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FISH PARASITOLOGY - ORIGINAL PAPER

A new species of *Myxobolus* (Myxozoa: Bivalvulida) infecting the medulla oblongata and nerve cord of brook trout *Salvelinus fontinalis* in southern Appalachia (New River, NC, USA)

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Abstract

Myxobolus neurofontinalis n. sp. infects the brain and medulla oblongata of brook trout (*Salvelinus fontinalis* [Mitchill, 1814]) in the New River, western NC. It is the first species of *Myxobolus* described from the brook trout and resembles another congener (*Myxobolus arcticus* Pugachev and Khokhlov, 1979) that infects nerve tissue of chars (*Salvelinus* spp.). The new species differs from *M. arcticus* and all congeners by myxospore dimensions and by having a mucous envelope and distinctive sutural markings. A phylogenetic analysis of the small subunit rDNA (18S) suggests that the new species shares a recent common ancestor with some isolates identified as *M. arcticus* and that the new species and its close relatives (except *Myxobolus insidiosus* Wyatt and Pratt, 1973) comprise a clade of salmonid nerve-infecting myxobolids. The phylogenetic analysis indicates that several isolates of "*M. arcticus*" (*sensu lato*) in GenBank are misidentified and distantly related to other isolates taken from the type host (*Oncorhynchus nerka* [Walbaum, 1792]) and from nearby the type locality (Kamchatka Peninsula, Russia). Serial histological sections of infected brook trout confirmed that myxospores of the new species are intercellular and infect nerve cord and medulla oblongata only. A single infected brook trout showed an inflammatory response characterized by focal lymphocytic infiltrates and eosinophilic granulocytes; however, the remaining 4 brook trout lacked evidence of a histopathological change or demonstrable host response. These results do not support the notion that this infection is pathogenic among brook trout.

Keywords *Myxobolus* · Salmonidae · Nerve tissue · Myxozoan · Taxonomy

Introduction

In general, there is a lack of taxonomic information on the species of *Myxobolus* Bütschli, 1882 (Bivalvulida: Myxobolidae) that infect salmonids in the Southeastern US and that infect chars (*Salvelinus* Richardson, 1836 [Salmoniformes: Salmonidae]) worldwide. Eighteen species of *Myxobolus* infect salmonids (Table 1) but only 1 (*Myxobolus cerebralis* Hofer, 1903) has so far has been reported from salmonids in the Southeastern US (Ruiz et al. 2017). Worldwide, 6 species of *Myxobolus* infect chars, compared with the 11 species of *Myxobolus* reported from species of *Oncorhynchus* Suckley, 1861 (Salmoniformes: Salmonidae) (Tables 1 and 2). Of these, only *M. cerebralis*, *Myxobolus neurobius* Schuberg and Schröder, 1905, and *Myxobolus ovoidalis* Fantham, 1930 reportedly infect the brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (Table 2). *Myxobolus cerebralis* has been confirmed infecting brook trout by morphological and molecular techniques (Baldwin et al. 1998; Kaeser et al. 2006; Lorz et al. 1989; Ruiz et al. 2017). *Myxobolus neurobius* was reported to be a common parasite of brook trout in the stream supplying water to a Pennsylvania trout hatchery, but no specimen was diagnosed nor was the tissue site of infection in the host specified

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Table 1 *Myxobolus* spp. that infect salmonids or that are morphologically and/or molecularly similar to *Myxobolus neurofontinalis* n. sp.

Species	Host	GenBank No.	MXL	MXW	MXT	PCL	PCW	PCE	PFC	IP	Site	LOC	Reference
*M. arcticus**	*Oncorhynchus nerka*	–	14.3–16.5	–	7.6–7.7	6.6–9.5	2.5–3.5	–	–	P	1	RUS	Pugachev and Khokhlov 1979
M. cerebralis	*Oncorhynchus mykiss*	EF370478	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	Y	5–6	A	2	GER	Lom and Hoffman 1971
M. evdokimovae	*Coregonus albula*	–	10.0–11.0	8.7–9.2	–	5.0–5.2	3.1–3.3	Y	–	P	3	RUS	Eiras et al. 2005
M. farionis	*Salmo trutta*	–	9.1; 8.5–10 0	6.6; 6.0– 7.5	4.7; 4.5–5.0	4.8; 4.5–5.5	2.3; 2.0–2.8	Y	8–9	P	1	SPA	Eiras et al. 2005
*M. fomenai**	*Oreochromis niloticus*	KX632947	15.0; 13.0–16.5	7.9; 6.2–9.0	6.3; 5.2–7.0	9.2; 8.4–10.0	2.7; 2.3–3.5	Y	6–7	P	4	EGY	Abdel-Ghaffar et al. 2008
*M. fryeri**	*Oncorhynchus kisutch*	EU346372	12.9; 11.1–14.8	8.6; 7.2–10.1	7.2; 6.4–7.7	6.9; 5.9–8.1	2.8; 2.0–3.3	N	8–10	–	5	OR, USA	Ferguson et al. 2008
M. ibericus	*Salmo trutta*	–	10.0; 9.0–11.0	8.6; 8.0–9.5	6.5; 6.0–7.0	4.9; 4.0–6.0	2.6; 2.2–3.5	N	7–8	P	6, 7	SPA	Eiras et al. 2005
M. insidiosus	*Oncorhynchus tshawytscha*	EU346373	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	–	–	–	4	OR, USA	Ferguson et al. 2008
M. kisutchi	*Oncorhynchus kisutch*	AB469988	9.4; 8.6–10.1	8.0; 7.2–9.0	5.6; 5.2–6.2	5.2; 4.5–6.0	2.8; 2.3–3.1	–	6–8	–	1	WA, USA	Urawa et al. 2009
M. krokhni	*Salvelinus alpinus*	–	9.6–12.0	7.5–10.5	6.6–6.9	5.0–6.6	2.4–4.0	Y	–	P	8	RUS	Eiras et al. 2005
M. murakamii	*Oncorhynchus masou*	AB469984	9.2; 10.3–12.1	9.9; 9.2–10.9	7.1; 6.2–7.8	5.1; 4.0–5.5	3.0; 2.4–3.9	Y	5–8	P	5	HKD, JPN	Urawa et al. 2009
M. neurobius	*Salmo trutta*	AB469986	9.2; 8.6–10.5	7.6; 7.0–8.2	6.1; 5.4–7.0	5.0; 3.9–5.5	2.4; 2.1–2.7	–	6–8	–	1	NOR	Urawa et al. 2009
***M. neurofontinalis* n. sp.**	***Salvelinus fontinalis***	**–**	**13.9; 12.0–16.0**	**9.6; 8.0–12.0**	**8.1; 7.0–10.0**	**7.8; 6.0–10.0**	**3.5; 3.0–5.0**	**Y**	**7–10**	**P**	**1**	**NC, USA**	**Present study**
M. neurotropus	*Ocorhynchus mykiss*	DQ846661	11.8; 11.2–13.0	10.8; 10.4–12.3	8.8; 8.4–9.1	5.9; 5.0–6.9	3.7	N	6–8	P	1	ID, USA	Hogge et al., b
M. salmonis	*Oncorhynchus keta*	–	8.2–10.4	7.4–9.5	5.5–8.3	3.6–5.8	2.1–3.4	Y	5–6	A	9	RUS	Eiras et al. 2005
M. soldatovi	*Oncorhynchus keta*	–	8.0–9.5	–	–	4.0–4.2	2.2–2.2	Y	–	–	10	AMB	Eiras et al. 2005
M. sphaeralis	*Coregonus fera*	–	9	9	–	–	–	–	8–18	–	12	SWI	Eiras et al. 2005
M. squamalis	*Oncorhynchus mykiss*	JX910362	7.3–10.2	6.1–9.6	5.2–7.2	3.3–5.1	2.1–3.9	Y	4–6	A	9	OR, USA	Polley et al. 2013
M. thymalli	*Thymallus arcticus*	–	9.0–11.0	8.0–10.5	5.9–7.2	5.2–6.5	2.7–3.6	Y	–	P	11	RUS	Eiras et al. 2005
M. vartanyanae	*Salmo ischan*	–	9.5–12.3	8.5–10.0	7.3–8.0	4.5–6.0	2.5–3.3	N	4–5	P	4	UKR	Eiras et al. 2005

