The Biology and Systematics of Myxozoan Parasites of Fish and Oligochaetes from Lake Sasajewun, Algonquin Park, Ontario

By

Chongxie Xiao

A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
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An abstract of a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy, 1999

Chongxie Xiao, Department of Zoology, University of Toronto

Since the discovery of the 2-host life cycle of myxozoans by Wolf and Markiw in 1984, numerous studies have contributed to the knowledge of myxozoan parasites, and at the same time, have raised even more questions. Most of these studies have only dealt with the experimental transmission of these parasites under laboratory conditions. In my dissertation, I have attempted to address some of the questions by looking at different aspects of these enigmatic parasites in a natural ecological setting.

To fully document myxosporeans in Lake Sasajewun, a survey of these parasites of fish was conducted which resulted in the description of 2 new species. An intensive survey of oligochaete hosts of actinosporeans in the lake revealed that the fauna consisted of 19 species. Upon examining more than 14,000 worms for actinosporeans, only 146 worms were found to be releasing spores. Despite a prevalence of only 1.0%, 22 new 'species' of actinosporeans of 8 collective groups were described. The number of actinosporean 'species' is less than half the number of myxosporean species from the same lake, suggesting that direct transmission might occur among fish. Water-borne actinosporean spores peaked when water

temperatures of the lake ranged from 18 to 24 C, which coincided with the feeding and growing season of larval fish.

The molecular variation of 18 myxozoans from Lake Sasajewun was analyzed by riboprinting, revealing a close relationship between *Myxobolus pendula* and *Myxobolus pellicides*, and between triactinomyxon 'C' and *Triactinomyxon ignotum*. Parsimony analysis of riboprint data lent further support to the 2-host life cycle hypothesis. The genetic diversity of selected myxozoans was further investigated by sequence comparisons of the 18S rRNA gene. The phylogenetic hypothesis generated from the sequence data in this study and those available from GenBank also supported the alternating life cycle hypothesis.

Phylogenetic analyses of the myxozoan species with known alternating life cycles, using morphological and developmental characters from myxosporean and actinosporean phases, were conducted through a total evidence approach. The analysis revealed that the suborder Variisporina and the family Myxobolidae were not monophyletic groups and that myxozoans were subject to thorough systematic revision.

La Biologie et les Systématiques de Parasites Myxozoa en les Poissons et les Oligochètes du Lac Sasajewun, Parc Algonquin, Ontario.

Par

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Résumé

Depuis la découverte du cycle de vie 2-hôte de myxosporidies par Wolf et Markiw en 1984, il y a de nombreuses études qui ont contribué au progrès de la connaissance de ces parasites et en même temps ont evoqué plus de questions. La plupart de ces études s'addressaient seulement aux transmissions expérimentales de ces parasites sous les conditions laboratoires . Pour addresser quelques unes de ces questions, on a étudié des aspects différents de ces parasites enigmatiques en ce qui concerne leur milieu écologique et naturel.

Pour bien documenter les myxosporidies dans le Lac Sasajewun, on a fait un levé qui a mené à une description de 2 espèces nouvelles. Un levé intensif de l'hôte oligochètes de ces parasites actinosporidies a révelé qu'il y a 19 espèces de 14 genres et 4 familles. En examinant plus de 14,000 vers pour les parasites on a seulement trouvé 146 vers qui libéraient des spores. Bien qu'il y avait une fréquence basse (1.0%), on a décrit 22 nouvelles espéces actinosporidies de 8 groupes collectifs. On a trouvé que le numéro d'espèces actinosporidies était moins de la moitié de celles trouvées dans le même lac, qui propose que la transmission directe peut avoir lieu parmi les poissons. Durant une étude écologique de ces parasites, les spores libérées

étaient prédominantes quand la range de la température était 18 à 24 C ce qui coincidait avec la saison d'alimentation et de croissance du poisson larvel.

La variation moléculaire de 18 Myxozoa du lac Sasajewun fut analysée par riboprinting, qui a montré une rélation évolutionnaire et proche entre *Myxobolus pendula* et *Myxobolus pellicides*, et entre Triactinomyxon 'C' et *Triactinomyxon ignotum*. L'analyse parsimonie avec les données riboprinting a montré plus d'évidence pour l'hypothèse du cycle 2-hôte.

Pour complémenter l'analyse riboprint, on a étudié plus la diversité génétique de Myxozoa selectionées par les comparisons séquences du gène 18S RNA. La phylogène produite par les données séquences de cette étude et ceux disponibles de GenBank aussi supportaient l'hypothèse du cycle de vie alternant.

Avec les cycles de vie alternants établises, une approche évidence-totale et utilisant les caractéristiques morphologiques et développementales, l'analyse phylogène d'espèces Myxozoa des phases myxosporidies et actinosporidies a fourni une hypothèse solide de leur phylogène et a revelé que le sous-ordre Variisporina et la famille Myxobolidae ne sont pas des groupes monophylétiques. Une fois que les systèmatiques des Myxozoa sont revisées, l'approche phylogénétique d'évidence-totale doit être adoptée pour refléter correctement les signaux phylogénétiques.

Acknowledgements

I am indebted to many people for their help and support during the course of my doctorate program.

First and foremost, I thank my supervisor, Dr. Sherwin S. Desser, for his guidance, patience, encouragement, financial support and for providing me with the opportunity to work in his lab. I thought you told me that I was going to be your last graduate student.

I also thank Dr. Lianqiang Li who highly recommended me to pursue my doctoral degree with Dr. Desser.

I thank my Advisory Committee members: Dr. Daniel R. Brooks for contributing to my knowledge of phylogenetic systematics with his cheerful and always interesting discussions; Dr. Rick Winterbottom, for his contributions to my knowledge of ecology and fish biology; he was always available when I needed any help.

I thank Dr. Maurice Ringuette for his help with my molecular techniques, Dr. Michael L. Kent for serving as my external examiner and for his valuable criticisms of my dissertation, and Dr. Kathryn Coates and Donald Stacey for their help with my study in Algonquin Park and their contributions to my knowledge of oligochaetes.

I am indebted to my colleagues in the lab, many of whom have come and gone over the years and will always be remembered. Henry Hong, Todd Smith, Betty Guest, Susan Kopko, Zhao-Rong Lun, Kowthar Salim, Luis Chiappetta, Sheri Kong, Nigel Thomas and Andy Han... thanks for all of your help and the good times.

Special thanks go to Henry Hong for his consistent help, encouragement and friendship.

I would also like to thank Dr. Lin Wu and Dr. Binhe Gu for their encouragement and financial support; Dr. Donald S. Martin, Dr. John R. Barta, Dr. Mark E. Siddall, Dr. Don Jackson, Dr. Anindo Choudhury, Dr. Robert Murphy, Dr. Douglas Currie, Dr. Yong Chen, Fernando Marques, Scott Monks, Jinzhong Fu and June Kim for their help, encouragement and friendship during my study at this great University.

I thank the Ontario Ministry of Natural Resources for the use of their facilities at the Wildlife Research Station in Algonquin Park; Dr. Mark Ridgway for his help in field sampling; Sean Blaney, Mark Johnson, Carl Rothfels and Gordon Lewer for their assistance in the identification of aquatic plants; and Cam Macdonald and Lisa Enright for the use of their water temperature data; the station managers (Kurt Schryer and Matt Cornish), the assistant managers and cooks, as well as all the other "Park People" who have lent their assistance to my research over the years.

I am indebted to the University of Toronto for subsidizing my graduate studies through the International Student Fee Waiver and the University of Toronto Open Doctoral Fellowship over the years, and Dr. Yoshio Masui for the University of Toronto Zoology International Student Award.

I thank all of the Faculty, Staff and Students at the Department of Zoology who have extended the hand of friendship over the years.

And last but not least, I am grateful for the love and support given to me over the years by my wife, Lixia Gao, my daughter, Jielin Xiao, my parents, Yuhao Xiao and Fengzhen Yang, and my parents-in-law, Haiming Gao and Shingying Zhou.

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CHAPTER 1

Historical Review

Historical Review

The phylum Myxozoa was thought to be comprised of 2 separate classes, Myxosporea Butschli, 1881, primarily of fishes and Actinosporea Noble, 1980, primarily of oligochaetes. Research on members of each class progressed independently until Wolf and Markiw's discovery (1984) that revealed their relationships. The history of research on the myxosporeans and actinosporeans will be dealt with separately prior to the time when their connection was established.

The myxosporeans of fish were first discovered in 1841 by Muller who called them "psorosperms". The term "myxosporidia" was coined in 1881 by Butschli, who was the first to document their trophozoite stage. In the following decades, numerous taxonomic studies and interpretations of myxosporean life cycles appeared. Interest in myxosporeans surged after World War II and resulted in additional studies on the pathogenicity, and later, in the 1960s, studies of their ultrastructure. Interest was renewed in 1984 following Wolf and Markiw's experimental transmission studies and their proposal of a 2-host life cycle involving alternation of myxosporeans and actinosporeans in their fish and worm hosts.

Major works on myxosporeans including those of Kudo (1920), Fantham et al. (1939), Hoffman (1967), Mitchell (1977), Schulman (1966, 1984), Lom and Dykova (1992) have been mainly taxonomic. Few studies were focused on the biology of these organisms under natural conditions. Species descriptions have been predominantly based upon the shape and structure of the spores in their vertebrate hosts, because of the lack of distinctive characters of their trophozoites. Host species and site of infection have also been taken into consideration in the designation of parasite species, hundreds of which have been described since the end of the 19th century, with most of the work being concentrated on fresh-water fish because of their accessibility. Myxosporeans have been reported from almost every tissue and

organ of fish, and usually show some degree of tissue specificity (Csaba, 1976; Mitchell, 1977; Lom & Dykova, 1992). For example, coelozoic species are found in the lumen of hollow organs such as the gall bladder or kidney tubules, while histozoic species form intercellular "cysts" in tissues such as the body wall musculature, gills, and integument. Although myxosporeans are generally believed to be host specific, several species have been described that infect different host species (see Lom and Dykova, 1992).

The taxonomy of the conventional myxosporeans has been and remains to be troublesome (Schulman, 1966; Mitchell, 1977; Lom and Noble, 1984; Lom and Arthur, 1988; Moser and Kent, 1994), but this is in no way due to a lack of effort by scientists to apply meaningful taxonomic principles and practices to this group. Schulman (1966) presented a classification based in part on the spore shape. Mitchell (1977) described the major taxonomic schemes based on descriptive criteria. Lom and Noble's recent classification (1984), which has been widely accepted, represents the results of a collective consideration of the available knowledge of this group and provides a basis for future revision.

The actinosporeans of oligochaetes were first discovered in 1889 by Stolc. The actinosporeans received far less attention than the myxosporeans, which explains the paucity of studies of their biology. Interest in these parasites was renewed following publication of the 2-host life cycle hypothesis by Wolf and Markiw (1984).

Studies on actinosporeans have been mainly taxonomic. Major works include those of Stolc (1899), Leger (1904), Ikeda (1912), Granata (1922, 1925), Georgevitch (1940), Janiszewska (1957), and Marques and Ormieres (1982). Species descriptions have been based predominantly on the shape and structure of the spores, many on a single random observation of this stage. Most actinosporeans have been reported from the intestinal wall of the worm host (Marques, 1984). Like the myxosporeans,

host species are important for the designation of parasite species. About 40 species have been described since the end of the 19th century, predominantly from oligochaetes.

The taxonomy of the actinosporeans is relatively stable because to date, only a small number of 'species' have been recorded. Historically, Stolc (1899) placed this group of parasites in the Mesozoa. Leger (1904) considered actinosporeans to be closely related to the myxosporeans. Caullery and Mesnnil (1904) grouped the Actinosporidies, Myxosporidies, Microsporidies and Sarcosporidies together based on the similarities of their spores. The Actinosporidia, Myxosporidia, and Microsporidia were defined as different orders of the subclass Cnidosporidia in the class Sporozoa of the phylum Protozoa in the 1950s (see Janiszewska, 1957). The notion that the Actinosporidia and Myxosporidia, which are multicellular, should not be grouped together with the Microsporidia, which is unicellular, reappeared in the 1960s (Ormieres and Frezil, 1969), and was further supported by the recognition of profound ultrastructural differences between these parasites. This led to the establishment of the phylum Myxozoa Grasse, 1970 in the kingdom Protista, consisting of two classes, Myxosporea and Actinosporea (Levine et al., 1980). This scheme was accepted until recently, when the 2-host life cycle data were published.

Attempts to determine the complete life cycles of myxosporeans and actinosporeans were unsuccessful for over 80 years. Ingestion of fresh myxosporean spores by fish and the exposure of fresh actinosporean spores by oligochaetes have not produced infection. Although there have been a few reports of the direct transmission of myxosporeans (Hoffman and Putz, 1971; Uspenskaya, 1978; Diamant, 1997), these have not been confirmed (Wyatt, 1978; Wolf and Markiw, 1984). Despite the fact that attempts to transmit infection by direct passage of spores have failed, myxosporean infections have been transferred by intraperitoneal injection of the trophozoites from infected tissues (Kent and Hedrick, 1985; Bower,

1985). Infections have also been induced under laboratory and field conditions by exposing susceptible fish to the contaminated water (Ching, 1984; Kent and Hedrick, 1986; Hendrickson et al., 1989). In 1984, Wolf and Markiw demonstrated a 2-host life cycle involving the transformation of myxosporeans into actinosporeans in the worm host and actinosporeans into myxosporeans in the fish host based on the completion of the life cycle of *Myxobolus cerebralis* Hofer, 1903 under laboratory conditions. Since their pioneering work, compelling evidence indicates that the myxosporeans and the actinosporeans do not constitute different classes, but different developmental stages of the same organisms (see Kent et al., 1994; Uspenskaya, 1995; Yokoyama, 1997).

