Research Article

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A new species of *Myxobolus* Bütschli, 1882 (Bivalvulida: Myxobolidae) infecting *stratum spongiosum* of the imperiled sicklefin redhorse, *Moxostoma* sp. (Cypriniformes: Catostomidae) from the Little Tennessee River, North Carolina, USA

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Abstract: The sicklefin redhorse, *Moxostoma* sp. (Cypriniformes: Catostomidae), is an innominate imperiled catostomid endemic to the Hiwassee and Little Tennessee river basins, which has been restricted to a few tributaries of these systems by impoundments. During collections to propagate sicklefin redhorse for reintroduction, a myxozoan, described herein, was observed infecting sicklefin redhorse in the Little Tennessee River Basin, North Carolina. *Myxobolus naylori* Ksepka et Bullard sp. n. infects the *stratum spongiosum* covering the scales of sicklefin redhorse. Myxospores of the new species differ from all congeners by the combination of having a mucous envelope, intercapsular process, and sutural markings as well as lacking an iodophilic vacuole in the sporoplasm. A phylogenetic analysis of the 18S rDNA gene recovered the new species in a polytomy with *Myxobolus marumotoi* Li et Sato, 2014 and a clade comprised of species of *Myxobolus* Bütschli, 1882; *Thelohanellus* Kudo, 1933, and *Dicauda* Hoffman et Walker, 1973. Histological sections of infected sicklefin redhorse skin revealed myxospores within a plasmodium in the *stratum spongiosum* dorsal to scales, encapsulated in collagen fibres, and associated with focal erosion of scales directly beneath the plasmodium; in some instances, the scale was perforated by the plasmodium. The specificity of the new species to sicklefin redhorse may make it a useful biological tag to differentiate sicklefin redhorse from morphologically similar species. The new species is the first parasite reported from sicklefin redhorse, a species of concern to the United States Fish and Wildlife Service. No species of *Myxobolus* has been reported from species of *Moxostoma* in the Southeast United States. As it was observed that *Myxobolus minutus* Rosser, Griffin, Quiniou, Alberson, Woodyard, Mischker, Greenway, Wise et Pote, 2016 is a primary junior homonym of *Myxobolus minutus* Nemeczek, 1911, we propose the replacement name *Myxobolus diminutus* (Rosser, Griffin, Quiniou, Alberson, Woodyard, Mischker, Greenway, Wise et Pote, 2016).

Keywords: Myxozoa, taxonomy, pathology, phylogeny

Myxobolus Bütschli, 1882 (Bivalvulida: Myxobolidae) is the most species-rich myxozoan genus, having > 800 species that infect predominately freshwater fishes, have a worldwide distribution, and generally exhibit high host and tissue specificity (Eiras et al. 2005, Molnár and Eszterbauer 2015, Liu et al. 2019). Thirty-two species of *Myxobolus* infect suckers (Catostomidae), with most records sourcing from the Great Lakes of the United States and Southeast Canada (Table 1). Five nominal species of *Myxobolus* infect species of *Moxostoma* Rafinesque (Cypriniformes: Catostomidae): *Myxobolus congesticus* (Kudo, 1934), *Myxobolus conspicuus* (Kudo, 1929), *Myxobolus gravidus* Kudo, 1934, *Myxobolus moxostomi* Nigrelli, 1948, and *Myxobolus vas-*

tus Kudo, 1934 (Table 1). *Myxobolus congesticus* infects the fins of the silver redhorse (*Moxostoma anisurum* [Rafinesque]) in the Rock River, Illinois (Kudo 1934). *Myxobolus conspicuus* was described infecting the dermis of the cranium of the smallmouth redhorse (*Moxostoma breviceps* [Cope]) in the Rock River, Illinois (Kudo 1929). It has subsequently been reported from that site of infection in smallmouth redhorse from the Fox River, Illinois, and shorthead redhorse (*Moxostoma macroleptidotum* [Lesueur]) from the St. Lawrence River and Lake Erie (Kudo 1934, Fantham et al. 1939, Dechtiar 1972). *Myxobolus gravidus* was described from the skin of the silver redhorse in the Fox River (Kudo 1934). *Myxobolus moxostomi* infects the epithelium of the

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shorthead redhorse from the New York Aquarium (Nigrelli 1948), and *M. vastus* infects the skin of shorthead redhorse from the Fox River (Kudo 1934). No species of *Myxobolus* has been reported from a species of *Moxostoma* in the Southeast United States.

Catostomids are almost entirely endemic to North America: the only exception being the Chinese high-fin banded shark (*Myxocyprinus asiaticus* [Bleeker] [Cypriniformes: Catostomidae]) that is endemic to Southeast Asia (Barton and Bond 2006). *Moxostoma* consists of 22 species endemic to North America (Fricke et al. 2019). Although most of these are relatively common and abundant throughout their range, several *Moxostoma* spp. are endemic to the southeastern USA. The robust redhorse (*Moxostoma robustum* [Cope]) is endemic to three river drainages in North Carolina and Georgia and was previously thought to be extinct. However, it was rediscovered in the 1990's and is currently listed as endangered in Georgia and North Carolina (Walsh et al. 1998, Peterson et al. 2013).

The sicklefin redhorse (*Moxostoma* sp.) is endemic to the Hiwassee and Little Tennessee river basins and most closely resembles the sympatric river redhorse (*Moxostoma carinatum* [Cope]) but can be differentiated by the placement of the breeding tubercles and presence of elongated dorsal fin rays (Favrot and Kwak 2018, Moyer et al. 2019). Habitat fragmentation has limited the range of the sicklefin redhorse to a few tributaries within these river basins; however, collection records indicate the sicklefin redhorse used to inhabit the majority of rivers in the Blue Ridge portion of these river basins (United States Fish and Wildlife Service 2020).

