



Morphological and molecular characterization of a novel *Myxobolus* species from the gastrointestinal tract of brown trout (*Salmo trutta*) in Spain

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Abstract

The genus *Myxobolus* Bütschli, 1882 is the largest group within the class Myxosporea and includes 905 nominal species, 18 of which have been found to infect fish belonging to the family Salmonidae. In the present study, microscopic analysis enabled detection of myxospores in 43 of 613 (7.0%) gastrointestinal tracts from brown trout (*Salmo trutta*) captured in several rivers in the northwest of the Iberian Peninsula. Measurement of the whole myxospores, polar capsules and other morphological characteristics, together with identification of the site of infection, has led us to propose a novel salmonid-myxobolid species, *Myxobolus compostellanus* n. sp. Molecular analysis of the small subunit ribosomal RNA (SSU-rRNA) gene yielded the same consensus sequence of 2039 bp in 14 fish specimens. A BLAST search indicated 97.6% similarity to *Myxobolus neurobius*. Phylogenetic analysis revealed that *M. compostellanus* n. sp. is clustered with other salmonid-infecting myxobolids. The present findings contribute to the existing knowledge about the genus *Myxobolus*, providing both morphological and molecular data on a novel species of *Myxobolus* found to infect the gastrointestinal tract of salmonids, *M. compostellanus* n. sp. in the brown trout (*S. trutta*).

Keywords *Myxobolus compostellanus* n. sp. · Brown trout · Morphological analysis · Molecular characterization · Northwest Spain

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Introduction

The phylum Myxozoa is a highly diverse group of metazoan parasites comprised by more than 2200 species belonging to 64 genera (Canning and Okamura 2004; Chang et al. 2015; Okamura et al. 2015). The group is continuously revisited, and as a consequence, more than 140 new species have been described in recent years (2014–2019). Although species infecting amphibians, reptiles, birds and even mammals have been reported, the myxozoans are mainly obligate endoparasites of fish. These parasites are characterized by complex life cycles, alternating between a vertebrate host (mainly a fish) and an invertebrate host (oligochaetes and bryozoans for the classes Myxosporea and Malacosporea, respectively) (Chang et al. 2015; Holzer et al. 2018).

The genus *Myxobolus* Bütschli, 1882 is the largest within the Myxosporea and includes 905 nominal species, 18 of which have been described in species of fish belonging to

the family Salmonidae (Eiras et al. 2005, 2014). The brown trout (*Salmo trutta* Linnaeus, 1758) is the most widely distributed salmonid species in the world and is indigenous in the Iberian Peninsula. It is an important angling species and is consequently of high socio-economic value, although it is not cultured for commercial purposes (MacCrimmon and Marshall 1968; Doadrio 2002; Almodóvar and Nicola 2004; Cobo et al. 2015).

Four *Myxobolus* species have been described in brown trout: *Myxobolus neurobius* Schuberg and Schröder, 1905, which infects the nervous system (Schuberg and Schröder 1905); *Myxobolus vartanyanae* Donec et al., 1973, which infects muscle (Donec et al. 1973); *Myxobolus farionis* González-Lanza and Álvarez-Pellitero, 1984, detected in the brain and the spinal cord; and *Myxobolus ibericus* González-Lanza and Álvarez-Pellitero, 1984, found in kidneys, spleen, liver and ureter and less frequently in gills, gonads, intestine, muscle, swim bladder, heart and peritoneum (González-Lanza and Álvarez-Pellitero 1984). However, only three of these are currently recognized as valid species, as *M. farionis* is considered a synonym of *M. neurobius* (Lom and Dyková 1992; Eiras et al. 2005; Urawa et al. 2009).

In a previous study focused on *Cryptosporidium* in brown trout (Couso-Pérez et al. 2019), microscopic analysis revealed the presence of spores of *Myxobolus* sp. in the gastrointestinal tract of fish specimens. In the present study, we carried out morphological and molecular analysis of these myxospores. Based on the findings obtained, we describe here a novel myxobolid species infecting the pyloric caeca and intestine of brown trout (*S. trutta*).

Material and methods

Sample collection and processing

Brown trout (*S. trutta*) specimens ($n=613$) were captured by local anglers for their own consumption in 44 Galician rivers (NW Spain) during the 2015 fishing season (15 March–15 August) (Table S1). The anglers removed the gastrointestinal tracts from the fish and placed them in individual plastic bags, which were then sealed hermetically and maintained at $-20\text{ }^{\circ}\text{C}$ until processing. The anglers also reported

the length of each specimen, the date of capture and the name of the river where the fish were captured (see Couso-Pérez et al. 2019). Considering that the minimum legal catch size is 19.0 cm, the fish were classified according to Sánchez-Hernández et al. (2012) as follows: 19.0–19.1 cm (<2 years; $n=160$), 19.2–25.9 cm (2–3 years; $n=355$) and 26.0–50.6 cm (>3 years; $n=98$).

