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RESEARCH COMMUNICATION

First report of *Myxobolus neurofontinalis* (Bivalvulida: Myxobolidae) infecting anadromous Brook Trout from Prince Edward Island, Canada

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Abstract

Objective: During routine histological examination of tissues from mortality events of anadromous Brook Trout *Salvelinus fontinalis* from Prince Edward Island (PEI), Canada, myxospores consistent with *Myxobolus* were observed infecting the central nervous system. The objective of this study was to identify the species of *Myxobolus* infecting the nervous system of anadromous Brook Trout from PEI, Canada.

Methods: Myxospore morphology, small subunit (SSU) ribosomal DNA (rDNA) sequence data, and histology were used to identify myxospores isolated from infected Brook Trout.

Result: Myxospore measurements from the PEI samples matched those reported in the description of *Myxobolus neurofontinalis* from North Carolina. A 1057-bp fragment of the SSU rDNA from myxospores collected from Brook Trout in PEI was identical to an isolate of *M. neurofontinalis* (MN191598) collected previously from the type locality, New River basin, North Carolina. Histological sections confirmed infections were intercellular in the central nervous system. Minimal host response was observed, with only sparse mononuclear inflammatory infiltrates present at the periphery of and within dispersed myxospores, suggesting that infections are not pathogenic to Brook Trout.

Conclusion: Myxospores were identified as *M. neurofontinalis*, which was previously described from the central nervous system of Brook Trout from the New River basin, North Carolina, USA. This constitutes the first time *M. neurofontinalis* has been documented outside of the New River basin in North Carolina.

KEYWORDS

morphology, Myxozoa, pathology

INTRODUCTION

Eight species of *Myxobolus* (Bivalvulida: Myxobolidae) infect the central nervous system of salmonids: *M. farionis* Gonzalez-Lanza and Alvarez-Pellitero 1984; *Myxobolus neurobius* Schuberg and Schroder 1905; *Myxobolus kisutchi* Yasutake and Wood 1957; *Myxobolus neurotropus* Hogge et al. 2008; *Myxobolus murakamii* Urawa et al. 2009; *Myxobolus arcticus* Pugachev and Khokhlov 1979; *Myxobolus fryeri* Ferguson et al. 2008; and *Myxobolus neurofontinalis* Ksepka and Bullard 2019. *Myxobolus farionis* infects Brown Trout *Salmo trutta* Linnaeus 1758 in the Duero River basin, Spain (Gonzalez-Lanza and Alvarez-Pellitero 1984). *Myxobolus kisutchi* infects Coho Salmon *Oncorhynchus kisutch* Walbaum 1792 in Washington (Hogge et al. 2008a). *Myxobolus neurobius* infects Brown Trout in Norway (Urawa

et al. 2009). *Myxobolus neurotropus* infects Rainbow Trout *Oncorhynchus mykiss* Walbaum 1792 in Idaho (Hogge et al. 2008b). *Myxobolus murakamii* infects Masu Salmon *Oncorhynchus masou* Brevoort 1856 in Hokkaido, Japan (Urawa et al. 2009). *Myxobolus arcticus* infects *Oncorhynchus* spp. in the Kamchatka Peninsula, Russia (Pugachev and Khokhlov 1979). *Myxobolus fryeri* infects Coho Salmon in Oregon (Ferguson et al. 2008). *Myxobolus neurofontinalis* infects Brook Trout *Salvelinus fontinalis* Mitchill 1814 in the New River basin, North Carolina (Ksepka et al. 2019).

Salmonid central nervous system-infecting species are not typically pathogenic or immunogenic. Only *M. murakamii*, the causative agent of salmonid sleeping disease, which impairs swimming, has been reported as pathogenic in cultured Masu Salmon in Japan (Urawa et al. 2009). Given the morphological similarity of *Myxobolus* spp. and the significant impacts that false positives for pathogenic species of *Myxobolus* can have on culture facilities and government agencies, documenting the range of nonpathogenic salmonid central nervous system-infecting *Myxobolus* species will reduce the potential for false positives for *Myxobolus cerebralis* Hofer 1903.