The United States Fish and Wildlife Service (USFWS) has ongoing projects to monitor populations of this species throughout its range and to propagate it for reintroduction into its historical habitat, which are currently focused on reintroducing sicklefin redhorse above impoundments in the Oconaluftee and Tuckasegee rivers (Little Tennessee River Basin) (Moyer et al. 2009, United States Fish and Wildlife Service 2020). As such, information about the specific taxonomic identity, pathological effects, and life cycles of the parasites and potential pathogens of these fishes are relevant to biosecurity associated with these planned stock enhancement activities.

The recent discovery of the causative agent of salmonid whirling disease in the southeastern United States (Ruiz et al. 2017) has raised awareness about the critical importance of conventional and molecular diagnostic approaches to identify species of *Myxobolus*. Based upon light microscopy of myxospores and histological sections of infected host tissue, we herein describe a new species of *Myxobolus* from the sicklefin redhorse. The new species is the sixth species of *Myxobolus* reported from a species of *Moxostoma*, the first reported from a species of *Moxostoma* in the Southeast USA, and the first parasite reported from the sicklefin redhorse.

MATERIALS AND METHODS

On 26 June 2018, myxospores consistent with a species of *Myxobolus* were excised from the skin of two sicklefin red-

horses within a closed recirculating aquaculture system at the Warm Springs National Fish Hatchery, Warm Springs, Georgia. These fish were sourced from the Oconaluftee River (35.446329, -83.382937), Little Tennessee River Basin, North Carolina. Myxospores were fixed in 10% neutral buffered formalin (NBF) for morphology or preserved in 95% ethanol for DNA extraction and shipped to the Aquatic Parasitology Laboratory at Auburn University.

After discovering the infection in the captive fish, we initiated field efforts to obtain additional conspecific myxospores for morphology and nucleotide analysis by non-lethal sampling, i.e., biopsy of infected scales and adjacent skin. A total of 136 sicklefin redhorses were collected from four riverine sites in North Carolina and Georgia during April through May 2019: 25 from Brasstown Creek (34.981111, -83.887806), Hiwassee River Basin, Georgia (9–12 April 2019); 42 from Valley River (35.190100, -83.967000), Hiwassee River Basin (29 April–3 May 2019); 46 from Oconaluftee River (35.446329, -83.382937), Little Tennessee River Basin (7–8 May 2019); and 23 from Tuckasegee River (35.363800, -83.245700), Little Tennessee River Basin (9 May 2019). All sicklefin redhorse were identified in the field by the presence of elongated dorsal fin rays and the placement of breeding tubercles. Any fish that could not be confidently identified was not sampled. After collection, each fish was measured (mm), weighed (g), tagged, examined for infection (biopsied or not), and released. Infected scales were excised with fine forceps and plasmodia were parsed equally in 10% NBF and 95% ethanol for morphology and DNA extraction, respectively.

Myxospore measurements were based on wet mounted, fresh, formalin-fixed myxospores using a 63× oil immersion objective and a 1.6× magnification changer. All measurements are reported in micrometres unless otherwise indicated. Lugol's iodine and India ink were used to stain the iodophilic vacuole and mucous envelope, respectively (Lom and Arthur 1989). To illustrate myxospores they were vortexed to suspend myxospores before a drop of this suspension was cover-slipped, inverted, and placed onto a thin layer of 1% agar (Lom 1969). Illustrations were made using a 100× oil immersion objective on an Olympus BX51 compound scope equipped with 1.6× magnifier, DIC, and drawing tube.

After fixation, formalin-fixed, infected scales were rinsed in distilled water for two hours, decalcified in EDTA for one month, dehydrated in an ethanol series, embedded in paraffin, sectioned at 4 µm, and stained with Harris's hematoxylin and eosin (Luna 1968). Thirty slides were cut from each portion yielding > 180 sections on 60 slides.

DNA was extracted from one microscopically/morphologically confirmed isolate of myxospores from each locality (Oconaluftee River and Tuckasegee River), each isolate consisted of myxospores from a single plasmodium, using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol. Two replicates were sequenced to identify any potential variability in the 18S rRNA gene. DNA concentration was measured using a NanoDrop-1000 spectrophotometer (Thermo Scientific, Nanodrop Technologies), diluted to 20 ng/µl, and stored at -20°C. A 1,651 and 1,669 base pair fragment of the 18s (rDNA) was amplified using primers M153-F (5'-CATTGGATAACCGTGGGAAATCT-3') and M2192-R (5'-TAGTAGCGACGGGCGGTGT-3') (Ksepka et al. 2019, 2020).

PCR amplification used the following thermocycler parameters: initial denaturation step of 95 °C for four min, followed by 35 cycles of 95 °C for 30 s, 62 °C for 30 s, and 72 °C for one min, with a final extension step of 72 °C for four min. Additionally, two sequencing primers M473-F (5'-GCTTGAGAAWCGGCTAC-CAC-3') and M1480-R (5'-GTGGTGCCCTTCCGTCAAT-TCC-3') were used to improve sequencing coverage (Ksepka et al. 2019, 2020). DNA sequencing was performed by ACGT incorporated (Wheeling, IL). Chromatograms were proofread by eye and assembled based on sequence overlap in Geneious version 2019.2.3 (<http://www.geneious.com>). Due to uneven sequencing and trimming of ambiguous base pairs at the 5' and 3' end of sequences, resulting sequences for the new species were 1,651 and 1,669 base pairs in length.

