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Myxobolus lepomis n. sp. (Cnidaria: Myxobolidae), a gill myxozoan infecting *Lepomis marginatus* Holbrook and *Lepomis miniatus* Jordan (Perciformes: Centrarchidae), in the Big Thicket National Preserve, Texas, USA

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Abstract A parasitological survey of freshwater fishes in the Big Thicket National Preserve in southeast Texas revealed myxozoan infections in two species of sunfish, *Lepomis marginatus* Holbrook and *Lepomis miniatus* Jordan (Perciformes: Centrarchidae). Pseudocysts were elongate-oval, 988 × 485 µm (ex *L. marginatus*) and 800 × 606 µm (ex *L. miniatus*) and demonstrated a predilection to the edge of the primary gill lamellae. Myxospores consistent with the genus *Myxobolus* were oblong, 16.8–21.3 (19.0 ± 0.9) µm long, 7.0–8.8 (7.9 ± 0.5) µm wide and 5.3–6.1 (5.8 ± 0.3) µm thick (ex *L. marginatus*) and 17.2–20.3 (18.8 ± 0.7) µm long, 7.5–9.9 (8.7 ± 0.6) µm wide, and 6.8–7.2 (7.0 ± 0.2) µm thick (ex *L. miniatus*); with 2 pyriform polar capsules 8.3–9.8 (9.0 ± 0.5) µm long, 2.2–2.7 (2.5 ± 0.2) µm wide (ex *L. marginatus*) and 9.2–10.5 (10.0 ± 0.4) µm long, 2.2–3.0 (2.8 ± 0.2) µm wide (ex *L. miniatus*). Statistically, the measurements of spore body width, polar capsule length, and polar capsule width were significantly different between myxospores from *L. marginatus* and *L. miniatus*. However, intraspecific genetic variability between isolates at the 18S rRNA gene was negligible, with < 0.8% variability across > 2,000 bp of sequence. The isolates shared no significant sequence similarity with any myxozoan deposited in the GenBank nucleotide database. Phylogenetic analysis inferred from the 18S rRNA gene from both *L. marginatus* and *L. miniatus* placed the isolates within a clade of myxozoan parasites of perciform fishes. Based on shared tissue and host family tropism, overlapping morphological characters and high degrees of sequence conservation at the 18S rRNA gene, we propose these isolates as

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morphologically distinct, genetically conspecific representatives of *M. lepomis* n. sp. from the gills of *L. marginatus* and *L. miniatus* in the Big Thicket National Preserve in Texas, USA.

Introduction

Myxozoans are cosmopolitan metazoan parasites of fresh, marine and brackish water fish with a wide range of host and tissue predilections. The typical biphasic life-cycle involves a planktonic actinospore stage released from annelid hosts and a myxospore stage infecting the fish host (Wolf & Markiw, 1984; Kent et al., 2001). As a function of accessibility and the economic importance of fish hosts, myxospore stages dominate the literature (Eiras, 2002; Eiras et al., 2005). Of the 64 known genera, *Myxobolus* Bütschli, 1882 is perhaps the most commonly encountered myxozoan genus and contains the largest number of described species (Eiras et al., 2005; Fiala et al., 2015).

Sunfish, *Lepomis* spp. (Perciformes: Centrarchidae) are popular freshwater sport fish endemic to North America waterways. In the southeastern United States, the dollar sunfish *Lepomis marginatus* Holbrook and redspotted sunfish *Lepomis miniatus* Rafinesque inhabit freshwater ponds, creeks, swamps and river systems (Lee et al., 1980). The myxozoan fauna of *Lepomis* spp. of North America have been well studied, but to date no myxozoans have been described from *L. marginatus* or *L. miniatus*. At least thirteen species of *Myxobolus* have been reported from *Lepomis* spp. from North America (Kudo, 1919; Hoffman et al., 1965; Cone & Anderson, 1977; Li & Desser, 1985; Cone et al., 1990; Cone & Overstreet, 1997, 1998). However, many of these reports occurred prior to the rise of molecular methods and the recent practice of supplementing morphological, host and geographic records with gene sequence data (Atkinson et al., 2015). Molecular sequence data serve two purposes: (i) they assist in the identification of cryptic species and/or provide molecular differentiation of morphologically ambiguous isolates from different hosts or tissue sites (Eszterbauer, 2002; Ferguson et al., 2008); and (ii) aid in the elucidation of life-cycles by direct sequence comparisons (Lin et al., 1999; Anderson et al., 2000; Kent et al., 2001; Bartholomew et al., 2006; Rosser et al., 2015). Herein we describe *Myxobolus lepomis* n. sp. infecting the gills of *L. marginatus* and *L. miniatus* from the Big Thicket National Preserve in Texas, USA based on novel morphology, histology and 18S rRNA sequence data.