*A species that has a myxospore that is pyriform, has overlapping measurements with *Myxobolus neurofontinalis* n. sp., and has an intercapsular process

MXL, myxospore length; *MXW*, myxospore width; *MXT*, myxospore thickness; *PCL*, polar capsule length; *PCW*, polar capsule width; *PCE*, polar capsules equal (yes or no); *PFC*, polar filament coils; *IP*, intercapsular process (present or absent)

Site: *1*, central nervous system; *2*, cranial cartilage; *3*, buccal cavity; *4*, somatic muscle; *5*, lateral line nerves; *6*, kidney; *7*, spleen; *8*, serosa; *9*, scales; *10*, epidermis; *11*, gall bladder; *12*, gill

LOC, locality; *NOR*, Norway; *USA*, United States of America; *ID*, Idaho; *WA*, Washington; *OR*, Oregon; *NC*, North Carolina; *RUS*, Russia; *JPN*, Japan; *HKD*, Hokkaido; *GER*, Germany; *SPA*, Spain; *EGY*, Egypt; *AMB*, Amur Basin; *SWI*, Switzerland; *UKR*, Ukraine

Table 2 Records of *Myxobolus* spp. infecting chars (*Salvelinus* spp.)

Myxozoan species	Host species	Locality	Site of infection	Morph	Nucl	Reference
M. arcticus	*S. alpinus*	Norway	Central nervous system (CNS)	Yes	18S	Urawa et al. 2009
M. arcticus	*S. alpinus*	Quebec, Canada	Brain	No	No	Desdevises et al. 1998
M. arcticus	*S. leucomaenis*	Hokkaido, Japan	CNS	Yes	18S	Urawa et al. 2009
M. arcticus	*S. malma*	Russia	Brain	Yes	No	Nagasawa et al. 1994
M. arcticus	*S. neiva*	Russia	CNS	Yes	No	Pughachev & Khokholov 1979
M. cerebralis	*S. confluentus*	Lab exposure	Cartilage	Yes	18S	Hedrick et al. 1999
M. cerebralis	*S. fontinalis*	Oregon, USA	Cartilage	Yes	18S	Baldwin et al. 1998
M. krokhini	*S. alpinus*	Russia	Serosa	Yes	No	Eiras et al. 2005
M. neurobius	*S. alpinus*	Newfoundland, Canada	Brain	Yes	No	Maloney et al. 1991
M. neurobius	*S. fontinalis*	Pennsylvania, USA	Not specified	No	No	O'Grodnick 1979
M. neurobius	*S. namaycush*	Yukon Territory, Canada	Brain	No	No	Arthur et al. 1976
M. neurofontinalis n. sp.	*S. Fontinalis*	North Carolina, USA	CNS	Yes	18S	Present study
M. neurotropus	*S. confluentus*	Idaho, USA	CNS	Yes	18S	Hogge et al. 2008b
M. ovoidalis	*S. fontinalis*	Quebec, Canada	Skin	Yes	No	Fantham et al. 1939

The columns "Morph" (morphological features) and "Nucl" (nucleotide sequences) indicate how myxospores were diagnosed in the cited reference

(O'Grodnick 1979). *Myxobolus ovoidalis*, which infects skin of South African minnows (Cyprinidae), was reported from the skin of a single brook trout in Quebec (Fantham et al., 1939); its myxospore dimensions were reported, but no voucher material or molecular data exists. Since then, no confirmed infection of *M. ovoidalis* has been reported in a salmonid.

Three salmonid genera include species commonly known as "trouts." *Salmo* spp. (Salmoniformes: Salmonidae) are endemic to northern hemisphere rivers that drain into the Atlantic Ocean; *Oncorhynchus* spp. are endemic to northern hemisphere rivers draining into the Pacific Ocean; and the chars (*Salvelinus* spp.) have a circumpolar distribution in the northern hemisphere (Bean 1888; Power 2002). Rainbow trout (*Oncorhynchus mykiss* [Walbaum, 1792]) and brown trout (*Salmo trutta* Linnaeus, 1757) have been introduced to the Southeastern US (Flebbe 1994), whereas brook trout is the only salmonid endemic to that region (Flebbe 1994). For that reason, resource managers in the Southeastern US are particularly interested in the parasites and potential pathogens of native brook trout populations, especially if those parasites are related to demonstrable pathogens of wild trout populations, e.g., *M. cerebralis*.

On 11 April 2018, 17 brook trout were collected from the New River near Boone, NC, and processed using the pepsin–trypsin digest (PTD) (Markiw and Wolf 1974) and the nested PCR for the detection of infection by *M. cerebralis* (see Andree et al. 1998). During this process, we observed a myxospore morphotype in the digested samples that was distinct from that of *M. cerebralis* and that subsequently did not amplify by the nested PCR for *M. cerebralis* (Fig. 1). Motivated by those results and based upon the examination of additional brook trout for infections using light microscopy and serial sectioning, we herein describe a new species of *Myxobolus* from the central nervous system (CNS; medulla oblongata and nerve cord) of brook trout in North Carolina. The new species comprises the 2nd species of *Myxobolus* reported from a salmonid in the Southeastern US, the 2nd reported from a char in the region, and the 7th from a char worldwide.

Materials and methods

On 11 April 2018, 17 brook trout, 5 brown trout, and 9 rainbow trout were collected from the New River (36° 10′ 58.56″ N, 081° 36′ 37.96″ W), placed on ice, and shipped overnight to Auburn University. Upon arrival, fish were measured (standard length [SL, mm], total length [mm]), weighed (g), and processed for detection of *M. cerebralis* (as per the animal health inspection guidelines in Fish Health Section 2, Chapter 5.2 of the Blue Book [USFWS and AFS-FHS 2014]). The head of each fish was removed and longitudinally bisected, with 1 half being frozen overnight then subjected to PTD (Markiw and Wolf 1974) and the other half being archived. Each PTD contained no more than 5 pooled half heads with no mixing of species. Each pellet resulting from the PTD was examined for 10 min using a Zeiss Axioskop with 40× objective and differential interference contrast (DIC) optical components. Pellets were subjected to the confirmatory *M. cerebralis*–specific nested PCR (Andree et al. 1998).

On 16 Aug 2018, 30 brook trout, 15 brown trout, and 4 rainbow trout were collected from the New River (36° 10′ 58.56″ N, 081° 36′ 37.96″ W), placed on ice, and shipped

Fig. 1 Myxospores of *Myxobolus neurofontinalis* Ksepka and Bullard n. sp. (Bivalvulida: Myxobolidae) from a pepsin–trypsin digested head of brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (Salmoniformes: Salmonidae) from the New River, NC; photographed using differential interference contrast optical components

overnight to Auburn University. Upon arrival, 6 brook trout, representing a range of sizes (83–150 mm standard length [SL]) were immediately fixed in 10% neutral buffered formalin (n.b.f) for histopathology. Biopsies (approximately 5 mm^3) of forebrain, midbrain, hindbrain, and nerve cord were examined for the presence of myxospores using a compound light microscope with aid of a 40× objective and DIC. Infected tissue was fixed in 10% n.b.f. or refrigerated in deionized water (myxospores intended for morphology) and 95% ethanol (myxospores for DNA extraction). Prevalence of the parasite was determined from the 16 Aug sample only because the previous sample was processed by pooling individual hosts as per AFS Blue Book.