Additional taxon selection for phylogenetic analysis was based on a recent phylogenetic review of the Myxobolidae, which prioritised sequences > 1,300 nucleotides tethered to a morphological diagnosis and included 74 species of the Myxobolidae from "clade E" of Zhang et al. (2019), all available sequences of *Myxobolus* spp. infecting catostomids, four species of *Henneguya* Thélohan, 1892 (Bivalvulida: Myxobolidae), and three species of Myxidiidae as an outgroup (Zhang et al. 2019). Sequences were aligned using MAFFT using an E-INS-i alignment strategy with a maxiterate value of 1,000 in Geneious version 2019.2.3 (Katoh and Standley, 2013). The alignment was trimmed to the length of sequences presented herein (1,867 base pairs) to minimise missing data at the 5' and 3' end of the alignment. 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 (nst=mixed) and a gamma distribution to model rate-heterogeneity. Defaults were used in all other parameters. Three runs with four 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 sumt command in MrBayes. The phylogenetic tree was visualised in FigTree v1.4.3 (Rambaut et al. 2014) and rendered for publication with Adobe Illustrator (Adobe Systems).

DESCRIPTION

Myxobolus naylori Ksepka et Bullard sp. n. Figs. 1–3

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Diagnosis of myxospores (based on 52 myxospores using differential interference contrast microscopy [25 stained with Lugol's iodine; 12 stained with India ink]; USNM coll. Nos. 1618813–1618818): Myxospore comprising 2 smooth symmetrical valves juxtaposed at sutural rim, 2 polar capsules, and sporoplasm; myxospore ellipsoid, 10.0–12.0 (mean $\pm$ SD = 10.8 ± 0.6 ; N = 52) long (Fig. 2A–C), 7.0–9.0 (8.2 ± 0.6 ; 38) wide (Fig. 2A–C), 5.0–6.0 (5.5 ± 0.5 ; 14) thick (Fig. 2D); sutural rim with prominent seam (Fig. 2D), 1.0 (39) thick (lateral margin in

frontal view) (Fig. 2A–C), 1.0 (39) thick (posterior margin in frontal view) (Fig. 2A–C), without flanking lateral ridges (sutural), 1.0–9.0 (5.0 ± 2.0 ; 38) sutural markings; polar capsules equal (Fig. 2A–C), clavate (Fig. 2A–C), 4.0–6.0 (4.9 ± 0.6 ; 76) long (Fig. 2A–C), 2.0–3.0 (2.9 ± 0.2 ; 76) wide (Fig. 2A–C), with 7–10 polar filament coils (Fig. 2A–C); sporoplasm lacking iodophilic vacuole (Fig. 2A–C), having 2 nuclei (Fig. 2A–C); 1–2 (1.8 ± 0.4 ; 12) thick mucous envelope prominent on the rounded posterior margin (Fig. 2A–C).

Type and only reported host: *Moxostoma* sp. (Cypriniformes: Catostomidae), sicklefin redhorse.

Type locality: Oconaluftee River (35.446329, -83.382937), Little Tennessee River Basin, North Carolina.

Other localities: Tuckasegee River (35.363800, -83.245700), Little Tennessee River Basin, North Carolina.

Specimens deposited: Myxospores of *M. naylori* fixed in 10% NBF (1 vial; syntype; USNM 1618813), and within paraffin sections (4 slides; syntypes; USNM 1618814–1618818); GenBank No. (18S: MN99711–MN99712).

Site in host: Intercellular plasmodia infecting the stratum spongiosum covering scales.

Prevalence: Myxospores were detected in 15 of 46 (prevalence = 33%) sicklefin redhorses collected from the Oconaluftee River (35.446329, -83.382937), Little Tennessee River Basin, North Carolina and 2 of 23 (prevalence = 9%) sicklefin redhorses collected from the Tuckasegee River (35.363800, -83.245700), Little Tennessee River Basin, North Carolina.

Etymology: The specific epithet "naylori" honors Mr. Kenneth Joseph Naylor, SPK's friend, whose influence helped cultivate SPK's interest in biology.

Differential diagnosis: The myxospore of the new species differs from those of its 32 congeners infecting catostomids by dimensions and the polar filament count as well as by the presence of a mucous envelope, sutural markings, and intercapsular process. Further, the new species lacks an iodophilic vacuole (Table 1). Of those 32 species, the myxospore of the new species differs from all but four (*M. congesticus*, *M. conspicuus*, *Myxobolus ellipticoides* [Fantham, Porter et Richardson, 1939], and *Myxobolus subcircularis* [Fantham, Porter et Richardson, 1939]) by myxospore dimensions and polar filament coil number (Table 1). Of those, the myxospore of the new species differs from that of *M. congesticus* and *M. conspicuus* by having a mucous envelope and intercapsular process as well as by lacking an iodophilic vacuole. The myxospore of the new species differs from that of *M. ellipticoides* by having a mucous envelope, sutural markings, and an intercapsular process. The myxospore of the new species differs from that of *M. subcircularis* by having a mucous envelope, sutural markings, and intercapsular process as well as by lacking an iodophilic vacuole (Fig. 2A–C).

A total of 17 species of *Myxobolus* that infect non-catostomid intermediate hosts have overlapping myxospore measurements with the new species (i.e. *Myxobolus clariae* Hemananda, Mohilal, Bandyopadhyay et Mitra, 2009, *Myxobolus corneus* Cone, Horner et Hoffman, 1990, *Myxobolus gallaicus* Iglesias, Parama, Alvarez, Leiro et Sanmartin, 2001, *Myxobolus hyderabadense* [Lalitha Kumari,

Fig. 1. A – sicklefin redhorse, *Moxostoma* sp. (Cypriniformes: Catostomidae) scale infected with a plasmodium of *Myxobolus naylori* Ksepka et Bullard sp. n. (Bivalvulida: Myxobolidae) from the Oconaluftee River, Little Tennessee River Basin, North Carolina; B – Myxospores of *Myxobolus naylori* Ksepka et Bullard sp. n. (Bivalvulida: Myxobolidae) collected from sicklefin redhorse, *Moxostoma* sp. (Cypriniformes: Catostomidae) from Oconaluftee River, Little Tennessee River Basin, North Carolina; differential interference contrast optical components; in water; C – stained with India ink.