The gastrointestinal tracts were processed as previously described (Couso-Pérez et al. 2019). Briefly, the samples were differentiated into pyloric caeca and intestine, which were homogenized separately. Each homogenate was individually filtered and subjected to diphasic concentration in 0.04 M phosphate-buffered saline (PBS) (pH 7.2)/diethyl ether (2:1). Finally, the pellets were resuspended in 500–1000 μL of 0.04 M PBS (pH 7.2) and stored at $-20\text{ }^{\circ}\text{C}$.

Microscopic analysis

The sediments were defrosted, and aliquots of 10 μL were examined under bright field and differential interference contrast (DIC) microscopy ($\times 400$ magnification). For the morphological characterization, a total of 40 *Myxobolus* mature myxospores from the homogenates of pyloric caeca (9 fish) and intestine (1 fish) were measured separately, and microphotographs were taken in an AxioPhot microscope (Carl Zeiss, Oberkochen, Germany), equipped with a digital camera (AxioCam ICc3, Carl Zeiss). Mature myxospores were measured following the recommendations of Lom and Dyková (1992).

Molecular characterization

Samples from the gastrointestinal tracts of 43 trout previously shown by microscopy to harbour spores of *Myxobolus* sp. were subjected to molecular analysis. Genomic DNA was individually extracted from 200 μL of homogenated samples of pyloric caeca ($n=30$), intestine ($n=2$) and both organs ($n=11$) by using the Stool DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. The nucleic acids thus extracted were stored at $-20\text{ }^{\circ}\text{C}$ until use. A PCR technique was used to amplify a fragment of ~ 2081 bp of the small subunit ribosomal RNA (SSU-rRNA) gene with

Table 1 Primers used for the amplification and sequencing of a fragment of ~ 2081 bp of the small subunit ribosomal RNA (SSU-rRNA) gene of *Myxobolus compostellanus* n. sp.

Primer	Direction	Sequence (5'→3')	Reference
18e	Forward	CTGGTTGATCCTGCCAGT	Hillis and Dixon (1991)
18r	Reverse	CTACGGAAACCTTGTTACG	Whipps et al. (2003)
ACT3f	Forward	CATGGAACGAACAAT	Hallett and Diamant (2001)
ACT3r	Reverse	ATTGTTTCGTTCCATG	Hallett and Diamant (2001)
Myx4r	Reverse	CTGACAGATCACTCCACGAAC	Hallett and Diamant (2001)
MyxospecF	Forward	TTCTGCCCTATCAACTTGTG	Fiala (2006)

myxosporean-specific primer pairs 18e-ACT3r, ACT3f-18r and MyxospecF-Myx4r (Table 1). The reaction mixture contained 2 μ L of extracted genomic DNA, 0.2 mM deoxyribonucleotide triphosphates (dNTPs; NZYTech Lda., Lisbon, Portugal), 0.1 pmol of each primer, 2.5 μ L of 10 \times Taq polymerase buffer, 2.5 mM MgCl₂ and 1.25 U of Taq DNA polymerase (NZYTech Lda.) in a 25 μ L reaction volume. The PCR conditions consisted of an initial denaturation of 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 52 °C for 45 s and 72 °C for 2 min, with a final extension at 72 °C for 7 min. Five-microliter aliquots of all PCR products were electrophoresed through a 1% agarose 1 \times Tris–acetate-EDTA (TAE) buffer gel and then stained with GreenSafe Premium (NZYTech Lda.). Positive PCR products were purified using the PCR Purification Kit (Norgen Biotek Corp.) and sequenced in both directions. The resulting consensus sequences were compared with other sequences of *Myxobolus* spp. infecting salmonids and other fish families deposited in GenBank® (Sayers et al. 2019, 2020) by using the public web interface of the BLAST® 2.2.31 program (Johnson et al. 2008). DNA sequences were aligned in MAFFT v7.471 using the L-INS-i multiple alignment strategy and other default parameters (Kato and Standley 2013). Phylogenetic analysis was performed using the maximum likelihood (ML) method in MEGA 7 (Kumar et al. 2016), and the Bayesian inference (BI) method in MrBayes v3.2.6 (Ronquist et al. 2012). For ML and according to the Akaike information criterion, the general time reversal (GTR) method was used, with discrete gamma distribution and invariant sites. The bootstrap value was estimated from 1000 replicates. The ML phylogenetic tree was generated using a total of 826 positions in the final data set. For BI, the posterior probabilities were estimated with the Markov chain Monte Carlo (MCMC) method, from 1,000,000 generations with 4 simultaneous MCMC chains and 2 independent runs,

with trees sampled at intervals of 100 trees and with 25% burn-in.

