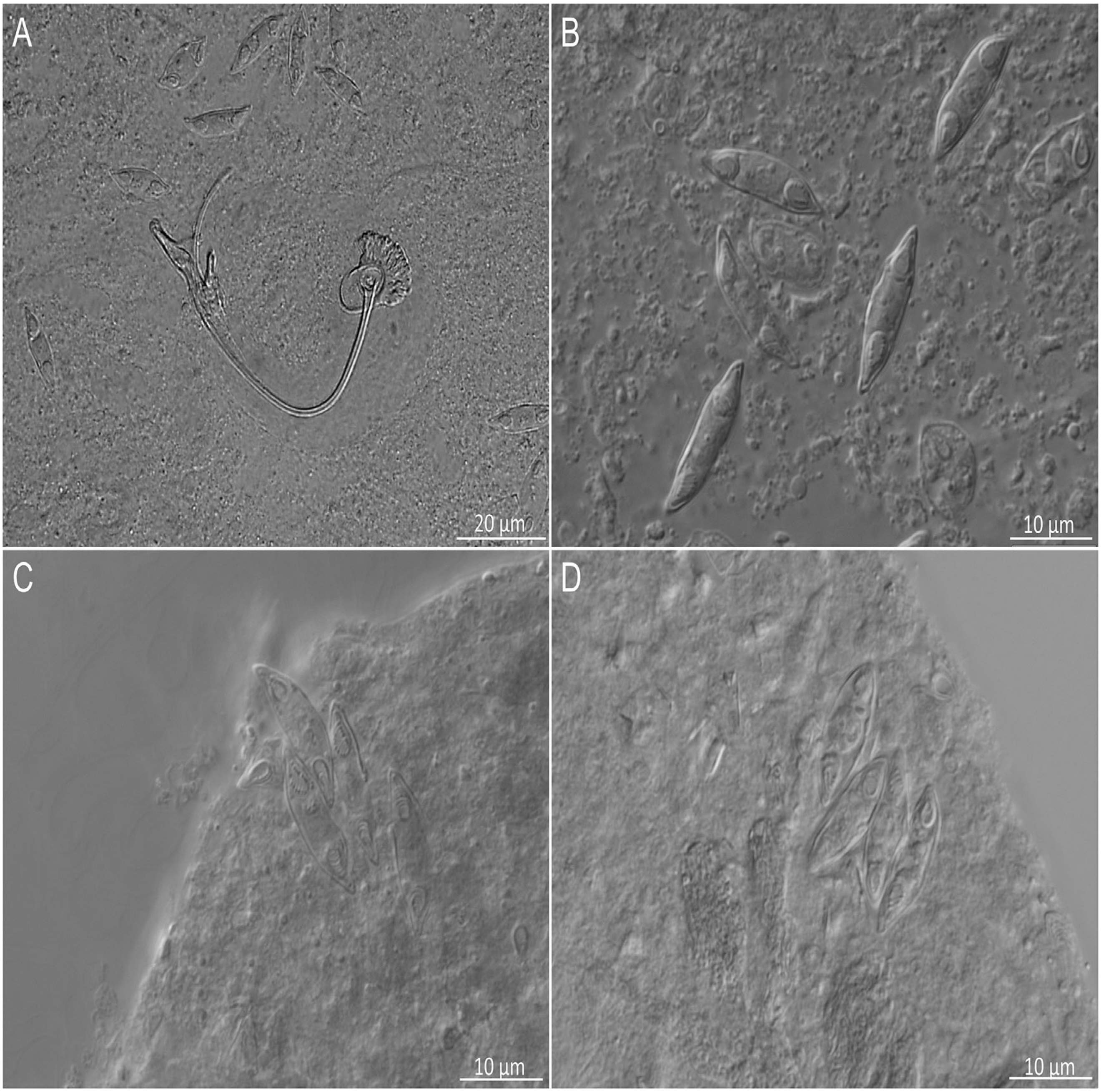

**Figure 1.** Microphotographs of myxospores of *Monomyxum ligophori* n. sp. scattered within the parenchym of A) *Ligophorus saladensis* ex *Mugil curema* with sclerotized parts of the male copulatory organ of the monopisthocotylan, B) *Ligophorus saladensis* ex *Mugil curema*, C) spores released from ruptured parenchymal tissues of *Ligophorus saladensis* ex *Mugil cephalus*, D) *Ligophorus mugilinus* ex *Mugil cephalus.*