Measurements of myxospores sourced from wet mounts of fresh (in water) and n.b.f.-fixed myxospores using a 63× oil immersion objective. Myxospores were prepared with Lugol's iodine and India ink to visualize the iodinophilic vacuole and mucous envelope, respectively (Lom and Arthur 1989). To illustrate myxospores, infected tissue was homogenized in water before a drop of that solution was cover-slipped, inverted, and placed onto a thin layer of 1% agar (Lom 1969). Illustrations were generated using 100× oil immersion objective on a Leica DMR compound scope equipped with 1.6× magnifier, DIC, and drawing tube.

After fixation, the 6 n.b.f.-fixed brook trout were grossed to fit into standard tissue processing cassettes. Fish < 100 mm SL were decapitated and the trunk cut into 1-cm-long portions. Fish ≥ 100 mm SL were processed with the additional step of removing the lower jaw from the head and removing the somatic muscle dorsal and ventral to the vertebral column. Collectively, this yielded 41 portions of tissue that were rinsed with distilled water for 2 h, immersed in EDTA for 1 month, processed routinely for histology by dehydration in an ethanol series, embedded in paraffin, sectioned at 4–7 μm, and stained with hematoxylin and eosin. The neurocranium of each brook trout was serially sectioned yielding 660 slides and approximately 2640 paraffin sections. Ten slides were cut from each trunk portion yielding > 1400 paraffin sections on 350 slides.

DNA was extracted from microscopically confirmed, infected nerve tissue using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's protocol. Before DNA extraction and to confirm the morphological identity of the myxospores, myxospores were whole-mounted as a fresh preparation on a glass slide, cover-slipped, and examined with a compound microscope equipped with DIC. DNA concentration was measured using a NanoDrop ND–1000 spectrophotometer (Thermo Scientific, Nanodrop Technologies), diluted to 50 ng/μl, and stored at − 20 °C. A 1113 base pair region of the 18s (rDNA) was amplified using primers M153-F (5′-CATTGGATAACCGTGGGAAATCT-3′) and M1480-R (5′-GTGGTGCCCTTCCGTCAATTCC-3′). The following thermocycle was used: initial denaturation

step of 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, with a final extension step of 72 °C for 5 min. DNA sequencing was performed by ACGT, Incorporated (Wheeling, IL, USA).

The phylogenetic analysis included 1 isolate from each salmonid-infecting *Myxobolus* species available in GenBank (Table 1), all isolates of *Myxobolus arcticus* Pugachev and Khokalov, 1979 that are linked to morphologically diagnosed myxospores within the source publication (Table 3), 20 species of Myxozoa from clade B in Naldoni et al. (2019), and 2 species of *Parvicapsula* Shulman, 1953 (Bivalvulida: Parvicapsulidae) as an outgroup as per Naldoni et al. (2019). We prioritized the use of myxozoan sequences tethered to a morphological diagnosis to avoid error cascades and erroneous conclusions. Sequences were aligned using MAFFT and trimmed to the length of the shortest sequence (874 base pairs) in the analysis (Katoh and Standley 2013). JModelTest2 version 2.1.10 selected the best-fit models of nucleotide substitution based on Bayesian information criteria (Darriba et al. 2012). Bayesian inference (BI) was performed in MrBayes version 3.2.5 (Ronquist and Huelsenbech 2003) using substitution model averaging (Bnst-mixed) and a gamma distribution to model rate-heterogeneity. Three runs with 4 Metropolis-coupled chains were run for 5,000,000 generations. Stationarity was checked in Tracer 1.7 (Rambaut et al. 2018), and an appropriate burn-in of 25% of generations was determined. Evidence of convergence was further checked with the "sump" command in MrBayes. All parameters had a potential scale reduction factor of 1.00. A majority rule consensus tree of the post burn-in posterior distribution was generated with the Bsumt^ command in MrBayes. The phylogenetic tree was visualized in FigTree v1.4.3 (Rambaut et al. 2014) and edited with Adobe Illustrator (Adobe Systems).

Results

Myxobolus neurofontinalis Ksepka and Bullard n. sp. (Figs. 2, 3, 4, and 5)

Diagnosis of myxospores (based on 87 myxospores using differential interference microscopy [25 stained with Lugol's iodine; 15 stained with India ink]; USNM coll. Nos. 1578999–1579006): Myxospore comprising 2 asymmetrical valves juxtaposed at sutural rim (1 smooth valve plus 1 valve more convex), 2 polar capsules, sporoplasm, and mucous envelope; Myxospore pyriform, 12.0–16.0 μm (mean ± SD = 13.9 ± 0.9; N = 83) long, 8.0–12.0 (9.6 ± 0.8; 74) wide, 7.0–10.0 (8.1 ± 0.7; 17) thick; sutural rim with prominent seam, 1.0–2.0 (1.1 ± 0.1; 75) thick (lateral margin in frontal view), 1.0–2.0 (1.6 ± 0.5; 75) thick (posterior margin in frontal view), without flanking lateral ridges (sutural), having sutural markings (0–4 [2.8 ± 0.8; 69] in number); polar capsules clavate, equal (28

Table 3 Records and associated morphological and nucleotide information for *Myxobolus arcticus*

Isolate	Host species	GenBank No.	MXL	MXW	MXT	PCL	PCW	PFC	Locality	Reference
M. arcticus 1	*Oncorhynchus nerka*	AB353129	13.2; 11.9–14.4	8.4; 7.8–9.4	6.6; 5.5–7.0	8.3; 7.0–9.5	3.1; 2.3–3.7	9–13	Hokkaido, Japan	Urawa et al. 2009
M. arcticus 2	*Oncorhynchus nerka*	HQ113227	13.2; 11.9–14.4	8.4; 7.8–9.4	6.6; 5.5–7.0	8.3; 7.0–9.5	3.1; 2.3–3.7	9–13	British Columbia, Canada	Urawa et al. 2009
M. arcticus 3	*Oncorhynchus masou*	AB469990	14.1; 13.3–15.6	8.7; 7.8–9.4	–	8.4; 7.5–9.0	3.1; 2.6–3.5	–	Hokkaido, Japan	Urawa et al. 2009
M. arcticus 4	*Oncorhynchus keta*	AB469991	14.0; 13.3–14.8	9.3; 8.6–10.1	6.3; 5.9–7.0	7.3; 6.6–7.8	3.1; 2.7–3.9	8–11	Hokkaido, Japan	Urawa et al. 2009
M. arcticus 5	*Salvelinus alpinus*	AB469992	14.4; 13.1–16.0	10.6; 9.8–11.1	7.8; 7.2–8.	7.8; 7.2–8.6	3.8; 3.1–4.3	8–10	Norway	Urawa et al. 2009
M. arcticus 6	*Salvelinus leucomaenis*	AB469993	13.5; 11.9–15.0	9.8; 8.4–10.9	7.7; 6.9–8.9	7.2; 5.8–8.5	3.7; 3.0–4.2	–	Hokkaido, Japan	Urawa et al. 2009

MXL, myxospore length; *MXW*, myxospore width; *MXT*, myxospore thickness; *PCL*, polar capsule length; *PCW*, polar capsule width; *PFC*, number of polar filament coils

of 70 myxospores; 40%) or subequal (42 of 70 myxospores; 60%) in size, 6.0–10.0 (7.8 ± 0.8; 153) long, 3.0–5.0 (3.5 ± 0.5; 159) wide, with 7–10 polar filament coils; sporoplasm containing spheroid iodinophilic vacuole, having 2 nuclei; intercapsular process present, 1.0–3.0 (2.3 ± 0.5; 124) long, 1.0–2.0 (1.5 ± 0.5; 124) wide; mucous envelope prominent on rounded posterior margin, 2.0 μm (2.0 ± 0.0; 3) thick.