Thusfar, fourteen myxozoan species have been shown to undergo a 2-host life cycle involving the alternation of myxosporeans and actinosporeans. Based on these discoveries, Kent et al. (1994) proposed higher-level taxonomic changes of the Myxozoa. They recommended that the class Actinosporea be suppressed and its genera provisionally reduced to the status of collective groups, except for *Tetractinomyxon* Ikeda, 1912, which was tentatively shifted to the myxosporean order Multivalvulida Schulman, 1959 due to one character, the single binucleate sporoplasm, which is shared with the conventional myxosporeans. Recently, Bartholomew et al. (1997) revealed that *Ceratomyxa shasta* Noble, 1950 alternates with a form of *Tetractinomyxon* to complete its life cycle. Despite the recent advances in the understanding of the Myxozoa, the traditional approach to unravelling their within-group relationships by utilizing only the overall similarity of the myxosporean stages, and ignoring the corresponding actinosporean stages, still persists.

Metazoan affinities for Myxozoa was suggested by Stolc a century ago (see Lom et al., 1997), but the Myxozoa have long been considered protistans. Recent molecular analyses (Smothers et al., 1994; Siddall et al., 1995; Schlegel et al., 1996;

Anderson et al., 1998) support Stolc's claim. Smothers et al. (1994) and Schlegel et al. (1996) hypothesized a close relationship between Myxozoa and Bilateria. Based on morphological, developmental, and molecular data, Siddall et al. (1995) postulated the inclusion of myxozoans in the Cnidaria.

CHAPTER 2

General Introduction

General Introduction

The myxozoan parasites have been known since the early 19th century. More than 1,200 species of myxosporean parasites have been described based exclusively on the spore stage in the vertebrate host, mainly fish (Lom & Dykova, 1992), and about 40 'species' of actinosporean parasites, based on the spore stage in the invertebrate host, mainly oligochaetes (Marques, 1984; Wolf & Markiw, 1984). Only recently have life histories been elucidated for a few species. Since Wolf and Markiw (1984) postulated the 2-host life cycle hypothesis of myxosporean parasites, most studies have been focused on the demonstration of the alternation between the actinosporean stage in the worm host and the myxosporean stage in the fish host under laboratory conditions (Markiw and Wolf, 1983; El-Matbouli and Hoffmann, 1989; Ruidisch et al., 1991; El-Matbouli et al., 1992, 1995; Trouillier et al., 1996; Bartholomew et al., 1997) and on their unity of identical ultrastructural patterns (Lom and Dykova, 1992, 1997; Lom et al., 1997). Despite this renewed interests in myxozoans, there is still a huge discrepancy between the number of described myxosporean species (about 1,200, see Lom and Dykova, 1992) and actinosporean 'species' (about 40, see Marques, 1984; Wolf & Markiw, 1984). In addition, the biology, diversity and ecology of myxozoan parasites, especially of the actinosporean stages of worms in their natural ecological setting, remain poorly known.

The myxosporean parasites of fish in Lake Sasajewun have been surveyed and documented by Gowen (1983), Li and Desser (1985), Lom et al. (1989), and Xiao and Desser (1997). Although these earlier surveys revealed a rich fauna of myxosporeans, their corresponding actinosporean stages and their oligochaete hosts have not been studied.

The purpose of this research was to: a) characterize the actinosporean

parasites of oligochaetes, not only by examining their morphology, ultrastructure and developmental stages, but also by investigating their host-specificity and molecular biological characteristics of representative 'species', and b) study the association among these parasites, their oligochaete hosts, and habitats in Lake Sasajewun, Algonquin Park, Ontario. This thesis was designed in part to test the hypothesis that, since the myxosporeans of fish are a highly diverse group in Lake Sasajewun, their corresponding actinosporeans should exhibit a similar diversity. To date, this is the most extensive study on the biology, ecology, and taxonomic characterization of actinosporean parasites of oligochaetes in a complex ecological setting.

In order to fully document the diversity of the myxosporeans, an additional survey was conducted to examine all species of fish in the lake. This resulted in 2 new species descriptions (Chapter 3). The diversity of actinosporean parasites was investigated by an extensive survey of the oligochaetes in the lake (Chapters 4 and 5). The longevity of actinosporean spores and their reaction to fish mucus were also investigated (Chapter 6). The associations among actinosporean parasites, their hosts, and their habitat revealed the synchronization between the feeding and growing season of susceptible larval fish and the maturation of most actinosporean spores (Chapter 7).

Molecular characterization of 9 myxosporeans and 9 actinosporeans from the lake was conducted by riboprinting (Chapter 8). DNA sequence analysis of 18S ribosomal DNA of species with identical riboprints revealed sequence differences between them and, in conjunction with 18S ribosomal DNA sequences of other myxozans from GenBank, phylogenetic analyses were performed (Chapter 9). In order to reflect the phylogenetic signals and the diverse range of life stages of myxozoan parasites, the phylogenetic total evidence approach was applied to the systematics of myxozoans (Chapter 10). Finally, a discussion of the general

conclusions and implications of these findings is presented (Chapter 11).

This thesis has been organized into self-contained chapters, each intended to reflect a discrete aspect of the biology of myxozoan species. Each chapter contains an abstract of its contents, an introduction, materials and methods, results, and discussion. Chapters 3, 4, 5, and 7 are all based on published works of which I was the primary author. The status of each of these is indicated on the cover page of each chapter. As such, I assume all responsibility for any errors of fact or form and for the conclusions, interpretations, and suggestions drawn therein.

CHAPTER 3

A Light and Electron Microscopic Study of *Sphaerospora ovophila* and *Myxobolus algonquinensis* of Fish from Lake Sasajewun, Algonquin Park

(Adapted from Xiao, C. & Desser, S.S. 1997. *Sphaerospora ovophila* n. sp. and *Myxobolus algonquinensis* n. sp. (Myxozoa, Myxosporea), ovarian parasites of fish from Algonquin Park, Ontario, Canada. J. Euk. Microbiol., **44**, 157-161.)

ABSTRACT

Sphaerospora ovophila n. sp. and *Myxobolus algonquinensis* n. sp., found in the ovary of the pumpkinseed (*Lepomis gibbosus*) and the golden shiner (*Notemigonus crysoleucas*) respectively from Algonquin Park, Ontario, are described using light and electron microscopy. Ovoid cysts of *S. ovophila* measured up to 500 µm in length. Monosporic pseudoplasmodia were ovoid or ellipsoid in shape and measured up to 12.5 µm in length. Spores were 7.2--8.4 µm long X 6.0--7.0 µm wide (in sutural plane) X 7.4--8.2 µm thick (perpendicular to sutural plane), with two subspherical polar capsules of equal size measuring 2.7--3.2 X 2.6--3.1 µm and each of which contained a polar filament with 6--7 coils. The spore had a straight sutural ridge which protruded slightly at the anterior end and contained two uninucleate sporoplasms. The spore valve had ornate folds on the posterior end. Cysts of *M. algonquinensis* ranged from ovoid to elongated ellipsoid in shape and measured up to 800 µm in length. Mature spores measured 13.6--15.7 µm long X 10.1--12.1 µm wide X 5.0--6.9 µm thick, with two pyriform polar capsules of equal size measuring 5.1--5.5 µm X 2.5--2.9 µm, each of which contained a polar filament with 4--6 coils. The spores of *M. algonquinensis* had smooth valves, a straight sutural ridge and a distinct small intercapsular appendix.

INTRODUCTION

The myxosporean genera *Sphaerospora* Thelohan, 1892 and *Myxobolus* Butschli, 1882 include more than 40 and 450 species respectively (Kudo, 1920; Desser et al., 1983; Landsberg & Lom, 1991; Lom & Dykova, 1992). The importance of species of these two genera as pathogens of fish is well documented (Lom et al., 1985; Fisher-Scherl et al., 1986; Lom, 1987; Lom & Dykova, 1992). Although numerous and detailed descriptions of different species of these two genera have been reported from nearly all tissues of fish, little is known of those species specifically infecting ovaries, with the exception of a single species of *Sphaerospora* (Chen & Hsieh, 1984; Lom & Dykova, 1992). During the study of life cycles of myxosporean parasites of fish in Algonquin Park, Ontario, cysts were found in the ovaries of the pumpkinseed, *Lepomis gibbosus*, and the golden shiner, *Notemigonus crysoleucas*. Examination of these cysts and spores revealed that the parasites in both fish hosts were new species, which are described herein.

MATERIALS AND METHODS

Fifty-three pumpkinseeds, *Lepomis gibbosus* and 45 golden shiners, *Notemigonus crysoleucas* from Lake Sasajewun (45°35'N, 78°30'W) were examined for myxosporean parasites. These fish, ranging in size from 3 to 12 cm, were caught in a wire mesh Gee Minnow Trap baited with Purina® dog chow.

Fish were examined immediately after capture or kept alive in aquaria for several days before examination. Tissue smears stained with Diff-Quik® and squash preparations of fresh tissues were examined, and measurements of spores and cysts were made of fresh material or glycerine-gelatin semi-permanent mounts (Lom & Arthur, 1989). Spore measurements were based on 25 specimens unless otherwise indicated and are given as a mean followed by the range in micrometers. Samples

of infected ovaries were fixed in Bouin's solution, and embedded in Paraplast®. Histological sections were stained with hematoxylin-eosin (H & E). Spores and tissue sections were photographed using brightfield or differential interference contrast (DIC) microscopy with a Zeiss Universal I photomicroscope or a Leitz Dialux 22.

Ovaries containing cysts were fixed using 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) at 4° C, postfixed with 1.0% osmium tetroxide in 0.125 M phosphate buffer with 0.8% potassium ferricyanide for 1 h in the dark and dehydrated through a graded series of ethanol. For transmission electron microscopy (TEM), the tissues were embedded in Spurr's epoxy resin and polymerized at 65° C for 8 h. Ultrathin sections were stained with 50% methanolic uranyl acetate and Reynolds lead citrate and examined with a Hitachi H7000 transmission electron microscope. For scanning electron microscopy (SEM), the tissues were cryofractured, dried by infiltration with Peldri II and sublimation, mounted on aluminum stubs, coated with gold-palladium and examined with a Hitachi S2500 scanning electron microscope.

RESULTS

Sphaerospora ovophila n. sp. (Figs. 1--15)

Host. Pumpkinseed, *Lepomis gibbosus*.

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

Site of infection. Ovary.

Prevalence. Eight of 44 (18.2%) female fish infected.

Cysts and trophozoites. Small cysts containing aggregates of pseudoplasmodia developed in interstitial tissues among eggs (Fig. 8). Large cysts around eggs, visible with naked eyes, whitish, ovoid or irregularly-shaped,

measuring up to 500 µm in length and containing spherical inclusions, aggregates of monosporic pseudoplasmodia and a variable number of spores (Figs. 6–9). Monosporic pseudoplasmodia either ovoid or ellipsoid, measuring up to 12.5 µm in diameter (Fig. 5).

Spores. Mature spores subspherical in sutural view, with slightly protruding anterior end and rounded posterior end (Figs. 1–4, 12, 14), measuring 8.2 (7.2–8.4) long (sutural view), 6.2 (6.0–7.0) wide (valve view), 7.9 (7.4–8.2) thick (sutural view, perpendicular to sutural plane). Spores with elevated straight sutural ridge (Figs. 1--3, 14). TEM and SEM showed a distinct thickening of the sutural ridge at the anterior end of spore (Figs. 12, 14). Spore valve with 5–6 ridges on surface of posterior end in sutural view (Figs. 1–3). TEM and SEM revealed that these ridges were in fact interconnected ornate folds on valve surface of posterior end of spore (Figs. 11–14). Two subspherical polar capsules equal in size (Fig. 1–3), 3.0 (2.7–3.2) long and 2.8 (2.6–3.1) wide, occupying anterior 2/3 of spore. Polar filament wound tightly into 6–7 coils (Figs. 1, 10, 12). Two uninucleate sporoplasms occupying the posterior part of spore (Figs. 1–3, 15).

Types. Slides and fixed specimens of holotypes were deposited in the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada [catalogue numbers CMNPA1996-0085 (spores), CMNPA1996-0086 (in ovary section), and CMNPA1996-0087 (ovary tissue)].

Etymology. *Sphaerospora ovophila* is named for its site of development in the fish host.

Myxobolus algonquinensis n. sp. (Figs. 16–25)

Host. Golden shiner, *Notemigonus crysoleucas*.

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

Site of infection. Ovary.

Prevalence. Five of 31 (15.1%) female fish infected.

Cysts and trophozoites. Cysts large, visible with naked eyes, whitish, ovoid to elongated ellipsoid shape, measuring up to 800 μm in length. Histological sections revealed that the cysts, found in connective tissues of ovaries or interstitial tissue among eggs, were polysporic plasmodia encased with host tissue (Figs. 21--23). Polysporic plasmodia in ovaries variable in shape and size, containing vegetative forms, developing sporonts, maturing and mature spores.

Spores. Mature spores ovoid with rounded ends in front view (Figs. 16, 18, 20), spindle-shaped in sutural view (Figs. 17, 19) and end view (Fig. 24), measuring 14.7

(13.6--15.4) long, 10.9 (10.1--12.1) wide, and 5.8 (5.0--6.9) thick (n=12). Spores with straight sutural ridge of uniform width (Figs. 17, 19). TEM revealed that the elevated sutural ridge was formed by a thickening of the valve along the sutural ridge (Figs. 24, 25). Spore with smooth valve surface of uniform thickness and with small intercapsular appendix (Figs. 16, 18, 20). Two equal-sized pyriform polar capsules, with short neck region, measuring 5.3 (5.1--5.5) by 2.7 (2.5--2.9) and occupying anterior half of spore (Figs. 16, 18, 20). Anterior tips of polar capsules slightly separated, abutting with sutural ridge (Figs. 16, 18). Polar filament wound into 4--6 coils. Single sporoplasm occupying posterior part of spore, extending between and around periphery of polar capsules with a distinct iodinophilous vacuole and two nuclei (Figs. 16, 18, 20).