### *Taxonomic summary and type material*

Class Myxosporea Bütschli, 1881
Order Bivalvulida Shulman, 1959
Suborder Variisporina Lom and Noble, 1984
Family Monomyxidae Freeman and Kristmundsson, 2015
Genus *Monomyxum* Freeman and Kristmundsson, 2015
*Monomyxum ligophori* n. sp. (with morphological characters of the genus)
Type (intermediate) host: *Ligophorus saladensis* (Monopisthocotyla, Dactylogyridae) (symbiotype parasitizing *Mugil curema*, symbioparatype infecting *Mugil cephalus* (Actinopterygii, Mugilidae))
Additional host: *Ligophorus mugilinus* (Monopisthocotyla, Dactylogyridae) parasitizing *Mugil cephalus* (Actinopterygii, Mugilidae)
Definitive host: *Streblospio benedicti* Webster, 1879 (Polychaeta, Spionidae)
Type locality: Stono Preserve, South Carolina, USA (32.733475 N, –80.177849 W)
Localisation of myxospores: Parenchymal tissues of monopisthocotylan hosts.
Localisation of actinospores: Tegument of middle to posterior segments of annelid host.

**Table 3.** Morphometric characterisation of myxospores infecting *Ligophorus saladensis* ex *Mugil curema* and ex *Mugil cephalus*, and *Ligophorus mugilinus* ex *Mugil cephalus* in the present study, and of the previously reported *Monomyxum incomptavermi* infecting *Diplectanocotyla gracilis* ex *Megalops cyprinoides* as published in Freeman and Shinn (2011). All measurements are presented in micrometres, as average, standard deviation, and range where available

| Species of *Monomyxum* | Monopisthocotylan host species | Fish host species | Spore | | | Polar capsule | |
|---|---|---|---|---|---|---|---|
| | | | length | width | thickness | length | width |
| *Monomyxum ligophori* n. sp. type host | *Ligophorus saladensis* | *Mugil curema* | 15.4 ± 0.8 (13.5–16.5) | 4.7 ± 0.6 (4.2–6.0) | 4.4 ± 0.3, (3.8–4.8) | 4.5 ± 0.4 (3.7–4.9) | 2.4 ± 0.3 (1.8–2.9) |
| *Monomyxum ligophori* n. sp. other record | *Ligophorus saladensis* | *Mugil cephalus* | 15.0 ± 0.6 (13.9–16.2) | 4.7 ± 0.4 (4.2–5.2) | 4.3 ± 0.6 (3.4–5.2) | 4.3 ± 0.5 (2.8–4.9) | 2.1 ± 0.3 (1.5–2.9) |
| *Monomyxum ligophori* n. sp. other record | *Ligophorus mugilinus* | *Mugil cephalus* | 13.1 ± 1.1 (10.6–14.5) | 4.3 ± 0.5 (3.5–5.1) | 4.2 ± 0.4 3.7–4.6) | 3.5 ± 0.4 (2.8–4.0) | 1.9 ± 0.2 (1.6–2.3) |
| *Monomyxum incomptavermi* | *Diplectanocotyla gracilis* | *Megalops cyprinoides* | 11.6 (11.3–11.8) | 4.9 (4.2–5.6) | – | 2.9 (2.4–3.3) | 2.0 (1.8–2.1) |