Statistical analysis

All statistical tests were carried out using Statgraphics® Centurion XVIII v18.1.12 statistical software (©2019 Stat-Point Technologies, Inc., Warrenton, VA, USA). Fisher's exact test and the chi-square test were used to determine any differences in the prevalence rates between anatomical locations and fish size classes, and the Mann–Whitney test was used to compare the intensities of infection. Differences were considered statistically significant at $P < 0.05$.

Results

Species description and taxonomic summary

A single brown trout (*S. trutta*) of length 21.5 cm (2–3 years old), captured in River Escádebas, was designated as the type host in which only mature myxospores were observed (Figs. 1 and 2). Thus, we obtained measurements of 20 mature myxospores found exclusively in pyloric caeca. The type morphometrics of the myxospores were as follows: length, 10.8 ± 0.3 (10.2–11.7) μ m; width, 8.8 ± 0.7 (7.4–10.0) μ m; and thickness, 6.8 ± 0.1 (6.7–6.8) μ m, with equal polar capsules of length 4.8 ± 0.1 (4.2–5.5) μ m and width 2.6 ± 0.5 (1.9–3.5) μ m and 5–6 tubule coils (Table 2). Moreover, a type SSU-rDNA sequence of 2039 bp was also obtained (GenBank® accession number MT396577).

Myxobolus compostellanus n. sp.

Family: Myxobolidae Thélohan, 1892.

Genus: *Myxobolus* Bütschli, 1882.

Type host: brown trout, *Salmo trutta* Linnaeus, 1758.

Type site of infection: only in pyloric caeca.

Fig. 1 Line drawing of the mature myxospores of *Myxobolus compostellanus* n. sp. **a** Valvular view. **b** Sutural view. Bar = 5 μ m

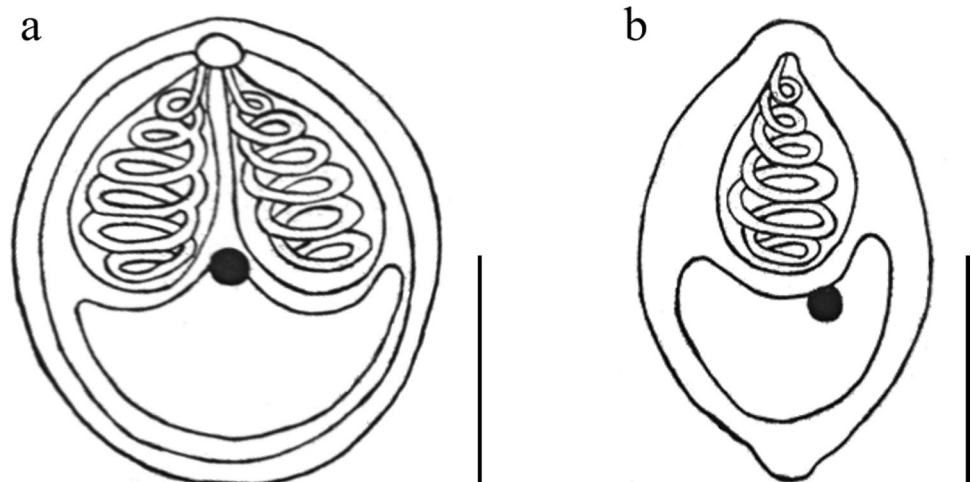


Fig. 2 Microphotographs of *Myxobolus compostellanus* n. sp. mature myxospores in a homogenized sample of pyloric caeca from brown trout (*Salmo trutta*), observed by differential interference contrast (DIC) microscopy. **a** Valvular view. **b** Sutural view. Bar = 5 μ m



Other sites of infection: less frequently observed in the intestine.
Type locality: River Escádebas, Galicia, northwest Spain.
Other localities: River Cabalar, River Xallas, River Furelos, River Mandeo, River Paradela, River Parga, River Roca, River Samo, River Sionlla and River Tambre, all in Galicia, northwest Spain (Table S1).

Prevalence: mature myxospores were detected in 43 of 613 brown trout (prevalence = 7.0%).

