**DESCRIPTIONS AND PHYLOGENETIC SYSTEMATICS OF
MYXOBOLUS SPP. FROM CYPRINIDS IN ALGONQUIN PARK, ONTARIO**

by

Kowthar Y. Salim

**A thesis submitted in conformity with the requirements
for the degree of Master of Science
Graduate Department of Zoology
University of Toronto**

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Descriptions and Phylogenetic Systematics of *Myxobolus* spp. from Cyprinids in Algonquin Park, Ontario

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Eight species of *Myxobolus*, of which five are described as new and two are redescrbed, were collected from cyprinids in Algonquin Park. The evolutionary relationships among these eight species using morphological and partial ssu-rDNA sequence data were studied. The resulting trees allowed for the evaluation of spore morphology, host specificity, and tissue tropism, criteria traditionally used in species identification. These criteria, recently criticized as creating artificial rather than natural taxonomic groupings, were evaluated for their reliability in the systematics of the species examined. The data showed that distantly related species often infect the same host and tissue, and closely related species often occur in different hosts. Morphologically similar species are more closely related to each other and the taxonomy based on spore morphology is consistent with the phylogenetic relationships. These results suggest that spore morphology is better

than host and tissue specificity for determining evolutionary relationships and species identity.

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Historical Review

The phylum Myxozoa Grassé, 1970 is comprised of two classes, the Myxosporea Bütschli, 1881 and the Actinosporea Noble, 1980. The Myxosporea, obligate parasites that predominantly infect fish, have also been found in other vertebrates (amphibians, reptiles), and rarely in invertebrates. The majority of these parasites are commensal; however, some myxosporeans are of major concern to the aquaculture industry because they cause disease in fish. Members of the class Actinosporea are primarily parasites of annelids. Past studies of myxozoans have focussed on taxonomy, with major works on the myxosporeans by Kudo (1920), Fantham, Porter, and Richardson (1939), Hoffman (1967), Mitchell (1977), Shulman (1966, 1984), Lom and Dyková (1992), and those on the actinosporeans by Stolc (1899), Leger (1904), Ikeda (1912), Granata (1922, 1925), Georgevitch (1940), Janiszewska (1957), and Marques and Ormieres (1982). Spore morphology is the major criterion used for the identification and description of new species of myxozoans, with host specificity, site of infection, and geographical locality used as additional criteria.

The myxosporeans were first discovered by Müller (1843). Later, Bütschli (1881) provided accurate descriptions of these parasites, referred to them as "myxosporidia" and included them in the class Sporozoa, which also consisted of microsporidians and members of the present day phylum Apicomplexa Levine, 1970. Stolc (1899), who discovered the actinosporeans, suggested that they should

be included within the Metazoa, however, this view was not adopted. Leger (1904) proposed a close historical association between the myxosporeans and the actinosporeans. Based on the similarity of spore morphology the Actinosporidies, Myxosporidies, Microsporidies and Sarcosporidies were grouped together in the class Sporozoa by Caullery and Mesnil (1904). With increasing knowledge about the life histories of these parasites, the class Sporozoa came to include only members of the phylum Apicomplexa, and the distinctive structural and biological differences between the microsporidians and myxozoans were eventually recognized. This was followed by the establishment of the phylum Myxozoa Grassé, 1970, which consists of the myxosporeans and actinosporeans in the kingdom Protista (Levine et al. 1980).

Traditionally, the myxozoans were grouped with the protozoans because of their small size. Despite the multicellular developmental stages of the myxozoans, Cavalier-Smith (1993) argued that the resemblance between myxozoans and metazoans is only superficial, hence myxozoans should remain within the kingdom Protozoa. This view has subsequently been rejected by others (Schlegel et al. 1996; Siddall et al. 1995; Smothers et al. 1994) who support the inclusion of the myxozoans within the kingdom Animalia. Although the latter point of view appears to be widely accepted, the position of the myxozoans within the metazoan lineage is presently controversial. Some authors (Anderson et al. 1998; Schlegel et al. 1996; Smothers et al. 1994) proposed that myxozoans are more closely related to the bilateria based on phylogenetic analysis of ribosomal DNA. However, Siddall

et al. (1995) using ultrastructural, developmental and molecular (ribosomal DNA) data, proposed that the myxozoans are cnidarians and sister taxa to the parasitic cnidarian, *Polypodium hydriforme*. These conflicting results have been attributed to long branches and low taxon density by Zrzavy et al. (1998).

Two hypotheses have been proposed regarding the mode of transmission of the Myxozoa: a direct life cycle, involving fish-to-fish transmission, and a two-host life cycle, with alternating development in a fish and an annelid. The direct life cycle hypothesis proposed by Hoffman and Putz (1971), has dominated transmission studies for several years. The failure of many attempts at direct transmission via peroral experimental infections was attributed to the requirement of spores to "age" for 3 to 4 months in the mud. The direct life cycle hypothesis is not generally accepted because studies reporting this phenomenon were poorly designed and lacked repeatability (El-Matbouli and Hoffman 1989; Grossheider and Korting 1992; Wyatt 1978). Wolf and Markiw (1984) first presented evidence for a two-host life cycle, and this was later confirmed by El-Matbouli and Hoffmann (1989), as well as in several other studies. Despite criticisms of the two-host life cycle hypothesis (Hamilton and Canning 1987), it has gained wide acceptance. To date, approximately 15 myxozoans have been shown to possess two-host life cycles (see Kent and Palenzuela 1999). The direct life cycle hypothesis has, however, not been dismissed entirely as two recent studies have demonstrated direct infection of marine fish (Diamant 1997; Swearer and Robertson 1999). Kent and Palenzuela (1999) suggested that invertebrates, other than annelids, might act

as hosts for actinosporeans, particularly in marine environments. There is increasing evidence to support this hypothesis (see Lester et al. 1998). Xiao (1999) proposed the simultaneous existence of several modes of transmission for these parasites including annelid-to-fish, fish-to-fish, annelid-to-annelid, and fish-to-invertebrate (other than an annelid). Clearly, the elucidation of the life cycle patterns of the Myxozoa is far from complete.

The discovery of the two-host life cycle has prompted some workers to suggest that the myxosporeans and actinosporeans should no longer be considered as two different classes (Kent et al. 1994; Lester et al. 1998; Lom et al. 1997; Uspenskaya 1995; Yokoyama 1997). The "new" taxonomy of this group is a subject of many debates. Kent et al. (1994) suggested that the class Actinosporea be suppressed in favor of the class Myxosporea. Lom et al. (1997) proposed that new actinosporeans should not be named using the Linnean system but that group names should designate the genera, followed by a number or letter to designate the species. Lester et al. (1998) rejected this scheme for several reasons, including the difficulty of finding actinosporean counterparts to the myxosporeans, the possible existence of different variations of life cycle, and the loss of taxonomic information for distinguishing members within the actinosporeans. Hence, Lester et al. (1998) proposed that the actinosporean name be suppressed only when a corresponding myxosporean stage is found. It appears that the naming scheme for the actinosporeans suggested by Lom et al. (1997) is currently being employed (Hallett

et al. 1998; Xiao and Desser 1998a, b), although support for it is far from unanimous among workers in the field.

Introduction

The genus *Myxobolus*, comprised of approximately 450 species, is the most speciose in the class Myxosporea (Lom and Dyková 1992). Myxosporeans, which infect almost any organ and tissue of their fish hosts, have been identified primarily on the basis of their spore morphology, and secondarily on host specificity and tissue tropism (Lom 1969; Lom and Arthur 1989). Although most studies on myxosporeans have been taxonomic, the systematics of this group remains problematic. The taxonomy at the generic level has been questioned as some genera are shown to be paraphyletic and some morphological characters used to distinguish the genera are unreliable (Desser and Paterson 1978; Landsberg and Lom 1991; Mitchell 1989; Smothers et al. 1994). Spore morphology tends to be variable for spore shape and the dimensions of the spore and polar capsules both within and between species are often continuous. This apparent plasticity of the morphological features of spores presents difficulties in identifying populations belonging to the same or different species. As a result, some authors have suggested that spore morphology provides unsuitable characters for hypothesizing evolutionary relationships and that some of the morphological features used in species identifications appear to be arbitrary (Andree et al. 1999; Mitchell 1989; Smothers et al. 1994).

Myxosporean taxonomists have long believed that species of these parasites exhibit a high degree of host and tissue specificity. Host and site of infection have

often been used as the guidelines for recognizing previously described species and identifying new species (Lom and Arthur 1989). Some authors (Lom and Arthur 1989; Lom and Cone 1996; Lom and Dyková 1992) have indicated that little is known about the extent of host and tissue specificity for most species, and that much of the available data are not dependable because of the poor descriptions of these parasites. Current evidence indicates a varying degree of host and tissue specificity for myxosporeans, this prompted some workers to question the use of these features as criteria for distinguishing species (Lom and Dyková 1995; Lom et al. 1989).

In this study, eight species of *Myxobolus* from four species of cyprinids in Algonquin Park, Ontario, were studied morphologically, of these five species were described as new (*M. bartai*, *M. martini*, *M. siddalli*, *M. smithi*, and *M. xiaoi*) and two were redescribed (*M. pendula* and *M. pseudokoi*). To determine the evolutionary relationships among the species examined and to assess the reliability of spore morphology, host specificity, and tissue tropism as dependable taxonomic criteria, phylogenetic analyses were conducted using combined morphological and ssu-rDNA sequence data.