Materials and methods

Fish collection

On July 1, 2010, fish were collected by seine from an unnamed tributary of Big Sandy Creek, near Sunflower Road in the Big Sandy Creek Unit of the Big Thicket National Preserve in Polk County, Texas, USA. After seining fish were maintained in aerated stream water until dissected. Myxozoan-infected gill filaments were excised and fixed in 70% ethanol for later morphological and molecular analyses. Fish hosts were identified to species using appropriate keys (Thomas et al., 2007).

Morphological description

Discrete pseudocysts (n = 2) were excised from ethanol-fixed gill tissues of individual specimens of *L. marginatus* and *L. miniatus.* Excised pseudocysts were placed on separate glass microscope slides with a drop of 0.09% saline, covered with coverslips and mechanically ruptured. Myxospores were examined using an Olympus BX-50 microscope (Olympus Optical Co. Ltd., Tokyo, Japan) and imaged using an Olympus DP72 camera and DP-2-Twain/cellSens software (Olympus Optical Co. Ltd.).

Digital measurements were obtained from photomicrographs of 30 individual myxospores from each fish species according to established guidelines for myxozoan species descriptions (Lom & Arthur, 1989). Normality of the data was confirmed and two-tailed *t*-tests were performed to determine whether differences between the morphological characters measured for myxospores from *L. marginatus* and *L. miniatus* were significant (Statistical Analysis Software, SAS Institute, Inc., Cary North Carolina, USA). It is important to note the measurements reported here were averaged from myxospores derived from ethanol-fixed tissues and could differ from fresh or unfixed specimens as a function of prolonged fixation (Kudo, 1921; Parker & Warner, 1970). All measurements are in micrometres and are given in the text as the range followed by the mean and standard deviation in parentheses.

A single infected gill arch from *L. marginatus* fixed in 10% neutral buffered formalin was submitted for routine histological processing. The tissue was embedded in paraffin and sectioned into 5 µm thick ribbons, then subsequently stained with hematoxylin and eosin for routine histological assessment.

Molecular data

Myxospores were rinsed from their respective microscope slides into individual 1.5 ml-microcentrifuge tubes and concentrated by centrifugation at 10,000×g for 10 min. Total genomic DNA was extracted from pelleted myxospores using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, California) and stored at −20 °C until analysis.

The 18S rRNA gene was amplified using primers routinely used for the molecular characterization of the Myxobolidae (Table 1). All 25-µl reactions contained 22 µl of Platinum Taq Supermix (Invitrogen, Carlsbad, California, USA), 10 pmol of each primer, and 1 µl of gDNA (∼10 ng/µl) suspension. Primers were paired as follows to generate overlapping fragments: ERIB1/ACT1R, Myxo1F/Myxgen3R, MyxospecF/MyxospecR, Genmyxo3/H2, H9/H2, and H9/ERIB10. Thermal cycling protocols were the same for all reactions and consisted of 95°C for 10 min, followed by 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 10 min. All reactions were performed on an MJ Research PTC-200 thermocycler (GMI, Ramsey, Minnesota, USA). Amplification products were electrophoretically passed through 1.2% agarose gels in the presence of ethidium bromide (0.5 µg/ml) and examined under ultraviolet light. A concurrently run molecular weight ladder (HyperLadder™ 50 bp, Bioline, London, UK) was used to identify appropriate sized bands for excision and purification using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, California). Direct sequencing of purified PCR amplicons was performed commercially using the forward and reverse primers from each respective reaction (Eurofins MWG Operon LLC, Huntsville, Alabama, USA). Sequencing reads were aligned and edited using the SeqMan™ utility of the Lasergene software package (DNAStar, Madison, Wisconsin, USA). Contiguous 18S rDNA sequences generated for each respective isolate were compared against each other and to other myxozoan sequences deposited in the GenBank non-redundant nucleotide database (Altschul et al., 1990).

Sequences (> 1,500 nt) from closely related myxozoans were identified by BLASTn search, downloaded, Clustal W aligned and trimmed using Molecular Evolutionary Genetic Analysis v6.0 (MEGA6) (Tamura et al., 2013). The final dataset contained 1,298 nt positions and all positions containing gaps or missing data were eliminated, resulting in an alignment length of 1,298 nt. The Akaike and Bayesian Information Criterion were used to determine the best evolutionary model for the data and phylogenetic placement of the isolates were assessed using the maximum likelihood method and Bayesian inference with the General Time Reversible model (GTR) (Nei & Kumar, 2000). Topology of the maximum likelihood analysis was assessed by bootstrapping with 1,000 pseudoreplicates (Felsenstein, 1985). Bayesian inference was implemented using MrBayes version 3.2.6 by Markov chain Monte Carlo