Taxonomic summary

Type host: *Salvelinus fontinalis* (Mitchill, 1814) (Salmoniformes: Salmonidae).

Site in host: Intercellular, infecting nerve cord and medulla oblongata.

Type locality: East Fork South Fork New River (36° 10′ 58.56″ N, 081° 36′ 37.96″ W), NC.

Prevalence: Myxospores were detected in 23 of 30 (prevalence = 77%) brook trout

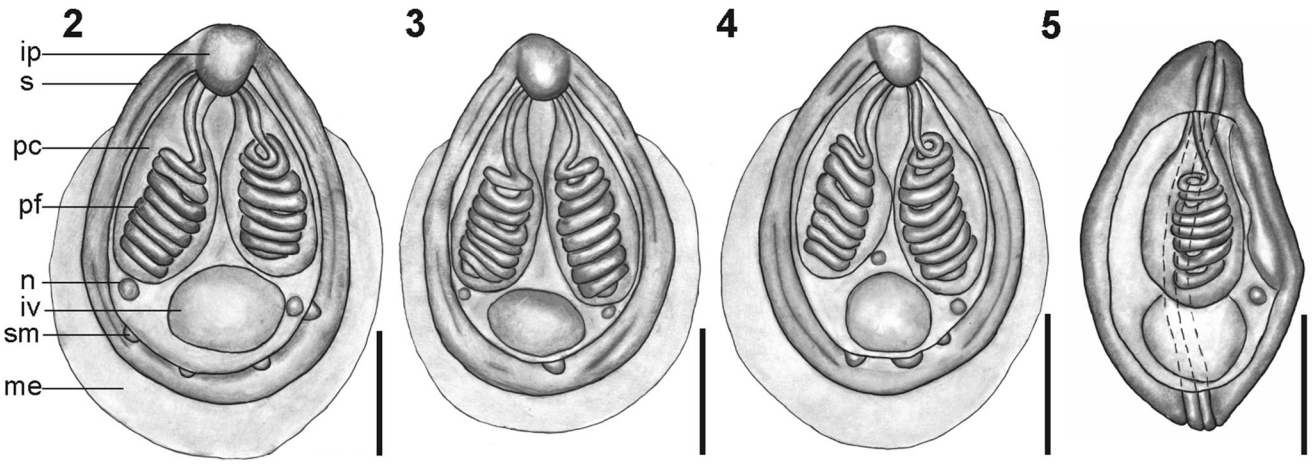
Specimens deposited: Myxospores of *M. neurofontinalis* fixed in 10% n.b.f. (1 vial; syntype; USNM 1578999), fixed in 10% n. b. f. within infected nerve cord (1 vial; syntype; USNM 1579000), and within paraffin sections (5 slides; syntypes; USNM 1579001–1579006); GenBank No. (18S: MN191598).

Etymology: The specific epithet refers to the apparent site and host specificity of this myxozoan species to the nerve cord and the medulla oblongata of brook trout.

Taxonomic remarks

The myxospore of the new species differs from that of all congeners by its dimensions and by having a mucous envelope and distinctive sutural markings. Of the 7 nominal species of *Myxobolus* that infect salmonid nerve tissue, 5 have a spheroid myxospore (*Myxobolus farionis* Gonzalez-Lanza and Alvarez-Pellitero, 1984, *M. neurobius*, *Myxobolus kisutchi* Yasutake and Wood, 1957, *Myxobolus neurotropus* Hogge, Campbell, and Johnson, 2008, and *Myxobolus murakamii* Urawa, Iida, Freeman, Yanagida, Karlsbakk, and Yokoyama, 2009), and the remaining 2 species (*M. arcticus* and *Myxobolus fryeri* Ferguson, Atkinson, Whipps and Kent, 2008) have a pyriform myxospore (Table 1). *Myxobolus arcticus* and *M. fryeri* have been diagnosed using a range of myxospore dimensions that variously overlap with the new species (Table 1). Of the species of *Myxobolus* that do not infect salmonid nerve tissue, only the myxospore of *Myxobolus fomenai* Abdel-Ghaffar, El-Toukhy, Al-Quraishy, Al-Rasheid, Abdel-Baki, Hegazy, and Bashtar, 2008 is pyriform, has similar dimensions and polar filament counts, and has an intercapsular process (Table 1). The new species differs from *M. fryeri*, which infects the lateral line nerve of *Oncorhynchus* spp., by having polar capsules of equal length and by having a myxospore with sutural markings and a mucous envelope and by lacking flanking lateral ridges on the sutural rim (Figs. 2, 3, 4, and 5). The myxospore of the new species differs from that of *M. fomenai* by having an iodinophilic vacuole, sutural markings, and a mucous envelope (Figs. 2, 3, and 4).

The myxospore of the new species is morphologically most similar to those described by Pugachev and Khokhlov (1979) as *M. arcticus* from the CNS of several salmonids in the Kamchatka Peninsula, Russia (Table 1). Subsequent reports have identified myxospores recovered from salmonids in other parts of the world as "*M. arcticus*," but we question those identifications based on the results of our phylogenetic analysis (Fig. 6). The new species and *M. arcticus* (*sensu stricto*), as diagnosed by Pugachev and Khokhlov (1979), both have a similarly sized myxospore having an iodinophilic vacuole



Figs. 2–5 Myxospores of *Myxobolus neurofontinalis* Ksepka and Bullard n. sp. (Bivalvulida: Myxobolidae) from prepared nerve tissue of brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (Salmoniformes: Salmonidae) from the New River, NC. **2**–**4** Frontal view. **5** Sutural view. ip, intercapsular process; sr, sutural rim; pc, polar capsules; pf, polar filaments; n, nuclei of the sporoplasm; iv, iodinophilic vacuole; sm, sutural markings; me, mucous envelope. Scale bars = 5 μm

and intercapsular process (Table 1). The myxospores diagnosed by Pugachev and Khokhlov (1979) lack a mucous envelope and sutural markings; however, both of these features are present in the myxospore of the new species (Figs. 2, 3, and 4), i.e., these myxozoans are clearly morphologically distinct. Regarding the taxonomic status of "*M. arcticus*" (*sensu lato*), a large range of myxospore measurements are associated with this identification: those from chars (*Salvelinus* spp.) are wider and thicker than those from Pacific salmon and trout (*Oncorhynchus* spp.) (Table 3). Myxospores of the new species are nearly the size of those of the myxospores previously reported from chars, but they are easily differentiated by the presence of a mucous envelope and sutural markings (Figs. 2, 3, and 4). Additional taxonomic comments regarding the status of the GenBank isolates ascribed to *M. arcticus* are detailed below (see sequence comparison and phylogenetic results).