Materials and Methods

Collection and identification of myxosporeans

Four species of cyprinids, *Phoxinus eos* Cope, 1862, *Notemigonus crysoleucas* Mitchill, 1814, *Notropis cornutus* Mitchill, 1817, and *Semotilus atromaculatus* Mitchill, 1818, were caught from Lake Sasajewun (45°36'N, 78°32'W), Kathlyn Lake (45°36'N, 78°33'W), and Broad Wing Lake (45°36'N, 78°32'W) in Algonquin Park, Ontario, using Gee minnow traps baited with Purina dog chow. These cyprinids were selected because they are abundant at the study sites and are infected with morphologically diverse species of *Myxobolus* with varying host and tissue specificity (Gowen 1983; Li and Dessler 1985; Lom et al. 1989; Xiao and Dessler 1997). A total of 77 *P. eos*, 315 *N. crysoleucas*, 211 *N. cornutus*, and 171 *S. atromaculatus* were examined from late May to early September 1998. Some of the fish were dissected soon after they were caught and others were kept in aquaria for several days before dissection. All the organs and tissues of the fish were examined for cyst-forming species of *Myxobolus*. The cysts were excised and stored in 1.6-ml microcentrifuge tubes with double distilled water at -20° C for further morphological and molecular analysis. Semi-permanent slides of spores were prepared with glycerine-gelatin (Lom and Arthur 1989) for morphometric analysis and photography. The presence or absence of a mucus envelope on the spore surface was determined using India ink. Measurements, based on 25 spores per species of *Myxobolus*, are given in micrometers (µm) with the mean followed by the

range in parentheses. Spores were photographed with a Zeiss Universal I photomicroscope using Kodak TMAX[®] 100 film and drawings were made using an Olympus BX50 microscope with a U-DA drawing attachment. To facilitate identification of morphologically similar species of *Myxobolus*, a statistical analysis was conducted employing an unpaired t-test assuming unequal variance.

Genomic DNA extraction

Spores were pelleted by centrifugation for 5 min, washed twice with 1X PBS, and resuspended in 200 µl of STE buffer (pH 8.0) with 1% SDS (Xiao and Desser 1999a). The spore suspension was boiled for 10 min and then plunged into liquid nitrogen. This sequence of boiling and freezing was repeated three times. The suspension was examined by light microscopy to verify that most of the spores had released their sporoplasm. Some spores were digested with proteinase K at 37° C for approximately 3 h after the boiling and freezing cycle (Xiao and Desser 1999a). Subsequently, the DNA was extracted three times with phenol:chloroform and precipitated overnight with ethanol at -20° C (Sambrook et al. 1989). Following centrifugation at 12,000 g for 15 min, the resulting DNA pellet was washed twice with 70% ethanol, air-dried, and resuspended in 10–20 µl of double distilled water.

Amplification of ssu-rDNA

Parasite ssu-rDNA was amplified with a forward primer A (5'-CCGAATTCCAGCTGCAGATCTGGTTGATCCTGCCAGT-3') and a reverse primer

B (5'-CCAAGCTTCCGCTGCAGGATCCTTCCGCAGGTTCACCT-3') which bind to conserved regions in the 5' and 3' end of the gene, respectively (Xiao and Desser 1999a). Polymerase chain reactions (PCRs) were performed in a total volume of 100 μ l containing 2 μ l of template DNA (approximately 150 ng of genomic DNA/ μ l), 10 μ l of 25 mM dNTP solution, 10 μ l of 10X PCR reaction buffer (500 mM KCl, 15 mM MgCl₂, and 100 mM tris-HCl, Pharmacia Biotech, Quebec, Canada), 4 μ l of each primer (5 μ M/ μ l), 0.5 μ l of 5 units/ μ l Taq DNA polymerase (Pharmacia Biotech, Quebec, Canada), and 69.5 μ l of double distilled water. This mixture was overlaid with approximately 50 μ l of mineral oil and placed in a Perkin Elmer DNA Thermal Cycler 480 for amplification. Initially all the reactions were preheated for 5 min at 94° C, then subjected to 35 cycles of denaturation at 94° C for 1 min, annealing at 56° C for 1 min, and extension at 72° C for 2 min, followed by a final cycle of an extended elongation step at 72° C for 5 min.

Purification and sequencing of amplified products

The PCR products were run on 1.5-% agarose gels in 1X TAE for approximately 2 h at 100 V. Myxosporean ssu-rDNA fragments were cut from the gels and purified using the Geneclean II Kit (BIO 101 Incorporated, La Jolla, California, USA). In addition to the forward primer A, a reverse internal primer 840R was used for sequencing the ssu-rDNA utilizing an ABI Prism 377 DNA sequencer. The 840R primer (5'-AAGCCTGCTTTGAGCACT-3') was designed based on conserved sequences within the ssu-rDNA gene of species of *Myxobolus*

from GenBank (Accession Nos. U96492, U96494, U96495, and AF085177–AF085182).

Phylogenetic analyses

Phylogenetic analyses were performed with PAUP version 3.1.1 (Swofford 1993) using three outgroups; *Myxidium* sp. (Accession No. U13829), *Myxidium lieberkuehni* reverse strand, mp19 (Accession No. X76639), and *Ceratomyxa shasta* (Accession No. AF001579). *Myxidium lieberkuehni* and *Myxidium* sp. were chosen as outgroups because previous analyses of the myxosporeans have shown species of *Myxidium* to be basal to members of the genus *Myxobolus* (Siddall et al. 1995; Smothers et al. 1994). An analysis conducted by Xiao and Desser (1999b) investigating myxozoan life cycle data in a phylogenetic context, resulted in two equally parsimonious trees, one of which showed that species of *Myxobolus* were more closely related to *Ceratomyxa shasta* than to *Myxidium giardi*. *Ceratomyxa shasta* was therefore included as an additional outgroup.

The morphological and molecular data were combined in a total evidence approach (Kluge 1989). Character states in the morphological data set are unordered and missing data are coded as '?' (Table 1). In total, there were 14 morphological characters (listed below) used in the analysis, some of which were obtained from Xiao and Desser (1999b).

1. Spore shape (front view): fusiform (0), ellipsoidal (1), subspherical (2), pyriform (3), arched (4).

2. Spore width (valvular view) to spore thickness (sutural view): width = thickness (0), width > thickness (1), width < thickness (2).
3. Polar capsule shape (front view): pyriform (0), elongated bulb (1), spherical (2).
4. Polar capsule position: at both ends of spore (0), only at anterior end of spore (1).
5. Spore with two polar capsules at one end: absent (0), present without intercapsular appendix (1), present with intercapsular appendix (2).
6. Sutural folds: absent (0), present (1).
7. Mutual size relation of polar capsules: equal (0), unequal (1).
8. Sporoplasm position: in mid part of spore body (0), not in mid part of spore body (1).
9. Location of spore in host: coelozoic (0), histozoic (1).
10. Striated valve surface: absent (0), present (1).
11. Direction of polar filament discharge: opposite along the longitudinal spore axis (0), anterior (1).
12. Spore valves: with pointed anterior end (0), with rounded anterior end (1).
13. Spore valves: with pointed posterior end (0), with rounded anterior end (1).
14. Position of polar capsules in relation to valves: corresponded to each other and located at the anterior end (0), at both ends and not corresponded to each other (1), located at anterior end and not corresponded to each other (2).

Table 1. Morphological character matrix of *Myxidium lieberkuehni*, *Myxidium* sp., *Ceratomyxa shasta*, and species of *Myxobolus*.

TAXA	CHARACTERS													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Myxidium lieberkuehni</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Myxidium</i> sp.	?	?	?	0	0	?	?	0	?	?	0	?	?	1
<i>Ceratomyxa shasta</i>	4	2	2	1	?	0	0	1	1	1	1	1	1	0
<i>Myxobolus pendula</i>	1	1	0	1	2	1	0	1	1	1	1	1	1	2
<i>Myxobolus smithi</i>	1	1	0	1	2	1	0	1	1	1	1	1	1	2
<i>Myxobolus siddalli</i>	1	1	0	1	2	1	0	1	1	1	1	1	1	2
<i>Myxobolus xiaoi</i>	1	1	0	1	2	1	0	1	1	1	1	1	1	2
<i>Myxobolus pseudokoi</i>	3	1	1	1	1	0	0	1	1	1	1	0	1	2
<i>Myxobolus bartai</i>	2	1	0	1	2	1	1	1	1	1	1	1	1	2
<i>Myxobolus martini</i>	1	1	0	1	2	1	0	1	1	1	1	1	1	2
<i>Myxobolus algonquinensis</i>	1	1	0	1	2	1	0	1	1	1	1	1	1	2

The ssu-rDNA sequences were aligned using ClustalW software, version 1.5 (Thompson et al. 1994) with default gap penalty settings. Ambiguous regions and non-informative sites were excluded from the phylogenetic analyses by visual assessment of the aligned sequences, and gaps were treated as missing data. Sensitivity analysis was performed to test the robustness of the clades to a combination of transition-to-transversion cost ratios of 1:1, 1:2, 1:3, 1:5, and 1:10. The most parsimonious trees were found using the branch and bound option. Bootstrap resampling (500 replicates) was used to estimate the statistical confidence in the branch points of the phylogenies resulting from increased transversional costs. To study the evolution of the mucus envelope, this character was optimized on the resulting tree (Fig. 32).

Results

Species descriptions and redescrptions

Five new species of *Myxobolus* are described, and two previously described species, *Myxobolus pendula* Guilford, 1967 and *Myxobolus pseudokoi* Li and Desser, 1985 are redescrbed using Lom and Arthur's (1989) guidelines for myxosporean descriptions. *Myxobolus pendula* and *M. pseudokoi* have been redescrbed to provide additional information and photomicrographs, which were absent from the original descriptions. Note that the species names are not to take availability from this thesis.

Myxobolus bartai n. sp.

(Fig. 1–4)

Host. Common shiner, *Notropis cornutus*.

Locality. Lake Sasajewun and Kathlyn Lake, Algonquin Park, Ontario, Canada.

Site of infection. Body wall musculature.

Prevalence. Two of 211 (0.95%) fish examined were infected.

Description. Elongate, whitish-yellow cysts, approximately 1 cm in length. Spores spherical in shape with approximately 5–6 distinct sutural folds at the posterior end. Spores 11.0 (10.3–11.4) long, 10.8 (10.0–11.3) wide, 7.1 (6.1–7.5) thick. Two pyriform, unequal polar capsules. Longer polar capsule is 6.3 (5.9–7.0) by 3.8 (3.2–4.2). Shorter polar capsule is 5.7 (4.9–6.3) by 3.3 (3.0–4.0). Polar filaments coiled 3–

4 times. Small, round intercapsular appendix present. Mucus envelope on spore surface absent.