Table 1 Primers used in amplification of the 18S rRNA gene of myxospores

Primer ID	Sequence (5′–3′)	Reference
ACT1R	AATTTCACCTCTCGCTGCCA	Hallet & Diamant (2001)
ERIB1	ACCTGGTTGATCCTGCCAG	Barta et al. (1997)
ERIB10	CCTCCGCAGGTTCACCTACGG	Barta et al. (1997)
Genmyxo4	GGATGTTGGTTCCGTATTGG	Griffin et al. (2008)
H2	CGACTTTTACTTCCTCGAAATTGC	Hanson et al. (2001)
H9	TTACCTGGTCCGGACATCAA	Hanson et al. (2001)
Myxo1F	CTGCCCTATCAACTWGTT	Kent et al. (2000)
Myxogen3R	TGCCTTCGCATTYGTTAGTCC	Kent et al. (2000)
MyxospecF	TTCTGCCCTATCAACTWGTTG	Fiala (2006)
MyxospecR	GGTTTCNCDGRGGGMCCAAC	Fiala (2006)

searches of two simultaneous runs of four chains for 1,000,000 generations with every 100th tree sampled (Ronquist & Huelsenbeck, 2003). The first 25% of trees were discarded as 'burn-in' and the posterior probabilities were calculated from the remaining trees.

Family Myxobolidae Thélohan, 1892
Genus *Myxobolus* Bütschli, 1882

***Myxobolus lepomis* n. sp.**

Type-host: *Lepomis marginatus* Holbrook, dollar sunfish (Centrarchidae).
Other host: *Lepomis miniatus* Jordan, redspotted sunfish (Centrarchidae).
Type-locality: Unnamed tributary of Big Sandy Creek (30.61378N, 94.67595W), Big Thicket National Preserve, Polk County, Texas, USA.
Type-material: Syntypes are deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska (HWML 103047–103049).
Site in host: Gills.
Prevalence of infection: 1 of 1 *L. miniatus*; 1 of 8 (12.5%) *L. marginatus.*
Representative DNA sequences: 18S rDNA, GenBank KY203390–KY203391.
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID). The LSID for *Myxobolus lepomis* n. sp. is urn:lsid:zoobank.org:act:790B9FFB-112C-4ED2-A918-B8E8C59BEA8B.
Etymology: The specific epithet is in reference to the genus of the host(s).

Description (Figs. 1, 2, 3)

Pseudocyst (ethanol fixed) ex *L. marginatus*, large, along the edge of the primary gill lamellae, 988 × 485. Pseudocyst (ethanol fixed) ex *L. miniatus*, large, along the edge of the primary gill lamellae, 800 × 606. Myxospores consistent with the genus *Myxobolus*, oblong; spores ex *L. marginatus* 16.8–21.3 (19.0 ± 0.9) long, 7.0–8.8 (7.9 ± 0.5) wide, 5.3–6.1 (5.8 ± 0.3) thick (Figs. 1A, C, E, F, 2, 3); spores ex *L. miniatus* 17.2–20.3 (18.8 ± 0.7) long, 7.5–9.9 (8.7 ± 0.6) wide, 6.8–7.2 (7.0 ± 0.2) thick (Fig. 1B, D, G, H). Polar capsules 2, equal, pyriform, 8.3–9.8 (9.0 ± 0.5) long, 2.2–2.7 (2.5 ± 0.2) wide (ex *L. marginatus*); 9.2–10.5 (10.0 ± 0.4) long, 2.2–3.0 (2.8 ± 0.2) wide (ex *L. miniatus*). Polar filament coils 10–12 times in the polar capsule. Intercapsular process absent. Sutural edge markings absent. Mucous envelop and iodinophilous vacuole not observed.

Morphometric variation

Statistically, the measurements of spore body width (t-test, t = -5.51, $P < 0.0001$; n = 30), polar capsule length (t-test, t = -9.12, $P < 0.001$ (n = 30), and polar capsule width (t-test, t = -7.89, $P < 0.0001$ (n = 30) were significantly different between myxospores from *L. marginatus* and *L. miniatus*. Meanwhile spore body length was not significantly different (t-test, t = 0.70, P = 0.49 (n = 30).

Histological data

Histologically, the gill arch examined had a single myxozoan plasmodium, approximately 400 µm wide, within the epithelium of the filament replacing lamellae (Fig. 3A). Such a nodule correlates to the filamental, epithelial type described previously in a review of the subject (Molnár, 2002). The plasmodium was covered by a 25 µm thick capsule composed of 6–8 layers of mesenchymal cells with an outer simple squamous epithelium. A delicate connective tissue capsule composed of 1–2 layers of elongate fibroblasts in a compact collagen matrix surrounded the parasitic core. This core had a thin peripheral rim of botryoid immature forms, each 2–5 µm wide, encircling the inner population of mature elongate spores (Fig. 3B).

Remarks

Overall, myxospores of the isolate are unusually oblong, compared to other species of *Myxobolus* from centrarchid fish of North America (Supplementary Table S1), but are most similar in morphology to *Myxobolus mississippiensis* Cone & Overstreet, 1997 from the gills of *Lepomis macrochirus* Rafinesque, 1810 from the Pascagoula River, Jackson County, Mississippi (Cone & Overstreet, 1997). Although similar, spores of the isolates described in this paper are longer (18.8 and 19.0 *vs* 17.7 µm) and wider (7.9 and 8.7 *vs* 5.2 µm) than those of *M. mississippiensis*.