We herein use myxospore morphology, small subunit (SSU) ribosomal DNA (rDNA), and histology to report infections of *M. neurofontinalis* in Brook Trout collected as periodic mortalities from Prince Edward Island (PEI), Canada, watersheds for histologic screening for neurotropic myxozoan infections (Table 1). This is the first report of *M. neurofontinalis* outside of the New River basin.

Impact statement

This study documents *Myxobolus neurofontinalis* outside of North Carolina for the first time and, given the morphological similarities to *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, highlights the importance of detailed morphological diagnosis coupled with nucleotide sequence data to myxozoan disease diagnostics.

METHODS

From July 10, 2014, to June 24, 2021, 27 wild Brook Trout mortalities were submitted by either private citizens or provincial conservation officers from 12 localities in PEI and were necropsied at the Atlantic Veterinary College (Table 1). Following necropsy, tissues were fixed in 10% neutral buffered formalin (NBF) for histology and morphology and were preserved in 95% ethanol for DNA extraction. After fixation, the brain was trimmed to fit into tissue processing cassettes, dehydrated in an ethanol series, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined histologically for potential pathological conditions. Samples containing tissues from individual fish that were confirmed by histology to be infected by myxozoans were shipped to Auburn University for further processing. Myxospores were isolated by macerating infected tissue in a small stender dish filled with sterilized, deionized water and pipetting the released myxospores into formalin (for morphology) or lysis buffer (for DNA extraction).

Measurements were generated at 1000× magnification from 50 NBF-fixed myxospores sourced from three infected fish. Micrographs of NBF-fixed myxospores from these same infected fish were taken at 1000× magnification. Lugol's iodine and India ink were used to stain the iodinophilic vacuole and mucous envelope, respectively, per Lom and Arthur (1989). All measurements are reported

TABLE 1 Results of histology screening for *Myxobolus neurofontinalis* infections in Brook Trout from Prince Edward Island, Canada. For histology results, *N* is the number of fish sampled and *H+* is the number of fish in which infections were detected.

			Histology results	
Date	**Locality**	**Coordinates**	***N***	***H+***
Jul 18, 2014	Morell River	–	3	3
Jul 26, 2016	Clyde River	46°14′35.0″N, 63°15′59.0″W	2	0
Nov 24, 2016	West River	46°14′47.0″N, 63°21′30.3″W	1	1
Sep 8, 2017	Morell River	46°24′55.8″N, 62°41′58.2″W	1	1
Oct 20, 2017	Hyde Creek	46°14′13.6″N, 63°12′46.6″W	3	0
Nov 7, 2017	Dunk River	46°21′13.3″N, 63°33′19.7″W	1	0
Nov 14, 2017	MacLure's Pond	46°00′41.9″N, 62°38′20.0″W	2	1
Aug 14, 2018	Whitlock's Pond	46°21′00.8″N, 62°31′40.6″W	1	1
Jun 5, 2020	Cousin's Pond tributary	46°31′37.2″N, 63°34′25.3″W	4	0
Nov 10, 2020	West River	46°13′00.5″N, 63°21′25.6″W	2	2
Nov 15, 2020	Trout River	46°41′36.6″N, 64°08′53.9″W	6	6
Jun 24, 2021	Pond, Dougan Road	46°21′15.1″N, 63°00′59.8″W	1	0

in micrometers unless otherwise specified. To photograph myxospores, we suspended the myxospores by vortexing, and a drop of the suspension was then cover-slipped, inverted, placed onto a thin layer of 1% agar (Lom and Arthur 1989), and photographed using a 100× oil immersion objective on an Olympus BX51 compound scope equipped with differential interference contrast components.