Types. Slides and fixed specimens of holotypes were deposited in the Canadian Museum of Nature, Invertebrate Zoology Collection, Ottawa, Canada [catalogue numbers CMNPA1996-0088 (spores), CMNPA1996-0089 (in ovary section), and CMNPA1996-0090 (ovary tissue)].

Etymology. *Myxobolus algonquinensis* is named for the location in which it was first observed.

DISCUSSION

The majority of the described species of *Sphaerospora* infect the kidneys of their hosts and undergo disporous sporogenesis (Desser et al., 1983; Arthur & Lom, 1985; Lom et al., 1985; Lom & Dykova, 1992; Kent et al., 1993). *Sphaerospora ovophila* is the second recorded species of this genus that produces cysts in the ovaries of fish. The other species, *S. plagiognathopis* (Chen & Hsieh, 1984; Lom & Dykova, 1992), was described from *Plagiognathopis microlepis* in China. The spores and polar capsules of *S. ovophila* are larger than those of *S. plagiognathopis*, which has spores 7.2 in length and 7.6 in thickness (sutural view) and polar capsules 2.4 in diameter. The posterior part of spore valves of *S. ovophila* has ridges, whereas that of *S. plagiognathopis* lacks ridges but instead has two membranous appendages projecting from the posterior end. *Sphaerospora ovophila* also differs from *S. plagiognathopis* in that the latter species is disporous.

Sphaerospora ovophila is morphologically distinct from *S. diminuta*, the only previously described species of this genus from renal tubules of the same pumpkinseed host and from other species with striated shell valves described from percid fish (Li & Desser, 1985; Lom et al., 1989; Lom & Dykova, 1992). The spores of *S. ovophila* are larger than those of *S. diminuta*, with the latter having a spore length of 7.0 and a thickness of 7.6 (sutural view, perpendicular to the sutural plane). The ornate folds on the posterior ends of the valves of *S. ovophila* (Figs. 1–3, 11–14) differ from the striations on the valves of *S. diminuta* and other species from percid fish in the pattern and number of the ridges. *Sphaerospora ovophila* also differs from *S. diminuta* in that the latter is disporous.

Monosporous sporogenesis has been described in 11 other species of *Sphaerospora*: *S. dogieli* (Bykhovskaya-Pavlovskaya et al., 1964), *S. epinepheli*

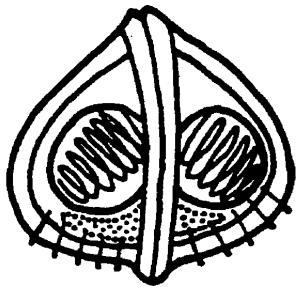
(Supamattaya et al., 1991), *S. galinae* (Evlanov, 1981), *S. hankai* (Lom et al., 1989), *S. ictaluri* (Hedrick et al., 1990), *S. inaequalis* (Landsberg, 1986), *S. molnari* (Lom & Dykova, 1992), *S. notropis* (Fantham et al., 1939), *S. oncorhynchi* (Kent et al., 1993), *S. petruschewskii* (Bykhovskaya-Pavlovskaya et al., 1964), and *S. scardinii* (El-Matbouli & Hoffmann, 1992). The spores of *S. ovophila* are most similar to those of *S. dogieli* and *S. galinae;* however, *S. dogieli* has two pyriform polar capsules and *S. galinae* two unequal polar capsules. In addition, *S. ovophila* has more polar filament coils and a unique pattern of ornate folds on the posterior part of the valves. Differences in host, site of infection and geographic distribution, along with differences in the shape of polar capsules and type of ornate ridges, justify establishing *S. ovophila* as a distinct species.

Numerous species in the genus *Myxobolus* have been documented from fish (Kudo, 1920; Li & Desser, 1985; Landsberg & Lom, 1991; Lom & Dykova, 1992). *Myxobolus algonquinensis* closely resembles *M. argenteus* (Lewis, 1968), *M. cycloides* (Lom & Dykova, 1992), *M. cyprinicola* (Bykhovskaya-Pavlovskaya et al., 1964), *M. ellipsoides* (Lom & Dykova, 1992), *M. notemigoni* (Lewis & Summerfelt, 1964) and *M. pendula* (Guilford, 1967). However, the spores of *M. algonquinensis* are larger than those of *M. argenteus, M. cyprinicola* and *M. notemigoni* in length and width, which measure 13.9 by 8.6, 9--12 by 7--9 and 11.8 by 9.0 respectively. In addition, the polar capsules of *M. algonquinensis* are longer than those of *M. cyprinicola,* polar capsules of which measure 4.2--5.2 by 2.5--3.0. The number of polar filament coils of *M. algonquinensis* are fewer than those of *M. argenteus* and *M. notemigoni,* both of which have 6--8 coils. *Myxobolus algonquinensis* differs from *M. argenteus* in that the latter does not have an intercapsular appendix. *Myxobolus algonquinensis* also differs from *M. notemigoni* in the shape of spores and polar capsules and in the lack of notches along the posterior part of the sutural ridge. The polar capsules of *M. algonquinensis* are smaller than those of *M. cycloides* and

M. pendula, which measure 6.6 by 3.6 and 6.8 by 3.4 respectively. Furthermore, *M. algonquinensis* differs from *M. cycloides* which has notches along the posterior part of the sutural ridge and from *M. pendula* which does not have an intercapsular appendix. *Myxobolus algonquinensis* differs from *M. ellipsoides* in the shape of spores and polar capsules in that the latter has a spore with a rounded anterior end, a slightly narrowed posterior end, its greatest width at the anterior part of the spore and its polar capsule with a shorter length measuring 4.5. Thus, the characteristic features and the unique infection site are sufficient to establish *M. algonquinensis* as a new species.

Figure **1.** *Sphaerospora ovophila* n. sp. Line drawing of mature spore in sutural view. Bar = 10 μm.

Figures **16, 17.** *Myxobolus algonquinensis* n. sp. Line drawings of mature spores. Bar = 10 μm. **16.** Front view. **17.** Sutural view.



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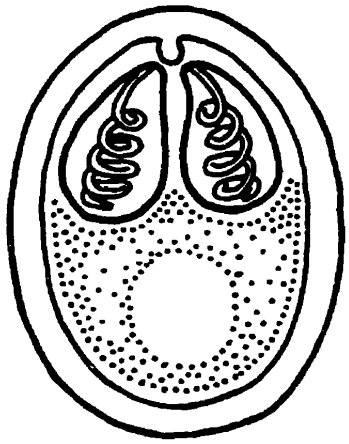

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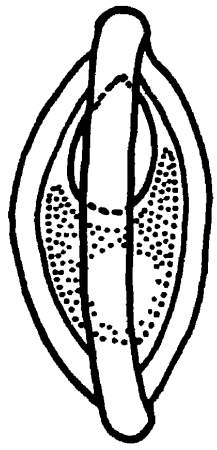

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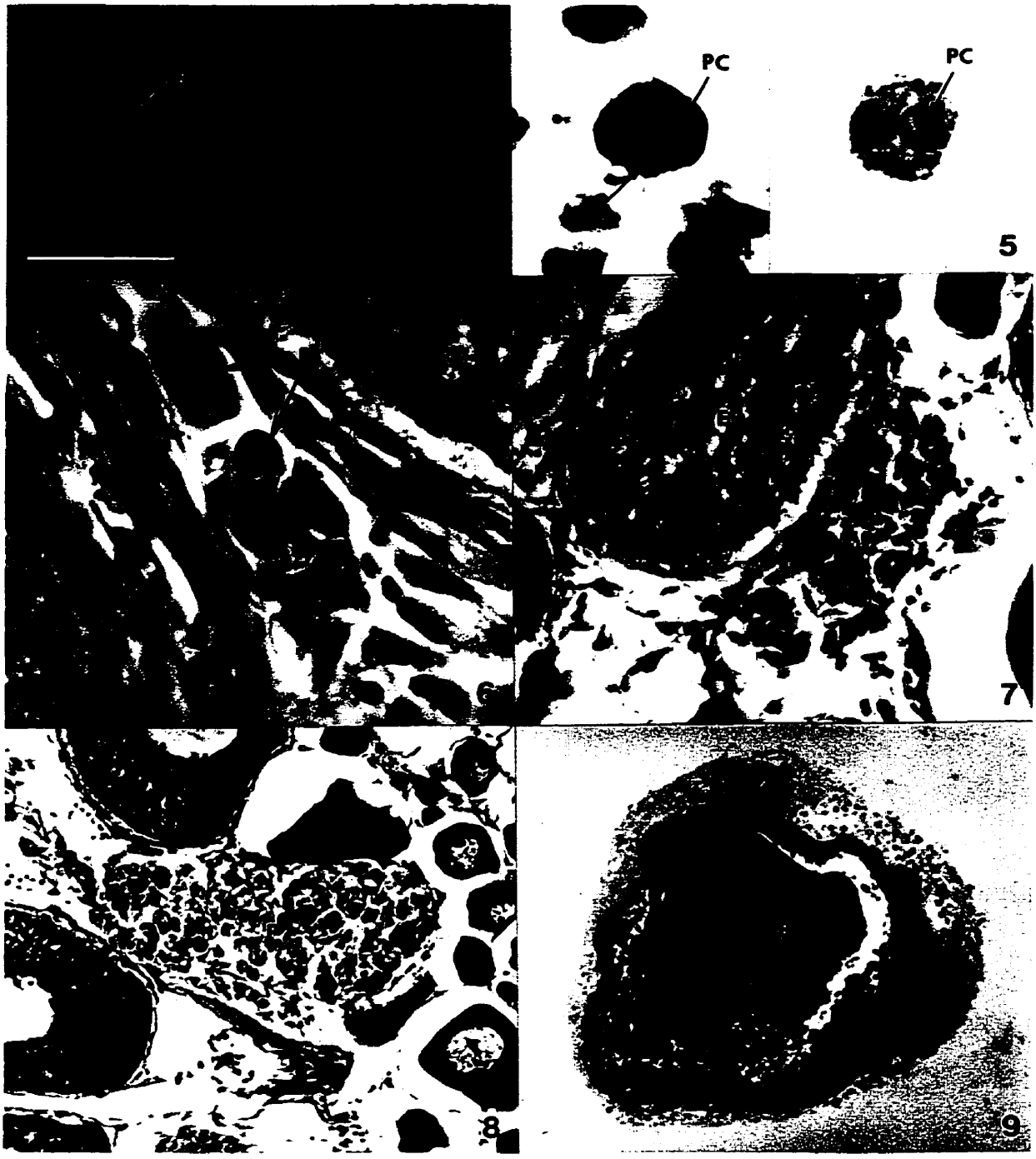
Figures **2--9**, spore (Figs. **2--4**), pseudoplasmodium (Fig. **5**) and plasmodium (Figs. **6--9**) of *S. ovophila* n. sp. **2, 3.** DIC photomicrographs of mature spores (sutural view) showing the sutural ridge (SR) and ornate ridges (OR). x 2,650. Bar = 10 µm. **4.** Spore stained with Diff-Quik® showing subspherical polar capsules (PC) and sporoplasm with two nuclei (Nu). x 2,440. **5.** Diff-Quik® stained monosporic pseudoplasmodium with two developing polar capsules (PC). x 2,440. **6.** Plasmodia (P) in the interstitial tissue (stained with H & E). x 1,450. **7.** Developing plasmodium (P) adjacent to egg (E) in ovary (stained with H & E). x 580. **8.** Maturing ellipsoid plasmodium (P) in the interstitial tissue of the ovary (stained with H & E). x 360. **9.** Semithin section of an egg (E) surrounded by a mature cyst (C). x 150.


PC
PC
5
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8
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Figures **10--15.** Transmission and scanning electron micrographs of *S. ovophila* n. sp. **10.** Longitudinal section of a polar capsule (PC), showing the tightly coiled polar filament (PF). x 13,700. Bar = 1 μm. **11.** Oblique longitudinal section of two maturing spores, showing the two valves (V) of each spore, the ornate folds (OF) at the posterior end, and the sutural ridge (SR). x 6,100. Bar = 5 μm. **12.** Mature spore with thickened anterior end (arrowhead). x 8,100. Bar = 5 μm. **13.** An immature aberrant spore with three developing polar capsules. x 8,700. Bar = 1 μm. **14.** SEM of a mature spore, showing ornate folds (OF) on the posterior part of the valve, and a straight sutural ridge (SR) which thickens and protrudes at the anterior end (arrowhead). x 12,000. Bar = 1 μm. **15.** SEM of an opened mature spore showing a spherical polar capsule (PC) and sporoplasm (SP). x 12,000. Bar = 1 μm.


C
OF
SR
OF
PC
SR

Figures **18--25.** Spore (Figs. **18--20**), plasmodium (Figs. **21--23**) and transmission electron micrographs (Figs. **24, 25**) of *M. algonquinensis* n. sp. **18.** DIC photomicrograph of mature spore (front view) showing polar capsules, polar filament and small intercapsular appendix (arrow). x 2,650. Bar = 10 µm. **19.** DIC photomicrograph of mature spore (sutural view) showing sutural ridge (arrow). x 2,600. **20.** DIC photomicrograph of spore with extruded polar filaments (PF). x 1,320. Bar = 10 µm. **21.** Several plasmodia (P) growing in the connective tissue of the ovary. x 360. **22.** Spindle-shaped plasmodium (P) in the connective tissue of the ovary. x 360. **23.** An elongated ellipsoidal cyst (C) in the connective tissue of the ovary. x 360. **24.** Section through a polysporic plasmodium. x 1,750. Bar = 10 µm. **25.** Transverse section of a maturing spore showing thickening of the sutural ridge (SR). x 12,000. Bar = 1 µm.


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CHAPTER 4

A Light Microscopic Study of Triactinomyxon and Raabeia Forms of Oligochaetes from Lake Sasajewun, Algonquin Park

(Adapted from Xiao, C. & Desser, S.S. 1998. Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of triactinomyxon and raabeia. J. Parasitol., **84**, 998-1009.)