Types. A slide with stained spores of *Myxobolus bartai* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Etymology. *Myxobolus bartai* is named for Dr. J. R. Barta in recognition of his contribution to the knowledge of parasitic protozoa.

Remarks. Spores of *M. bartai* are similar in appearance to those of *M. microlatus* Lee and Nie, 1965 (see Chen 1973), *M. amurensis* Achmerov, 1960 (see Shulman 1966), and *M. petenensis* Frey et al., 1998. Spores of *M. microlatus* are smaller in length and width, and also possess smaller polar capsules as compared to those of *M. bartai*. Although there is overlap in spore and polar capsule measurements between *M. amurensis* and *M. bartai*, spores of *M. amurensis* are narrower at the anterior pole, whereas spores of *M. bartai* have rounded anterior and posterior poles. In addition, spores of *M. amurensis* do not possess sutural folds. Unlike *M. bartai*, spores of *M. petenensis* are wider and lack an intercapsular appendix.

Fig. 1–4. Mature spores of *Myxobolus bartai* n. sp. 1. Frontal view of a spore with a circular intercapsular appendix (arrow) and unequal polar capsules. 2. Frontal view of a spore illustrating the sutural folds (arrow). 3. Fresh spore with India ink. Note absence of the mucus envelope on the spore surface. 4. Line drawing of a mature spore. Bar = 10 μ m.

4

Myxobolus martini n. sp.

(Fig. 5–8)

Host. Golden shiner, *Notemigonus crysoleucas*.

Locality. Lake Sasajewun, Algonquin Park, Ontario, Canada.

Site of infection. Eye socket.

Prevalence. Thirteen of 315 (4.13%) fish examined were infected.

Description. Spherical, creamy-white cysts approximately 3–4 mm in diameter.

Ellipsoidal spores with sutural folds. Spores 17.9 (16.4–19.5) long, 12.1 (10.3–13.5) wide, 8.4 (7.3–9.5) thick. Two equal-sized pyriform polar capsules measuring 6.0 (5.2–6.4) by 3.2 (3.0–3.6). Polar filaments coiled 6–7 times. Small, round intercapsular appendix present. Mucus envelope on spore surface absent.

Types. A slide with stained spores of *Myxobolus martini* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Etymology. *Myxobolus martini* is named for Dr. D. S. Martin in recognition of his contribution to the knowledge of parasitic protozoa.

Remarks. Spores of *M. martini* resemble those of *M. algonquinensis* Xiao and Desser, 1997, which were originally described from the gonads of *N. crysoleucas* from Lake Sasajewun. In this study, spores of *M. algonquinensis* were found in the mesentery and within cartilage of the gill arch, as well as the gonads. There is no significant difference in the length of spores of *M. algonquinensis* in these various tissues, whereas spores of *M. martini* (mean spore length = 17.9 μm) are significantly longer than those of *M. algonquinensis* (mean spore length = 14.7 μm) (Table 2). Spores of

M. cycloides Gurley, 1893 (see Lom and Dyková 1992), though similar in appearance to *M. martini*, are narrower and possesses a relatively large, triangular intercapsular appendix unlike the smaller, circular intercapsular appendix of *M. martini*.

Table 2. A standard t-test comparison of the spore length of *M. martini* and isolates of *M. algonquinensis*.

Comparison of spore length	Respective means		t-stat	P value	Difference in length
<i>M. algonquinensis</i> (all isolates) vs. <i>M. martini</i>	15.13	17.87	-14.54	< 0.001	significant
<i>M. algonquinensis</i> (gonad) vs. <i>M. martini</i>	15.34	17.87	-13.24	< 0.001	significant
<i>M. algonquinensis</i> (mesentery) vs. <i>M. martini</i>	14.98	17.87	-11.29	< 0.001	significant
<i>M. algonquinensis</i> (gill arch) vs. <i>M. martini</i>	15.05	17.87	-10.78	< 0.001	significant
<i>M. algonquinensis</i> (gonad) vs. <i>M. algonquinensis</i> (mesentery)	15.34	14.98	1.60	> 0.05	not significant
<i>M. algonquinensis</i> (gonad) vs. <i>M. algonquinensis</i> (gill arch)	15.34	15.05	1.26	> 0.05	not significant
<i>M. algonquinensis</i> (mesentery) vs. <i>M. algonquinensis</i> (gill arch)	14.98	15.05	-0.24	> 0.05	not significant

Fig. 5–8. Mature spores of *Myxobolus martini* n. sp. 5. Frontal view of a spore with a small, circular intercapsular appendix (arrow). 6. Frontal view of a spore illustrating the sutural folds (arrow). 7. Frontal view of a spore with a discharged polar filament coil and a coiled polar filament. 8. Line drawing of a mature spore. Bar = 10 μ m.

8

Myxobolus siddalli n. sp.

(Fig. 9–12)

Host. Common shiner, *Notropis cornutus*.

Locality. Lake Sasajewun, Kathlyn Lake, and Broad Wing Lake, Algonquin Park, Ontario, Canada.

Site of infection. Kidney.

Prevalence. Eleven of 211 (5.21%) fish examined were infected.

Description. Cysts round, pigmented, approximately 250 µm in diameter. Spores ellipsoidal to oval in shape with sutural folds visible on posterior end. Spores 10.3 (9.3–11.2) long, 8.9 (8.2–9.7) wide, 6.3 (5.4–7.1) thick. Two polar capsules equal in size occupying approximately half the length of spore. Polar capsules measuring 5.4 (4.1–6.2) by 3.1 (2.9–3.5). Polar filaments coiled 5–7 times. Intercapsular appendix distinct, large/long and triangular. Mucus envelope present on spore surface.

Types. A slide with stained spores of *Myxobolus siddalli* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Etymology. *Myxobolus siddalli* is named for Dr. M. E. Siddall in recognition of his contribution to the knowledge of parasitic cnidarians.

Remarks. Spores of *M. siddalli* are most similar to those of *M. smithi*. Both species were located in the kidneys of their respective hosts, *M. siddalli* from *N. cornutus* and *M. smithi* from *P. eos*. Spores of *M. siddalli* differ from those found in *P. eos* in the shape of the intercapsular appendix and the length of the polar capsules.

Spores of *M. siddalli* possess longer polar capsules and a distinctly longer and pointed intercapsular appendix in comparison to those of *M. smithi*. *Myxobolus siddalli* is also similar in appearance to *M. schuberti* Li and Desser, 1985, and *M. enoblei* Lom and Cone, 1996. Li and Desser (1985) described *M. schuberti* from the kidneys and other organs of *N. cornutus* from Lake Sasajewun. Although *M. schuberti* and *M. siddalli* were located in the same host and tissue, *M. schuberti* differs from the latter in spore shape. Unlike spores of *M. siddalli*, those of *M. schuberti* have narrower anterior ends; moreover, the intercapsular appendix indicated by the illustrations of *M. schuberti* appears to be smaller than that of *M. siddalli*. Although spores of *M. enoblei* are similar in shape to those of *M. siddalli*, the spores and polar capsules of the latter species are smaller.

Fig. 9–12. Mature spores of *Myxobolus siddalli* n. sp. 9. Frontal view of a spore with a long, triangular intercapsular appendix (arrow). 10. Frontal view of a spore illustrating the sutural folds (arrow). 11. Fresh spore with India ink indicating presence of a mucus envelope on the spore surface (asterisk). 12. Line drawing of a mature spore. Bar = 10 μ m.

12

Myxobolus smithi n. sp.

(Fig. 13–16)

Host. Redbelly dace, *Phoxinus eos*.

Locality. Kathlyn Lake and Broad Wing Lake, Algonquin Park, Ontario, Canada.

Site of infection. Kidney.

Prevalence. Ten of 77 (12.99%) fish examined were infected.

Description. Cysts round, pigmented, approximately 250 μ m in diameter. Spores ellipsoidal to oval in shape measuring 10.6 (9.9–11.4) long, 8.8 (8.3–9.3) wide, 6.2 (5.4–6.7) thick. Sutural folds present on posterior end of spores. Two equal-sized polar capsules occupying approximately half the length of spore. Polar capsules measuring 4.5 (4.1–5.1) by 2.9 (2.2–3.1). Polar filaments coiled 5–7 times.

Intercapsular appendix present. Mucus envelope present on spore surface.

Types. A slide with stained spores of *Myxobolus smithi* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Etymology. *Myxobolus smithi* is named for Dr. T. G. Smith in recognition of his contribution to the knowledge of parasitic protozoa.

Remarks. Spores of *M. smithi* most closely resemble those of *M. siddalli* in shape and dimensions, however, differ markedly in the shape of the intercapsular appendix and in the size of the polar capsules (discussed above). Based on measurements of spore length and width, *M. smithi* also resembles *M. sinensis* Nie and Lee, 1964 (see Chen 1973). However, according to the diagrams by Chen (1973) it appears that, unlike *M. smithi*, spores of *M. sinensis* tend to be tapered anteriorly.

The intercapsular appendix appears to be absent in some spores of *M. sinensis*, whereas it appears to be a fixed character in *M. smithi* spores. When present, the intercapsular appendix in spores of *M. sinensis* is smaller than that of *M. smithi*.

Fig. 13–16. Mature spores of *Myxobolus smithi* n. sp. 13. Frontal view of a spore with an intercapsular appendix (arrow). 14. Lateral view of a spore illustrating a sutural ridge (arrow). 15. Fresh spore with India ink indicating a mucus envelope surrounding the spore surface (asterisk). 16. Line drawing of a mature spore. Bar = 10 μ m.

16

Myxobolus xiaoi n. sp.

(Fig. 17–20)

Host. Common shiner, *Notropis cornutus* and golden shiner, *Notemigonus crysoleucas*.