Fig. 2. Myxospores of *Myxobolus naylori* Ksepka et Bullard sp. n. (Bivalvulida: Myxobolidae) collected from sicklefin redhorse, *Moxostoma* sp. (Cypriniformes: Catostomidae) from Oconaluftee River, Little Tennessee River Basin, North Carolina. A–C – frontal view; D – sutural view. Abbreviation: sr – sutural rim; pc – polar capsule; pf – polar filaments; me – mucous envelope; sm – sutural markings, ip – intercapsular process; n – nuclei (n). Scale bars = 5 µm.

1969], *Myxobolus irinae* Daniyarov, 1975, *Myxobolus kalarfi* [Kalantan et Arfin, 1991], *Myxobolus leshanensis* Ma et Zhao, 1992, *Myxobolus macrocapsularis* Reuss, 1906, *Myxobolus mongolicus* Pronin, 1973, *Myxobolus naffari* Abdel-Ghaffar, Ibraheim, Bashtar et Ali, 1998, *Myxobolus njoyai* Nchoutpouen et Fomena, 2011, *Myxobolus percari-nae* Iskov et Karatev, 1982, *Myxobolus smithi* Salim et Dessler, 2000, *Myxobolus squamae* Keysselitz, 1908, *Myxobolus strelkovi* Kostarev et Kulemina, 1971, *Myxobolus zhujiangensis* [Chen et Zhou, 1998], and *Myxobolus zillli* Sakiti, Blanc, Marques et Bouix, 1991) (Table 1).

The presence of sutural markings differentiates the new species from *M. clariae*, *M. hyderabadense*, *M. kalarfi*, *M. macrocapsularis*, *M. naffari*, *M. njoyai*, *M. strelkovi*, and *M. zhujiangensis*. The remaining eight species of *Myxobo-*

lus infecting non-catostomids (*M. corneus*, *M. gallaicus*, *M. irinae*, *M. leshanensis*, *M. mongolicus*, *M. percari-nae*, *M. smithi*, and *M. squamae*) have sutural markings and, with exception to *M. corneus*, are distinct from the new species by lacking an iodophilic vacuole. The myxospore of the new species differs from that of *M. corneus* by having a mucous envelope (Fig. 2A–C).

While reviewing *Myxobolus* spp. that infect catostomids, it was observed that *Myxobolus minutus* Rosser, Griffin, Quiniou, Alberson, Woodyard, Mischker, Greenway, Wise et Pote, 2016 is a primary junior homonym of *Myxobolus minutus* Nemeczek, 1911 (Nemeczek 1911, Rosser et al. 2016). Per the code of International Code of Zoological Nomenclature (ICZN) article 53.3, two taxa erected within a genus with the same specific epithet constitutes primary ho-

Fig. 3. Transverse histological sections (hematoxylin and eosin) of sicklefin redhorse, *Moxostoma* sp. (Cypriniformes: Catostomidae) scales infected with *Myxobolus naylori* Ksepka et Bullard sp. n. (Bivalvulida: Myxobolidae). **A** – infected *stratum spongiosum* showing plasmodia of myxospores (P); **B** – infected *stratum spongiosum* showing host encapsulation (E), scale loss (*), lymphocytic inflammatory infiltrates (arrows), and proliferating fibroblasts (F) associated with plasmodium (P); **C** – infected *stratum spongiosum* showing host encapsulation (E) and absence of mucous producing cells (*) associated with plasmodium.

monymy. Per ICZN articles 60.1 and 60.3, the junior homonym must be rejected and replaced with an available substitute name (Ride et al. 1999). We consulted with two of the taxonomic authorities of the latter proposed “*M. minutus*” (Graham Rosser, Mississippi State University, Starkville, Mississippi, and Matthew Griffin, Mississippi State University, Stoneville, Mississippi), who agreed with our replacement name, *Myxobolus diminutus* (Rosser, Griffin, Quini-

ou, Alberson, Woodyard, Mischker, Greenway, Wise et Pote, 2016) **nom. nov.**, for this taxon. This satisfies the ICZN and maintains the original etymology regarding the small size of the myxospores (Rosser et al. 2016).

Phylogenetic analysis

The two 18S sequence fragments generated for the new species of *Myxobolus* comprised 1,867 base pairs after

Fig. 4. Phylogenetic relationships of species of Myxobolidae infecting catostomids, cyprinids, and genetically similar species to *Myxobolus naylori* Ksepka et Bullard sp. n. (Bivalvulida: Myxobolidae) reconstructed with the 18S rRNA gene using Bayesian inference. Scale bar is in substitutions per site. New species in bold. *Myxobolus* spp. infecting catostomids boxed in grey.

Table 1. The *Myxobolus* spp. that infect fish from the family Catostomidae or that are morphologically similar to *Myxobolus naylori* sp. n. Mean measurements with range provided in μm . *Abbreviation:* MXL – myxospore length; MXW – myxospore width; MXT – myxospore thickness; PCL – polar capsule length; PCW – polar capsule width; PFC – polar filament coils; ME – mucous envelope (present/absent); IV – iodophilic vacuole in sporoplasm (present/absent); SM – sutural markings (present/absent); IP – intercapsular process (present/absent).