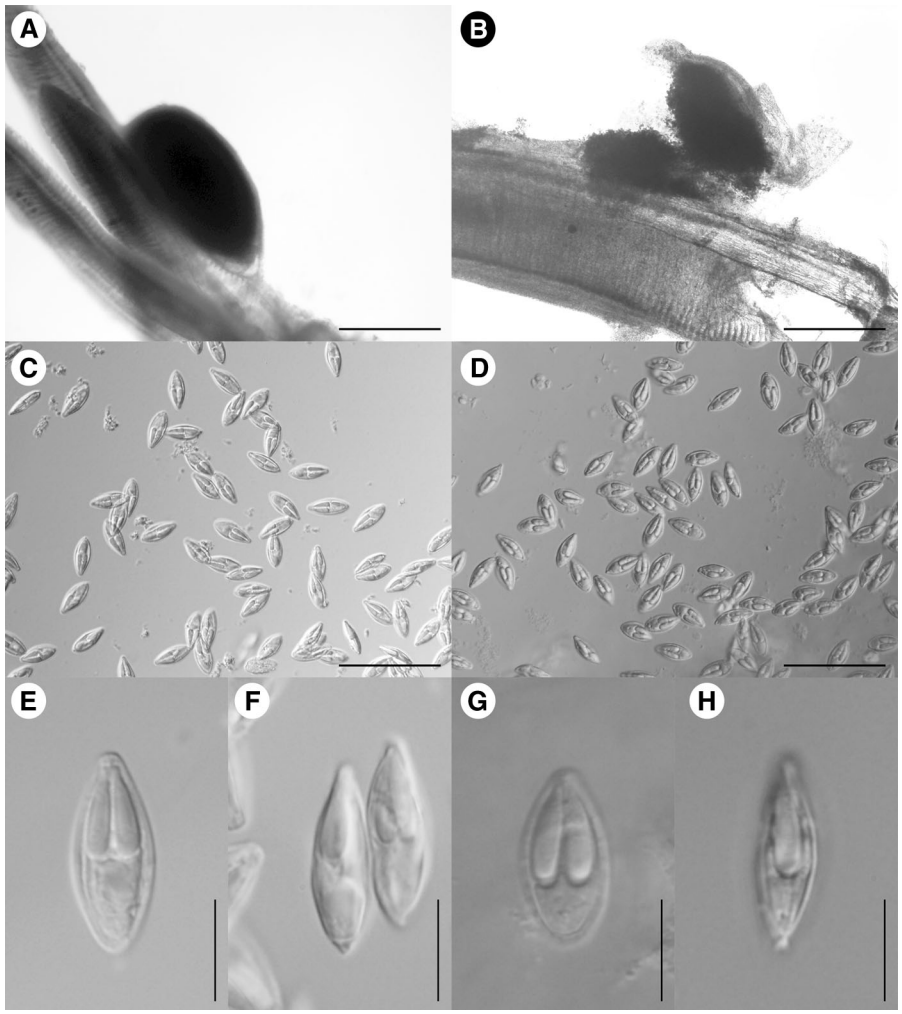

Fig. 1 Photomicrographs of myxospores of *Myxobolus lepomis* n. sp. ex *Lepomis marginatus* (A, C, E–F) and *Lepomis miniatus* (B, D, G–H). A, Pseudocyst in gill filament of *L. marginatus*; B, Pseudocyst in gill filament of *L. miniatus*; C, Myxospores ex *L. marginatus*; D, Myxospores ex *L. miniatus*; E, Myxospore ex *L. marginatus*, valvular view; G, Myxospore ex *L.* miniatus, valvular view; F, Myxospore ex *L. marginatus*, sutural view; H, Myxospore ex *L. miniatus*, sutural view. *Scale-bars*: A, B, 500 μm; C, D, 50 μm; E–H, 10 μm

Furthermore, polar capsules are longer (9.0 and 10.0 *vs* 7.2 μm), but narrower (2.5 and 2.8 *vs* 6.3 μm) than those in *M. mississippiensis*. Similar to *M. mississippiensis*, the posterior end of some myxospores bent away from the sutural plane. All other species of *Myxobolus* from *Lepomis* spp. and other centrarchid fishes of North America differ considerably in size and possess the typical rounded *Myxobolus* shape (Supplementary Table S1).

It should be noted, however, that these differences could be associated with shrinkage of the myxospores as a result of ethanol fixation and should be verified with freshly collected (unfixed) myxospores when encountered. Furthermore, although significantly different across multiple morphological characters, the ranges of all features overlapped (Supplementary Table S1).