Locality. Lake Sasajewun, Kathlyn Lake, and Broad Wing Lake, Algonquin Park, Ontario, Canada.

Site of infection. Within cartilage of the gill arch.

Prevalence. Six of 211 (2.84%) *Notropis cornutus* and five of 315 (1.59%) *Notemigonus crysoleucas* examined were infected.

Description. Small, white, oval to irregular cysts. Spores ellipsoidal to oval in shape with rounded anterior and posterior ends. Spore valves thickened posteriorly and sutural folds present. Spores measuring 11.0 (9.8–12.2) long, 8.5 (8.1–9.2) wide, 6.0 (5–6.9) thick. Two pyriform polar capsules equal in size, 4.8 (4.2–5.4) by 2.8 (2.1–3.1). Polar filaments coiled 5–7 times. Intercapsular appendix present. Mucus envelope present on spores from *Notemigonus crysoleucas* and absent from those in *Notropis cornutus*.

Types. A slide with stained spores of *Myxobolus xiaoi* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Etymology. *Myxobolus xiaoi* is named for Dr. C. Xiao in recognition of his contribution to the knowledge of parasitic cnidarians.

Remarks. Spores of *M. xiaoi* resemble those of *M. opsariichthyi* Lee and Nie, 1965 (see Chen 1973), *M. nemachili* Weiser, 1949 (see Shulman 1966), *M. siddalli*, and *M.*

smithi in shape, dimensions, and presence of sutural folds. Diagrammatic representations of *M. opsariichthyi* and *M. nemachili* indicate that the intercapsular appendix is absent in both species, whereas this feature is present in all the spores of *M. xiaoi* examined. Both *M. siddalli* and *M. smithi* possess larger intercapsular appendices as compared to *M. xiaoi*. A characteristic feature unique to *M. xiaoi* is the thickening of the spore valves at the posterior end of the spore. *Myxobolus bibullatus* Kudo, 1934 (redescribed by Grinham and Cone 1990) possesses posteriorly thickened spore valves, however, spores of this species are larger and also lack an intercapsular appendix. The only difference between spores of *M. xiaoi* from *Notemigonus crysoleucas* and those from *Notropis cornutus*, is the presence of a mucus envelope surrounding spores isolated from the gill arch of *Notemigonus crysoleucas*. Mitchell (1989) proposed that the mucus envelope is an unreliable morphological character because it varies among spores of the same species. Since the taxonomic reliability of the mucus envelope is undetermined, using this character to distinguish otherwise morphologically identical taxa is inadvisable.

Fig. 17–20. Mature spores of *Myxobolus xiaoi* n. sp. 17. Frontal view of a spore with an intercapsular appendix (arrow) and sutural folds (arrowhead). 18. Frontal view of a spore illustrating posteriorly thickened spore valves (arrow). 19. Fresh spore with India ink indicating presence of a mucus envelope on the spore surface (asterisk). 20. Line drawing of a mature spore. Bar = 10 μ m.

20

Myxobolus pendula Guilford, 1967

Syn. *Myxobolus pellicides* Li and Desser, 1985

(Fig. 21–24)

Host. Creek chub, *Semotilus atromaculatus*.

Locality. Lake Sasajewun, Algonquin Park, Ontario.

Site of infection. Gill arch.

Prevalence. Fourteen of 171 (8.19%) fish examined were infected.

Description. Cysts white, smooth and sac-like, vary in size from 1–10 mm in diameter, attached to gill arch. Spores ellipsoidal to oval in shape with sutural folds. Spores 14.4 (13.4–15.3) long, 10.7 (9.8–12.1) wide, 7.4 (7.0–8.2) thick. Equal-sized, pyriform polar capsules occupying approximately a third of spore length. Polar capsules measuring 6.2 (5.3–6.8) by 3.6 (3.1–4.1). Polar filaments coiled 7–8 times. Small, circular intercapsular appendix present. Mucus envelope present in some isolates but absent in others.

Voucher specimen. A slides with stained spores of *Myxobolus pendula* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Remarks. *Myxobolus pendula*, which was first identified in sac-like cysts attached to the gill arch of *S. atromaculatus* by Guilford (1967) and later reported by Gowen (1983) in Lake Sasajewun, Algonquin Park, has been redescribed due to the lack of photomicrographs in the original description and also to provide additional information. In 1985, Li and Desser isolated spores from the same host and tissue

with identical spore morphology to *M. pendula*, except for the presence of the mucus envelope, which led these authors to assign it a new species designation *M. pellicides*. A synonymy is proposed for *M. pendula* and *M. pellicides* as these taxa possess similar spore morphology, identical host and tissue specificity, identical riboprint patterns, high sequence similarity, and are sister taxa (Xiao 1999; Xiao and Dessler 1999a, b). Furthermore, the taxonomic reliability of the mucus envelope, the only character used to distinguish these taxa, is uncertain.

Fig. 21–24. Mature spores of *Myxobolus pendula* Guilford, 1967. 21. Frontal view of a spore with a small, round intercapsular appendix (arrow). 22. Frontal view of a spore illustrating sutural folds (arrow). 23. Fresh spore with India ink indicating presence of a mucus envelope on the spore surface (asterisk). 24. Line drawing of a mature spore. Bar = 10 μm .

Myxobolus pseudokoi Li and Desser, 1985

(Fig. 25–28)

Host. Common shiner, *Notropis cornutus*.

Locality. Lake Sasajewun, Kathlyn Lake, and Broad Wing Lake, Algonquin Park, Ontario.

Site of infection. Gill filaments.

Prevalence. Thirty-three of 211 (15.64%) fish examined were infected.

Description. Oval, white cysts ranging in size from 100–800 μm in diameter located in primary and secondary gill lamellae. Spores elongated and pyriform with pointed anterior ends and rounded posterior ends. Sutural folds absent. Spores 13.2 (12.4–14.3) long, 6.4 (5.6–7.0) wide, 5.4 (5.0–6.1) thick. Two equal-sized, elongated bulb-shaped polar capsules occupying approximately half the length of spore. Polar capsules measuring 6.2 (5.4–7.0) by 2.4 (2.0–2.8). Polar filaments coiled 6–8 times. Intercapsular appendix absent. Mucus envelope present on posterior surface of spore.

Voucher specimen. A slides with stained spores of *Myxobolus pseudokoi* was submitted to the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada.

Remarks. *Myxobolus pseudokoi* has been redescribed to provide additional information and photomicrographs, which were absent from the previous description of this parasite by Li and Desser (1985).

Fig. 25–28. Mature spores of *Myxobolus pseudokoi* Li and Desser, 1985. 25.

Frontal view of a spore illustrating the lack of an intercapsular appendix. 26.

Lateral view of a spore illustrating the sutural ridge (arrow). 27. Fresh spore with India ink indicating presence of a mucus envelope at the posterior end of the spore surface (asterix). 28. Line drawing of a mature spore. Bar = 10 μ m.

Sequencing and phylogenetic analyses

Following PCR amplification of the ssu-rDNA of each species of *Myxobolus*, a fragment of approximately 2.1–2.2 kb was isolated. In some cases, host DNA was also amplified during the PCR reaction, however, this fragment was easily distinguished from parasite ssu-rDNA due to the latter's larger molecular weight. Approximately 800 bp of ssu-rDNA was sequenced for each species of *Myxobolus*.

Sensitivity analysis of the combined morphological and molecular data set to test the robustness of the clades involved alterations of the transition-to-transversion cost ratios. The parsimony analysis, when the transition-to-transversion cost ratio was 1:1, resulted in two equally parsimonious trees that differed only in the relative positions of *M. siddalli* and *M. smithi* (Fig. 29–30). Each tree was 837 steps long and had a consistency index of 0.72 and a retention index of 0.73. Of the 772 characters in the data matrix only 261 characters were informative and the rescaled consistency index was 0.52. The strict consensus tree of the two equally parsimonious trees is portrayed in Fig. 31.

Increasing the cost of transversional changes from 2 to 10 produced only one most parsimonious tree (Fig. 32) that has an identical topology to the tree shown in Fig. 29. Of the 772 characters in the matrix only 273 characters were informative. The tree length and consistency index varied with increasing transversional costs, the rescaled consistency index ranged from 0.43 to 0.49. Since the transition-to-transversion cost ratio of 1:1 is an unrealistic interpretation of genome evolution, the topology in Fig. 32 has been chosen to represent the phylogeny of the species

examined. This tree consists of one monophyletic group composed of *M. smithi*, *M. siddalli*, *M. martini*, and *M. algonquinensis*. *Myxobolus bartai*, *M. pseudokoi*, and *M. pendula* form another monophyletic group. Both of the aforementioned clades form sister clades, with *M. xiaoi* basal to them. When the mucus envelope was mapped on this tree, six optimizations were produced. One optimization, which is shown in Fig. 32, indicates the convergence of this character in *M. pseudokoi*, *M. siddalli*, *M. smithi*, and isolates of *M. pendula* and *M. xiaoi*. Another optimization indicates the repeated loss of the mucus envelope in *M. bartai*, isolates of *M. xiaoi* and *M. pendula*, and the ancestor of *M. martini* and *M. algonquinensis*. The remaining four optimizations consist of different combinations of convergence and loss of the mucus envelope.

Fig. 29–31. Phylogenetic trees derived from morphological and ssu-rDNA sequence data, based on transition-to-transversion cost ratio of 1:1. **29–30.** Two equally parsimonious trees. **31.** A strict consensus tree of the aforementioned phylogenies.

29

30

31

Fig. 32. The single most parsimonious tree derived from morphological and ssu-rDNA sequence data, based on transition-to-transversion cost ratios of 1:2, 1:3, 1:5, and 1:10. The numerical value at each node represents the bootstrap value following 500 replicates, and slash marks on terminal branches designate the presence of the mucus envelope. The corresponding host and site of infection is indicated for each taxon within the ingroup.