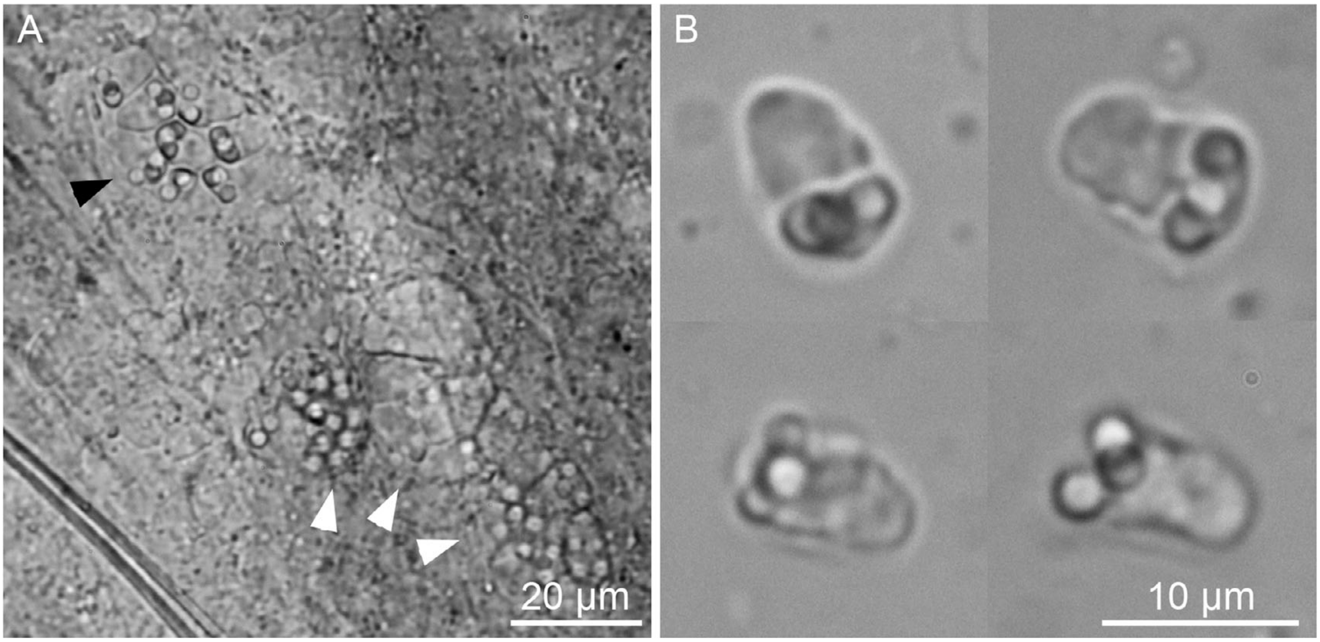

**Figure 2.** Microphotographs of saccimyxon-type actinospores of *Monomyxum ligophori* n. sp. within the tegument of the annelid *Streblospio benedicti*: A) 8-spore pansporocyst (dark arrowhead) and other less developed stages (light arrowheads). B) composite image showing four actinospores after release from host.

Prevalence of myxospores: 16.7% of *L. saladensis* ex *M. curema*, 14.3% of *L. saladensis* ex *M. cephalus*, 2.8% of *L. mugilinus* ex *M. cephalus*

Prevalence of actinospores: 0.8% (1/128) of *S. benedicti*; 0.2% (1/345) of all polychaetes examined.

Etymology: The species epithet 'ligophori' refers to the genus name of the monopisthocotylan host species.

Zoobank registration number: urn:lsid:zoobank.org:pub:DC2B07B9-7F8C-4945-A28E-C818961A61FC

Type material, myxospores: A single specimen, mounted in glycerine ammonium picrate, of *L. saladensis* ex *Mugil curema* infected by *Monomyxum ligophori* n. sp. serves as holotype. A hologenophore (HU 1095) corresponding to a DNA voucher (GenBank accession number PX612002) and two other specimens of *L. saladensis* ex *M. cephalus* (HU 1096) and *L. mugilinus* ex *M. cephalus* (HU 1097) infected by *Monomyxum ligophori* n. sp. are assigned as paratypes. All types are deposited at the institutional collection of the Research Group Zoology: Biodiversity and Toxicology of Hasselt University (Diepenbeek, Belgium).

Description of myxospores: Spore fusiform, with binucleate sporoplasm, two equal pyriform polar capsules (nematocysts) located at ends of spore, polar tubules with four coils, spore valves smooth with suture line inconspicuous; morphometrics are presented in Table 3. Line drawings are presented in Figure 3.