Species	Host	MXL	MXW	MXT	PCL	PCW	PFC	ME	IV	SM	IP	Site*	Locality	Reference
<i>M. bellus</i> Kudo, 1934	<i>Carpiodes carpio</i> (Rafinesque)	10.0–11.0	6.5–7.0	4.0–5.0	4.0–5.0	1.5–2.0	9–11	–	P	P	A	1	Illinois, USA	Kudo 1934
<i>M. bibullatus</i> (Kudo, 1934)	<i>Catostomus commersonii</i> (Lacepède)	14.0–15.0	11.5–12.5	6.0–7.5	7.0	3.5	–	–	A	P	A	1	Illinois, USA	Kudo 1934
<i>M. bubalis</i> Otto et Jahn, 1943	<i>Ictiobus bubalis</i> (Rafinesque)	13.1–14.7	10.2–11.7	–	5.8–6.3	2.2–2.9	6–7	–	P	P	P	9	Iowa, USA	Otto and Jahn 1943
<i>M. clariae</i> Hemananda, Mohilal, Bandyopadhyay et Mitra, 2009	<i>Clarias batrachus</i> (Hamilton)	10.5; 10.2–11.5	7.0; 6.8–7.7	–	3.7; 3.4–4.3	2.4; 1.7–2.6	–	A	P	A	P	13	India	Hemananda et al. 2009
<i>M. commersoni</i> (Fantham, Porter et Richardson, 1939)	<i>Catostomus commersonii</i>	9.5–16.5	7–11.4	–	7.7	3.2	–	–	A	A	A	1	Québec, Canada	Fantham et al. 1939
<i>M. congesticus</i> Kudo, 1934	<i>Moostoma anisurum</i> (Cope)	9.0–10.0	8.5–9.5	6.0	5.0–6.0	2.5–3.5	–	–	P	P	A	4	Illinois, USA	Kudo 1934
<i>M. conspicuus</i> Kudo, 1929	<i>Moostoma brevicaeps</i> (Cope)	9.0–11.5	6.5–8.0	4.5–5.5	5.0–7.0	2.0–2.5	10	–	P	P	A	2	Illinois, USA	Kudo 1929
<i>M. cornutus</i> Cone, Horner et Hoffman, 1990	<i>Lepomis macrochirus</i> Rafinesque	9.4; 8.0–10.5	8.0; 6.5–9.0	–	5.3; 4.0–5.5	2.4; 2.5–3.0	7–8	A	A	P	P	13	Illinois, USA	Cone et al. 1990
<i>M. diminutus</i> nom. nov. (Rosser, Griffin, Quiniou, Alberson, Woodyard, Mischke, Greenway, Wise et Pote, 2016)	<i>Ictiobus bubalis</i>	8.6; 7.4–9.6	8.8; 7.5–9.9	6.7; 6.5–7.3	4.3; 3.6–4.9	3.3; 2.8–3.8	5–6	–	A	A	A	3	Mississippi, USA	Rosser et al. 2016
<i>M. discrepans</i> Kudo, 1919	<i>Carpiodes velifer</i> (Rafinesque)	11.4–13.5	9.5–11.0	8.5–9.5	5.5–6.0	3.5–4.0	–	–	P	P	A	3	Illinois, USA	Kudo 1919
<i>M. ellipticoides</i> (Fantham, Porter et Richardson, 1939)	<i>Catostomus commersonii</i>	11.4–14.1	6.8–8.2	–	4.5–5.9	1.8–3.2	–	–	A	A	A	2	Québec, Canada	Fantham et al. 1939
<i>M. endovusius</i> (Davis, 1947)	<i>Ictiobus bubalis</i>	14.3; 13.1–15.4	13; 12.2–13.8	8.5	8.7; 7.7–9.6	5.5; 5.0–6.2	5–7	A	P	A	P	3	Illinois, USA	Lom and Cone 1996
<i>M. enoblei</i> Lom et Cone, 1996	<i>Ictiobus bubalis</i>	14.4; 13.5–15.0	11.1; 10.5–11.5	7.5	8.3; 7.9–8.5	4.8; 4.5–5.5	6–7	A	A	P	P	3	Illinois, USA	Lom and Cone 1996
<i>M. exallatus</i> Pugachev, 1980	<i>Catostomus catostomus</i> (Forster)	9.7–9.9	9.0–9.1	–	5.4–5.6	3	–	–	P	A	P	3	Russia	Pugachev 1980
<i>M. filamentus</i> (Rice et Jahn, 1943)	<i>Ictiobus bubalis</i>	16.3	13.2	–	7.8	6.2	8	–	A	A	P	3	Iowa, USA	Rice and Jahn 1943
<i>M. galliaicus</i> Iglesias, Parama, Alvarez, Leiro et Sammartin, 2001	<i>Pseudochondrostoma polylepis</i> (Steindachner)	10.0; 8.5–11	8.8; 8.2–9.5	5.7; 5.0–6.0	4.9; 4.5–5.5	2.9; 2.7–3.0	7–8	P	P	P	P	3	Spain	Iglesias et al. 2001
<i>M. globosus</i> Gurley, 1893	<i>Erimyzon succetta</i> (Lacepède)	7.0–8.0	6.0	5.0	–	–	–	–	P	A	A	3	Illinois, USA	Gurley 1893
<i>M. gravidus</i> Kudo, 1934	<i>Moostoma anisurum</i>	12.0–14.0	9.5–10.0	7.0	5.0–5.5	2.5	–	–	P	P	A	1	Illinois, USA	Kudo 1934
<i>M. hyderabadense</i> (Lalitha Kumari, 1969)	<i>Systomus sarana</i> (Hamilton)	10.1; 9.3–11.5	5.9; 5.0–8.0	–	5.8; 5.0–7.3	2.2; 1.4–3.0	8–9	–	A	P	P	3	India	Lalitha Kumari 1969
<i>M. ictiobus</i> Rosser, Griffin, Quiniou, Alberson, Woodyard, Mischke, Greenway, Wise et Pote, 2016	<i>Ictiobus bubalis</i>	13.9; 12.7–14.5	12.5; 10.7–13.6	12.6; 10.3–14.8	6.6; 5.6–7.4	4.5; 3.7–4.9	5–6	–	A	A	A	3	Mississippi, USA	Rosser et al. 2016
<i>M. irinae</i> Daniyarov, 1975	<i>Luctibarbuis capito</i> (Güldenstädt)	9.4–10.6	7.0–7.7	–	4.0–5.9	2.4–3.0	–	–	P	A	P	6	Central Asia	Eiras et al. 2005
<i>M. kalariffi</i> (Kalanian et Arfin, 1991)	<i>Garrus tibanicus</i> Trewavas	13.0; 11.3–15.5	7.3; 6.8–8.3	5.5; 5.0–7.0	5.6; 4.7–6.5	2.4; 2.4–3.0	6–8	–	P	A	P	5	Saudi Arabia	Kalanian and Arfin 1991
<i>M. kozloffii</i> Wyatt, 1979	<i>Delistius lucatus</i> (Cope)	13.5; 13.5–15.5	8.6; 8.0–9.5	7.2; 6.5–7.5	7.7; 7.5–8.5	3.2; 3.0–3.5	–	–	P	P	A	6	Oregon, USA	Wyatt 1979
<i>M. lamellus</i> Grinham et Cone, 1990	<i>Catostomus commersonii</i>	12.0; 9.5–13.5	10.5; 9.0–12.0	7.0; 5.0–8.0	6.0; 5.0–7.0	3.5; 3.0–4.5	5–6	A	P	A	P	3	Nova Scotia, Canada	Grinham and Cone 1990
<i>M. lesbanensis</i> Ma et Zhao, 1993	<i>Onychostoma angustistomatum</i> (Fang)	10.4; 9.6–12.0	8.5; 8.0–8.8	5.2; 4.8–5.6	4.9; 4.8–5.2	2.0; 1.8–2.4	–	–	P	A	P	6	China	Ma and Zhao 1992
<i>M. macrocapularis</i> Reuss, 1906	<i>Blicca bjoerkna</i> (Linnaeus)	9.0–14.5	6.0–9.9	4.5–6.0	5.0–8.6	2.4–3.6	–	A	P	A	P	3	Russia	Eiras et al. 2005
<i>M. meglitschi</i> (Meglitsch, 1937)	<i>Carpiodes cyprinus</i> (Lesueur)	12.0–14.0	11.0–13.0	7.0–8.5	6.0–7.0	3.0–4.0	6	–	A	A	A	3	Illinois, USA	Meglitsch 1937
<i>M. microthecus</i> (Meglitsch, 1942)	<i>Minytrema melanops</i> (Rafinesque)	11.7; 10.0–12.5	10.2; 8.3–11.4	4.5; 4.3–5.2	5.5; 3.8–6.3	2.4; 1.9–3.2	5–7	–	A	A	P	10	Illinois, USA	Meglitsch 1942
<i>M. mongolicus</i> Pronin, 1973	<i>Oreoleuciscus potanini</i> (Kessler)	11.0–12.0	8.0–11.0	4.0–6.0	3.5–5.0	2.2–3.2	–	–	P	A	P	6, 9, 11, 12	Russia	Eiras et al. 2014
<i>M. morrissonae</i> Lom et Cone, 1996	<i>Ictiobus bubalis</i>	10; 9.6–10.5	9.5; 9.1–10.3	5.0	5.5; 5.3–5.8	3.7; 3.4–4.0	6	A	A	P	P	3	Illinois, USA	Lom and Cone 1996
<i>M. moxostomi</i> Nigrelli, 1948	<i>Moxostoma macrolepidotum</i> (Lesueur)	7.6; 6.2–9.4	7.2; 5.5–9.4	3.9; 3.1–4.7	3.6; 2.3–3.9	2.3; 1.6–3.2	3–5	–	P	A	A	1	New York Aquarium	Nigrelli 1948
<i>M. multiplicatus</i> (Reuss, 1906)	<i>Ictiobus bubalis</i>	12.0; 12.0–12.5	9.5; 9.2–9.5	6.0; 6.0–6.5	4.0; 3.5–4.5	2.2; 2.0–2.5	–	–	A	P	A	3	Iowa, USA	Rice and Jahn 1943