ABSTRACT

Eight forms of triactinomyxon and 6 forms of raabeia of oligochaetes from Lake Sasajewun, Algonquin Park are described. Of the 8 forms of triactinomyxon, 2 designated as triactinomyxon 'B' and 'E' were found in both *Limnodrilus hoffmeisteri* and *Tubifex tubifex*. Three additional forms, designated as triactinomyxon 'A', 'C', and 'D', occurred in *L. hoffmeisteri*. One form infecting *L. hoffmeisteri* was identified as *Triactinomyxon ignotum* Stolc, 1899, and another infecting *T. tubifex* was tentatively identified as *Triactinomyxon dubium* Granata, 1924. One form infecting *L. hoffmeisteri* and *Rhyacodrilus coccineus* was designated as triactinomyxon 'F'. Of the 6 forms of raabeia, 4 designated as raabeia 'A', 'B', 'C' and 'F' were found in *L. hoffmeisteri*, and 2 designated as raabeia 'D' and 'E' were recorded in *T. tubifex*. The sporoplasmic mass of each form of triactinomyxon usually moved posteriorly within the cavity of the spore axis and was released through its posterior end, whereas the sporoplasmic mass of each form of raabeia was released through the anterior end of the epispore. An increase in the number of germ cells was observed in the floating spore stage of triactinomyxon 'B', 'D', 'E', and raabeia 'B'.

INTRODUCTION

Since Wolf and Markiw (1984) postulated a 2-host life cycle, myxozoan parasites of both oligochaetes and fish have received increased attention (El-Matbouli and Hoffmann, 1989; Hedrick et al., 1989; Yokoyama et al., 1991; Kent et al., 1993; El-Matbouli et al., 1995; Uspenskaya, 1995; Trouillier et al., 1996; Andree et al., 1997; Bartholomew et al., 1997). In addition to documenting new myxosporean species from fish hosts, recent studies have been focused on the demonstration of the alternation between actinosporean and myxosporean cycles under laboratory conditions (Markiw and Wolf, 1983; El-Matbouli and Hoffmann, 1989; Ruidisch et al., 1991; El-Matbouli et al., 1992, 1995; Trouillier et al., 1996; Bartholomew et al., 1997). Based on the life cycle data, Kent et al. (1994) proposed a major revision of the phylum Myxozoa, namely the "demise of a class of protists", the Actinosporea. Following the view of Kent et al. (1994), Lom et al. (1997) provided guidelines for the uniform characterization of the actinosporean stages. However, with the exception of a few studies (Marques, 1984; Yokoyama et al., 1993; McGeorge et al., 1997; Hallett et al., 1998), little attention has been focused on the biology of actinosporean stages. Even in the few studies where 2-host life cycles have been demonstrated (see Kent et al., 1994), documentation of the actinosporean stage has been incomplete, and only the stages of myxosporean development in the fish host are described, with the notable exceptions of *Myxobolus cerebralis* (see Wolf and Markiw, 1984) and *M. cultus* (see Yokoyama et al., 1995).

Extensive study of the actinosporean phase is essential for understanding the biology of myxozoan parasites and to allow for meaningful taxonomic revisions based on the matching of appropriate myxosporeans and actinosporeans. Resolution of the gross imbalance between the number of described myxosporean species (about 1,200, see Lom and Dykova, 1992) and actinosporean 'species' (about

38, see Marques, 1984) is also required.

As a primary component of a comprehensive investigation of the biology of myxozoan parasites in their natural environment, the actinosporean stages of oligochaetes in Lake Sasajewun, Algonquin Park, Ontario, are documented adopting the taxonomic system of Kent et al. (1994) and following the guidelines of Lom et al. (1997). In this chapter, 8 forms of triactinomyxon, 6 of which are new, and 6 new forms of raabeia are described.

MATERIALS AND METHODS

A total of 14,100 worms, from the 19 species of oligochaetes collected from various sites in Lake Sasajewun, was examined for actinosporean parasites in 1995, 1996 and 1997. Oligochaetes were kept in 24 x 2 ml cell-well plates (Yokoyama et al., 1991). Plates, with 2 worms of the same species in each well, were filled with lake water and kept at temperatures ranging from 15 to 25 C. Worms in plates were examined microscopically every other day for the release of actinosporean spores over a 4 wk period. When spores were found, the 2 worms were separated into new wells and examined daily.

Samples of freshly released actinosporean spores of each form were transferred to separate wells filled with lake water and observed daily until their sporoplasmic masses were released. Fresh spores examined by differential interference contrast (DIC) microscopy, and Diff-Quik® stained smears of spore suspensions observed by bright field and phase contrast microscopy, were photographed using a Zeiss Universal I photomicroscope. Measurements of spores taken from Diff-Quik® stained smears were based on 25 specimens, and given as a mean followed by the range in micrometers (μm).

Samples of the anterior, mid and posterior portions of infected worms were

fixed in Bouin's solution, and embedded in Paraplast®. Histological sections were stained with hematoxylin-eosin and examined under the light microscope to identify the location of the infection within the worm.

The prevalence of infection of actinosporeans was defined as the percentage of oligochaetes releasing mature actinosporean spores into the water. The prevalence of infection in its strict sense (Margolis et al., 1982) could not be determined, because the immature stages infecting the oligochaete could not be counted by the cell-well plate method (Yokoyama et al., 1991).

RESULTS

A schematic diagram illustrating the various components of the actinosporean spores is shown in Figure 1.

Descriptions of forms of triactinomyxon

Triactinomyxon 'A'

(Figs. 2, 16)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped; spore axis, with an increase in diameter posteriorly, composed of epispore containing sporoplasmic mass and style; sporoplasmic mass usually moves posteriorly in cavity of spore axis; length of spore axis 215.0 (190.0-250.0), width 19.0 (17.0-23.0); length of sporoplasmic mass 55.0 (40.0-70.0), width 11.0-13.0; 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 370.0 (340.0-380.0), width of bases 21.0 (20.0-23.0); 3 pyriform polar capsules equal in size, 6.0 (5.7-6.2) in length and 4.0 (3.8-4.1) in width, situated at top of spore axis; small nuclei of polar

capsules close to their bottom; polar filament coiled 3-5 times; sporoplasmic mass contains about 256 small germ cells during the entire spore stage; small spherical germ cells nearly equal in size, about 0.8-1.0 in diameter; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 4 of 6,550 worms (0.06%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0023).

Remarks

This form of triactinomyxon differs from all known members of the conventional genus *Triactinomyxon* (Marques, 1984) and the form of triactinomyxon described by McGeorge et al. (1997) because it has a unique number of germ cells. It is also similar to the triactinomyxon stage (El-Matbouli and Hoffmann, 1993) of *Myxobolus carassii* in morphology, but triactinomyxon 'A' has much longer processes (370.0 versus 277.0) and more germ cells (about 256 versus about 150).

Triactinomyxon 'B'

(Figs. 3, 17)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical spore axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped; spore axis composed of epispore containing sporoplasmic mass, and style; freshly released spores with a slightly enlarged anterior region containing elongating sporoplasmic mass, followed by a short

narrow region of style, then with style of slightly increasing in diameter posteriorly; length of spore axis 130.0 (120.0-145.0), width 20.0 (18.0-23.0); length and width of sporoplasmic mass 23.0 (20.0-25.0) and 18.0 (16.0-20.0) respectively for freshly released spores, 45.0 (40.0-50.0) in length and 15.0 (14.0-16.0) in width after its elongation; 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 205.0 (200.0-210.0), width of bases 20.0 (18.0-21.0); 3 pyriform polar capsules equal in size, 5.0 (4.7-5.2) in length and 3.0 (2.8-3.1) in width, situated at top of spore axis; small, ovoid nuclei of polar capsules close to their bottom; polar filament coiled 3-5 times; sporoplasmic mass in freshly released spores contains 16, but occasionally 8, germ cells; spores released 2 days or longer contain about 32 germ cells; spherical germ cells vary in size ranging from 2.0 to 4.5 in diameter; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Hosts: *Limnodrilus hoffmeisteri* Claparede, 1862 and *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 1 of 6,550 *L. hoffmeisteri* (0.02%) and 1 of 1,430 *T. tubifex* (0.07%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0024).

Remarks

This form is similar to *Triactinomyxon dubium* Granata, 1924, but it has an epispore with a slightly enlarged anterior portion. It is also similar to *Triactinomyxon magnum* Granata, 1922 (see Marques, 1984) and *Triactinomyxon legeri* Mackinnon and Adam, 1924. However, it has a larger spore axis and shorter spore processes than *T.*

magnum, and a larger spore axis and longer spore processes than *T. legeri*. Furthermore, this form has a characteristic number of germ cells, 16 for freshly released spores and 32 for spores released 2 days or longer, whereas the other 3 have a different number of germ cells, *T. dubium* with 32, *T. magnum* with 16, and *T. legeri* with 24 germ cells.

Triactinomyxon 'C'

(Figs. 4, 18)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped (Fig. 4); spore axis, with an increase in diameter posteriorly, composed of epispore containing sporoplasmic mass and style, while sporoplasmic mass elongating slightly after spore released from oligochaete host; length of spore axis 195.0 (185.0-205.0), width 32.0 (26.0-38.0); length of sporoplasmic mass 18.0 (16.0-20.0) for freshly released spores and about 28.0-31.0 after elongation, width 10.0-13.0; 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 290.0 (280.0-300.0), width of bases 25.0 (23.0-26.0); 3 pyriform polar capsules equal in size, 5.0 (4.7-5.2) in length and 3.0 (2.8-3.1) in width, situated at top of spore axis; ovoid nuclei of polar capsules small, close to their bottom; polar filament coiled 3-5 times; sporoplasmic mass contains 8 germ cells during the entire spore stage, arranged into 2 columns of 4 each (Fig. 18); spherical germ cells nearly equal in size, 4.5-6.0 in diameter; small ovoid polar bodies, usually 4 in number, present in the majority of spores; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 6 of 6,550 worms (0.09%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0025).

Remarks

This form, although similar to *Triactinomyxon ignotum* Stolc, 1899 with 8 germ cells, has much longer processes (290.0 versus 193.0), a smaller sporoplasmic mass (18.0-30.0 versus 40.0 in length), a different arrangement of germ cells, and obvious cytoplasmic remnants of the capsulogenic cells.

***Triactinomyxon ignotum* Stolc, 1899**

(Figs. 5, 19)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical spore axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped (Fig. 5); spore axis composed of epispore containing sporoplasmic mass and style; sporoplasmic mass usually moves posteriorly in cavity of spore axis; length of spore axis 175.0 (170.0-180.0), width 26.0 (25.0-27.0); length of sporoplasmic mass 40.0 (38.0-44.0), width 14.0 (13.0-15.0); 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 193.0 (175.0-210.0), width of bases 22.0 (20.0-23.0); 3 pyriform polar capsules, equal in size, 5.0 (4.7-5.2) in length and 3.0 (2.8-3.1) in width, situated at top of spore axis; 3 elongated ovoid remnants of capsulogenic cell cytoplasm with small nuclei laying between the sporoplasm and the polar capsules; polar filament coiled 4-5 times; cylindrical sporoplasmic mass occupies epispore cavity, contains 8 spherical germ cells during the entire spore stage (Fig. 19), equal in

size, about 5.0 in diameter, and usually with 4 round polar bodies, 2.0 in diameter; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Type host: *Tubifex* sp.

Site of infection: Intestinal epithelium

Type locality: Moldau, Prague, Czech Republic.

Other host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun, Algonquin Park, Ontario.

Prevalence of infection: 1 of 6,550 worms (0.02%).

Voucher specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-30).

Remarks

This form is identical to *T. ignotum* Stolc, 1899 in the shape and size of the spore. Movement of the sporoplasmic mass within the spore axis has not been reported in the original description of spores of *T. ignotum*, whereas it was observed in the spores of this study. This is the first report of *T. ignotum* in *L. hoffmeisteri*.

Triactinomyxon 'D'

(Figs. 6, 20)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped; spore axis, with an enlarged anterior portion containing elongating sporoplasmic mass, composed of epispore and style; length of spore axis 63.0 (60.0-65.0), width 17.0 (16.0-18.0); length of sporoplasmic mass 22.0 (20.0-25.0)

for freshly released spores and 43.0 (40.0-50.0) after elongation, width 14.0 (13.0-15.0); 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 110.0 (105.0-115.0), width of bases 11.0 (10.0-12.0); 3 pyriform polar capsules equal in size, 6.0 (5.7-6.2) in length and 4.0 (3.8-4.1) in width, situated at top of spore axis; small nuclei of polar capsules, triangular or ovoid in shape, close to their bottom; polar filament coiled 4-5 times; discharged polar filament about 45.0-53.0 in length; sporoplasmic mass in freshly released spores contains 16 germ cells, 32 germ cells in spores released 2 days or longer; spherical germ cells nearly equal in size, 2.5-4.0 in diameter; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 4 of 6,550 worms (0.06%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0026).

Remarks

This form, although similar to triactinomyxon 'B' in morphology and the characteristic number of germ cells, has a much smaller epispore (63.0 versus 130.0 in length) and smaller processes (110.0 versus 205.0 in length). It is also similar to *T. dubium*, and *Triactinomyxon robustum* Marques, 1984, but it differs from *T. dubium* and *T. robustum* in having an epispore with an enlarged anterior portion and a characteristic number of germ cells, 16 for freshly released spores and 32 for spores released 2 days or longer, whereas *T. dubium* and *T. robustum* reported with a

constant number of 32 and 28, respectively.