Histopathology

The normal CNS of salmonids has been detailed by Nieuwenhuys and Pouwels (1983), Ferguson (2006), and Barton and Bond (2007). The nerve cord of uninfected brook trout is round in cross section and comprised of the central ependymal canal, a single dorsal horn, 2 ventral horns, and surrounding meninges. Dorsal and ventral horns are

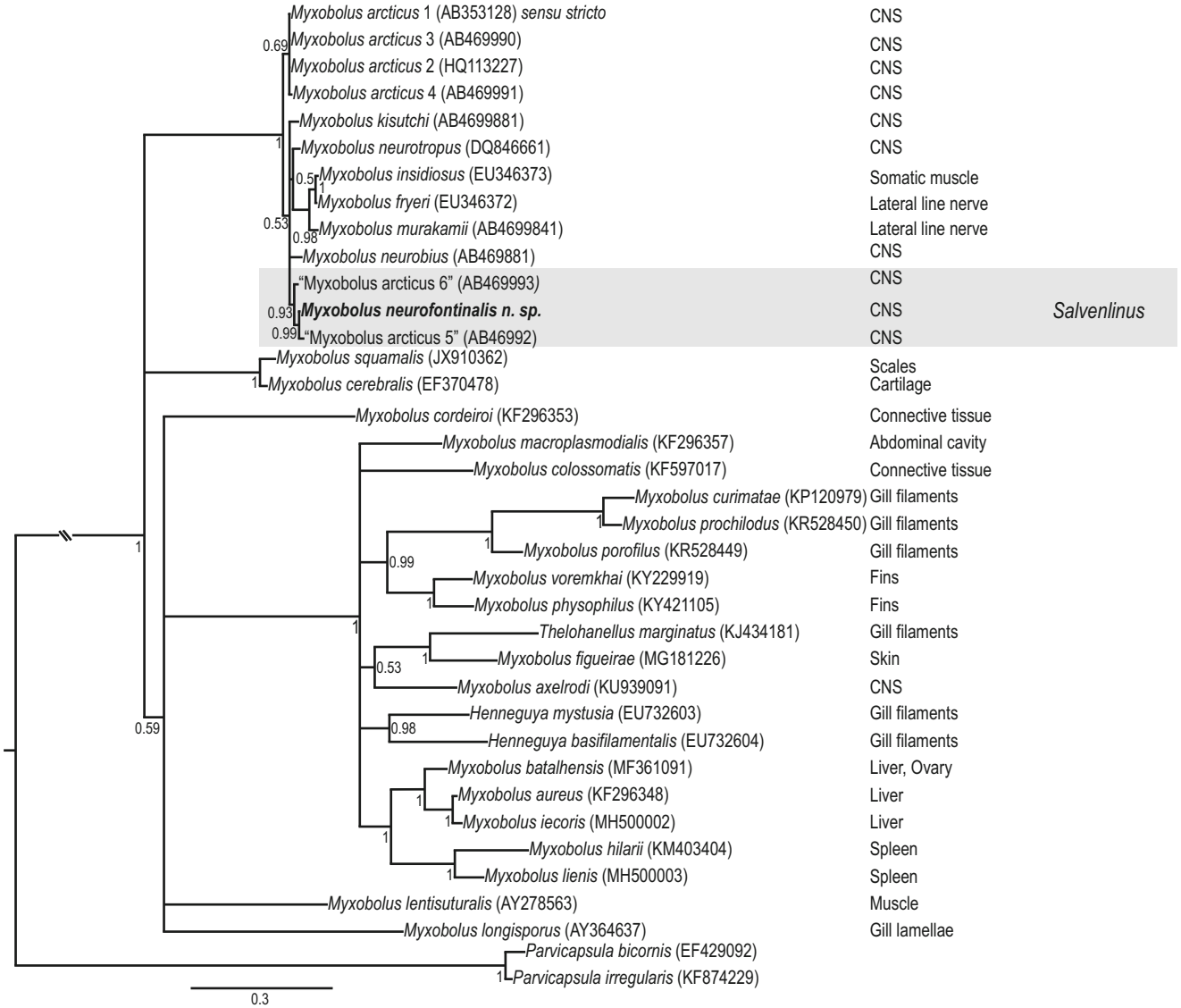


Fig. 6 Phylogenetic relationships (small subunit ribosomal DNA, 18S) of *Myxobolus* spp. Bütschli, 1882 (Bivalvulida: Myxobolidae) infecting salmonids and genetically similar species to *Myxobolus neurofontinalis* Ksepka and Bullard n. sp. reconstructed using Bayesian inference. *Salvelinus* Richardson, 1836 (Salmoniformes: Salmonidae) central nervous system (CNS) infecting species boxed in light gray. *Myxobolus arcticus* Pugachev and Khokhlov, 1979 isolates in quotes we consider not representative of the originally described species. Scale bar is in substitutions per site

comprised of gray matter and form an inverted Y shape, with gray matter being separated by regions of white matter. The medulla oblongata begins ventral to the cerebellum and extends caudally where it tapers into the nerve cord. It begins U shaped, the dorsal surface being the floor of the fourth ventricle, progressing caudally becomes circular in cross section with the fourth ventricle closing to become a hollow canal marking the beginning of the ependymal canal. The medulla oblongata is composed primarily of gray matter with a central area of white matter that contains the paired Mauthner cells (Nieuwenhuys and Pouwels 1983; Ferguson 2006; Barton and Bond 2007).

Of 6 brook trout processed for histopathology, 5 had myxospores in the CNS. Some myxospores were observed in variously sized intercellular masses (groups of myxospores) that were circular in outline but lacked a membranous enveloping structure (plasmodium). Others were loosely dispersed within the nerve tissue of the nerve cord and medulla oblongata (Figs. 7, 8, 9, 10, 11, and 12). No myxospore was detected in another tissue. In both nerve cord and medulla oblongata, compression of the neuropil surrounding myxospore masses was observed (Figs. 8 and 10). No demonstrable host cellular response was observed in the infected medulla oblongata (Figs. 7 and 8). Dispersed myxospores in the nerve cord were surrounded by an inflammatory response characterized by lymphocytic infiltrates and eosinophilic granulocytes (Figs. 11 and 12). Anecdotally, older (longer) brook trout were infected with a larger number of myxospore masses (foci), fish ≥ 100 mm SL having 1–51 foci and fish < 100 mm SL having 0–19 foci, perhaps suggesting that infections accumulate or proliferate over time but still do not seemingly exhibit demonstrable pathology even with relatively intense/dense infections.