<i>M. musculosus</i> (Kudo, 1923)	<i>Catostomus commersonii</i>	13.0–15.0	10.0–11.5	8.0–8.5	5.0–6.0	2.5–3.3	6	–	A	P	A	5	Michigan, USA	Kudo 1923
<i>M. naffari</i> Abdel-Ghaffar, Ibrahim, Bashir et Ali, 1998	<i>Labeo niloticus</i> (Linnaeus)	11.9; 10.8–13.2	8.8; 7.8–9.8	–	5.1; 4.5–6.2	2.6; 2.5–3.0	7–9	–	P	A	P	3	Egypt	Eiras et al. 2005
<i>M. naylori</i> n. sp. Ksepka et Bullard	<i>Moxostoma</i> sp., sicklefin redhorse	10.8; 10.0–12.0	8.2; 7.0–9.0	5.5; 5.0–6.0	4.8; 4.0–6.0	2.9; 2.0–3.0	7–10	P	A	P	P	14	North Carolina, USA	Present study
<i>M. nijoyi</i> Nhouptouen et Fomena, 2011	<i>Labeo parvus</i> Boulenger	9.7; 9.0–10.5	8.7; 7.8–9.0	–	5.2; 4.5–5.8	3.0; 2.8–3.5	6–8	–	P	A	P	3, 4, 6, 7	Cameroun	Fomena 2011
<i>M. obliquus</i> Kudo, 1934	<i>Carpiodes velifer</i>	8.0–9.0	7.0–8.0	5.0–6.0	4.5	2.0	6–7	–	P	P	P	5	Illinois, USA	Kudo 1934
<i>M. oblongus</i> Gurley, 1893	<i>Erimyzon sucetta</i>	14.0–17.0	8.5	5.5–6.0	–	–	–	–	A	A	A	1	Illinois, USA	Gurley 1893
<i>M. ovalis</i> (Davis, 1923)	<i>Itiobius bubalus</i>	15.0–17.0	15.0	11.0	8.0–9.0	6.0	4–6	–	A	A	A	3	Iowa, USA	Davis 1923
<i>M. ovata</i> Kudo, 1934	<i>Itiobius bubalus</i>	11.5–13.0	9.0–10.0	7.0	5.5–6.5	2.5–3.0	10	–	P	A	P	1	Illinois, USA	Kudo 1934
<i>M. percarinae</i> Iskov et Karatev, 1982	<i>Percarina demidoffi</i> (Nordmann)	10.8–13.0	7.2–9.6	–	3.6–4.8	2.0–2.4	–	–	P	A	A	3, 6	Ukraine	Eiras et al. 2005
<i>M. pratti</i> (Wyatt, 1979)	<i>Delistes laxatus</i>	18.2; 17.0–20.5	12.6; 11.0–14.0	7.9; 7.5–8.5	6.6; 5.5–7.5	3.2; 2.5–3.5	6–8	–	P	A	A	6	Oregon, USA	Wyatt 1979
<i>M. smithi</i> Salim et Desser, 2000	<i>Chrosomus eos</i> (Cope)	11.0; 9.8–12.2	8.5; 8.1–9.2	6.0; 5.2–6.9	4.8; 4.2–5.4	2.8; 2.1–3.1	5–7	P	A	P	P	6	Ontario, Canada	Salim and Desser 2000
<i>M. squamiae</i> Keysseltz, 1908	<i>Cyprinus carpio</i> (Linnaeus)	10.0–13.5	7.5–9.0	5.0–6.0	4.5–5.5	3.0	–	A	P	A	P	1	Russia	Eiras et al. 2005
<i>M. strelkovi</i> Kostarev et Kulemina, 1971	<i>Phoxinus phoxinus</i> (Linnaeus)	8.0–12.2	6.0–11.0	4.0–7.0	3.3–5.4	2.0–4.1	–	–	P	A	P	3, 8	Russia	Eiras et al. 2005
<i>M. subcircularis</i> Fantham, Porter et Richardson, 1939	<i>Catostomus commersonii</i>	9.1–11.8	8.2–10.0	–	3.2–5.0	1.8–3.0	–	–	P	A	A	5	Québec, Canada	Fantham et al. 1939
<i>M. transvalis</i> Gurley, 1893	<i>Clinostomus funduloides</i> Girard	6.0–7.0	8.0	–	–	–	–	–	P	A	A	2	Virginia, USA	Gurley 1893
<i>M. vastus</i> Kudo, 1934	<i>Moxostoma macrolepidotum</i>	9.5–10.5	7.5–8.0	4.0–4.5	4.5–5.5	1.5–2.5	–	–	P	P	A	1	Illinois, USA	Kudo 1934
<i>M. zhujiangensis</i> (Chen et Zhou, 1998)	<i>Clarias fuscus</i> (Lacépède)	12.2; 11.7–12.6	8.0; 7.2–8.6	5.6; 5.2–5.8	4.7; 4.2–5.0	2.4; 2.2–2.6	6–7	A	A	A	A	1, 4	China	Eiras et al. 2005
<i>M. zilli</i> Sakiti, Blanc, Marques et Bouix, 1991	<i>Coptodon zilli</i> (Gervais)	9.8; 8.0–11.0	7.5; 6.0–8.0	–	5.1; 4.0–6.0	2.5; 2.0–3.0	–	–	P	A	P	3	Benin	Eiras et al. 2005