Description of actinospores consistent with that provided in Atkinson et al. (2019).

Differential diagnosis: *Monomyxum ligophori* n. sp. shows similar morphology of the myxospores as the only other described congener, *M. incomptavermi* with a fusiform shape. The species differ in number of coils in the polar tubules: *Monomyxum ligophori* n. sp. (n = 4) compared to *M. incomptavermi* (n = 3).

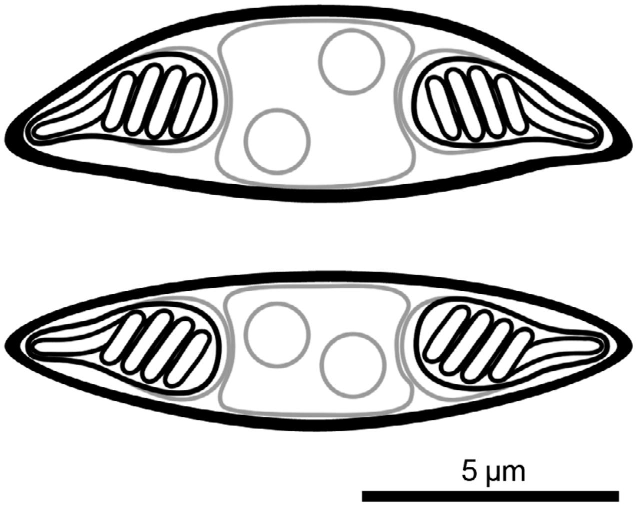

**Figure 3.** Line drawing of myxospores of *Monomyxum ligophori* n. sp. in frontal/valvular view (top) and side/sutural view (bottom).

The binucleate sporoplasm of *M. ligophori* n. sp. is in contrast with a single nucleus observed in *M. incomptavermi.* Myxozoan spores of *Monomyxum ligophori* n. sp. are longer (>12 µm; fixed) compared to *M. incomptavermi* (<12 µm; fresh) while having similar width. The size variation in the length of spores reported from individuals infecting *L. saladensis* and *L. mugilinus*, respectively, are of a similar magnitude as the difference between *M. ligophori* n. sp. and *M. incomptavermi* but with overlapping ranges is noted and should be further investigated from additional fresh material if possible.

### *Phylogenetic reconstruction*

Molecular characterisation of the SSU rDNA of the myxospore type infection in the monopisthocotylan yielded 870 bp, and from the actinospores in the annelid host yielded 1686 bp. Sequences were almost identical to each other (a difference of two base pairs over the entire length of the fragment [0.2%]) and to the previously published sequence (MH791159) of the saccimyxon type described by Atkinson et al. (2019) from the polychaete host *S. benedicti.* Further, *M. ligophori* n. sp. differed 9.3% with *M. incomptavermi* (GQ368246) and 6.3% with *Monomyxum* sp. (GQ368245). Phylogenetic reconstruction revealed the position of *M. ligophori* n. sp. as part of a clade including the other *Monomyxum* species hyperparasitic in monopisthocotylan flatworms (see Figure 4).

## Discussion

In the present study, *Monomyxum ligophori* n. sp. is described from hyperparasitic myxozoan infections observed in two monopisthocotylan species infecting *Mugil* spp. collected from the ParasiteBlitz organized at the Stono Preserve, South Carolina (de Buron et al. 2025). Furthermore, we inferred the complex life cycle of the myxozoan by matching molecular identities of the hyperparasitic myxospore stage with an actinospore stage in a benthic spionid polychaete collected also during the ParasiteBlitz, and described previously from the same area (Atkinson et al. 2019). Our study therefore provides evidence for a polychaete annelid host being part of the life cycle of a myxozoan hyperparasite of a fish-infecting monopisthocotylan flatworm, the first elucidated life cycle of a myxozoan that does not involve a vertebrate host (Figure 5). The branchial co-infection of myxozoan parasites in dactylogyrid monopisthocotylans showed no proliferation of the myxozoan in the fish host itself, as observed for other *Monomyxum* spp. (Freeman and Shinn 2011). However, given the limited sample size of potential fish hosts, the hypothesis of a non-fish myxosporean life cycle could be tested in future studies by screening a broader range of fish hosts in the area.