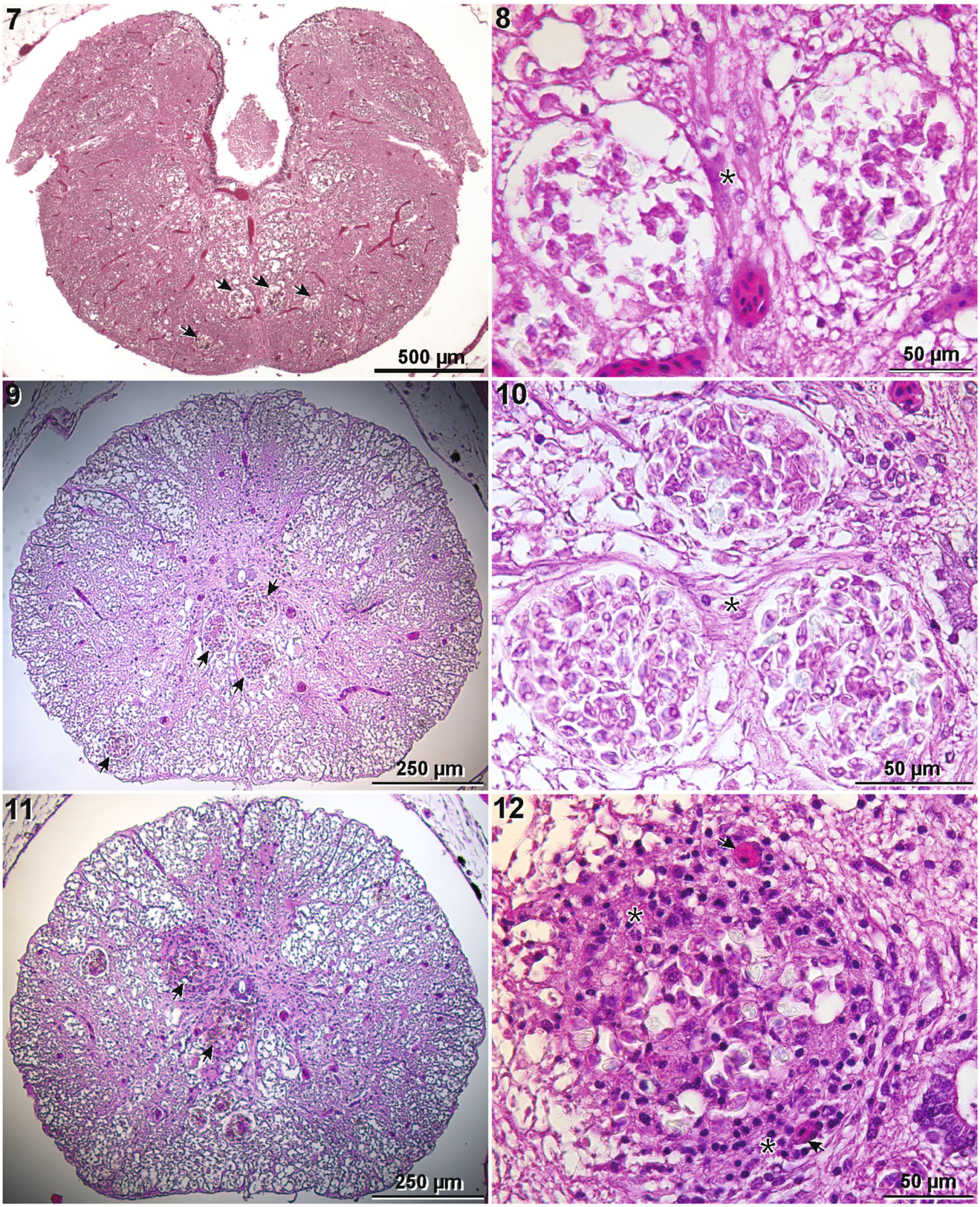
Sequence comparison and phylogenetic analysis

The amplified 18S fragment of the new species comprised 1113 nucleotides (aligned portion comprising 874 nucleotides). This sequence was > 96% similar to 9 sequences in NCBI GenBank that were collectively ascribed to 4 species of *Myxobolus*, all of which were included in the phylogenetic analysis (Fig. 6; Table 4). The 18S sequence of the new species differed from that of *M. neurobius* by 28 (3.3%) nucleotides, *M. neurotropus* by 25 (2.9%), *M. kisutchi* by 24 (2.8%), and "*M. arcticus*" (see above taxonomic issues) by 11–23 (1.3–2.4%) (isolate 4 by 23 [2.4%]; isolates 1, 2, and 3 by 21 [2.4%]; isolate 6 by 13 [1.4%]; isolate 5 by 11 [1.3%]) (Table 4).

Although the recovered tree slightly differs from others previously published, the placement of *Myxobolus* spp. infecting salmonid nerve tissue in a clade separate from *M. cerebralis* and the separation of myxospores previously identified as *M. arcticus* and infecting *Oncorhynchus* and *Salvelinus* is consistent with previously published trees (Urawa et al. 2009). The placement of *M. squamalis* as sister to *M. cerebralis* differs from published trees, which place it sister to *Myxobolus insidiosus* Wyatt and Pratt, 1963 (Carriero et al. 2013; Ferguson et al. 2008). We recovered 3 primary clades: (i) species that principally infect salmonid nerve tissue, (ii) *M. squamalis* + *M. cerebralis*, and (iii) remaining ingroup taxa (including 2 species of *Henneguya* Thélohan, 1892 [Bivalvulida: Myxobolidae]). The last clade was largely unresolved, comprising a polytomy with 3 lineages and a clade comprising a polytomy with 2 monotypic lineages and 4 clades. Low support values for *M. arcticus* isolates 1–4 (0.69) and the clade comprising all other salmonid CNS *Myxobolus* species (0.53) are worth noting because additional taxon and character sampling may alter the tree topology.

The species of *Myxobolus* infecting salmonid nerve tissue were monophyletic (except *M. insidiosus* infecting somatic muscle). The phylogenetic analysis recovered 2 clades comprising *Myxobolus* spp. that each infect the CNS only and that infect *Oncorhynchus* spp. (isolates 1–4 of "*M. arcticus*") and chars (the new species plus isolates 5–6 of "*M. arcticus*" infecting *Salvelinus* spp.), respectively (Fig. 6). *Myxobolus arcticus* isolates 1–4 have > 99.4% or greater sequence similarity and likely comprise a single species infecting *Oncorhynchus* spp. (Table 4). *Myxobolus arcticus* isolate 1 was sourced from Hokkaido, Japan, nearest the type locality (Kamchatka Peninsula, Russia) for *M. arcticus* and from the type host, *Oncorhynchus nerka* (Walbaum, 1792). We regard *M. arcticus* isolates 1–4 as representing *M. arcticus* (*sensu stricto*) (given that no voucher material exists from the original description of *M. arcticus*, given that isolate 1 sources from the type host nearby the type locality, and given that isolates 1–4 are nearly identical). *Myxobolus arcticus* (*sensu lato*) isolates 5 and 6 infect chars, have < 97.7% sequence similarity to *M. arcticus* isolates 1–4 (infecting *Oncorhynchus* spp.), do not share a recent common ancestor with *M. arcticus* isolates 1–4, and therefore comprise a species of *Myxobolus* that is clearly distinct from *M. arcticus*. The

Figs. 7–12 Transverse histological sections (hematoxylin and eosin) of brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (Salmoniformes: Salmonidae) medulla oblongata and nerve cord infected with *Myxobolus neurofontinalis* Ksepka and Bullard n. sp. (Bivalvulida: Myxobolidae). **7** Low magnification view of infected medulla oblongata showing foci of myxospores (arrows). **8** High magnification view of **7** showing compression of the neuropil surrounding myxospores (*). **9** Low magnification view of infected nerve cord showing circular foci of myxospores (arrows). **10** High magnification view of **9** showing compression of neuropil surrounding foci of myxospores (*). **11** Low magnification view of infected nerve cord showing dispersed myxospores (arrows). **12** High magnification view of dispersed myxospores in **11** showing surrounding lymphocytic infiltrates (*) and eosinophilic granulocytes (arrows)



new species is sister to myxospores previously identified as *M. arcticus* isolate 5, and together they share a recent common ancestor with myxospores previously identified as *M. arcticus* isolate 6. The identity of these isolates of *M. arcticus* is indeterminate, but clearly some sequences ascribed to this myxozoan have been misidentified and need resolution.