*Site: 1 – skin; 2 – dermis; 3 – gills; 4 – fins; 5 – muscle; 6 – kidney; 7 – spleen; 8 – liver; 9 – intestine; 10 – mesentery; 11 – brain; 12 – eye; 13 – testes; 14 – stratum spongiosum

alignment and were identical. We recovered two well-supported clades: (A) 27 species of *Myxobolus* and two species of *Thelohanellus* Kudo, 1933 (Bivalvulida: Myxobolidae) and (B) 38 species of *Myxobolus* (including the new species), 12 species of *Thelohanellus*, and one species of *Dicauda* Hoffman et Walker, 1973 (Bivalvulida: Myxobolidae) (Fig. 4). Consistent with Liu et al. (2019), *Myxobolus* spp. that infect catostomids were recovered paraphyletic (Fig. 4). The new species was recovered as a distinct lineage within clade B, but was part of a polytomy with *Myxobolus marumotoi* Li et Sato, 2014, which infects the muscle of the dark sleeper (*Odontobutis obscura* [Temminck et Schlegel] [Gobiiformes: Odontobutidae]), and a clade comprising the remainder of clade B (Fig. 4). The uncertain placement of the new species may be a result of undersampling of *Myxobolus* spp. infecting catostomids and odontobutids, which are represented only by seven and one species, respectively, in contrast to *Myxobolus* spp. infecting cyprinids, which are represented by 57 species. Future studies to illuminate the diversity of species of *Myxobolus* infecting catostomids and odontobutids may provide better placement of the new species in phylogenetic analyses.