Triactinomyxon 'E'

(Fig. 7)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped; spore axis, with an increase in diameter posteriorly, composed of epispore containing sporoplasmic mass and style; length of spore axis 200.0 (190.0-210.0), width 41.0 (40.0-42.0); length of sporoplasmic mass 50.0 (47.0-53.0), width 16.0 (15.0-17.0); 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 285.0 (270.0-300.0), width of bases 32.0 (30.0-33.0); 3 pyriform polar capsules equal in size, 5.0 (4.8-5.2) in length and 3.0 (2.9-3.1) in width, situated at top of spore axis; ovoid nuclei of polar capsules obvious, close to their bottom; polar filament coiled 4-5 times; discharged polar filament about 34.0-36.0 in length; sporoplasmic mass in freshly released spores contains 16 germ cells, 32 germ cells in spores released 2 days or longer; spherical germ cells nearly equal in size, 4.0-5.0 in diameter; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Hosts: *Limnodrilus hoffmeisteri* Claparede, 1862 and *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 1 of 6,550 *L. hoffmeisteri* (0.02%) and 1 of 1,430 *T. tubifex* (0.07%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue

No. CMNPA1998-0027).

Remarks

This form is similar to triactinomyxon 'B' and 'D' in the characteristic number of germ cells, but it has a significantly longer spore axis and longer processes than triactinomyxon 'B' (spore axis 200.0 versus 130.0, and processes 285.0 versus 205.0) and 'D' (spore axis 200.0 versus 63.0, and processes 285.0 versus 110.0), in addition, they differ in the shape of the epispore. This form is also similar to *T. dubium*, however, they differ in the characteristic number of germ cells (16 for freshly released spores and 32 for spores released 2 days or longer versus 32).

***Triactinomyxon dubium* Granata, 1924**

Triactinomyxon sp. Leger, 1904

(Fig. 8)

Mature spores composed of 3 transparent valves fused to form a hollow, vertical and cylindrical axis, separated proximally to form spore processes; spore valves and processes evertible, invaginated while spore in pansporocyst; released spores anchor-shaped; spore axis, with a slightly increase in diameter posteriorly, composed of epispore containing sporoplasmic mass and style; length of spore axis 145.0 (130.0-160.0), width 25.0 (22.0-28.0); length of sporoplasmic mass 31.0 (30.0-32.0), width 19.0 (18.0-20.0); 3 spore processes equal in size, pointed and slightly curved upwards; ovoid nuclei of processes close to their bases; length of processes 260.0 (250.0-270.0), width of bases 27.0 (26.0-28.0); 3 pyriform polar capsules equal in size, 5.5 (5.3-5.8) in length and 4.0 (3.8-4.1) in width, situated at top of spore axis; ovoid nuclei of polar capsules small, close to their bottom; polar filament coiled 3-5 times; discharged polar filament about 34.0-36.0 in length; sporoplasmic mass contains 32 germ cells during the entire spore stage; spherical germ cells equal in size, 4.0-4.5 in diameter; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Type host: *Tubifex tubifex*.

Site of infection: Intestinal epithelium.

Type locality: Grenoble, France

Other locality: Lake Sasajewun, Algonquin Park, Ontario.

Prevalence of infection: 2 of 1,430 worms (0.14%).

Voucher specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0029).

Remarks

This form found in *Tubifex tubifex* is similar to *T. dubium* Granata, 1924 = *Triactinomyxon* sp. Leger, 1904 (see Marques, 1984) in having 32 germ cells, but further comparisons could not be made due to insufficient information in the original descriptions. Therefore, it has been tentatively identified as *T. dubium*, which Kent et al. (1994) synonymized with *M. cerebralis*. However, the form described here is different from actinospores of *M. cerebralis* (see Wolf and Markiw, 1984).

Triactinomyxon 'F'

(Fig. 9)

Mature spores composed of style-less epispore with 3 processes of equal size, pointed and curved upwards at posterior end of epispore; released spore anchor-shaped; bases of spore processes surround posterior end of epispore cavity; ovoid nuclei of spore processes randomly located; length of spore processes 180.0 (160.0-200.0), and width of bases about 10.0-11.0; epispore cylindrical, 50.0 (46.0-56.0) in length and 6.4 (5.5-7.0) in width; 3 equal-sized pyriform polar capsules, 5.0 (4.6-5.2) in length and 3.0 (2.7-3.1) in width, situated on top of epispore with apex slightly inclined outwards; nuclei of polar capsules small, irregularly ovoid in shape, close to

bottom of polar capsules; polar filaments coiled 3-4 times; sporoplasmic mass cylindrical, occupies epispore cavity completely and contains 16 germ cells arranged in 1 or 2 columns during the entire spore stage; 16 small polar bodies associated with germ cells in sporoplasmic mass; pansporocysts developed in the intestinal epithelial cells of the worm; pansporocysts with 8 spores.

Taxonomic summary

Hosts: *Limnodrilus hoffmeisteri* Claparede, 1862 and *Rhyacodrilus coccineus* Vejdovsky, 1875.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 44 of 6,550 *L. hoffmeisteri* (0.67%), including 2 worms with mixed infection of a form of raabeia and a form of echinactinomyxon, respectively, and 5 of 910 *R. coccineus* (0.55%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0028).

Remarks

This form closely resembles members of the collective group triactinomyxon because of its anchor-shaped waterborne spore, the cylindrical epispore, and processes that are pointed and curved upwards, but differs from them by the absence of a style. It also resembles spores of the collective group raabeia because of its style-less epispore, and the pointed, upward-curved processes, but differs by having a cylindrical epispore, whereas the epispore of forms of raabeia is ovoid and cup-shaped. However, since the corresponding myxosporean stage is unknown, and there is no need to create a new actinosporean collective group (Kent et al., 1994; Lom et al., 1997), this form is therefore assigned to the nearest collective group, triactinomyxon.

Descriptions of forms of Raabeia

Raabeia 'A'

(Fig. 10)

Mature spores composed of a style-less epispore with 3 equal-sized processes which gradually tapered towards their pointed and slightly upcurved tips; processes evertible, invaginated while spore in pansporocyst; bases of processes embraced epispore cavity almost entirely; nuclei of processes close to their bases, usually ovoid in shape; length of processes 145.0 (135.0-165.0), and width of bases about 8.0-9.0; epispore cavity ovoid in shape, containing sporoplasmic mass; epispore dimension 16.0 (14.0-18.0) in length and 10.0 (8.0-11.0) in width; 3 pyriform polar capsules, equal in size with 4.0 (3.8-4.3) in length and 2.0 (1.9-2.2) in width, situated at anterior end of epispore with apex inclined outward; nuclei of polar capsules tiny, ovoid in shape, close to their bottom; polar filament coiled 3-4 times; discharged polar filament about 18.0 in length; style absent; sporoplasmic mass contains 8 germ cells during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 3 of 6,550 worms (0.05%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0031).

Remarks

This form is similar to *Raabeia gorlicensis* Janiszewska, 1955, *Raabeia furciligera* Janiszewska and Krztov, 1973, and *Raabeia magna* Janiszewska, 1957 in the shape of

the epispore, but it differs from them in having 8 germ cells, whereas *R. gorlicensis* and *R. furciligera* have 32, and *R. magna* 128 germ cells (also see Marques, 1984). It is also similar to a form of raabeia reported by McGeorge et al. (1997) in the size of the epispore, however, they differ in the size of their processes (145.0 versus 219.0 in length) and polar capsules (4.0 x 2.0 versus 7.0 x 5.0).

Raabeia 'B'

(Fig. 11)

Mature spores composed of a style-less epispore with 3 equal-sized processes which gradually tapered towards their pointed and slightly upcurved tips; processes evertible, invaginated while spore in pansporocyst; bases of processes embraced posterior part of epispore cavity; nuclei of processes close to their bases or at the anterior part of the processes, usually ovoid in shape; length of processes 230.0 (210.0-240.0), and width of bases 14.0 (13.0-15.0); epispore cavity elongated and ovoid in shape, containing sporoplasmic mass; epispore dimension 25.5 (24.0-26.5) in length and 9.0 (8.0-10.0) in width; 3 pyriform polar capsules, equal in size with 5.5 (5.0-5.8) in length and 2.7 (2.5-2.8) in width, situated at anterior end of epispore with apex inclined outward; nuclei of polar capsules tiny, ovoid in shape, close to their bottom; polar filament coiled 3-4 times; style absent; sporoplasmic mass in freshly released spores contains 8 germ cells, 16 germ cells in spores released for 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 3 of 6,550 worms (0.05%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the

Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0032).

Remarks

This form is similar to *R. gorlicensis* and *R. furciligera*, but it has a shorter epispore (25.5 versus 35.0) and longer processes (230.0 versus 170.0) than *R. gorlicensis*, and smoother and longer processes than *R. furciligera* (230.0 with pointed ends versus 100.0 to 150.0 with bifurcate or trifurcate ends). Furthermore, it differs from them in the characteristic number of germ cells, 8 for freshly released spores and 16 for spores released 2 days or longer, whereas *R. gorlicensis* and *R. furciligera* have 32 germ cells.

Raabeia 'C'

(Fig. 12)

Mature spores composed of a style-less epispore with 3 equal-sized processes which gradually tapered towards their pointed and slightly upcurved tips; processes evertible, invaginated while spore in pansporocyst; bases of processes embraced epispore cavity almost entirely; nuclei of processes close to their bases or at anterior part of processes, usually ovoid in shape; length of processes 210.0 (200.0-220.0), and width of bases 10.0-12.0; epispore cavity ovoid in shape containing sporoplasmic mass; epispore dimension 16.5 (15.5-17.5) in length and 9.0 (8.0-11.0) in width; 3 pyriform polar capsules, equal in size with 4.5 (4.0-5.0) in length and 2.3 (2.0-2.5) in width, situated at anterior end of epispore with apex slightly inclined outward; nuclei of polar capsules small, kidney-shaped, close to their bottom; polar filament coiled 3-4 times; style absent; sporoplasmic mass contains 8 germ cells and usually 2-3 polar bodies during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 2 of 6,550 worms (0.03%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0033).

Remarks

This form is similar to raabeia 'A' in the size of the epispore and in having 8 germ cells, but it differs from raabeia 'A' in having much longer processes (210.0 versus 145.0). It is also similar to the raabeia stage of *Myxobolus cultus* (Yokoyama et al., 1991, 1995) in the length of processes, however, they differ in the size of the epispore (16.5 versus 21 in length) and the characteristic number of germ cells (8 versus 11 to 16).

Raabeia 'D'

(Fig. 13)

Mature spores composed of a style-less epispore with 3 equal-sized processes which gradually tapered towards their pointed and slightly upcurved tips; processes evertible, invaginated while spore in pansporocyst; bases of processes embraced epispore cavity almost entirely; nuclei of processes close to their bases, usually ovoid in shape; length of processes 290.0 (270.0-310.0), and width of bases about 11.0-13.0; epispore cavity slightly elongated and ovoid in shape, containing sporoplasmic mass; epispore dimension 21.5 (20.0-23.0) in length and 9.5 (7.5-14.0) in width; 3 pyriform polar capsules, equal in size with 4.5 (4.0-5.0) in length and 3.0 (2.8-3.1) in width, situated at anterior end of epispore with apex inclined outward; nuclei of polar capsules small, ovoid in shape, close to their bottom; polar filament coiled 3-4 times; style absent; sporoplasmic mass contains 16 germ cells during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the

worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 1 of 1,430 worms (0.07%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0034).

Remarks

This form, although similar to *R. gorlicensis* in the shape of spores, has 16 germ cells, whereas *R. gorlicensis* has 32 germ cells. They also differ in the size of the epispore (21.5 x 9.5 versus 35.0 x 35.0) and processes (290.0 versus 170.0 in length).

Raabeia 'E'

(Fig. 14)

Mature spores composed of a style-less epispore with 3 equal-sized processes which gradually tapered towards their pointed and slightly upcurved tips; processes evertible, invaginated while spore in pansporocyst; bases of processes embraced epispore cavity almost entirely; nuclei of processes close to their bases, usually ovoid in shape; length of processes 215.0 (200.0-230.0), and width of bases about 9.0-11.0; epispore cavity slightly elongated and ovoid in shape, containing sporoplasmic mass; epispore dimension 24.0 (22.5-25.0) in length and 11.0 (8.5-13.0) in width; 3 pyriform polar capsules, equal in size with 4.5 (4.0-5.0) in length and 2.6 (2.3-3.0) in width, situated at anterior end of epispore with apex inclined outward; nuclei of polar capsules tiny, ovoid in shape, close to their bottom; polar filament coiled 3-4 times; style absent; sporoplasmic mass contains 12 germ cells during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the

worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 1 of 1,430 worms (0.07%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0035).

Remarks

This form differs from members of the conventional genus *Raabeia* and the raabeia stage of *M. cultus* (Yokoyama et al., 1995) in having 12 germ cells. It also differs from other forms of raabeia described in this report in the number of germ cells and in the size of spores.

Raabeia 'F'

(Figs. 15)

Mature spores composed of a style-less epispore with 3 equal-sized processes which gradually tapered towards their pointed and slightly upcurved tips; processes evertible, invaginated while spore in pansporocyst; bases of processes embraced posterior part of epispore cavity; nuclei of processes close to their bases, usually ovoid in shape; length of processes 145.0 (130.0-165.0), and width of bases about 6.0-7.0; epispore cavity ovoid in shape, containing sporoplasmic mass; epispore dimension 16.5 (15.0-18.0) in length and 8.5 (7.0-10.0) in width; 3 pyriform polar capsules, equal in size with 4.5 (4.3-4.8) in length and 2.5 (2.3-2.7) in width, situated at anterior end of epispore with apex inclined outward; nuclei of polar capsules small, elongated and ovoid in shape, close to their bottom; polar filament coiled 3-4 times; style absent; sporoplasmic mass contains 16 germ cells and many

small inclusions or particles during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the worm; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 3 of 6,550 worms (0.05%), including 1 worm with mixed infection of triactinomyxon 'F'.

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0036).