Table 4 Pairwise differences in 18S rDNA sequences among *Myxobolus neurofontinalis* n. sp. and all nominal congeners that infect the central nervous system of salmonids

Myxozoan species	GenBank No.		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Myxobolus neurofontinalis		(1)	–	98.7	98.6	97.6	97.6	97.6	97.3	97.2	97.1	96.7
Myxobolus arcticus 5	AB469993	(2)	11	–	97.3	97.6	97.6	97.6	97.6	97.5	97.3	96.5
Myxobolus arcticus 6	AB469992	(3)	13	24	–	96.4	96.4	96.4	96.2	95.8	95.9	95.5
Myxobolus arcticus 1	AB353129	(4)	21	21	32	–	100	100	99.5	97.3	96.9	96.7
Myxobolus arcticus 3	AB469990	(5)	21	21	32	0	–	100	99.5	97.3	96.9	96.7
Myxobolus arcticus 2	HQ113227	(6)	21	21	32	0	0	–	99.5	97.3	96.9	96.7
Myxobolus arcticus 4	AB469991	(7)	23	21	34	6	6	6	–	97.1	96.7	96.3
Myxobolus kisutchi	AB469988	(8)	24	22	37	24	24	24	26	–	97.0	96.5
Myxobolus neurotropus	DQ846661	(9)	25	23	36	26	26	26	29	26	–	96.7
Myxobolus neurobius	AB469986	(10)	28	30	39	28	28	28	32	30	28	–

Discussion

The description of *M. arcticus* is incomplete, and some GenBank sequences ascribed to this taxon evidently comprise misidentifications. Pugachev and Khokhlov (1979) reported myxospore length and thickness, polar capsule length and width, intercapsular process presence, and iodinophilic vacuole presence based on observations of myxospores taken from 4 host species (*O. nerka*, *Oncorhynchus kisutch* [Walbaum 1792], *Salvelinus malma* [Walbaum 1792], and *Salvelinus nieva* [Taranetz 1933]) (Tables 1 and 2). That description lacks detail of myxospore width, number of polar filament coils, or presence/absence of the sutural markings, the mucous envelope, and the flanking lateral ridge (Pugachev and Khokhlov 1979). Subsequently, myxospores identified as *M. arcticus* have been reported from 3 species of *Oncorhynchus* and 2 species *of Salvelinus* (Table 3) (Urawa et al. 2009). We inferred that *M. arcticus* isolate 1 is conspecific with Pugachev and Khokhlov's (1979) specimens because it was collected from the type host, nearest the type locality, and the same tissue in addition to having overlapping myxospore dimensions. Based on the incomplete description combined with the variability of myxospore dimensions and sequence similarity of isolates, we consider *M. arcticus* a *species inquirenda* that is nevertheless distinct from the new species. Hence, Urawa et al.'s (2009) identifications of "*M. arcticus*" isolates 5 and 6 as misidentifications, and pending a more detailed morphological diagnosis, perhaps are conspecific with the new species herein.

We detected 3 potential errors in GenBank pertaining to *Myxobolus* spp., each of which lacked a morphological diagnosis. First, the sequence representing the isolate of *M. squamalis* (U96495) used in previous analyses differs from another presumptive conspecific isolate (JX910362) by 22% (99 nucleotides) (Andree et al. 1997; Polley et al. 2013). Second, several authors (Andree et al. 1999; Bahri et al. 2003; Easy et al. 2005; Ferguson et al. 2008) have recovered "*M. arcticus*" (AF085176) sister to several cyprinid-infecting *Myxobolus* spp.; however, this isolate differs from *M arcticus* isolate 1 (AB353129) by 14% (106 nucleotides) (Urawa et al. 2009). Third, several authors (Andree et al. 1997; Easy et al. 2005; Yokoyama et al. 2007) recovered *M. insidiosus* (U96494) sister to *M. cerebralis*; however, that sequence differs from the morphologically diagnosed isolate of *M. insidiosus* (EU346373) by 14% (106 nucleotides) (Ferguson et al. 2008).

Misidentifying species of *Myxobolus* can lead to false positives in diagnostic screening. Several species of *Myxobolus* represent salmonid pathogens. *Myxobolus cerebralis*, infecting the cranial cartilage of salmonids and the causative agent of whirling disease, causes skeletal deformities and impaired swimming behavior (Bartholomew and Reno 2002). Whirling disease has been associated with declines in wild trout populations (Nehring and Walker 1996; Vincent 1996; Thompson et al. 1999). *Myxobolus murakamii*, infecting the nervous system of masu (*Oncorhynchus masou masou*) (Brevoort, 1856) and amago salmon (*Oncorhynchus masou ishikawae*) Jordan and McGregor, 1925, is the causative agent of myxosporean sleeping disease and impairs swimming in cultured pacific salmon in Japan (Urawa et al. 2009). Myxospores of the new species show a high degree of tissue tropism to nerve tissue of the medulla oblongata and nerve cord. Salmonid CNS-infecting species of *Myxobolus* are evidently not routinely pathogenic (Gonzalez–Lanza and Alvarez–Pellitero 1984; Maloney et al. 1991; Kent et al. 1993; Hogge et al., 2008a, b). Since CNS-infecting species can be detected while screening for *M. cerebralis*, reliable morphological identification (and readily differentiating them from *M. cerebralis*) is critically important in the context of biosecurity and hatchery checks. False positives can have significant economic and conservation impacts for culture facilities and state agencies: facilities positive for infection by *M. cerebralis*

can be forced to cease stocking or destroy trout in the hatchery (Hoffman 1990; Bartholomew and Reno 2002). Given the relative morphological similarity of the new species to *M. cerebralis*, the present work highlights the importance of detailed morphological diagnosis coupled with nucleotide sequences tethered to morphological vouchers in myxozoan disease diagnostics.

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Compliance with ethical standards All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Conflict of interest The authors declare they have no conflict of interest.

References

Abdel-Ghaffar F, El-Toukhy A, Al-Quraishy S, Al-Rasheid K, Abdel-Baki AS, Hegazy A, Bashtar AR (2008) Five new myxosporean species (Myxozoa: Myxosporea) infecting Nile tilapia *Oreochromis niloticus* in Bahr Shebin, Nile Tributary, Nile Delta, Egypt. Parasitol Res 103:1197–1205

Andree KB, Gresoviac SJ, Hedrick RP (1997) Small subunit ribosomal RNA sequences unite alternate actinosporean and myxosporean stages of *Myxobolus cerebralis* the causative agent of whirling disease in salmonid fish. J Eukaryot Microbiol 44:208–215

Andree KB, MacConnell E, Hedrick RP (1998) A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. Dis Aquat Org 34:145–154

Andree KB, Székely C, Molnár K, Gresoviac SJ, Hedderick RP (1999) Relationships among members of the genus *Myxobolus* (Myxozoa: Bivalvidae) based on small subunit ribosomal DNA sequences. J Parasitol 85:68–74

Arthur JR, Margolis L, Arai HP (1976) Parasites of fishes of Aishihik and Stevens Lakes, Yukon Territory, and potential consequences of their interlake transfer through a proposed water diversion for hydroelectrical purposes. J Fish Res Board Can 33:2489–2499

Bahri S, Andree KB, Hedrick RP (2003) Morphological and phylogenetic studies of marine *Myxobolus* spp. from mullet in Ichkeul Lake, Tunisia. J Eukaryot Microbiol 50:463–470

Baldwin TJ, Peterson JE, McGhee GC, Staigmiller KD, Motteram ES, Downs CC, Stanek DR (1998) Distribution of *Myxobolus cerebralis* in salmonid fishes in Montana. J Aquat Anim Heal 10:361–371

Bartholomew JL, Reno PW (2002) The history and dissemination of whirling disease. Am Fish Soc Symp 26:1–22