Deposited material: mature myxospores of *M. compostellanus* n. sp. fixed in 10% neutral buffer formalin have been deposited in the collection held in the Natural History Museum of the University of Santiago de Compostela, Santiago de Compostela, Spain (1 vial; syntype; CPZ_Myozoa_10501), and three sequences of the SSU-rRNA gene of the myxosporean stage with a total of 2039 bp have been deposited in the GenBank® under accession numbers MT396577, MT548607 and MT548608.

Etymology: the species epithet “compostellanus” refers to the city of Santiago de Compostela, which university was founded in 1495.

Morphological analysis

During the microscopic analysis of the individual homogenates, only mature myxospores of *M. compostellanus* n. sp. were observed. Measurement of a total of 20 mature myxospores isolated from eight pyloric caeca samples and one intestinal sample (from additional 9 fish) showed that the myxospores were ellipsoidal in shape in valvular view, of length 10.6 ± 0.4 (9.5–11.1) μ m ($n=20$), width 8.5 ± 0.6 (7.4–9.9) μ m ($n=17$) and thickness 6.7 ± 0.1 (6.7–6.8) μ m ($n=3$). The spores had two equal pyriform polar capsules of length 5.2 ± 0.2 (4.8–5.6) μ m ($n=11$) and width 2.8 ± 0.3 (2.5–3.3) μ m ($n=11$), occupying about half of the spore length. The polar tubules formed

5–6 coils ($n=2$), and the extended tubules ranged in length from 31.2 to 38.4 μ m ($n=4$).

Prevalence

Mature myxospores of *M. compostellanus* n. sp. were detected in 43 of 613 gastrointestinal tracts of brown trout examined under bright field and DIC microscopy (7.0%), with a mean intensity of 328.7 ± 540.6 (range 50–3320) myxospores/trout (Table 3). The positive trout were captured in 11 of the 44 rivers sampled, with prevalence values ranging between 3.5% (River Parga) and 50.0% (River Mandeo) (Table S1).

Regarding the anatomical location, the myxospores were detected in pyloric caeca (30 trout; prevalence, 4.9%; mean intensity, 176.7 myxospores/trout), intestine (2 trout; prevalence, 0.3%; mean intensity, 106.5 myxospores/trout) and in both locations (11 trout; prevalence, 1.8%; mean intensity, 783.6 myxospores/trout), with statistically significant differences between the prevalence rates in the two anatomical locations ($P < 0.01$) but not between the mean intensity values (Table 3).

Considering the age of the trout, the prevalence was higher in specimens < 2 years than in the other age groups considered (8.8%, 5.9% and 8.2% in trout < 2 years, 2–3 years and > 3 years, respectively), although the differences were not statistically significant. The mean intensity of parasitization increased with the age of the fish (267.4 myxospores/trout for specimens < 2 years, 336.5 for 2–3 years and 415.5 for > 3 years); again, the differences were not statistically significant (Table 3).