The DNA was extracted from one microscopically confirmed isolate of myxospores from one ethanol-preserved portion of infected tissue by using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol, with the exceptions that (1) the proteinase K incubation step was extended overnight and (2) 100 µL of AE buffer were used for the elution step to increase the DNA concentration. After extraction, the concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, NanoDrop Technologies) and the sample was stored at −20°C. A fragment of a variable region of the SSU rDNA was amplified using primers M153-F (5′-CATTGGATAACCGTGGGAAATCT-3′) and M1480-R (5′- GTGGTGCCCTTCCGTCAATTCC-3′; Ksepka et al. 2019). Polymerase chain reaction (PCR) amplification followed Ksepka et al. (2019). The PCR products were visualized on a 1% agarose gel and purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol, with the exception that sterile water was substituted for AE buffer for eluting DNA. The products were sequenced in both directions using primers from PCR amplification by Genewiz (South Plainfield, New Jersey). Chromatograms were assembled based on sequence overlap and were proofread by eye, and low-quality read ends were trimmed in Geneious version 2021.1.1 (http://www.geneious.com). The resulting sequence was compared to those in GenBank by using the nucleotide Basic Local Alignment Search Tool (National Center for Biotechnology Information).

RESULTS

Screening results

Myxobolus neurofontinalis was detected in Brook Trout from 7 of 12 collection events (Table 1). Myxospores were observed in histological sections of nerve tissue in one of one (100%) Brook Trout from the West River (2016 collection), the Morell River (2017 collection), and Whitlock's Pond; two of two (100%) Brook Trout from the West River (2020 collection); three of three (100%) Brook Trout from the Morell River (2014 collection); six of six (100%) Brook Trout from the Trout River; and one of two (50%) Brook Trout from MacLure's Pond (Table 1).