Discussion

Spore morphology, host specificity, and tissue tropism

Morphological characters, if determined by the environment within the host, will be highly susceptible to convergence and cannot be used as the primary criterion for species identification. The clade comprised of *M. smithi*, *M. siddalli*, *M. martini*, and *M. algonquinensis*, was chosen to evaluate the determinants of spore morphology within a phylogenetic context. The two basal taxa, *M. smithi* and *M. siddalli*, possess morphologically similar spores and occur in the same tissue of different hosts. *Myxobolus martini* and *M. algonquinensis* are also morphologically similar taxa that occur in different tissues of the same host. The occurrence of species with similar spore morphology (*M. smithi*-*M. siddalli*, and *M. martini*-*M. algonquinensis*) in similar environments (tissue and host, respectively), suggests that morphological similarity may be due to convergent evolution in adapting to similar environments. Alternatively, the close historical association between *M. smithi* and *M. siddalli* could account for the morphological similarity resulting from persistent plesiomorphies, and the morphological similarity between *M. martini* and *M. algonquinensis* would be homologies inherited from their most recent common ancestor. Hence, the tree (Fig. 32) appears to be ambiguous in providing support for whether spore morphology is homoplastic (environmentally determined) or homologous. If spore morphology were determined by divergent and stabilizing environmental selection within the host, then distantly related taxa should exhibit

similar spore morphology. However, *M. xiaoi* and *M. algonquinensis*, which are distantly related species that occur in the same tissue and host, possess distinct spore morphology. This evidence is contrary to the hypothesis that spore morphology is largely homoplastic or environmentally determined. Shulman (1966) and Moser (1977) have reported a correlation between spore morphology and environmental conditions within the host. In fact, the latter author proposed that environmental factors, specifically the physiology and behavior of the hosts, directly determine the spore shape. My analysis indicates that this hypothesis is false.

In order for host specificity to reflect close historical associations, one would expect parasites from identical host species to be more closely related to each other. The phylogenetic analysis revealed that monophyletic groups were not always based on the host specificity of the taxa. In fact, distantly related taxa, such as *M. bartai* and *M. siddalli*, were located in the same host, and the reverse was true for closely related taxa, such as *M. smithi* and *M. siddalli*. Furthermore, some taxa including *M. xiaoi*, *M. pseudokoi*, and *M. siddalli* possess overlapping host ranges. These results indicate that host specificity is not reliable in species identification in the absence of morphological information, nor is it an accurate indicator of evolutionary relationships.

Tissue specificity was also examined for its ability to reflect evolutionary relationships. To determine whether the species in this study grouped according to tissue tropism, the tissues were categorized as external and internal tissues.

External tissues include those in contact with the external environment of the host, such as the skin, gills, and fins. Internal tissues include those that are not exposed to the external environment of the host, such as the liver, heart, and muscles. The phylogenetic analyses indicate that the taxa did not group on the basis of tissue tropism. As an example, *M. bartai*, an internal-tissue-infecting myxosporean, is more closely related to species that infect external tissues, *M. pseudokoi* and *M. pendula*, rather than to the other species that infect internal tissues including *M. siddalli*, *M. smithi*, and *M. martini*. Furthermore, isolates of *M. xiaoi* and *M. algonquinensis* share identical tissue tropism, however, they are evolutionarily distantly related taxa. These results contradict the hypothesis by Andree et al. (1999), that tissue tropism is a better indicator of the phylogenetic relationships among species of *Myxobolus* than is spore morphology or host specificity. Hence, the infection of a particular tissue is not necessarily indicative of close historical associations.

Spore morphology, taxonomy, and evolutionary relationships

There have been numerous synonyms within the genus *Myxobolus* because of the morphological similarity among many species (Lom and Arthur 1989). Consequently, several workers have expressed concerns with the use of morphology in the systematics of these parasites (Andree et al. 1999; Mitchell 1989; Smothers et al. 1994). The goal of current taxonomic studies is to establish a natural classification and the need to assess the spore morphology of myxosporeans has

now become well justified (Lester et al. 1998; Smothers et al. 1994). Thus, I have assessed spore morphology for its significance in the taxonomy of this group and for its ability to reflect close historical associations among species.

The proposed taxonomy of the species in this study, based on spore morphology, is consistent with the relationships depicted in the tree (Fig. 32). Spores located in the gonads, mesentery, and gill arches of *N. crysoleucas* were designated as isolates of *M. algonquinensis* because they are morphologically indistinguishable, exhibit high sequence similarity (at least 98%), and form a monophyletic group. The genetic variation among these isolates could represent different subpopulations of the same species, natural variation, or the accumulation of errors during the PCR reaction and sequencing (Cline et al. 1996; Ewing and Green 1998). Similarly, differences in sequences of *Myxidium lieberkuehni* was suggested by Schlegel et al. (1996) to be a result of either two morphologically identical but genetically distinct species or genetically distinct and isolated lineages of *Myxidium lieberkuehni*. The taxonomic status of *M. martini* with reference to the phylogenetic tree (Fig. 32) and its relationship with *M. algonquinensis* could be interpreted in two ways. Either *M. martini* is another isolate of *M. algonquinensis* and should be synonymized with the latter or, *M. martini* is a different species that is sister taxon to *M. algonquinensis*. The morphological evidence, which supports the second scenario, indicates that the spore length can be used to differentiate these taxa (Table 2).

Xiao (1999), and Xiao and Desser (1999a, b) observed identical riboprint patterns, 98.8% similarity of ssu-rDNA sequences, and a sister taxon relationship between *M. pendula* and *M. pellicides*, but did not synonymize them. Although there is no absolute guide to assigning a taxonomic designation to a particular level of sequence divergences, I have proposed a synonymy between *M. pendula* and *M. pellicides*. Similarly, isolates of *M. xiaoi* with and without the mucus envelope have been designated as isolates and not distinct species based on morphological information. The taxonomic status of the mucus envelope, the only morphological character that distinguishes the aforementioned clades, is uncertain. Mitchell (1989) suggested that the mucus envelope is an unreliable character because its presence appears to be variable in spores of *M. muelleri* and in spores from a single cyst of *Myxobolus* sp. It is possible that the mucus envelope is a polymorphic character or, alternatively, that its presence may be ontogenetically determined. For example, smaller immature cysts may contain spores that lack mucus envelopes. The presence of the latter may be dependent on the environmental conditions outside the host, or its presence may be cyclic in time. Conversely, the mucus envelope could be a taxonomically informative character that evolved convergently in different taxa. Our analysis is ambiguous regarding the evolution of the mucus envelope as there are six possible evolutionary scenarios indicating varying instances of convergence and loss (see results). Clearly, there is a need for the assessment of this character employing life cycle and ecological studies. Until the taxonomic reliability of the mucus envelope has been determined, it is

inadvisable to use this character for distinguishing otherwise morphologically identical taxa.

The unreliability of some morphological features does not warrant the dismissal of all morphological characters in the identification of species and as good indicators of evolutionary relationships. In fact, phylogenetic analyses of the species of *Myxobolus* in this study indicate that species with similar spore morphology are more closely related to each other than to species with distinct spore morphology. The monophyly of *M. algonquinensis* and *M. martini*, both possessing ellipsoidal spores, indicates the close historical association between these morphologically similar taxa. Likewise, *M. smithi* and *M. siddalli* are two morphologically similar species that belong to the same monophyletic group. Other morphologically similar taxa forming monophyletic groups include isolates of *M. xiaoi* and isolates of *M. pendula*. These results suggest that spore morphology can reflect close historical associations among taxa.

This study has shown that spore morphology is useful in the systematics of species of *Myxobolus* as there were no apparent patterns that correlated host specificity or tissue tropism and evolutionary relationships. In fact, host specificity and tissue tropism should not be used as a means of identifying species and determining evolutionary relationships because they are dynamic and complex phenomena. The life cycle of these parasites is limited and contentious (Diamant 1997; Lester et al. 1998; Lom and Dyková 1995; Wolf and Markiw 1984), moreover conclusive species identification and determination of the range of host as well as

the tissue tropism for myxosporeans can be achieved by transmission experiments. Until the life cycles of species of *Myxobolus* have been elucidated, the continued use of spore morphology is recommended, with host and tissue specificity playing a secondary role in the identification of species. Future work on species of *Myxobolus* and other Myxosporea including sequencing of other genes, identification of additional useful morphological characters, and the elucidation of life cycles, will enhance the taxonomy and systematics of this group of parasites.

Summary

Eight species of *Myxobolus* were collected from four species of cyprinids (*P. eos*, *N. cornutus*, *N. crysoleucas*, *S. atromaculatus*) in three lakes (Lake Sasajewun, Kathlyn Lake, and Broad Wing Lake) in Algonquin Park, Ontario. Five new species of *Myxobolus* (*M. bartai*, *M. martini*, *M. smithi*, *M. siddalli*, and *M. xiaoi*) were described, and two species (*M. pseudokoi* and *M. pendula*) were redescribed, with supplemental photomicrographs and additional information. *Myxobolus algonquinensis*, described previously from this study site, was found in additional tissues (mesentery and within cartilage of gill arch) within the same host (*N. crysoleucas*). All the aforementioned species of *Myxobolus* were characterized on the basis of spore morphology.

Following species identification, parasite DNA was extracted and the ssu-rDNA was amplified and sequenced. Phylogenetic analyses were conducted using a combined data set comprised of morphological and molecular (ssu-rDNA) sequence data. Phylogenetic analyses using a range of transition-to-transversion cost ratios (1:1, 1:2, 1:3, 1:5, and 1:10), indicated that the molecular data were not sensitive to an increase in transversional costs because the resulting trees possessed identical topology. Analysis of the total evidence data set, when the transition-to-transversion cost ratio was 1:1, resulted in two equally parsimonious trees that differed in the relationships between *M. smithi* and *M. siddalli*. Discussion about evolutionary relationships, spore morphology, host specificity, and tissue tropism

was based on the tree resulting from increased transversional costs, because assuming a 1:1 transition-to-transversion cost ratio in genome evolution is known to be unrealistic.