Table 2 Comparison of species of *Myxobolus* that infect salmonid fishes

Species	Host	Site of infection	Material examined	Spore			Polar capsules				Location	GenBank number	Reference
				L	W	T	L	W	E	TC			
<i>Myxobolus arcticus</i>	<i>Oncorhynchus masou</i>	Medulla oblongata and spinal cord	Fresh	14.1±0.5 (13.3–15.6)	8.7±0.4 (7.8–9.4)	6.4±0.3 (5.6–7.0)	8.4±0.3 (7.5–9.0)	3.1±0.2 (2.6–3.5)	=	8–10	Japan	AB353130	Urawa et al. (2009)
<i>Myxobolus cerebralis</i>	<i>Oncorhynchus mykiss</i>	Brain cartilage	Fresh	8.7 (7.4–9.7)	8.2 (7.0–10.0)	6.3 (6.2–7.4)	5.1 (5.0–6.0)	3.2 (3.0–3.5)	=	5–6	Germany USA	EF370478	Lom and Hoffman (1971); Arsan et al. (2007)
<i>Myxobolus evdokimovae</i>	<i>Coregonus albula</i>	Wall of the mouth	–	10.0–11.0	8.7–9.2	–	5.0–5.2	3.1–3.3	=	–	Russia	–	Eiras et al. (2005)
<i>Myxobolus fryeri</i>	<i>Oncorhynchus kisutch</i>	Peripheral nerves of skeletal muscles	Fresh	12.9±0.8 (11.1–14.8)	8.6±0.7 (7.2–10.1)	7.2±0.4 (6.4–7.7)	6.9±0.6 (5.9–8.1)	2.8±0.3 (2.0–3.3)	≠	8–10	USA	EU346370	Ferguson et al. (2008)
<i>M. compositellanus</i> n. sp.	<i>S. trutta</i>	Pyloric caeca	Fresh	10.8±0.3 (10.2–11.7)	8.8±0.7 (7.4–10.0)	6.8±0.1 (6.7–6.8)	4.8±0.1 (4.2–5.5)	2.6±0.5 (1.9–3.5)	=	5–6	Spain	MT396577	Present study
<i>M. ibericus</i>	<i>S. trutta</i>	Kidney, spleen, gall bladder and ureter*	Fresh	10.0±0.5 (9.0–11.0)	8.6±0.5 (8.0–9.5)	6.6±0.3 (6.0–7.0)	5.0±0.5 (4.0–6.0)	2.7±0.3 (2.2–3.5)	≠	7–9	Spain	–	González-Lanza and Alvarez-Pellitero (1984)
<i>Myxobolus insidiosus</i>	<i>Oncorhynchus tshawytscha</i>	Skeletal muscles	Fresh	14.7 (13.3–15.9)	9.4 (7.9–10.5)	7.4 (6.8–8.3)	7.7 (5.3–9.3)	3.2 (1.9–3.9)	=	–	USA	EU346374	Ferguson et al. (2008)
<i>Myxobolus kisutchi</i>	<i>O. kisutch</i>	Brain, spinal cord and optic nerves	Fresh	9.0±0.5 (8.2–10.1)	7.7±0.4 (7.0–8.5)	6.1±0.4 (5.4–6.7)	3.9±0.3 (3.4–4.2)	2.2±0.2	=	5–6	USA	EF431919	Hogge et al. (2008a)
<i>Myxobolus krokhi</i>	<i>Salvelinus alpinus</i>	Abdominal serosa	–	9.6–12.0	7.5–10.5	6.6–6.9	5.0–6.6	2.5–4.0	=	–	Russia	–	Eiras et al. (2005)
<i>Myxobolus nurakamii</i>	<i>O. masou</i>	Lateral line nerves	Fresh	11.3±0.4 (10.3–12.1)	9.9±0.4 (9.2–10.9)	7.1±0.4 (6.2–7.8)	5.1±0.4 (4.0–5.5)	3.0±0.3 (2.4–3.9)	=	5–8	Japan	AB469984	Urawa et al. (2009)
<i>M. neurobius</i>	<i>S. trutta</i>	Nervous system	Fresh	9.2±0.4 (8.6–10.5)	7.6±0.3 (7.0–8.2)	6.1±0.5 (5.4–7.0)	5.0±0.4 (3.9–5.5)	2.4±0.1 (2.1–2.7)	=	6–7	Norway	AB469986	Urawa et al. (2009)
<i>Myxobolus neurofontinalis</i>	<i>Salvelinus fontinalis</i>	Nerve cord and medulla oblongata	Fixed	13.9±0.9 (12.0–16.0)	9.6±0.8 (8.0–12.0)	8.1±0.7 (7.0–10.0)	7.8±0.8 (6.0–10.0)	3.5±0.5 (3.0–5.0)	=	7–10	USA	MN191598	Ksepka et al. (2019)
<i>Myxobolus neurotropus</i>	<i>O. mykiss</i>	Brain and spinal cord	Fresh	11.8±0.5 (11.2–13.0)	10.8±0.5 (10.4–12.3)	8.8±0.3 (8.4–9.1)	5.9±0.6 (5.0–6.9)	3.7±0.4	≠	6–8	USA	DQ846661	Hogge et al. (2008b)
<i>Myxobolus salmonis</i>	<i>Oncorhynchus keta</i>	Surface under scales	Fixed	8.2–10.4	7.4–9.5	5.5–8.3	3.6–5.8	2.1–3.4	=	5–6	Russia	–	Hoshina (1949)
<i>Myxobolus soldatovi</i>	<i>O. keta</i>	Skin	–	8.0–9.5	–	–	4.0–4.2	2.0–2.2	=	–	China	–	Akhmerov (1960)
<i>Myxobolus spherialis</i>	<i>Coregonus fera</i>	Branchial mucosa	–	9	9	–	–	–	–	–	Switzerland	–	Gurley (1893)
<i>Myxobolus squamalis</i>	<i>O. mykiss</i>	Scales	Fresh	8.4±0.6 (7.4–9.4)	7.9±0.6 (7.1–9.0)	6.2±0.1 (6.2–6.3)	4.1±0.3 (3.3–4.7)	2.9±0.2 (2.5–3.3)	=	4–6	USA	JX910362	Polley et al. (2013)

Table 2 (continued)