Myxozoan hyperparasites of fish-infecting parasitic flatworms, trematodes or monopisthocotylans, have been reported in coastal

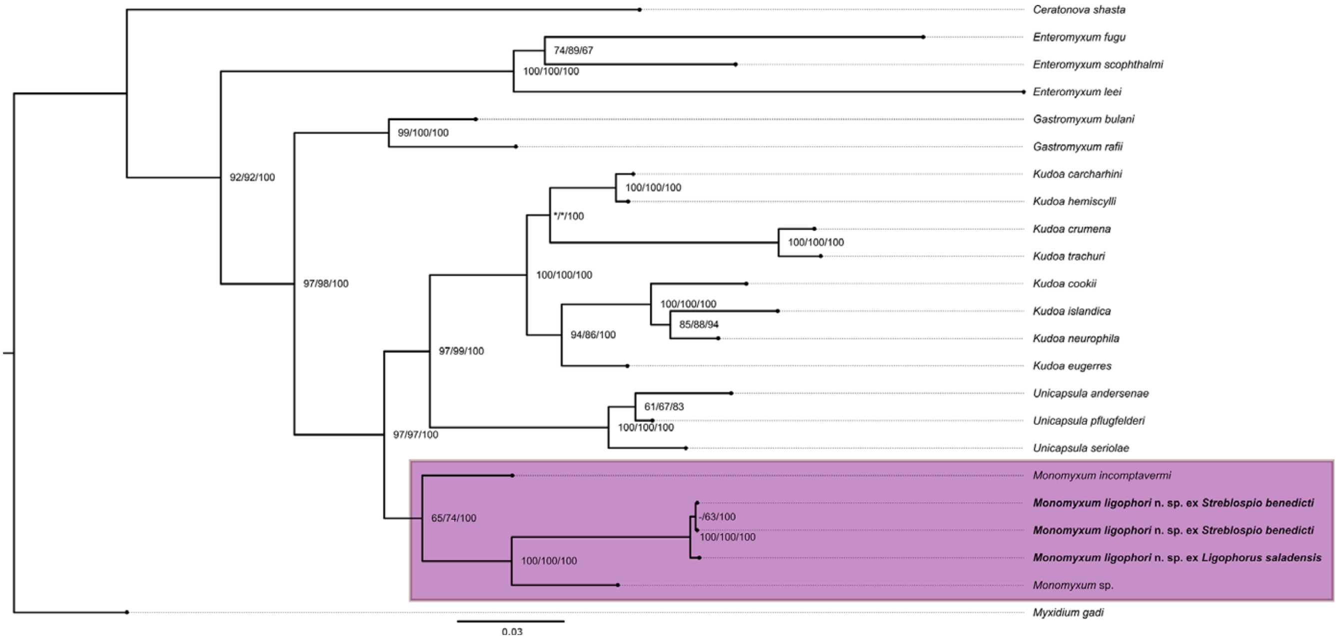

**Figure 4.** Phylogenetic reconstruction of the histozoic marine myxosporean lineages based on a portion of 18S rDNA region (1561 bp including gaps). The clade of hyperparasites of monopisthocotylan flatworms is highlighted. Support values are presented as Ultrafast bootstrap values/SH-aLRT/Bayesian posterior probabilities. The scale bar represents the estimated number of substitutions per site.