Remarks

This form is similar to raabeia 'D' described in this report in having 16 germ cells, however, it differs from raabeia 'D' in having much shorter processes (145.0 versus 290.0) and a shorter epispore (16.5 versus 21.5). It is also similar to raabeia 'A' reported here in the size of spores, but differs in the characteristic number of germ cells (16 versus 8).

DISCUSSION

Nine 'species' of the conventional genus *Triactinomyxon* Stolc, 1889 have been recorded to date (see Marques, 1984). These 'species' descriptions have been based on the morphology of the pansporocysts, the number of germ cells in, and dimensions of, the sporoplasmic mass, and measurements of the mature spore. Successive observations of the freshly released spores in this study revealed that 3 of the 8 forms of triactinomyxon (triactinomyxon 'B', 'D', and 'E') exhibited an increase in the number of germ cells and in the size of the sporoplasmic mass in free floating

spores, and only raabeia 'B' of the 6 forms of raabeia showed an increase in the number of germ cells in floating spores. These observations indicated that, although the number of germ cells and the size of the sporoplasmic mass are useful diagnostic characteristics, these features change as the germ cells divide and the sporoplasmic mass moves posteriorly within the style of free floating spores. Therefore, documentation of the number of germ cells and the size of the sporoplasmic mass based on single random observations of the spore stage is incomplete, unless successive observations of the spores are made to determine the characteristic number of germ cells and the size of the sporoplasmic mass for an actinosporean form.

Naidu (1956) postulated that the sporoplasmic mass was liberated through the posterior end of the spore axis of *Triactinomyxon naidanum*, whereas El-Matbouli et al. (1995) claimed that the sporoplasmic mass of the actinosporean stage of *Myxobolus cerebralis* was released through the anterior end of the spore axis. In situ observations of free floating spores of each form of triactinomyxon in this study revealed that spores kept in cell wells filled with lake water released their sporoplasmic mass usually through the posterior end of the spore axis (Figs. 2, 15).

The term style has been employed for the "empty" posterior region of the spore axis. The presence or absence of a style has been utilized as a taxonomic character at the generic level in the conventional classification of actinosporeans (Janiszewska, 1957), and the size of the style has, for a long time, been used as a feature for distinguishing among forms of triactinomyxon (Janiszewska, 1955, 1957, 1959, 1964; Lom et al., 1997). However, the increase in size of the sporoplasmic mass and its movement posteriorly in free floating spores in triactinomyxon forms in this study, indicated that the size of the style is an unreliable character.

Thus far, 3 'species' of the conventional genus *Raabeia* Janiszewska, 1955 have been described (Marques, 1984), each with a definite number of germ cells in their

spores. These are *R. gorlicensis* Janiszewska, 1955, with 32 germ cells, found in *T. tubifex*; *R. magna* Janiszewska, 1957, with 128 germ cells, and *R. furciligera* Janiszewska and Krztov, 1973, with 32 germ cells, both recorded in *L. hoffmeisteri*. The forms of raabeia described here were also found in either *T. tubifex* or *L. hoffmeisteri*, indicating a strong host preference.

The site of the sporoplasmic mass release of raabeia spores has never been recorded. In situ observations of free floating spores of each form of raabeia in this study revealed that spores kept in cell wells filled with lake water released their sporoplasmic mass through the anterior end of the epispore, similar to spores of members of the conventional genera *Aurantiactinomyxon*, *Synactinomyxon*, and *Echinactinomyxon* (Marques and Ormieres, 1982; McGeorge et al., 1997), and *Siedleckiella* (Uspenskaya, 1995), but different from spores of triactinomyxon forms (discussed above). Further studies are needed to understand the adaptive functions and taxonomic implications of the associations of a spherical epispore with the release of the sporoplasmic mass through the anterior end, and a cylindrical epispore with the release of the sporoplasmic mass through the posterior end.

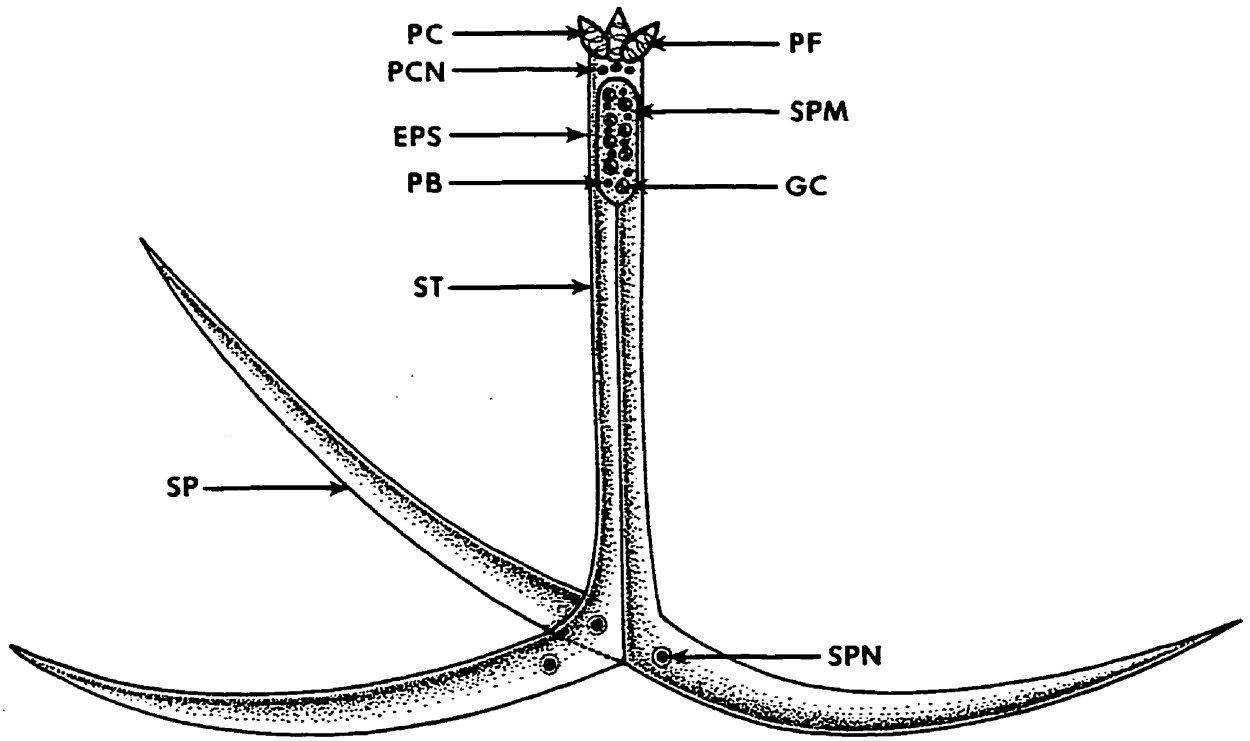
The shape and size of the spore and spore processes of actinosporean stages have been used as diagnostic characteristics since their discovery by Stolc (1899). Our observations confirmed that the shape and size of the spore axis, spore processes, and polar capsules are important and reliable taxonomic characters. These characters, along with the number of germ cells and the size of the sporoplasmic mass from successive observations of free floating spores, provide valuable data to formulate a meaningful description of actinosporean stages.

The gross prevalence (infected worms versus the total number of worms examined) of all forms of actinosporeans of oligochaetes from Lake Sasajewun was about 1%. This is in agreement with other published data of prevalence which ranges from 0.1% to 4% (Yokoyama et al., 1991). The prevalence of each form

described here was low, ranging from 0.02% to 0.67%. However, the natural prevalence was probably higher as some infected worms may not have released mature spores during the time they were being observed in the cell-well plates.

Based on our current knowledge of the myxozoan life cycle, it is reasonable to assume that all 14 actinosporean forms identified in this study have an alternate myxosporean stage in a fish host, although the corresponding myxosporean stage remains unknown for each. Triactinomyxon and raabeia stages have to date been associated with infections of fish with species of *Myxobolus* (see Lom et al., 1997), and previous studies have documented about 40 species of *Myxobolus* from fish of Lake Sasajewun (Gowen, 1983; Li and Desser, 1985; Xiao and Desser, 1997). Further studies to match these 14 described forms of triactinomyxon and raabeia to the known species of *Myxobolus* in the lake are needed.

Figure 1. Schematic line drawing of a water-borne spore of the conventional genus *Triactinomyxon* Stolc, 1899. PC: polar capsules; PF: polar filaments; PCN: polar capsule nuclei; EPS: epispore (epispore body or spore axis); SPM: sporoplasmic mass (endospore); GC: germ cell; PB: polar body; SP: spore process; ST: style; SPN: spore process nucleus.

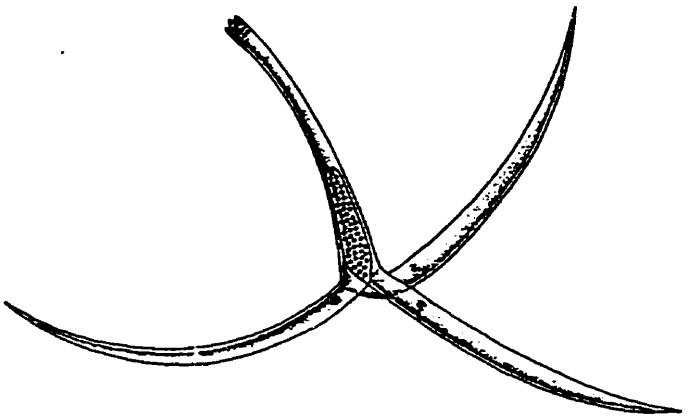


1

Figure 2. Line drawing of a 5 days old spore of triactinomyxon 'A'.
Scale bar = 100 μm.

Figure 3. Line drawing of a 2 days old spore of triactinomyxon 'B'.
Scale bar = 100 μm.

Figure 4. Line drawing of a 2 days old spore of triactinomyxon 'C'.
Scale bar = 100 μm.



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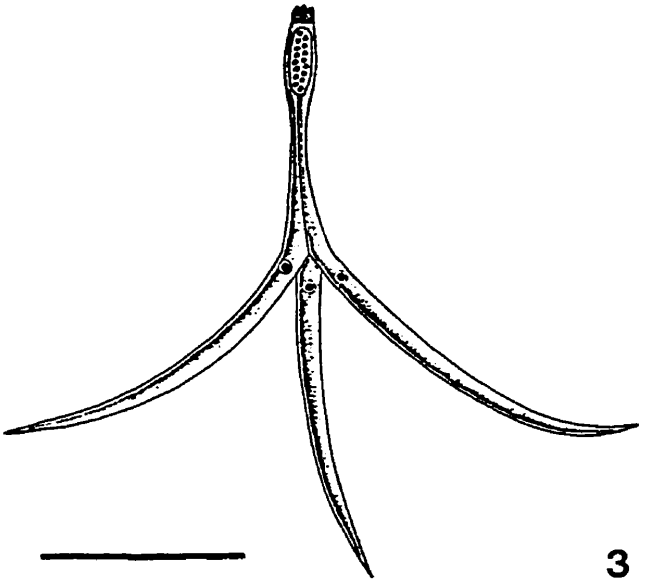

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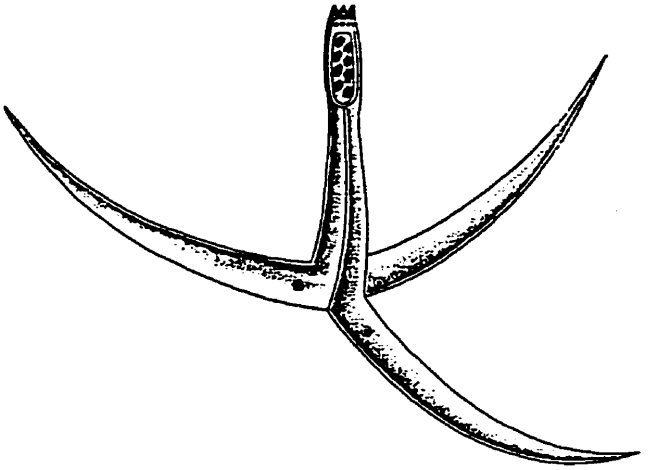


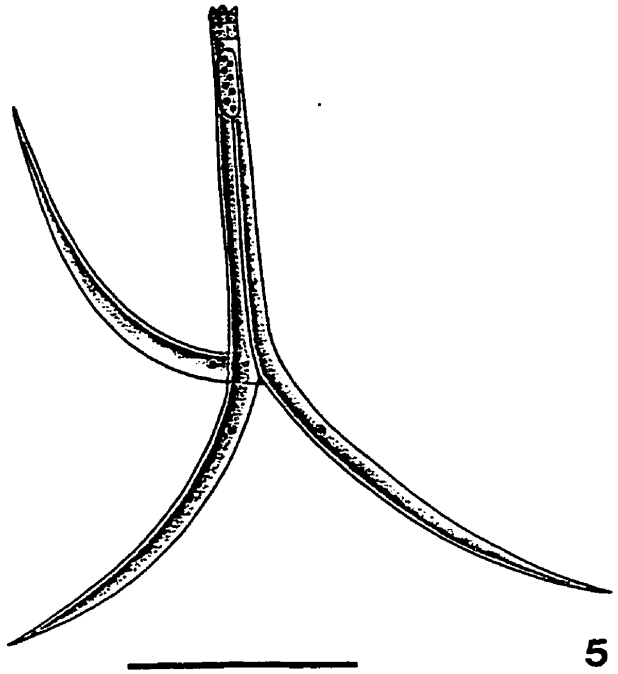
4

Figure 5. Line drawing of a freshly released spore of *Triactinomyxon ignotum* Stolc, 1899. Scale bar = 100 µm

Figure 6. Line drawing of a 3 days old spore of triactinomyxon 'D'. Scale bar = 50 µm.

Figure 7. Line drawing of a freshly released spore of triactinomyxon 'E'. Scale bar = 100 µm.