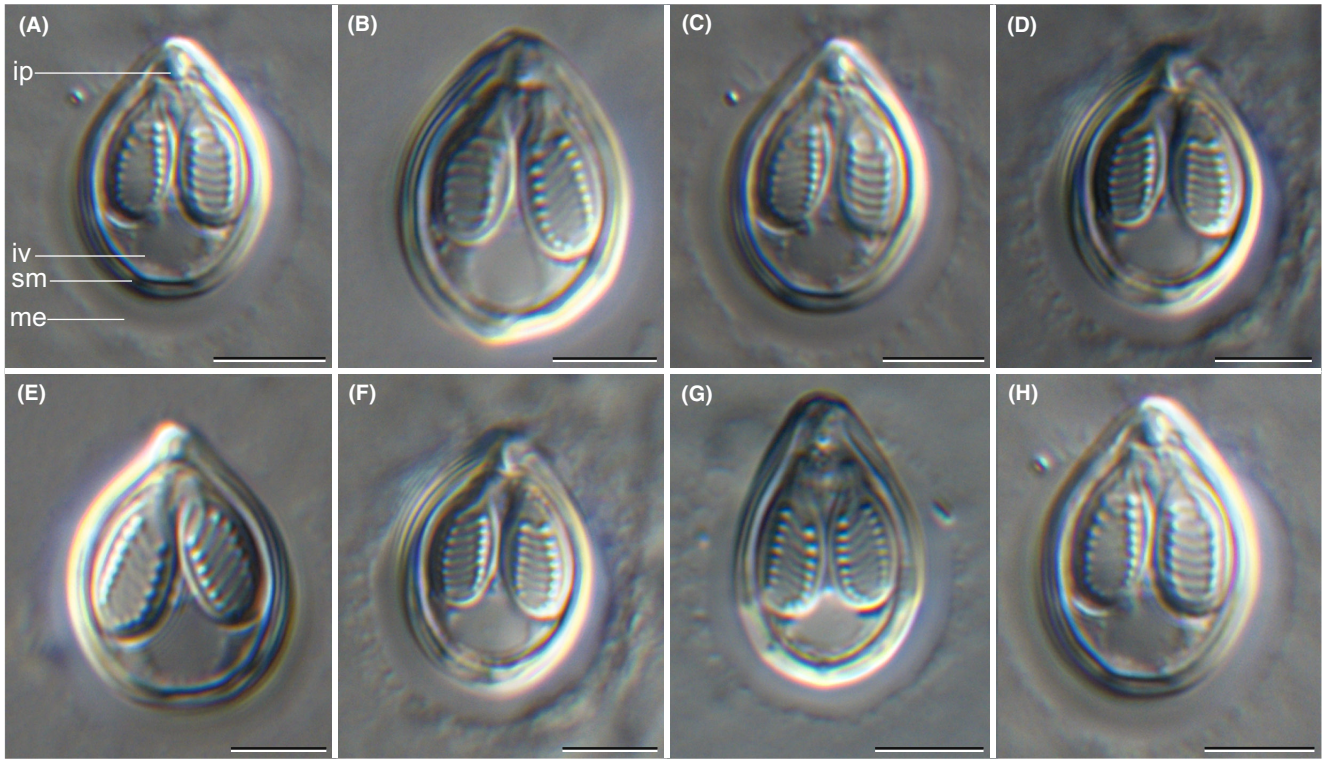

FIGURE 1 (A–H) Myxospores of *Myxobolus neurofontinalis* collected from the central nervous system of Brook Trout from Prince Edward Island, Canada, and photographed with differential interference contrast optical components (scale bars = 5 µm). ip, intercapsular process; iv, iodinophilic vacuole; me, mucous envelope; sm, sutural markings.

Myxospore morphology

Dimensions of NBF-fixed myxospores sourced from the central nervous system of Brook Trout from PEI were indistinguishable from those of NBF-fixed myxospores of *M. neurofontinalis*, suggesting that the samples collected herein are conspecific with *M. neurofontinalis* (see Ksepka et al. 2019). The myxospores from PEI Brook Trout were 13.0–15.0 μm (mean ± SD = 14.2 ± 0.6 μm; $N = 50$) long, 9.0–11.0 μm (10.4 ± 0.7 μm; $N = 40$) wide, and 8.0–10.0 μm (8.5 ± 0.7 μm; $N = 10$) thick (Figure 1). Polar capsules were 7.0–9.0 μm (8.2 ± 0.7 μm; $N = 80$) long and 3.0–4.0 μm (3.1 ± 0.4 μm; $N = 80$) wide, with 7–10 polar tubule coils (Figure 1). Like the specimens of *M. neurofontinalis* from North Carolina (Ksepka et al. 2019), an intercapsular process, iodinophilic vacuole in the sporoplasm, mucous envelope on the rounded posterior margin of the myxospore, and sutural markings were likewise present in the collected samples (Figure 1).

Sequence comparison

The 1057-base-pair SSU rDNA (18S) sequence fragment generated herein for *M. neurofontinalis* (GenBank accession number OR452314) was identical to the sequence of *M. neurofontinalis* (MN191598) from its type locality (New River basin), which supports the morphological assertion that samples collected herein are conspecific with *M. neurofontinalis* (Ksepka et al. 2019). Hence, we consider the samples collected herein to be conspecific with *M. neurofontinalis*, as they are morphologically and molecularly indistinguishable from isolates of *M. neurofontinalis* infecting Brook Trout in North Carolina.

Histological comparison

Myxospores of *M. neurofontinalis* were observed in the central nervous system of 15 of 27 (55%) Brook Trout sampled. Histological observations were consistent with those of Ksepka et al. (2019). Myxospores were observed in intercellular foci, with no defined plasmodium, and were loosely dispersed within the neuropil (Figure 2A,B). A mild inflammatory response was observed on the periphery of dispersed myxospores, characterized by the presence of sparse mononuclear inflammatory infiltrates within and on the periphery of the cluster of myxospores (Figure 2B). No host response was observed in association with developing presporogonic stages (Figure 2C).