The reliability of conventional criteria for species identification, such as spore morphology, host specificity, and tissue tropism, and their use in determining evolutionary relationships was evaluated in a phylogenetic context. The data revealed that neither host specificity nor tissue tropism accurately reflected close historical association, nor could these criteria be used for species identification in the absence of spore morphology. The close evolutionary relationships among morphologically similar taxa and the consistency of spore-based taxonomy with the phylogenetic hypothesis support the reliability of spore morphology in the systematics of the species examined.

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Appendix 1

Aligned ssu-rDNA sequences of *Myxidium lieberkuehni*, *Myxidium* sp., *Ceratomyxa shasta*, and species of *Myxobolus*.

	10	20	30	40	50	60	70	80
<i>Myxidium</i> sp.	TAAGATTAAG	CCATGCATGT	-CTGA-GTTC	ATACCTAGTT	AACG-TGAGA	CTGCGAAAGG	CTCAGTACAT	CAGTTATAGT
<i>Myxidium lieberkuehni</i>	TAAGCTTAAG	CCATGCATGT	-CTAA-GTTC	AAAC--ATTT	AATG-TGAGA	CTGCGAAAGG	CTCAGTAAAT	CAGCTATAAT
<i>M. xiaoi</i> (<i>N. cornutus</i>)	AAGGACTCAG	CCATGCAAGT	GTAAGAGTTC	ATACGATTTA	AAAG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGCGATGAT
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	AAGGACTCAG	CCATGCAAGT	GTAAGAGTTC	ATACGATTTA	AAAG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGCGATGAT
<i>M. pendula</i> (mucus present)	AAAGACTAAG	CC-TGCACGT	GCCAA-GTTC	ATACGATCTT	ATCG-TGAGA	CTGCGGACGG	CTCAGTATAT	CAGTGATTAT
<i>M. pendula</i> (mucus absent)	AAAGACTAAG	CCATGCACGT	GCCAA-GTTC	ATACGATCTT	ATCG-TGAGA	CTGCGGACGG	CTCAGTATAT	CAGTGATTAT
<i>M. pseudokoi</i>	GAAGATTAAG	CCATGCACGT	GCCAA-GTTC	ATACGATCTT	ATCG-TGAGA	CTGCGGACGG	CTCAGTATAT	CAGTGATTAT
<i>M. bartai</i>	GAAGATTAAG	CCATGCATGT	GCCAA-GTTC	AAACGTTTAT	ATCG-TGAGA	CTGCGGAAGG	CTCAGTATAT	CAGTGATTAT
<i>M. algonquinensis</i> (mesentery)	GAAGATTAAG	CCATGTATGT	GCCAA-GTTC	ATACGTTAGA	--CG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGTGACAAT
<i>M. algonquinensis</i> (gill arch)	GAAGATTAAG	CCATGTATGT	GCCAA-GTTC	ATACGTTAGA	--CG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGTGACAAT
<i>M. algonquinensis</i> (gonad)	-AAGATTAAG	CCATGTATGT	GCCAA-GTTC	ATACGTTAGA	--CG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGTGACAAT
<i>M. martini</i>	-AAGATTAAG	CCATGTATGT	GCCAA-GTTC	ATACGTTAGA	--CG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGTGATAAT
<i>M. siddalli</i>	GAAGATTAAG	CCATGTATGT	GCCAA-GTTC	AAACGTAATT	--CG-TAAGA	CTGCGGACGG	CTCAGTAAAT	CAGTGATAAT
<i>M. smithi</i>	GAAGATTAAG	CCATGTATGT	GCCAA-GTTC	AAACGTTATA	--CG-TGAGA	CTGCGGACGG	CTCAGTAAAT	CAGTGATAAT
<i>Ceratomyxa shasta</i>	AAAGATTAAG	CCATGCATGT	-CTAA-GTTC	ATATCATTTA	AATGATGAA	CTGCGAAGGC	GTCAGTAAAT	CAGTTATCGT
	90	100	110	120	130	140	150	160
<i>Myxidium</i> sp.	TTATTTCGATT	GTGAATCCAC	A-TGGATAAC	CGTGG-AAAT	CTAGAGCTAA	TACATGCGAA	AA	
<i>Myxidium lieberkuehni</i>	CTATTTGATT	GTTAACTCCC	AGTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCAAC	TA	
<i>M. xiaoi</i> (<i>N. cornutus</i>)	CTATCTGATT	GTACATCC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCGTT	GA	
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	CTATCTGATT	GTACATCC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCGTT	GA	
<i>M. pendula</i> (mucus present)	CTGTTTGATT	GTCTTACC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCAAA	TA	
<i>M. pendula</i> (mucus absent)	CTGTTTGATT	GTCTTACC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCAAA	TA	
<i>M. pseudokoi</i>	CTATTTGATT	GTCTTCCC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCAGT	TC	
<i>M. bartai</i>	CTGTTTGATT	GTCTAACC-T	AT-GGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACATGCAGT	TA	
<i>M. algonquinensis</i> (mesentery)	CTATTTGATT	TTCTCTCC-C	AATGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACGTGCGGT	AT	
<i>M. algonquinensis</i> (gill arch)	CTATTTGATT	TTCTCTCC-C	AATGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACGTGCGGT	AT	
<i>M. algonquinensis</i> (gonad)	CTATTTGATT	TTCTCTCC-C	ATTGGATAAC	CGTGGGAAAT	CTAAAGCTAA	TACGTGCGGT	AT	
<i>M. martini</i>	CTATTTGATT	TTCTCTCC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACGTGCGGT	AC	
<i>M. siddalli</i>	CTATTTGATT	TTCTCTCC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACGTGCGGT	AC	
<i>M. smithi</i>	CTATTTGATT	TTCTCTCC-C	ATTGGATAAC	CGTGGGAAAT	CTAGAGCTAA	TACGTGCGGT	AA	
<i>Ceratomyxa shasta</i>	CTGTTTCGATC	GATACATGCC	A-TGGATAAC	TGTGGCAAAC	CTAGAGCTAA	TACATGCAAA	AT	

	170	180	190	200	210	220	230	240
<i>Myxidium</i> sp.				GCATTTA	TTAGATTAA	CCAATCATTG		
<i>Myxidium lieberkuehni</i>				GCATTTA	TTGGAA-ATA	CCAA-CTTTG		
<i>M. xiaoi</i> (<i>N. cornutus</i>)				GCATTTA	TTAGAATAAA	CCAACGCTA		
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)				GCATTTA	TTAGAATAAA	CCAACGCTA		
<i>M. pendula</i> (mucus present)				GCATTTA	TTAGACTTAA	CCATCTACTG		
<i>M. pendula</i> (mucus absent)				GCATTTA	TTAGACTTAA	CCATCTACTG		
<i>M. pseudokoi</i>				GCATTTA	TTAGACTTAA	CCATCTACTG		
<i>M. bartai</i>				GCATTTA	TTAGTTTAA	CCAATTACTG		
<i>M. algonquinensis</i> (mesentery)				GCATTTA	TTAGACTAAA	CCAACACTA		
<i>M. algonquinensis</i> (gill arch)				GCATTTA	TTAGACTAAA	CCAACACTA		
<i>M. algonquinensis</i> (gonad)				GCATTTA	TTAGACTAAA	CCAACACTA		
<i>M. crysoluecas</i>				GCATTTA	TTAGACTAAA	CCAACACTA		
<i>M. siddalli</i>				GCATTTA	TTAGACTAAA	CCAACATTG		
<i>M. smithi</i>				GCATTTA	TTAGACTATA	CCAACATTG		
<i>Ceratomyxa shasta</i>				GCATTTA	TCCAGCTAA	CCAATCGGGC		

	250	260	270	280	290	300	310	320
<i>Myxidium</i> sp.		TTGTGGTGAG	TCTGGATAAC	TGTGCCGATC	GTA-TGGCCT	AG-AGCCGGC	GACATTTTCA	TTAAATTTCT
<i>Myxidium lieberkuehni</i>		ATA-GGTGAA	TCTGGATAAC	TG-GCTGATC	GTA-TGGCCT	TG-TGCTGAC	GACGTTTCAA	TTGAATTTCT
<i>M. xiaoi</i> (<i>N. cornutus</i>)		TTGTGGTGAG	TCTAGATAAC	TTTGCTGATC	GTA-TGGCCT	TAAAGCCGGC	GACATTTTCA	TTGAATTTCT
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)		TTGTGGTGAG	TCTAGATAAC	TTTGCTGATC	GTA-TGGCCT	AATAGCCGGC	GACATTTTCA	TTGAATTTCT
<i>M. pendula</i> (mucus present)		TAAGGCG-AA	TCTAGATAAC	TTTGCTGATC	GTA-TGGCCT	AG-TGCCGAC	GACGTTTCAA	TTGAGTTTCT
<i>M. pendula</i> (mucus absent)		TAAGGCG-AA	TCTAGATAAC	TTTGCTGATC	GTA-TGGCCT	AG-TGCCGAC	GACGTTTCAA	TTGAGTTTCT
<i>M. pseudokoi</i>		TAAGGCG-AA	TCTAGATAAC	TTTGCTGATC	GTA-TGGCCT	CG-TGCCGAC	GACGTTTCAA	TTGAGTTTCT
<i>M. bartai</i>		TAAG-GTGA-	TCTAGATAAC	TTTGCTGATC	GCA-TGGC-T	A--TGCCAGC	GACGTTTCAA	TTGAGTTTCT
<i>M. algonquinensis</i> (mesentery)		TAAGGGG-AA	TCTGGATAAC	TTTGCTGATC	GTA-TGGCCT	AG-TGCCGGC	GACGTTTCAA	TTGAGTTTCT
<i>M. algonquinensis</i> (gill arch)		TAAGGGG-AA	TCTGGATAAC	TTTGCTGATC	GTA-TGGCCT	AG-TGCCGGC	GACGTTTCAA	TTGAGTTTCT
<i>M. algonquinensis</i> (gonad)		TAAGGG--AA	TCTGGATAAC	TCTGCTGATC	GTA-TGGCCT	AG-TGCCGGC	GACGTTTCAA	TTGAGTTTCT
<i>M. martini</i>		TAAGGGG-AA	TCTGGATAAC	TTTGCTGATC	GTA-TGGCCT	AG-TGCCGGC	GACGTTTCAA	TTGAGTTTCT
<i>M. siddalli</i>		TAAGGGG-AA	TCTGGATAAC	TTTGCTGATC	GTA-TGGCCT	AG-TGCCGGC	GACGTTTCAA	TTGAGTTTCT
<i>M. smithi</i>		TAAGGGGGAA	TCTGGATAAC	TCTGCTGATC	GTA-TGGCCC	AG-TGCCGGC	GACGTTTCAA	TTGAGTTTCT
<i>Ceratomyxa shasta</i>		TA--GGTGAA	TCTAGATAAC	TGTGCCGATC	GTT--GCTT	TA-TGC-GGC	GATATTTTCA	TTGAGTTTCT