Figure 8. Line drawing of a freshly released spore of *Triactinomyxon dubium* Granata, 1924 = *Triactinomyxon sp.* Lager, 1904. Scale bar = 100 µm.



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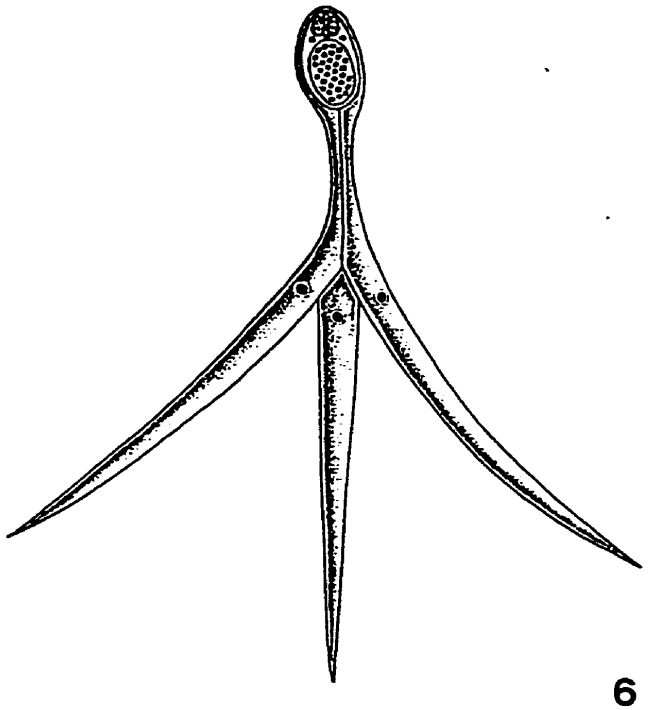

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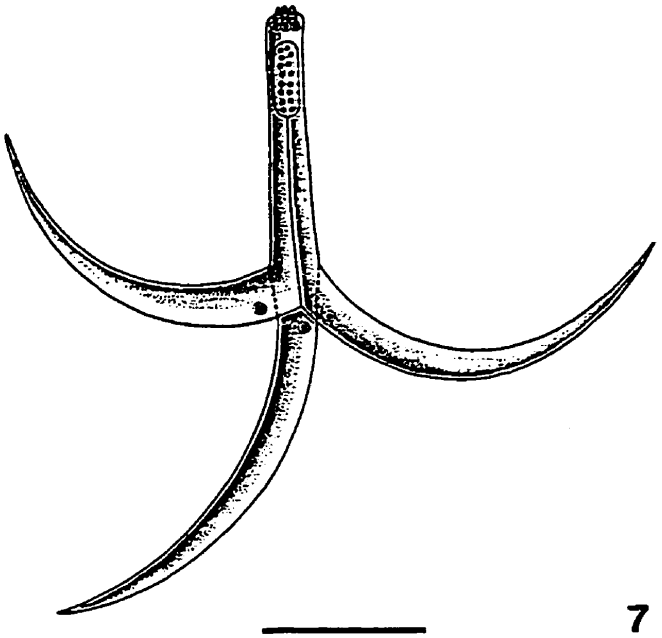

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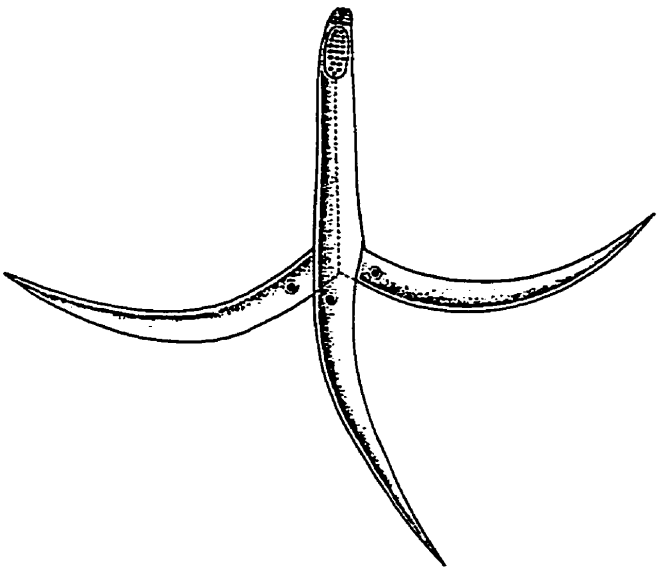
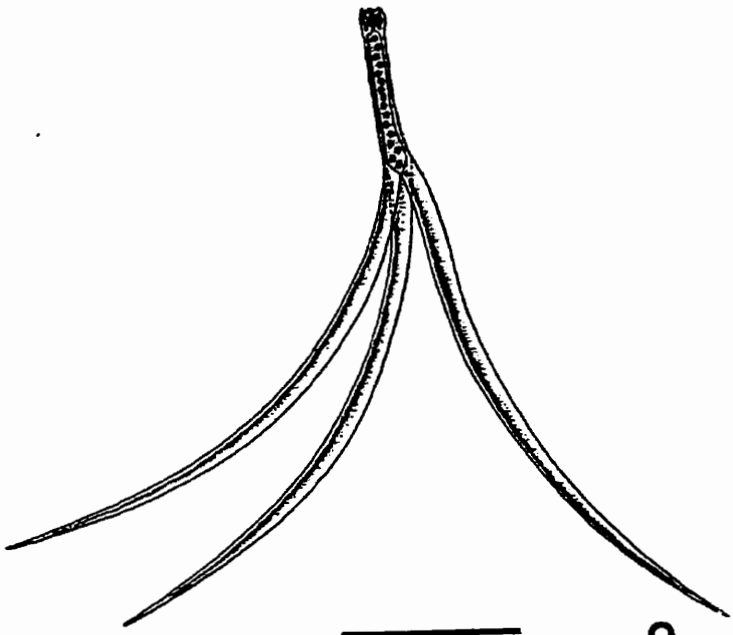


8

Figure 9. Line drawing of a freshly released spore of triactinomyxon 'F'.
Scale bar = 50 μm.

Figure 10. Line drawing of a freshly released spore of raabeia 'A'. Scale bar = 50 μm.

Figure 11. Line drawing of a freshly released spore of raabeia 'B'. Scale bar = 50 μm.



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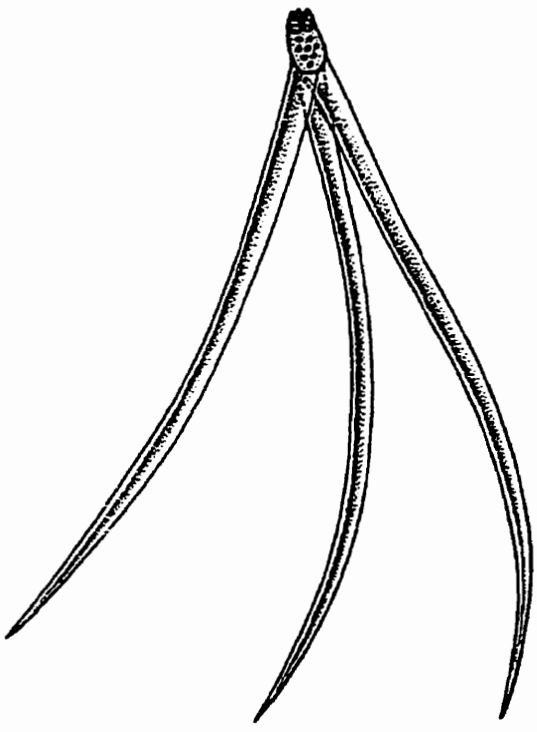

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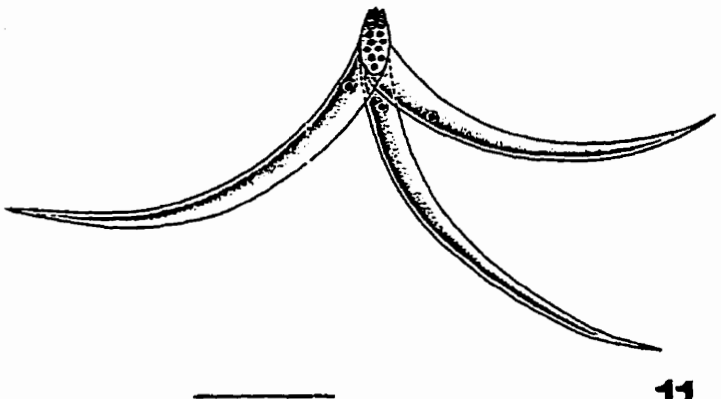

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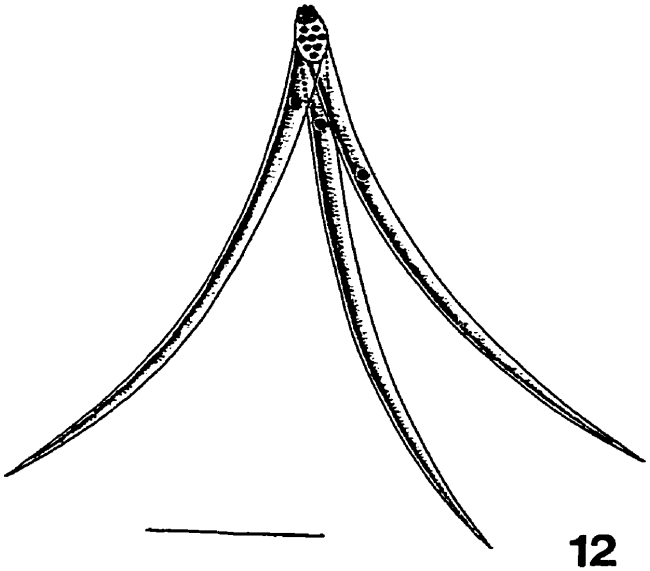
Figure 12. Line drawing of a freshly released spore of raabeia 'C'. Scale bar = 50 µm.

Figure 13. Line drawing of a freshly released spore of raabeia 'D'. Scale bar = 50 µm.

Figure 14. Line drawing of a freshly released spore of raabeia 'E'. Scale bar = 50 µm.

Figure 15. Line drawing of a freshly released spore of raabeia 'F'. Scale bar = 50 µm.



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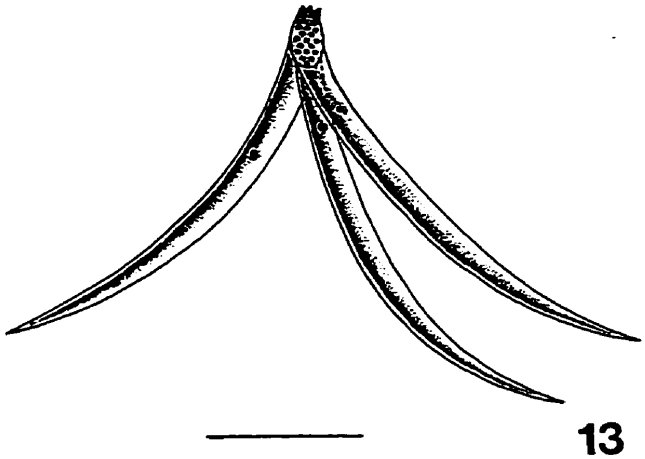

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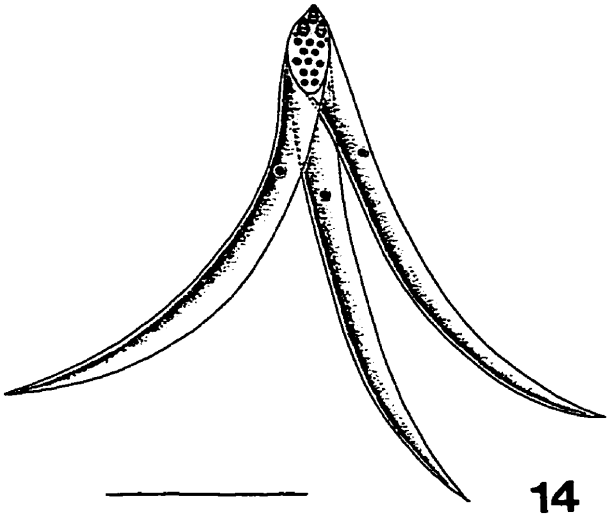

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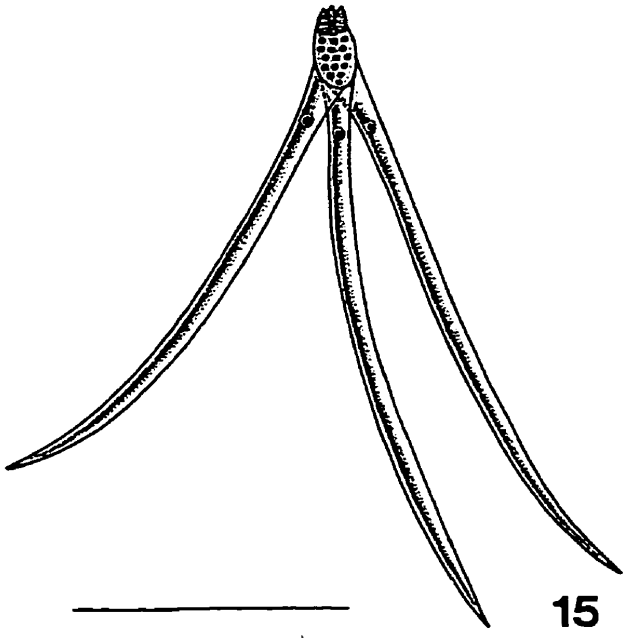