Molecular data

Phylogenetic analysis inferred from the 18S rRNA gene for myxospores of *Myxobolus lepomis* n. sp. from both *L. marginatus* and *L. miniatus* placed the isolates within a clade of myxozoan parasites of perciform fishes (Fig. 4). Within this clade, the two isolates fell

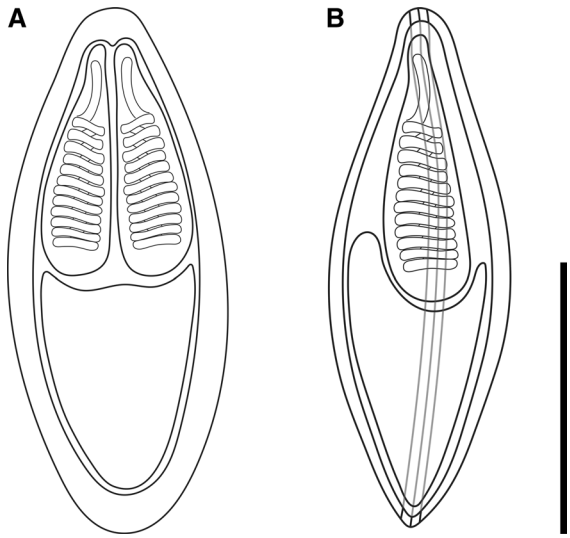

Fig. 2 Line drawing of *Myxobolus lepomis* n. sp. ex *Lepomis marginatus* (wet mount preparation of myxospores). A, Valvular view; B, Sutural view. *Scale-bar*: 10 µm

within a subclade containing *M. branchiarum* from the centrarchid smallmouth bass and a neoactinomyxum type actinospore from *L. hoffmeisteri*. The remaining subclades consisted of myxozoans from marine and freshwater perciform fishes.

Intraspecific genetic variability at the 18S rRNA gene was negligible between isolates, with < 0.8% variability across > 2,000 bp of sequence. This is consistent with previous accounts of intraspecific variability of *Myxobolus* species citing a range of variability from 0.1 to > 3.0 % (Andree et al., 1999; Whipps et al., 2004; Molnár et al., 2006; Arsan et al., 2007; Atkinson et al., 2015; Griffin et al., 2014; Scott et al., 2015). Alignment of the 18S rRNA gene sequences (Fig. 5) from both isolates demonstrated little variation (< 14 bp) especially in variable regions previously considered useful in species designation (Hallett et al., 2006; Iwanowicz et al., 2008; Griffin et al., 2009a, b; Camus & Griffin, 2010).

The isolates shared no significant sequence similarity with any myxozoan deposited in the GenBank database, but shared highest sequence similarity (96.3–96.4%; 75% coverage; JF714994) with *Myxobolus branchiarum* Walsh, Iwanowicz, Glenney, Iwanowicz & Blazer, 2012 from the gills of smallmouth bass *Micropterus dolomieu* Lacépède from Maryland, Virginia and West Virginia, USA, followed by a neoactinomyxum type actinospore (93.9–94.0%; 99% coverage; AF378353) from *Limnodrilus hoffmeisteri* Claparède from Ontario, Canada, and an aurantiactinomyxon type actinospore (87.1–87.3%; 100% coverage; AF378356) from *L. hoffmeisteri* from Ontario, Canada. Lastly, the isolates shared < 85.0% sequence similarity with seven species of *Henneguya* Thélohan, 1892 described from siluriform fishes from Mississippi, USA.

While the two isolates differed significantly across several morphological features, they share similar tissue predilection in closely related hosts. Moreover, the similarity in molecular sequence data of this species further indicates these two isolates represent a single novel species with morphological dissimilarity in different hosts, described here as *Myxobolus lepomis* n. sp.

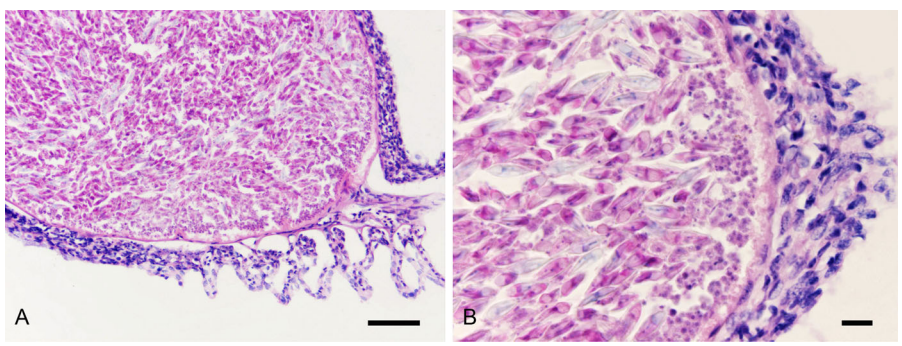

Fig. 3 Photomicrographs of a plasmodium of *Myxobolus lepomis* n. sp. in the gills of *Lepomis marginatus*. A, Pseudocyst in gill filament that replaces lamellae, is lined by a densely cellular mantle, and is well demarcated by a thin capsule (hematoxylin and eosin staining); B, The pseudocyst is composed of a thin peripheral rim of small immature forms that surround numerous mature spores (hematoxylin and eosin staining). *Scale-bars*: A, 50 µm; B, 10 µm