Barton M, Bond CE (2007) Bond's biology of fishes, 3rd edn. Thomson Brooks, Belmont, California

Bean TH (1888) Distribution and some characters of the Salmonidae. Am Nat 22:306–314

Carriero MM, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2013) Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. PLoS ONE 8:e73713. https://doi.org/10.1371/journal.pone.0073713

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics, and parallel computing. Nat Methods 9:772

Desdevises Y, Arthur JR, Pellerin-Massicotte J (1998) Parasites of anadromous arctic char (*Salvelinus alpinus*) from two sites in Ungava Bay (Quebec, Canada). J Helminthol Soc Wash 65:87–90

Easy RH, Johnson SC, Cone DK (2005) Morphological and molecular comparison of *Myxobolus procerus* (Kudo,1934) and *M. intramusculi* n. sp. (Myxozoa) parasitizing the muscles of the trout–perch *Percopsis omiscomaycus*. Syst Parasitol 61:115–122

Eiras JC, Molnár K, Lu YS (2005) Synopsis of species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 61:1–46

Fantham HB, Porter A, Richardson LR (1939) Some Myxosporidia found in certain freshwater fishes in Quebec province, Canada. Parasitol 31:1–77

Ferguson HW (2006) Systemic pathology of fish: a text and atlas of normal tissues in teleosts and their responses to disease, 2nd edn. Scotian Press, London

Ferguson JA, Atkinson SD, Whipps CM, Kent ML (2008) Molecular and morphological analysis of myxobolus spp. of salmonid fishes with the description of a new *Myxobolus* species. J Parasitol 94:1322–1334

Flebbe PA (1994) A regional view of the margin: salmonid abundance and distribution in the southern Appalachian Mountains of North Carolina and Virginia. Trans Am Fish Soc 123:657–667

Gonzalez-Lanza C, Alvarez-Pellitero MP (1984) *Myxobolus farionis* n. sp. and *M. ibericus* of *Salmo trutta* from the Duero basin (NW Spain). Description and population dynamics. Angew Parasitol 25:181–189

Hedrick RP, McDowell TS, Mukkatira K, Georgiadis MP, MacConnell E (1999) Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. J Aquat Anim Health 11:330–339

Hoffman GL (1990) *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. J Aquat Anim Health 2:30–37

Hogge CI, Campbell MR, Johnson KA (2008a) Redescription and molecular characterization of *Myxobolus kisutchi* Yasutake & Wood, 1957. J Fish Dis 31:707–712

Hogge CI, Campbell MR, Johnson KA (2008b) A new species of Myxozoan (Myxosporea) from the brain and spinal cord of rainbow trout (*Oncorhynchus mykiss*) from Idaho. J Parasitol 94:218–222

Kaeser AJ, Rasmussen C, Sharpe WE (2006) An examination of environmental factors associated with *Myxobolus cerebralis* infection of wild trout in Pennsylvania. J Aquat Anim Health 18:90–100

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Kent ML, Whitaker DJ, Margolis L (1993) Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, a myxosporean parasite of Pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (Lumbriculidae). Can J Zoology 71:1207–1211

Lom J (1969) On a new taxonomic character in Myxosporidia, as demonstrated in descriptions of two new species of *Myxobolus*. Folia Parasitol 16:97–103

Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. J Fish Dis 12:151–156

Lom J, Hoffman GL (1971) Morphology of the spores of *Myxosoma cerebralis* (Hofer 1903) and *M. cartilaginis* (Hoffman, Put, and Dunbar, 1965). J Parasitol 57:1302–1308

Lorz HV, Amandi A, Banner CR, Rohovec JS (1989) Detection of *Myxobolus* (*Myxosoma*) *cerebralis* in salmonid fishes in Oregon. J Aquat Anim Health 1:217–221

Maloney R, Crawford RJ, Markiw M, Groman D (1991) The occurrence of *Myxobolus neurobius* (Myxosporea) in wild young Atlantic salmon and arctic char in Newfoundland. J Aquatic Anim Health 3:146–147

Markiw ME, Wolf K (1974) *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements – sequential enzymatic digestions and purification by differential centrifugation. J Fish Res Board Can 31:15–20

Nagasawa K, Urawa S, Dubinin VA (1994) A parasitological survey of sockeye salmon (*Oncorhynchus nerka*) and dolly varden (*Salvelinus malma*) from the Ozernaya River System, Kamchatka. Sci Rep Hokkaido Salmon Hatch 48:17–21

Naldoni J, Zatti SA, Silva MRM, Maia AAM, Adriano EA (2019) Morphological, ultrastructural, and phylogenetic analysis of two novel *Myxobolus* species (Cnidaria: Myxosporea) parasitizing bryconid fish from São Francisco River, Brazil. Parasitol Int 71: 27–36

Nehring RB, Walker PG (1996) Whirling disease in the wild: the new reality in the intermountain west. Fish 21:28–30

Nieuwenhuys R, Pouwels E (1983) The brain stem of Actinopterygian fishes. In: Northcutt RG, Davis RE (eds) Fish Neurobiology. The University of Michigan Press, Ann Arbor, pp 25–88

O'Grodnick JJ (1979) Susceptibility of various salmonids to whirling disease (*Myxosoma cerebralis*). Trans Am Fish Soc 108:187–190

Polley TM, Atkinson SA, Jones GR, Bartholomew JL (2013) Supplemental description of *Myxobolus squamalis* (Myxozoa). J Parasitol 99:725–728

Power G (2002) Charrs, glaciation and seasonal ice. Environ Biol Fish 64:17–35

Pugachev ON, Khokhlov PP (1979) Myxosporea of the genus *Myxobolus*–parasites of the salmonids head and spinal brain. *In* Systematic and ecology of fish from continental water bodies of the Far East Region. Vladivostok, pp 137–139 (In Russian)

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) FigTree:v1.4.3 Available from http://tree.bio.ed.ac.uk/software/figtree

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in bayesian phylogenetics using Tracer 1.7. Syst Biol 67:901–904

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinform 19:1572–1574

Ruiz CF, Rash JM, Arias CR, Besler DA, Orélis-Ribeiro R, Womble MR, Roberts JR, Warren MB, Ray CL, Lafrentz S, Bullard SA (2017) Morphological and molecular confirmation of *Myxobolus cerebralis* myxospores infecting wild–caught and cultured trout in North Carolina (SE USA). Dis Aquat Org 126:185–198

Thompson KG, Nehring RB, Bowden DC, Wygant T (1999) Field exposure of seven species or subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. J Aquat Anim Health 11:312–329

Urawa S, Iida Y, Freeman MA, Yanagida T, Karlsbakk E, Yokoyama H (2009) Morphology and molecular comparisons of *Myxobolus* spp. in the nerve tissues of salmonids with the description of *Myxobolus murakamii* n. sp., the causative agent of myxosporean sleeping disease. Fish Pathol 44:72–80

US Fish and Wildlife Service and American Fisheries Society – Fish Health Section (2014) *Myxobolus cerebralis* (whirling disease). In: AFS-FHS Blue Book Section 2: USFWS/AFS-FHS standard procedure for aquatic animal health inspections. American Fisheries Society – Fish Health Section, Bethesda, MD, p. 1–13

Vincent ER (1996) Whirling disease and wild trout: the Montana experience. Fish 21:32–33

Yokoyama H, Kageyama T, Ohara K, Yanagida T (2007) *Myxobolus nagaraensis* n. sp. (Myxozoa: Myxozporea) causes abdominal distension of freshwater goby *Phinogobius* sp. OR type from the Nagara River. Fish Sci 73:633–639