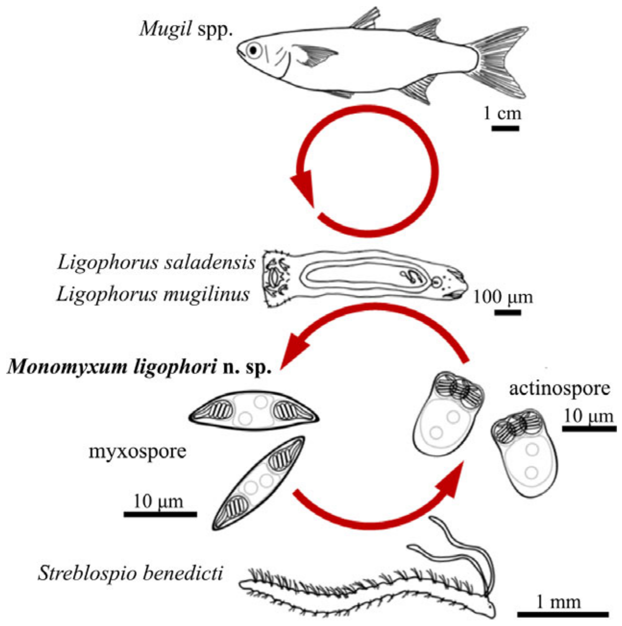

**Figure 5.** Schematic representation of the life cycle of *Monomyxum ligophori* n. sp. including the host species involved and line drawings of parasite myxospore and actinospore stages.

habitats of Peninsular Malaysia (*M. incomptavermi*), Lake Hamana in Japan (*Monomyxum* sp.), a river in North-West Spain (*M. giardi*), estuarine areas on the east coast of the USA (*Fabeospora vermicola* Overstreet, 1976), and a brackish lagoon at the Mediterranean coast (*Fabeospora* sp.) (summarized in Freeman and Shinn [2011]). Of these, four monopisthocotylan-myxozoan relationships have been described involving three species of monopisthocotylans. It is noteworthy that the above-mentioned reports stem mainly from coastal environments and euryhaline fishes. Our study at the Stono Preserve adds the first report of a hyperparasitic monomyxid myxozoan lineage from a coastal environment of the western Atlantic.

Reported prevalence of fish with myxozoan-infected monopisthocotylans varies among myxozoan species from 0.3% (*M. giardi* infecting *P. bini* ex *A. anguilla*, see Aguilar et al. 2004) up to 50% (*M. incomptavermi* infecting *D. gracilis* ex *M. cyprinoides*, Freeman and Shinn 2011). In the present study, the prevalence of hyperparasitic infection was 12.5% ex *M. curema* and 40% ex *M. cephalus*. The reported prevalence in the infrapopulation of the infected monopisthocotylan flatworms seems to vary between 30% in case of *M. giardi* infecting *P. bini* (Aguilar et al. 2004) and in *M. ligophori* n. sp., with 5% infecting *L. mugilinus* ex *M. cephalus*, 50% from *L. saladensis* ex *M. cephalus*, and 50% from *L. saladensis* ex *M. curema*, respectively. Unlike the apparent parasitic castration of their polychaete hosts (Atkinson *et al.* 2019), development of monopisthocotylan hosts seems not to be affected, as we observed copulatory organs in all individuals with overt *M. ligophori* n. sp. infections. However, that is not the case of *M. incomptavermi* as poor integrity of parenchym and lack of copulatory organs were reported (Freeman and Shinn 2011). In general, the sporadic nature of reports of myxozoan hyperparasitism in monopisthocotylan flatworms hinders any general conclusions on the frequency of this interparasitic type of relationship, and thus the degree of any pathogenic effect on the monopisthocotylan remains an open question.

Seasonal variations in infection by myxosporean stages have been reported across other marine histozoic myxozoan species including *Kudoa inornata* Dyková, de Buron, Fiala, and Roumillat, 2009, a species infecting seatrout *Cynoscion nebulosus* (Cuvier 1830), an economically and ecologically important fish in estuaries and harbours in southeastern North America (De Buron et al. 2017). The actinospore stages of *M. ligophori* n. sp. have been detected in the area of Charleston Harbour over the summer period (between May and July) and fall (November) (Atkinson et al. 2019). The peak of myxosporean infection of *K. inornata*, a species found in the same locality as *M. ligophori* n. sp., correlated positively with temperature and fish densities (De Buron et al. 2017), a pattern detected for other histozoic myxozoan species (Henning et al. 2019). Other studies reported the infection patterns of *Kudoa* spp., being influenced by salinity levels (Dos Santos et al. 2019; Jones and Long 2022). As reports on the myxosporean life stage of monomyxid myxozoans have all been one-time observations, including our study, seasonal patterns of the infection and the relation to seasonal peaks observed in other histozoic marine species hosts need to be verified.