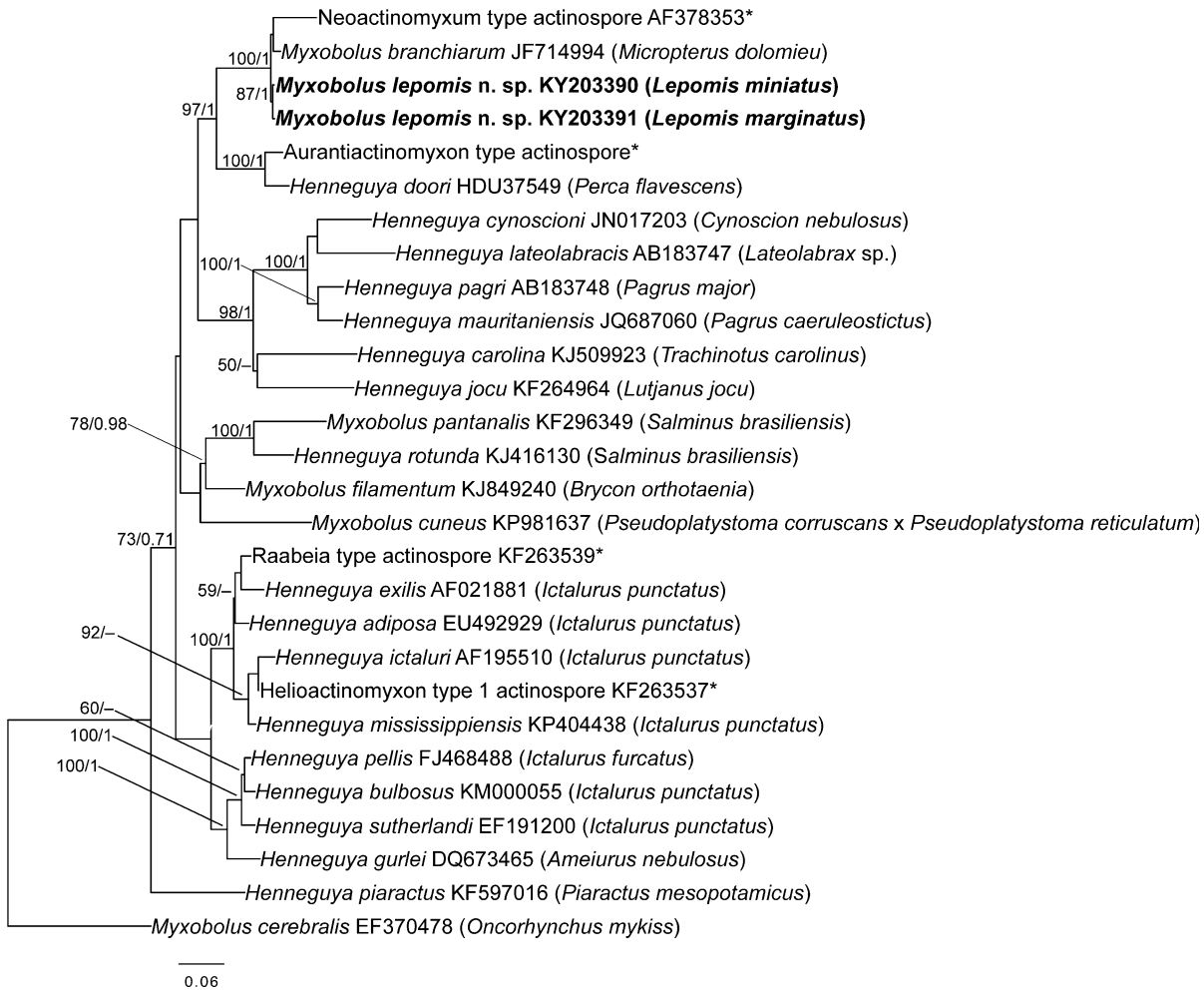

Fig. 4 Phylogenetic tree constructed from 18S rRNA gene sequences of *Myxobolus lepomis* n. sp. ex *Lepomis marginatus* and *L. miniatus* (*bold*) and other closely related members of the family Myxobolidae with the fish host in parentheses. Actinospore stages with unknown fish hosts are indicated by an asterisk. Topology shown is based on Maximum Likelihood analysis. Values above branches represent Maximum Likelihood analysis bootstrap confidence values obtained from 1,000 pseudoreplicates followed by Bayesian posterior probabilities. Values < 50% not shown; "–" represents differing topologies