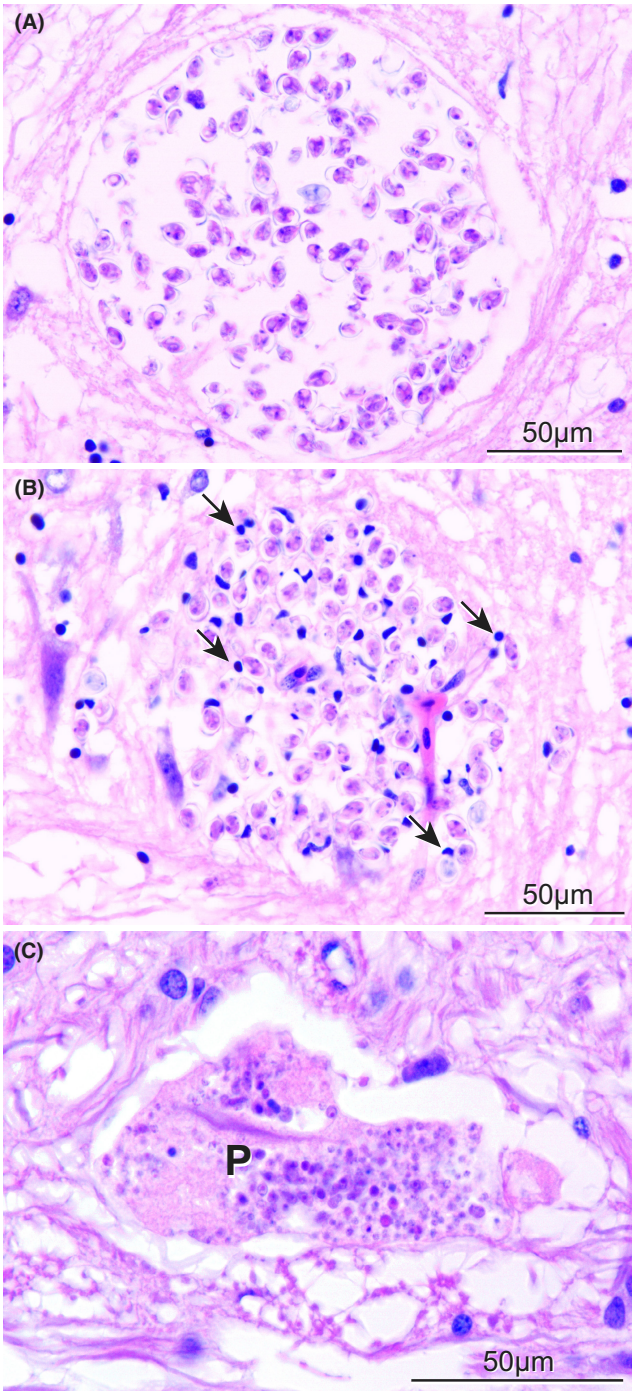

FIGURE 2 Histological sections (hematoxylin and eosin) of Brook Trout nerve tissue infected by *Myxobolus neurofontinalis*: (A) foci of myxospores; (B) dispersed myxospores, showing sparse mononuclear inflammatory infiltrates (arrows); and (C) presporogonic stage (P).

DISCUSSION

Myxobolids infecting catadromous and anadromous fishes have obligately freshwater definitive hosts (Eszterbauer et al. 2015). Of the 28 myxobolid life cycles known, four

infect fishes that can be anadromous or catadromous, and all four have freshwater definitive hosts: *M. arcticus*; *M. cerebralis*; *M. portucalensis* Saraiva and Molnar 1990; and *Henneguya nuesslini* Schuberg and Schroder 1905 (Eszterbauer et al. 2015). *Myxobolus arcticus*, which infects the central nervous system of *Oncorhynchus* spp. in the Pacific basin, matures in *Styolodrilus heringianus* Claparède 1862 (Clitella: Lumbriculidae) and *Lumbriculus variegatus* Muller 1774 (Clitella: Lumbriculidae; Kent et al. 1993; Urawa 1994; Urawa et al. 2011). The bone- and cartilage-infecting *M. cerebralis* has been introduced extensively outside of its theoretical native range; it infects a number of anadromous salmonids and matures in *Tubifex tubifex* sensu lato (Clitella: Lumbriculidae; Markiw and Wolf 1983; El-Matbouli et al. 1992; Hedrick and El-Matbouli 2002; Ksepka et al. 2021). *Henneguya nuesslini*, infecting the connective tissue of Brown Trout, matures in *T. tubifex* (Kallert et al. 2005). *Myxobolus portucalensis*, which infects the connective tissue of the catadromous European Eel *Anguilla anguilla* Linnaeus 1758, matures in "*T. tubifex*" (see El-Mansy et al. 1998). This, in combination with the landlocked status of Brook Trout from the type locality for *M. neurofontinalis* (New River basin), suggests that the Brook Trout sampled from PEI were infected in freshwater (Ksepka et al. 2019). No record exists of a marine invertebrate definitive host for a myxobolid.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The manuscript lacks shared data.

ETHICS STATEMENT

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

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