Histopathology

Plasmodia (ellipsoid in cross section) of *Myxobolus naylori* sp. n. were intercellular and infected the connective tissue of the *stratum spongiosum* of sicklefin redhorse skin. Myxospore development in the plasmodium was synchronous, with mature myxospores occupying almost the entire volume of the plasmodium (Fig. 3A). In both infected scales, plasmodia were encapsulated by a lightly eosinophilic layer of densely packed type I collagen fibres with active fibroblasts and sparse lymphocytic inflammatory infiltrates on the periphery of the encapsulation (Fig. 3B,C). Lateral to the plasmodia were regions of loosely packed proliferating fibroblasts (Fig. 3B). Scales beneath plasmodia showed varying degrees of erosion ranging from a marked thinning of the scale to a complete loss of the area of the scale beneath the plasmodium (Fig. 3A,B). Goblet and alarm substance cells were absent from the epithelium anterior to plasmodia (Fig. 3A,C). In fish infected with up to 14 plasmodia, no scale was observed having >1 plasmodium.

DISCUSSION

Particular parasite infections have been proposed as effective biological tags (MacKenzie 2002, Marcogliese and Jacobson 2014), and it is not uncommon to encounter proposals by parasitologists or fisheries biologists to use this or that parasite as a marker (“tag”) that helps a biologist identify to what species or population the host belongs. However, there are many pitfalls to this practice (MacKenzie and Abauza 2005). Parasites suitable for use as biological tags should (i) persist in host for a long period of time, (ii) exhibit a high degree of host specificity (ideally strict specificity; the parasite does not infect another host), (iii) be benign (non-pathogenic), and (iv) be relatively easy to identify in the field or easily observed as an ectoparasite (MacKenzie et al. 2013). Some myxozoans meet these criteria and represent a promising group of parasites to be

used as biological tags because they exhibit high host specificity and can reside in their host for many years (Cantatore et al. 2016). As such, a number of myxozoans have been used as biological tags in stock assessments (Catalano et al. 2014, Marcogliese and Jacobson 2014). *Myxobolus arcticus* (Pugachev et Khokhlov, 1979) has been used to assess homing precision and natal origin of Chinook salmon (*Oncorhynchus tshawytscha* [Walbaum] [Salmoniformes: Salmonidae]) (Quinn et al. 1987). *Kudoa nova* Naidenova, 1975 (Multivalvulida: Kudoidae) has been used to differentiate stocks of Atlantic horse mackerel (*Trachurus trachurus* [Linnaeus] [Carangiformes: Carangidae]) (MacKenzie et al. 2008). While many species of *Myxobolus* are extremely morphologically similar, the new species' unique combination of possessing a mucous envelope, intercapsular process, sutural markings, and lacking an iodophilic vacuole in the sporoplasm makes it relatively simple to identify, especially given the minute size of the myxospore. Although the plasmodia reside beneath the epithelium, their large size makes them readily apparent (presenting as an ectoparasite) such that no dissection of, and no killing of, the host is required to identify infections. In specific, the new species represents a potential biological tag for the sicklefin redhorse in the Little Tennessee River Basin due to its likely high level of host specificity (infecting only sicklefin redhorse) in that drainage basin. It is worth noting that it is possible the new species could infect closely related *Moxostoma* spp. However, after the initial detection in 2018 personnel were screening all *Moxostoma* spp. collected for infections and did not observe plasmodia on the body surface of any other species of *Moxostoma* suggesting the new species is specific to sicklefin redhorse. This could be useful in differentiating the sicklefin redhorse from the river redhorse, which inhabits the same system and can be difficult to differentiate from the sicklefin redhorse, particularly if the dorsal fin, the primary morphological difference between the species, is damaged.

No species of *Myxobolus* has been reported as a pathogen of a wild catostomid. The lesion we report herein is minor and probably benign to the overall health of infected

sicklefin redhorses. Conversely, obviously, several myxozoans are pathogenic if infecting hosts outside of their native range (Bartholomew and Reno 2002, Molnár 2002). For example, *Myxobolus cerebralis* (Bütschli, 1882), the causative agent of whirling disease, was introduced to North America from Europe (Bartholomew and Reno 2002) and is non-pathogenic to brown trout (*Salmo trutta* Linnaeus) in Europe. In North America, *M. cerebralis* led to mortality of wild and cultured salmonids and remains one of the most important salmonid pathogens in North America (Thompson et al. 1999, Bartholomew and Reno 2002). *Thelohanellus nikolskii* Akhmerov, 1954 was introduced from Asia to Europe with infected grass carp (*Ctenopharyngodon idella* [Valenciennes]) (Molnár 2002). Outside of its native range *T. nikolskii* forms clusters of plasmodia on the fins, impairing fin development, of common carp (*Cyprinus carpio* Linnaeus) representing one of the most common diseases of carp in Hungary (Molnár 1982, 2002). As the new species was not detected in the 41 fish from the Hiwassee River Basin, previous sicklefin redhorse survey work in the Hiwassee River Basin have not resulted in reports of infections by *Myxobolus*, and breeding and reintroduction/stock enhancement efforts are ongoing, it is important to consider the new species as a potential pathogen if introduced to the Hiwassee River Basin population or into a culture setting.

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