Discussion

Currently, thirteen *Myxobolus* species are described from *Lepomis* spp. in North America. Kudo (1919) recorded the first account, *Myxobolus mesentericus* Kudo, 1919 from multiple organ systems of the green sunfish *Lepomis cyanellus* Rafinesque in Illinois, USA. *Myxobolus cartilaginis* Hoffman, Putz & Dunbar, 1965 was later described infecting the head cartilage and cartilage aspects of gill arches of *L. cyannelus*, the bluegill *Lepomis macrochirus* Rafinesque, and *Micropterus salmoides* Lacépède from West Virginia, USA (Hoffman et al., 1965). Cone & Anderson (1977) described the myxozoan parasites infecting *L. gibbosus* from Algonquin Park, Ontario, Canada. In their study, three new *Myxobolus* species were characterized: *Myxobolus dechtiari* Cone & Anderson, 1977 was described from the gill tissue, *Myxobolus magnaspherus* Cone & Anderson, 1977 from kidney tissue and peritoneum, and *Myxobolus uvuliferis* Cone & Anderson, 1977, which was interestingly restricted to the connective tissue capsules surrounding metacercariae of *Uvulifer ambloplitis* (see Cone & Anderson, 1977). Additionally, *Myxobolus osburni* Herrick, 1936, originally described from the smallmouth bass, was reported from *L. gibbosus* in

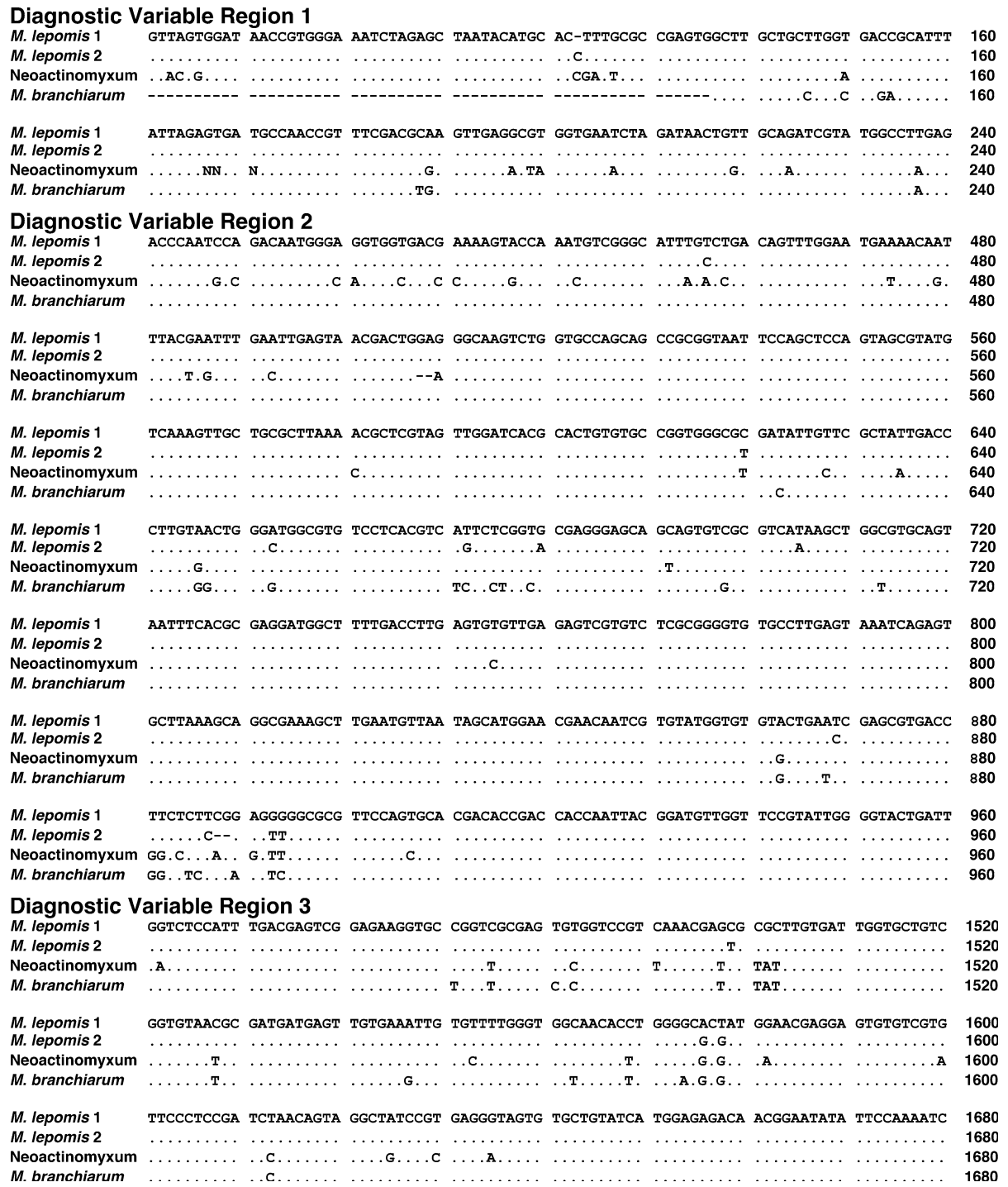

Fig. 5 18S rRNA gene sequence alignment of *Myxobolus lepomis* n. sp. isolates ex *Lepomis marginatus* and *L. miniatus*. Alignment shows diagnostic variable regions identified for the Myxobolidae. *Abbreviations*: *M. lepomis* 1, sequence for the isolate ex *L. miniatus*; *M. lepomis* 2, sequence for the isolate ex *L. marginatus*