*Monomyxum ligophori* n. sp. was the only myxozoan species found to infect polychaetes in the area, despite the presence of multiple other "marine clade" (likely polychaete hosted; Fiala et al. 2015) myxozoan genera (i.e. *Kudoa*, *Myxidium*) encountered in fish sampled during the ParasiteBlitz (de Buron et al. 2025). Specifically, the actinospore stage of *M. ligophori* n. sp. was encountered once, at a prevalence of 0.8% (1/128) in *S. benedicti* and 0.2% (1/345) in all marine annelids examined. This is an almost identical prevalence to that observed previously: 0.8% (6/734) of *S. benedicti*; 0.2% of 3,214 polychaetes examined (Atkinson et al. 2019). The difficulty of discovering the annelid hosts of marine fish myxosporeans is well established (e.g. Hallett et al. 1999, 2001; Rocha et al. 2020) and due to inherently low infection prevalence in the vast and diverse annelid biota in estuarine ecosystems.

As early phases of myxosporean infection are easily missed using visual examination alone, molecular methods have been developed for species of aquaculture or zoonotic importance (Funk et al. 2007; Grabner et al. 2012). This is exemplified in monopisthocotylans, where systematic barcoding revealed many morphologically cryptic infections compared with those monopisthocotylan individuals having fully developed myxospores (Morris and Freeman 2010). In the present study, we used molecular screening to confirm the absence of *M. ligophori* n. sp. in two other species of parasitic flatworms, *L. uruguayensis* and *M. macracantha*, co-infecting the fish hosts, thereby suggesting a certain level of host-specificity of *M. ligophori* n. sp. towards its monopisthocotylan host species even in the presence of a sympatric congener. However, given the relatively limited sample size of both fish and monopisthocotylan hosts, results on the host-specificity towards monopisthocotylans and absence in the vertebrate hosts should be further checked in the light of previously reported variable prevalences (Jones et al. 2019; MacKenzie et al. 2005) and seasonality (Alama-Bermejo et al. 2013) of myxosporean infections. The low prevalence of the *M. ligophori* n. sp. actinospore stage (a single infected individual out of 345 annelids examined) restricts any conclusions on its host-specificity towards the annelid host.

Our phylogenetic reconstruction shows the presence of three major myxosporean clades, corresponding with the results of previous studies (Fiala et al. 2015; Fiala and Bartošová 2010; Kodádková et al. 2015). *Monomyxum ligophori* n. sp. is part of a moderately supported clade of monomyxid histozoic myxozoans that infect monopisthocotylan flatworms (Figure 3).

According to the WoRMS database (Whipps et al. 2025), >3000 described myxozoan species exist whereas life cycles of only some 50 species have been resolved (Eszterbauer et al. 2015). Most myxozoans are highly specific with regard to their vertebrate hosts (Molnár and Eszterbauer 2015). It has been suggested that trophic relationships and relative abundance of alternative hosts drive these associations between parasites, including myxozoans, and their hosts (Lootvoet et al. 2013). The scarcity of known life cycles and corresponding unknown identity of non-fish hosts combined with the lack of genetic resources hindered tests on the origin of the hyperparasitic myxozoan lineages. Despite the close molecular similarity of species of *Monomyxum* and other marine histozoic lineages including *Kudoa* and *Gastromyxum*, our results confirm the previously suggested common origin of *Monomyxum* species infecting monopisthocotylan flatworms (Freeman and Kristmundsson 2015). In the present study, the first life cycle of any hyperparasitic myxozoan species is presented, supporting the previous suggestions on the involvement of additional invertebrate hosts in monomyxids as an alternative to teleosts (Freeman and Kristmundsson 2015). However, as infections of other fish hosts have been detected by molecular methods (Freeman and Kristmundsson 2015), the strict affinity of monomyxids towards monopisthocotylan hosts is supported only by a few taxa.

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