	330	340	350	360	370	380	390	400
<i>Myxidium</i> sp.	GCCCTATC-A	ACTTGTGGT	AAGGTAGTGG	CTTACCAAGG	TGATA-CGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>Myxidium lieberkuehni</i>	GCCCTATC-A	ACTTGTGGT	AAGGTAGTGG	CTTACCAAGG	TTGTAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. xiaoi</i> (<i>N. cornutus</i>)	GCCCTATC-A	ATTGGTTGGT	AAGGTAGTGG	CTTACCAAGG	TTGTAACGGG	TAACGGGGGA	TCAGGGTTCG	ATTCCGGAGA
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	GCCCTATC-A	ATTGGTTGGT	AAGGTAGTGG	CTTACCAAGG	TTGTAACGGG	TAACGGGGGA	TCAGGGTTCG	ATTCCGGAGA
<i>M. pendula</i> (mucus present)	GCCCTATC-A	ATTTGTTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. pendula</i> (mucus absent)	GCCCTATC-A	ATTTGTTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. pseudokoi</i>	GCCCTATC-A	ACTTGTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. bartai</i>	GCCCTATC-A	ATTTGTTGGT	AAGGTATTGG	CTTACCAAGG	TTGTAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. algonquinensis</i> (mesentery)	GCCCTATC-A	ACTTGTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. algonquinensis</i> (gill arch)	GCCCTATC-A	ACTTGTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGG-TTCG	ATTCCGGAGA
<i>M. algonquinensis</i> (gonad)	GCCCTATCCA	ACTTGTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGG-AA	TCAGGGTTCG	ATTCCGGAGA
<i>M. martini</i>	GCCCTATC-A	ACTTGTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. siddalli</i>	GCCCTATC-A	ATTTGTTGGT	AAGGTATTGG	CTTACCAAGG	TTGCAACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>M. smithi</i>	GCCCTATCCA	ACTTGTGGT	AAGGTATTGG	CTTACCAAGG	TTGCCACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA
<i>Ceratomyxa shasta</i>	GCCCTATC-A	ACTTGTGGT	ATGGTATTGG	CCTACCAAGG	TTTGTACGGG	TAACGGGGAA	TCAGGGTTCG	ATTCCGGAGA

	410	420	430	440	450	460	470	480
<i>Myxidium</i> sp.	GGGAGCCTCA	GAAACGGCTA	CCACATCCAA	GGACGGCAGC	AGGCGCGCAA	ATTACCCAAT	CCAGACACTG	GGAGGTGGTG
<i>Myxidium lieberkuehni</i>	GGGAGCCTGA	GAAATGGCTA	CCACATCCAA	GGGAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CCACAATGTG	GGAGGTGGTG
<i>M. xiaoi</i> (<i>N. cornutus</i>)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CCAGACAGTG	GGAGGTGGCG
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CCAGACAGTG	GGAGGTGGCG
<i>M. pendula</i> (mucus present)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGGAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. pendula</i> (mucus absent)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGGAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. pseudokoi</i>	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTA
<i>M. bartai</i>	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. algonquinensis</i> (mesentery)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAAC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. algonquinensis</i> (gill arch)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAAC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. algonquinensis</i> (gonad)	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAAC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. martini</i>	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAAC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. siddalli</i>	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAAC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>M. smithi</i>	GGGAGCCTGA	GAAACGGCTA	CCACATCCAA	GGAAGGCAAC	AGGCGCGCAA	ATTACCCAAT	CTAGACAGTA	GGAGGTGGTG
<i>Ceratomyxa shasta</i>	GGGAGCCTGA	GAAACGGCTA	CCACTTCTAA	GGAAGGCAGC	AGGCGCGCAA	ATTACCCAAT	CCAGACATTG	GGAGGTAGTG

	490	500	510	520	530	540	550	560
<i>Myxidium</i> sp.	ACGAGAAATA	CCAGGTCGTT	--CCCAAAAG	GATT-CGACA	CTGGAATGAG	TGCAATTAA	AAATTTCAAC	GAGTAACAAC
<i>Myxidium lieberkuehni</i>	ACGAGAAATA	TTAGGTCGTT	TTCCCTCAGG	GAATGCGATA	CTGAAATGAG	CACAATTAG	AACATTTGTC	GAGTAACAAC
<i>M. xiaoi</i> (<i>N. cornutus</i>)	ACGAGAATTA	CTAAGTGGTA	--GCCTTTAT	GGTTATCGAC	TTAGAATGAA	CGTGATTTAA	GAAATCCGTT	GAGTAACAAC
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	ACGAGAATTA	CTAAGTGGTA	--GCCTTTAT	GGTTATCGAC	TTAGAATGAA	CGTGATTTAA	GAAATCCGTT	GAGTAACAAC
<i>M. pendula</i> (mucus present)	AAGAGAAGTA	CTTAGTGGTG	--ACCAAATG	GTCCC--AAC	TTGGAATGAG	GCCAATTTAA	GAAATTCGTC	GAGTAACACTAC
<i>M. pendula</i> (mucus absent)	AAGAGAAGTA	CTTAGTGGTG	--ACCAAATG	GTCCC--AAC	TTGGAATGAG	GCCAATTTAA	GAAATTCGTC	GAGTAACACTAC
<i>M. pseudokoi</i>	AAGAGAAGTA	CTGAGTGGTG	--GCAAATTG	TTACC--AAC	TTGGAATGAA	CGTAATTTAA	GCAATTCGAT	GAGTAACACTAC
<i>M. bartai</i>	AAGAGAAGTA	CTAAGTGGTG	--GTTATTTA	CTACC--AGC	TTGGAATGGA	CGTAATTTAA	GCATTTTCGAT	GAGTAACACTAC
<i>M. algonquinensis</i> (mesentery)	AAGAGAATTA	CTAAATGGTG	--GTCTTATT	GATCATTTGT	TTGGAATGAA	CGTAACCTAA	GAAATTCGTT	GAGAAACAAC
<i>M. algonquinensis</i> (gill arch)	AAGAGAATTA	CTAAATGGTG	--GTCTTATT	GATCATTTGT	TTGGAATGAA	CGTAACCTAA	GAAATTCGTT	GAGAAACAAC
<i>M. algonquinensis</i> (gonad)	AAGAGAATTA	CTAAATGGTG	--GTCTTATT	GATCATTTGT	TTGGAATGAA	CGTAACCTAA	GAAATTCGTT	GAGAAACAAC
<i>M. crysoluecas</i>	AAGAGAATTA	CTAAATGGTG	--GTCAAATT	GATCATTTGT	TTGGAATGAA	CGTAACCTAA	GAAATTCGTT	GAGAAACAAC
<i>M. siddalli</i>	AAGAGAATTA	CTAAATGGTG	--GTCTTATT	GATCATTTGT	TTGGAATGAA	CGTAACCTAA	GAAATTCGTT	GAGAAACAAC
<i>M. smithi</i>	AAGAGAAATA	CTAAATGGTG	--GTCCAATG	GATCACTTGT	TTGGAATGAA	CGTAACCTAA	GAAATTCGTT	GAGAAACAAC
<i>Ceratomyxa shasta</i>	ACGAGAAATA	CCGGACTGGA	---TCTTACG	ATCCA--GTA	CTGGAATGAA	CGATATGTAA	TCATTTTCGAT	GAGGATCTAC

	570	580	590	600	610	620	630	640
<i>Myxidium</i> sp.	TGGAGGGCAA	GTC-TGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-GTATCC	AAACGTTGCT	GCGGTTAAAA
<i>Myxidium lieberkuehni</i>	TGAAGGGCAA	GTC-TGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTTC-AGT	AGT-GTATAT	TAAAGCTGCG	GCGGTTAAAA
<i>M. xiaoi</i> (<i>N. cornutus</i>)	TGGAGGGCAA	GTC-TGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGC-GTACCT	CAAAGTTGCT	GCGTTTAAAA
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	TGGAGGGCAA	GTC-TGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGC-GTACCT	CAAAGTTGCT	GCGTTTAAAA
<i>M. pendula</i> (mucus present)	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCCCG	CGGTAATTCC	AGCTCCAGT	GGCCGTGATT	TAAAGTTGCT	GCGTTTAAAA
<i>M. pendula</i> (mucus absent)	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCCCG	CGGTAATTCC	AGCTTC-AGT	GGCCGTGATT	TAAAGTTGCT	GCGTT-AAAA
<i>M. pseudokoi</i>	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	GGC-GTGATT	TAAAGTTGCT	GCGTTTAAAA
<i>M. bartai</i>	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	GGC-GTGATT	TAAAGTTGCT	GCGTTTAAAA
<i>M. algonquinensis</i> (mesentery)	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-TTGCTT	TAAAGTTGTT	GCGTTTAAAA
<i>M. algonquinensis</i> (gill arch)	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-TTGCTT	TAAAGTTGTT	GCGTTTAAAA
<i>M. algonquinensis</i> (gonad)	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-TTGCTT	TAAAGTTGTT	GCGTTTAAAA
<i>M. martini</i>	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-TTGCTT	TAAAGTTGTT	GCGTTTAAAA
<i>M. siddalli</i>	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-TTGCTT	TAAAGTTGTT	GCGTTTAAAA
<i>M. smithi</i>	TGGAGGGCAA	GTCCTGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-TTGCTT	TAAAGTTGTT	GCGTTTAAAA
<i>Ceratomyxa shasta</i>	TGGAGGGCAA	GTC-TGGTGC	CAGCAGCC-G	CGGTAATTCC	AGCTCC-AGT	AGT-GTATAT	CAACATTGTT	GCGGTTAAAA