the same study (Cone & Anderson, 1977). In a comprehensive survey of protozoan and myxozoan parasites of several freshwater fish from lakes in Algonquin Park, Li & Desser (1985) described three novel *Myxobolus* species from pumpkinseed. *Myxobolus gibbosus* Li & Desser, 1985 was found in multiple organ systems, such as the kidney, muscle, spleen, swimbladder and ureters (Li & Desser, 1985). Similarly, *Myxobolus lepomicus* Li & Desser, 1985 was described infecting the gall bladder, gills, intestinal tract, heart, muscle, swimbladder and ureters of pumpkinseed. Cardiac tissue of pumpkinseed was parasitized by *Myxobolus paralintoni* Li & Desser, 1985. *Myxobolus corneus* Cone, Horner & Hoffman, 1990 was described from the corneal tissue of bluegill collected from a pond in Illinois, USA (Cone et al., 1990). From the Pascagoula River, Mississippi, USA, *Myxobolus mississippiensis* Cone & Overstreet, 1997 was described from the gill tissue of the bluegill (Cone & Overstreet, 1997). And lastly, *Myxobolus jollimorei* Cone & Ovrestreet, 1998 was described infecting the bulbus arteriosus of bluegill collected from the Pascagoula River, Mississippi, USA and Lake Erie, Ontario, Canada (Cone & Overstreet, 1998).

Morphologically *M. lepomis* n. sp. was most similar to *M. mississippiensis* and even shared similar appearances at the posterior end, where some spores bend away from the sutural plane (Cone & Overstreet, 1998). However, myxospores of *M. lepomis* n. sp. were significantly larger than those of *M. mississippiensis* (Supplementary Table S1). The current study reports the first *Myxobolus* species infecting the dollar and redspotted sunfish, respectively, and is the first record for a *Myxobolus* species in a sunfish from Texas, USA.

Molecular sequence data suggests these isolates to be conspecific based on 18S rRNA gene sequence similarity ($< 0.8\%$ variability across $>$ 2,000 bp) between the two isolates. This variability is consistent with previous reports of intraspecific variation among *Myxobolus* spp. from different hosts and different geographical locales (Andree et al., 1999; Whipps et al., 2004; Molnár et al., 2006; Arsan et al., 2007; Atkinson et al., 2015; Griffin et al., 2014; Scott et al., 2015). Furthermore, variability in diagnostic regions of the 18S rRNA gene was low (average of 99.2% between isolates *vs* $< 97\%$ between *M. lepomis* and *M. branchiarum*). Phylogenetic analysis of the 18S rRNA gene sequences obtained from the two isolates and other closely related myxozoans revealed the distinct clustering of the isolates into a clade containing *M. branchiarum* from the centrarchid smallmouth bass. This clade also contained a neoactinomyxum actinospore from *L. hoffmeisteri*. It is thought these isolates represent a novel, undersampled clade of myxozoans that predominantly infect centrarchid fish. Just the same, until further myxozoans from centrarchid fish are described and sequenced, this is merely conjecture. However, host family and order have been deemed as strong phylogenetic signals for the Myxobolidae (see Griffin et al., 2009a, b; Carriero et al., 2013; Rosser et al., 2015, 2016). As such, a discrete clade of centrarchid infecting myxobolids is highly plausible.

Although the isolates of *M. lepomis* n. sp. described here from *L. marginatus* and *L. miniatus* differed significantly in spore body width, polar capsule length and polar capsule width, these ranges overlapped between isolates. Additionally, these parasites demonstrate similar tissue tropism in two closely related fish hosts. Shrinkage associated with ethanol fixation could account for the disparities between the two case isolates, although both isolates were fixed similarly. In any respect, this putative morphological dissimilarity will only be resolved once fresh material is collected and examined. Based on shared tissue and host family tropism, overlapping morphological characters and a high degree of sequence conservation at the 18S rRNA gene, we propose these isolates to be *M. lepomis* n. sp. infecting the gills of *L. marginatus* and *L. miniatus* from the Big Thicket National Preserve.

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Compliance with ethical standards

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

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed (IACUC # 16-08-22-1016-3-01; collecting permit BITH-2016-SCI-0001).

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