Species	Host	Site of infection	Material examined	Spore		Polar capsules				Location	GenBank number	Reference	
				L	W	T	L	W	E				TC
<i>Myxobolus thymalli</i>	<i>Thymallus arcticus</i>	Gall bladder	–	9.0–11.0	8.0–10.5	5.9–7.2	5.2–6.5	2.7–3.6	=	–	Russia	–	Eiras et al. (2005)
<i>M. vartanyanae</i>	<i>S. trutta</i>	Muscles	–	9.5–12.3	8.5–10.0	7.3–8.0	4.5–6.0	2.5–3.3	≠	–	Russia	–	Donec et al. (1973)
<i>Myxobolus</i> sp.	<i>S. trutta</i>	Nervous tissue in the musculature	Fresh	8.8 (7.8–9.2)	6.4 (5.8–7.0)	5.1 (4.7–5.5)	4.0 (3.2–4.7)	2.6 (2.1–3.2)	–	–	UK	AM042702	Holzer et al. (2006)

Numerical values indicate mean \pm standard deviation (SD) (range). All measurements are expressed in micrometres. Data from the species described in the present study are highlighted in bold

L length, W width, T thickness, E equal, TC tubule coils

*Less frequent in gills, gonads, intestine, muscle, swim bladder, heart and peritoneum

Molecular characterization

Consensus sequences of 2039 bp of the SSU-rRNA gene were obtained from 17 different fish specimens. In 14 trout, including the type host, the consensus sequences were 100% identical and no differences were observed between the sequences from pyloric caeca and intestinal isolates. In the remaining 3 specimens, the consensus sequences, obtained from pyloric caeca samples, showed one nucleotide difference at position 646 bp (C/T in 2 fish) and at position 1520 bp (A/T in 1 trout). Therefore, molecular data indicate that the isolates obtained belong to the same myxozoan species. These three representative sequences showed a similarity of 97.5–97.6% relative to the sequence of *M. neurobius* deposited in the GenBank® database under accession number AB469986. The phylogenetic analysis of the same consensus sequence isolated from 14 trout specimens revealed that this sequence was genetically distinct and grouped within the species clade that infects salmonids; the novel species appears most similar to *M. neurobius* (Fig. 3).

The consensus sequence of the *M. compostellanus* n. sp. isolated from 14 fish, which were 100% identical, and the two consensus sequences, which showed one nucleotide difference at one position relative to the former consensus sequence, have been deposited in the GenBank® database under accession numbers MT396577, MT548607 and MT548608, respectively.

Discussion

The present study reports the identification of a novel *Myxobolus* species, *M. compostellanus* n. sp. in brown trout (*S. trutta*). The novel species shares the same morphological features as described for the genus (Lom and Dyková 2006), and it is phylogenetically clustered among the other species of *Myxobolus* that infect salmonids.

Generally, myxozoan species that infect fish are host-, tissue- and organ-specific parasites (Molnár and Eszterbauer 2015; Rocha et al. 2019b). To our knowledge, only three species of *Myxobolus* are recognized to parasitize the brown trout (*S. trutta*): *M. neurobius*, *M. vartanyanae* and *M. ibericus*, of which only *M. ibericus* has been reported in the intestine of this host but less frequently in other organs (kidney, spleen, liver, gall bladder, ureter, gills, gonads, muscle, swim bladder, heart and peritoneum) (see Table 2).

In 1984, González-Lanza and Álvarez-Pellitero described *M. ibericus* in brown trout from the Duero basin (León, NW Spain), a different watershed than those sampled in the present study (see Couso-Pérez et al. 2019). The authors observed ellipsoidal or nearly spherical

Table 3 Prevalence, intensity and anatomical location of *Myxobolus compostellanus* n. sp. myxospores in specimens of brown trout (*Salmo trutta*) from northwest Spain grouped according to length and estimated age of the fish