	650	660	670	680	690	700	710	720
<i>Myxidium</i> sp.	CGCTCGTAGT	TGG	AGAG	AGTAGCTTGG				AGGAAGTGTC
<i>Myxidium lieberkuehni</i>	CGCTCGTAGT	TG-	ATGA	CGAAGCTCGG				CTGTAGTATT
<i>M. xiaoi</i> (<i>N. cornutus</i>)	CGCTCGTAGT	TGG	ATCA	CATGGTTAAG				CTCAAGTGTA
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	CGCTCGTAGT	TGG	ATCA	CATGGTTAAG				CTCAAGTGTA
<i>M. pendula</i> (mucus present)	CGCTCGTAGT	TGG	ATCA	TGCAGTGATA				TACCATTTTT
<i>M. pendula</i> (mucus absent)	CGCTCGTAGT	TGG	ATCA	TGCAGTGATA				TACGATTTT-
<i>M. pseudokoi</i>	CGCTCGTAGT	TGG	ATCA	CGCAGTAGTA				TTTCATGAT-
<i>M. bartai</i>	CGCTCGTAGT	TGG	ATCA	AGCGGTG---				TGACATTTGA
<i>M. algonquinensis</i> (mesentery)	CGCTCGTAGT	TGG	ATCA	CGCAGTGATG				CAAATGTATC
<i>M. algonquinensis</i> (gill arch)	CGCTCGTAGT	TGG	ATCA	CGCAGTGATG				CGAATGTATC
<i>M. algonquinensis</i> (gonad)	CGCTCGTAGT	TGG	ATCA	CGCAGTGATG				CAAATGTATC
<i>M. martini</i>	CGCTCGTAGT	TGG	ATCA	CGCAGCGATG				TTAATGTATC
<i>M. siddalli</i>	CGCTCGTAGT	TGG	ATCA	CGCAGCGATA				TACTTGAATC
<i>M. smithi</i>	CGCTCGTAGT	TGG	ATCA	CGCAGCGACA				CATTTGTATC
<i>Ceratomyxa shasta</i>	CGCTCGTAGT	TGG	ATAA	CGAGGGTAAA				TTTATTACAA

	730	740	750	760	770	780	790	800
<i>Myxidium</i> sp.	GCTCGCTATC	GAAAGTTAG-	CAAGTACTAT	TC	CTTGTTGTAG	C		GCTCC
<i>Myxidium lieberkuehni</i>	GCGAAGCAGA	CCACGTCTGT	CTAGTGGTGC	GG	CGTGCGGTAT	G		--TCC
<i>M. xiaoi</i> (<i>N. cornutus</i>)	GATGTAGCGG	GGTATTATCC	CCGATGCAAT	TG	TGGAGTACTG	C		GAAC
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	GATGTAGTGG	GGTCTTATCC	CTACTACAAT	TG	TGGAGTACTG	C		GAAC
<i>M. pendula</i> (mucus present)	CTCCAACGAG	TTTATTGAAT	CTGTACCATT	TG	CGTGTATTATG	C		GAATG
<i>M. pendula</i> (mucus absent)	CTCCACCGAG	TTTATTGAAT	CTGT-CCATT	TG	CGTGTATTATG	C		GAATG
<i>M. pseudokoi</i>	-GCCTCAGGG	TTTCT-GAAT	TTCA-TTAGT	TG	AATGTTAACG	C		GAATG
<i>M. bartai</i>	CTGCTTC-AG	TT----GATG	-TGAGCCA--	AG	GATGAAAACG	C		AACTG
<i>M. algonquinensis</i> (mesentery)	CGTCACGTAC	GGCCATTTGA	CGTCACCCGC	TC	AGATATCATG	C		GGACG
<i>M. algonquinensis</i> (gill arch)	CGTCACGTAC	GGCCATTTGA	CGTCACCCGC	TC	AGATATCATG	C		GGACG
<i>M. algonquinensis</i> (gonad)	CGTCACGTAC	GGCCATTTGA	CGTCACCCGC	TC	AGATATCATG	C		GGACG
<i>M. martini</i>	CGTCACGTAC	GGCCATTTGA	CGTCACCCGC	TC	AGATATCATG	C		GGACG
<i>M. siddalli</i>	CGTCACGTAC	GGCCATTTAT	CGGTGTCAGC	TT	AGATGCCATG	C		GAATA
<i>M. smithi</i>	CGTCACGTAC	GGCCATTTGG	CGGCATTTCGT	TC	AGATGCCATG	C		GAGTG
<i>Ceratomyxa shasta</i>	GGGTCAATAC	TTTGCTTAAT	TGAATTGTAT	TG	TATAGCGTGC	C		CAAGC

	810	820	830	840	850	860	870	880		
<i>Myxidium</i> sp.	AGGCTACTTG	T	G	TGAGAATGGT	AT	ATGGT	CTTCATTGTA	CTGTGTATTA	TGTCTTACGT	AGT-GTGCCT
<i>Myxidium lieberkuehni</i>	GGGCTACTAG	A	G	TGAGAATGGT	GA	ATGGT	CTTTACCG-A	CTGCTCATCG	TGTCTCGCAG	AGT-GTGCCT
<i>M. xiaoi</i> (<i>N. cornutus</i>)	TAACTGATTG	C	A	TGGGGATGGT	AA	TTGAC	CTTAAGTGCG	TCGATTATTG	TGTCCCATGG	AGT-GTGCCT
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	TAACTGATTG	C	A	TGGGGATGGT	AA	TTGAC	CTTAAGTGCG	TCGATTATTG	TGTCCCATGG	AGT-GTGCCT
<i>M. pendula</i> (mucus present)	TTACTATTTG	C	A	CAAGTATGGC	AT	TTGGC	CTTTAGTGAG	TTGAGTGTC	TGTCTTGTGG	AGT-GTGCCT
<i>M. pendula</i> (mucus absent)	TTACTATTTG	C	A	CAAGTATGGC	AT	TTGGC	CTTTAGTGAG	TTGAGTGTC	TGTCTTGTGG	AGT-GTGCCT
<i>M. pseudokoi</i>	CTACTATTTG	C	A	CAAGTGTGGT	AT	TTGGC	CTTTAGTGAG	TTGAGTATCA	TGCCTTGTGG	AGT-GTGCCT
<i>M. bartai</i>	TCACTATTTA	A	G	CAAACATGTT	AG	TTGGC	CTTTAATGAG	TTGATTATCA	TGTTTTGCAG	AGTTGTGCCT
<i>M. algonquinensis</i> (mesentery)	TCGCTAGTTG	C	G	TGAGATAGAT	TG	TTGGC	CTTGAATGTG	CCGGTATTCT	TGTCTTGCAG	GGT-GTGCCT
<i>M. algonquinensis</i> (gill arch)	TCGCTAGTTG	C	G	TGAGATAGAT	TG	TTGGC	CTTGAATGTG	CCGGTATTCT	TGTCTTGCAG	GGT-GTGCCT
<i>M. algonquinensis</i> (gonad)	TCGCTAGTTG	C	G	TGAGATAGAT	TG	TTGGC	CTTGAATGTG	CCGGTATTCT	TGTCTTGCAG	GGT-GTGCCT
<i>M. crysoluecas</i>	TCGCTAGTTG	C	G	TGAGATAGAT	TG	TTGGC	CTTGAATGTG	CCGGTATTCT	TGTCTTGCAG	GGT-GTGCCT
<i>M. siddalli</i>	TTGCTAGTTG	C	G	TGAGATAGAT	TG	TTGGC	CTTTAATGAG	CCGGTATTCT	TGTCTTGCAG	GGT-GTGCCT
<i>M. smithi</i>	TTGCTAGTTG	C	G	TGAGATAGAT	TG	TTGGC	CTTTAATGAG	CCGGTATTCT	TGTCTTGCAG	GGT-GTGCCT
<i>Ceratomyxa shasta</i>	GTAACGCTAG	A	G	CATGGAACGA	AT	TTGAC	CTGAATCAGT	TTGTTGGTAA	AAAGTTACGC	AA--GTAAC

	890	900	910	920
<i>Myxidium</i> sp.	TGAATAAATC	AG		
<i>Myxidium lieberkuehni</i>	TGAATAAACC	AG		
<i>M. xiaoi</i> (<i>N. cornutus</i>)	TGAATAAAAC	AG		
<i>M. xiaoi</i> (<i>N. crysoleucas</i>)	TGAATAAAAC	AG		
<i>M. pendula</i> (mucus present)	TGAATAAA-C	AG		
<i>M. pendula</i> (mucus absent)	TGAATAAAAC	AG		
<i>M. pseudokoi</i>	TGAATAAAAC	AG		
<i>M. bartai</i>	TGAATAAAAC	AG		
<i>M. algonquinensis</i> (mesentery)	TGAATAAAAC	AG		
<i>M. algonquinensis</i> (gill arch)	TGAATAAAAC	AG		
<i>M. algonquinensis</i> (gonad)	TGAATAAAAC	AG		
<i>M. martini</i>	TGAATAAAAC	AG		
<i>M. siddalli</i>	TGAATAAAAC	AG		
<i>M. smithi</i>	TGAATAAAAC	AG		
<i>Ceratomyxa shasta</i>	AACCTGAAAC	GG		