Age group	Anatomical location			Total
	PC	INT	PC + INT	
19.0*–19.1 cm (< 2 years), n = 160				
Positive samples (n/%)	10/6.3	1/0.6	3/1.9	14/8.8
Intensity (mean ± SD) (range, myxospores/trout)	244.9 ± 295.6 (71–980)	93.0 ± 0.0 (93)	400.7 ± 248.1 (140–634)	267.4 ± 277.1 (71–980)
19.2–25.9 cm (2–3 years), n = 355				
Positive samples (n/%)	15/4.2	1/0.3	5/1.4	21/5.9
Intensity (mean ± SD) (range, myxospores/trout)	146.2 ± 111.0 (50–390)	120.0 ± 0.0 (120)	950.8 ± 1300.9 (143–3320)	336.5 ± 686.3 (50–3320)
26.0–50.0 cm (> 3 years), n = 98				
Positive samples (n/%)	5/5.1	0/0	3/3.1	8/8.2
Intensity (mean ± SD) (range, myxospores/trout)	132.0 ± 63.2 (55–200)	–	888.0 ± 571.9 (272–1402)	415.5 ± 498.8 (55–1402)
Total, n = 613				
Positive samples (n/%)	30/4.9	2/0.3	11/1.8	43/7.0
Intensity (mean ± SD) (range, myxospores/trout)	176.7 ± 189.9 (50–980)	106.5 ± 19.1 (93–120)	783.6 ± 903.3 (140–3320)	328.7 ± 540.6 (50–3320)

PC pyloric caeca, INT intestine, PC + INT pyloric caeca and intestine, SD standard deviation

*Minimum legal catch size

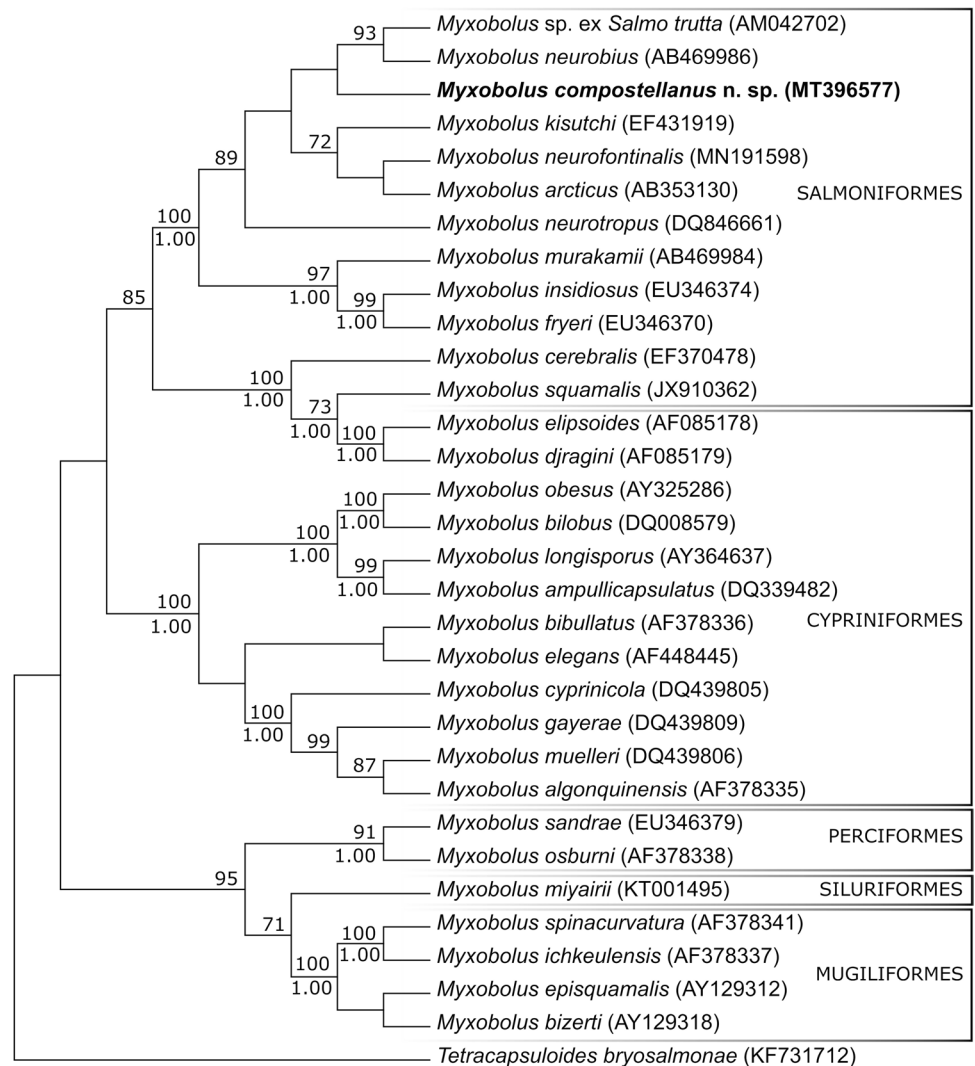
myxospores (length $10.0 \pm 0.5 \mu\text{m}$; width $8.6 \pm 0.5 \mu\text{m}$) with a relatively thick sutural ridge ($6.6 \pm 0.3 \mu\text{m}$) and pyriform polar capsules (length $5.0 \pm 0.5 \mu\text{m}$; width $2.7 \pm 0.3 \mu\text{m}$), which occupied about half of the spore length and were often of different sizes. The polar tubules formed 7–9 coils and the extended tubules measured 21–45 μm in length (González-Lanza and Álvarez-Pellitero 1984). The morphometric features of *M. ibericus* overlap with those of *M. compostellanus* n. sp. except in the number of tubule coils (7–9 vs. 5–6, respectively) (see Table 2). However, the extraordinarily extensive tissue tropism and the absence of molecular data compromise the validity of the species *M. ibericus* described by González-Lanza and Álvarez-Pellitero (1984), and it is possible that the original description may correspond to observations of several species of the genus.

In the present study, only mature myxospores were observed and they were mainly located in the pyloric caeca of brown trout (*S. trutta*), differing from *M. ibericus* and other salmonid-infecting *Myxobolus* species in spore morphology and tissue tropism. Therefore, we propose a novel species *M. compostellanus* n. sp. infecting the gastrointestinal tract of the brown trout (see Table 2). Although tissue samples were not available for histological studies, the larger number of positive samples, higher prevalence rates and higher intensities determined in pyloric caeca samples than in intestinal samples (30 vs. 2 positive samples; prevalence of 4.9 vs. 0.3%; and maximum intensity of 980 vs. 120 myxospores/trout,

respectively) suggest that the pyloric caeca is the main anatomical location of the parasite. The presence of myxospores in both anatomical locations in 11 trout may be a consequence of intestinal transit (see Table 3).

Considering that most species of *Myxobolus* have been described using mainly phenotypic features, such as spore morphometry, tissue tropism and host specificity, and that the myxospores are often morphologically very similar, the inclusion of molecular characterization is essential for reliable species identification (Holzer et al. 2004; Eiras et al. 2005, 2014; Atkinson et al. 2015). Moreover, the use of molecular data enables the study of the phylogenetic relationships between the different species of the genus, with amplification and sequencing of the SSU-rRNA gene being a well-known and the most extensively used marker for molecular characterization of myxosporean parasites (Rocha et al. 2019a). The molecular and phylogenetic results obtained in the present study show that *M. compostellanus* n. sp. is genetically distinct from other existing species grouped within the clade of myxobolid species that infect salmonids and that it is most closely related to *M. neurobius*. Although *M. compostellanus* n. sp. and *M. neurobius* parasitize the same host (*S. trutta*), the morphometric characteristics and site of infection of these species are different. Thus, *M. compostellanus* n. sp. is mainly located in the pyloric caeca and less frequently in the intestine, while *M. neurobius* infects the brain and the spinal cord (Eiras et al. 2005; Urawa et al. 2009).

Fig. 3 Phylogenetic relationships among *Myxobolus compostellanus* n. sp. isolated from brown trout (*Salmo trutta*) and other species of *Myxobolus* as inferred by maximum likelihood analysis of the small subunit ribosomal RNA (SSU-rRNA). Nodal support values are shown for bootstrapping (1000 replicates) above the branches and for Bayesian posterior probabilities below the branches. Bootstrap values < 70% or nodes not represented in Bayesian inference are not shown. The species *M. compostellanus* n. sp. and its corresponding accession number obtained in this study are highlighted in bold



Previous phylogenetic research on myxozoans and fish revealed that vertebrate host family specificity has a stronger effect on the evolution of myxobolid species (Ferguson et al. 2008; Carriero et al. 2013; Zhang et al. 2018; Rocha et al. 2019b). The phylogenetic analysis conducted in the present study shows that the novel species, *M. compostellanus* n. sp., is clustered with other salmonid-infecting myxobolids. Moreover, considering the topology of the internal salmonid clade, a tendency for species grouping according to host genus is observed. Thus, in general, species of *Myxobolus* that infect salmonids form three well-supported subclades. Two of these subclades comprise species that infect members of the genus *Oncorhynchus*, and one comprises species of the genus *Salmo*, which, in addition, only includes species of *Myxobolus* that infect *S. trutta*, probably owing to the scarce number of species described for this fish genus and the lack of genetic data.

In conclusion, the present report contributes to the existing knowledge about the genus *Myxobolus*, providing both morphological and molecular data on a novel species of *Myxobolus*, *M. compostellanus* n. sp., infecting the gastrointestinal tract of a salmonid, the brown trout (*S. trutta*), and confirming that the species is closely related to other species of *Myxobolus* that infect salmonids.

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Declarations

Conflict of interest The authors declare no competing interests.

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