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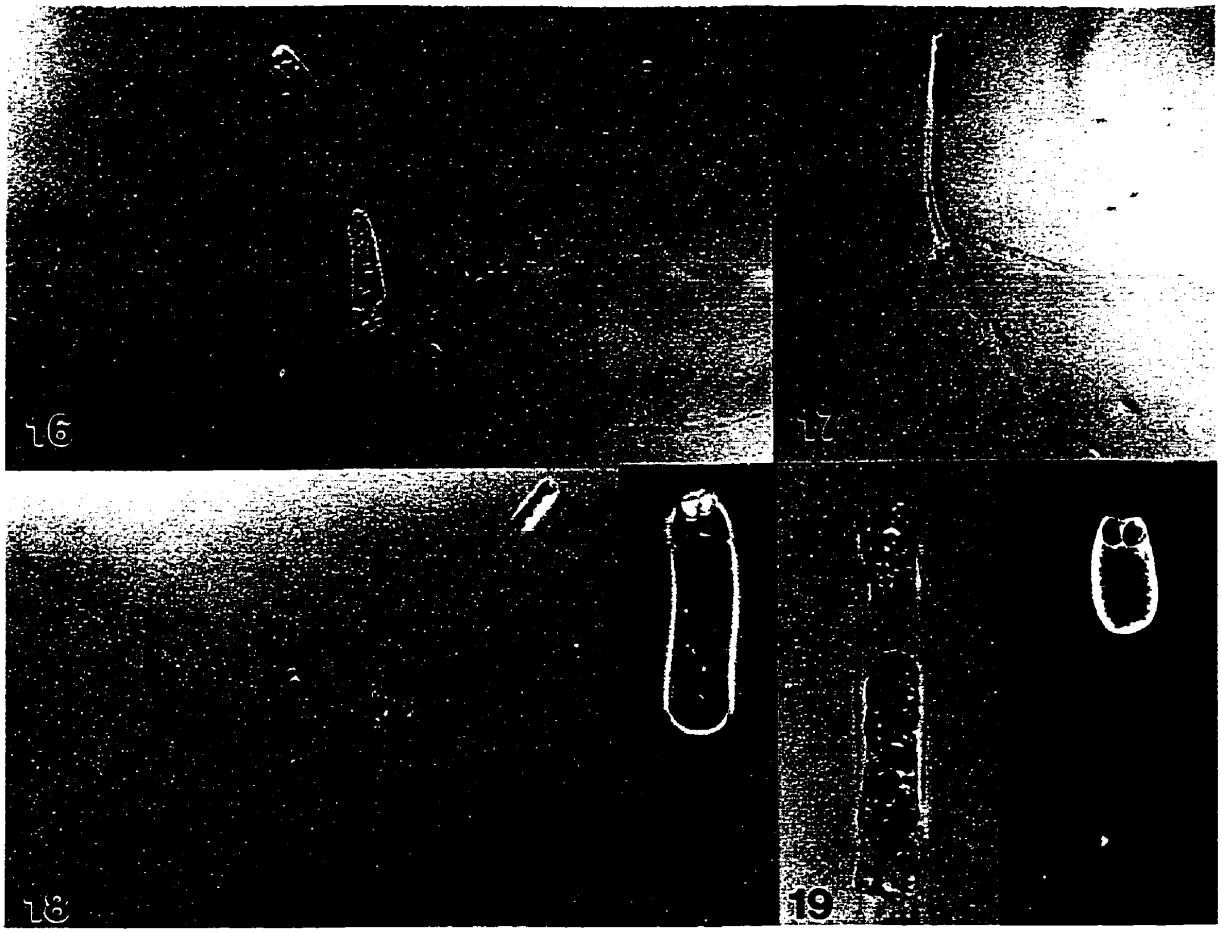
Figures 16-20. Micrographs of forms of triactinomyxon 'A', 'B', 'C', *T. ignotum*, and triactinomyxon 'D'. Fig. 16, a 5 days old spore of triactinomyxon 'A' with the sporoplasmic mass at the posterior end of the epispore, x 220; Fig. 17, a 2 days old spore of triactinomyxon 'B', x 260; Fig. 18, a freshly released spore of triactinomyxon 'C', x 230, and its sporoplasmic mass at the anterior part of the epispore with 8 germ cells in 2 columns (inset, x 1500); Fig. 19, anterior part of a freshly released spore of *T. ignotum*, showing discharged polar filaments and 8 germ cells in the sporoplasmic mass, x 820; Fig. 20, a 2 days old spore of triactinomyxon 'D', x 880.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CHAPTER 5

A Light Microscopic Study of Echinactinomyxon, Neoactinomyxum, Aurantiactinomyxon, Guyenotia, Synactinomyxon and Antonactinomyxon Forms of Oligochaetes from Lake Sasajewun, Algonquin Park, Ontario

(Adapted from Xiao, C. & Desser, S.S. 1998. Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new froms of echinactinomyxon, neoactinomyxum, aurantiactinomyxon, guyenotia, synactinomyxon and antonactinomyxon. J. Parasitol., **84**, 1010-1019.)

ABSTRACT

Six forms (5 of which are new) of echinactinomyxon, and 5 new forms of the collective groups neoactinomyxum, aurantiactinomyxon, guyenotia, synactinomyxon and antonactinomyxon of oligochaetes in Lake Sasajewun, Algonquin Park are described. Five forms of echinactinomyxon designated as echinactinomyxon 'A', 'B', 'C', 'D' and 'E', a form of neoactinomyxum, and a form of aurantiactinomyxon were found in *Limnodrilus hoffmeisteri*. *Echinactinomyxon radiatum*, a form of synactinomyxon, and a form of antonactinomyxon were recorded from *Tubifex tubifex*. A form of guyenotia was found in *Lumbriculus variegatus*. The sporoplasmic mass of these actinosporeans was released through the anterior end of the epispore. An increase in the number of germ cells was observed in the floating spore stage of all forms except echinactinomyxon 'C' and the form of antonactinomyxon.

INTRODUCTION

Since Wolf and Markiw (1984) introduced the 2-host life cycle hypothesis of myxosporean parasites, actinosporean parasites of oligochaetes have received increased attention. Other than the recent reports of Hallett et al. (1998) and McGeorge et al. (1997) who described forms of actinosporeans of annelids, Yokoyama et al. (1993) who studied some biological characteristics of spores of actinosporeans infecting *Branchiura sowerbyi*, and Lom and Dykova (1992) and Lom et al. (1997a) who described the ultrastructure of actinosporean spores, most studies have been focused on demonstrating the alternation between actinosporean and myxosporean cycles under laboratory conditions (Markiw and Wolf, 1983; El-Matbouli and Hoffmann, 1989; Hedrick et al., 1989; Ruidisch et al., 1991; El-Matbouli et al., 1992, 1995; Kent et al., 1993; Uspenskaya, 1995; Trouillier et al., 1996; Andree et al., 1997; Bartholomew et al., 1997). Despite this renewed interests in myxozoans, the diversity and biology of actinosporeans in their natural ecological setting remains poorly known.

The discovery of 56 species of myxosporean parasites of fish in Lake Sasajewun (Gowen, 1983; Li and Desser, 1985; Lom et al., 1989; Xiao and Desser, 1997) led to a survey of the actinosporean stages of myxozoan parasites of oligochaetes in this lake. The survey resulted in the descriptions of 12 new forms of triactinomyxon and raabeia (Xiao and Desser, 1998a). In this chapter, 6 forms (5 of which are new) of echinactinomyxon, and 5 new forms of the collective groups neoactinomyxum, aurantiactinomyxon, guyenotia, synactinomyxon and antonactinomyxon, are described following the taxonomic system of Kent et al. (1994) and the guidelines of Lom et al. (1997b).

MATERIALS AND METHODS

A total of 14100 worms from 19 species of oligochaetes collected from various sites in Lake Sasajewun were examined for actinosporean parasites in 1995, 1996 and 1997. Oligochaetes were checked every other day using the cell-well plate method (Yokoyama et al., 1991) over a 4-wk period. Waterborne actinosporean spores examined by differential interference contrast (DIC) microscopy, and Diff-Quik® stained smears of spore suspensions observed by bright field and phase contrast microscopy, were photographed using a Zeiss Universal I photomicroscope. Samples of freshly released spores of each form were transferred to separate wells filled with lake water and observed daily until their sporoplasmic masses were released. Measurements of spores taken from Diff-Quik® stained smears were based on 25 specimens, and given as an average followed by its range in micrometers. Samples of infected oligochaetes were fixed in Bouin's solution and prepared for subsequent histological examination (for detailed methodology, see Xiao and Desser, 1998).

DESCRIPTION

***Echinactinomyxon radiatum* Janiszewska, 1957**

(Fig. 1)

Mature spores composed of a style-less epispore with 3 straight, equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes enclosed posterior part of epispore body; single ovoid nucleus close to base of each process; length of processes 90.0 (80.0-95.0), and width of bases 7.5 (7.0-8.0); epispore cavity subspherical, contained sporoplasmic mass; epispore body 25.0 (23.0-26.0) in length and 16.5 (15.5-18.0) in width; 3 pyriform polar capsules, equal in size, 5.5 (5.0-6.0) in length and 3.0 (2.8-3.1)

in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, triangular or ovoid, close to bottom of capsules; polar filament coiled 4-5 times; discharged polar filament about 65.0 in length; style absent; sporoplasmic mass in freshly released spores contained 16 germ cells, 32 germ cells in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Type host: *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Type locality: River Oder, Poland.

Other locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 1 of 1430 worms (0.07%).

Voucher specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0042).

Remarks

This form of echinactinomyxon is identical to *E. radiatum* Janiszewska, 1957 in the shape of the spore, the number of germ cells, and the size of the epispore body and processes.

Echinactinomyxon 'A'

(Fig. 2).

Mature spores composed of a style-less epispore with 3 straight, equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes enclosed posterior part of epispore body; single ovoid nucleus located randomly in each process; length of processes 75.0 (65.0-85.0), and width of bases 5.0 (4.0-6.0); epispore cavity subspherical, contained sporoplasmic mass; epispore body 14.0 (12.5-15.0) in length and 9.5 (8.5-

11) in width; 3 pyriform polar capsules, equal in size, 5.0 (4.8-5.4) in length and 3.5 (3.2-3.6) in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filament coiled 3-5 times; discharged polar filament about 25.0 in length; style absent; sporoplasmic mass in freshly released spores contained 8 germ cells and usually 8 polar bodies, 16 germ cells and usually 8 polar bodies in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 8 of 6550 worms (0.12%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0037).

Remarks

This form of echinactinomyxon, although similar to *E. radiatum* in spore shape, has fewer germ cells (8 for freshly released spores and 16 for spores released 2 days or longer versus 16 for freshly released spores and 32 for spores released 2 days or longer), a smaller epispore body (14.0 versus 25.0 to 30.0 in length) and shorter epispore processes (75.0 versus 100.0 to 125.0). It is also similar to *Echinactinomyxon astilum* in the number of germ cells for spores released 2 days or longer, but differs in the size of the epispore body (14.0 versus 25.0 to 35.0 in length) and processes (75.0 versus 45.0 to 60.0 in length).

Echinactinomyxon 'B'

(Fig. 3)

Mature spores composed of a style-less epispore with 3 straight, equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes nearly enclosed epispore body; single ovoid nucleus close to base of each process; length of processes 50.0 (45.0-55.0), and width of bases 5.0 (4.0-6.0); epispore body subspherical, contained sporoplasmic mass; epispore body 12.0 (11.5-12.5) in length and 9.5 (9.0-10.5) in width; 3 pyriform polar capsules, equal in size, 3.8 (3.5-4.0) in length and 2.7 (2.5-3.0) in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filament coiled 3-4 times; style absent; sporoplasmic mass in freshly released spores usually contained 8 germ cells, 16 germ cells in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 2 of 6550 worms (0.03%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0038).

Remarks

This form, although similar to echinactinomyxon 'A' in the characteristic number of germ cells, has a shorter epispore body (12.0 versus 14.0), shorter processes (50.0 versus 75.0), smaller polar capsules (3.8 x 2.7 versus 5.0 x 3.5), and without obvious polar bodies.

Echinactinomyxon 'C'

(Figs. 4)

Mature spores composed of a style-less epispore with 3 straight, occasionally with slight curve, and equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes enclosed posterior part of epispore body; single ovoid nucleus located randomly in each process; length of processes 83.0 (70.0-95.0), and width of bases about 5.5 (5.0-6.0); epispore cavity subspherical, contained sporoplasmic mass; epispore body 13.0 (12.0-14.0) in length and 10.0 (8.5-12.0) in width; 3 pyriform polar capsules, equal in size, 3.8 (3.5-4.0) in length and 2.2 (2.0-2.5) in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, oval, close to bottom of capsules; polar filament coiled 3-4 times; style absent; sporoplasmic mass with 16 germ cells during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 22 of 6550 worms (0.34%), including 1 worm with mixed infection of triactinomyxon 'F'.

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0039).

Remarks

This form is similar to echinactinomyxon 'A' in the size of the epispore body and processes, but they differ in the characteristic number of germ cells (16 for the entire spore stage versus 8 for freshly released spores and 16 for spores released 2

days or longer), in the size of polar capsules (3.8 x 2.2 versus 5.0 x 3.5), and in the presence of obvious polar bodies in spores of triactinomyxon 'A'. This form is also similar to echinactinomyxon 'B' in the size of the epispore body and polar capsules, but has longer epispore processes (83.0 versus 50.0).

Echinactinomyxon 'D'

(Fig. 5)

Mature spores composed of a style-less epispore with 3 straight, equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes enclosed posterior part of epispore body; single ovoid nucleus close to base of each process; length of processes 100.0 (85.0-105.0), and width of bases about 6.0-7.0; epispore cavity subspherical, contained sporoplasmic mass; epispore body 16.0 (15.0-17.0) in length and 11.5 (11.0-12.5) in width; 3 pyriform polar capsules, equal in size, 3.9 (3.8-4.0) in length and 2.4 (2.3-2.5) in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filament coiled 3-5 times; style absent; sporoplasmic mass in freshly released spores contained 16 germ cells and usually 16 polar bodies, 32 germ cells and 16 polar bodies in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 16 of 6550 worms (0.24%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0040).

Remarks

This form, although similar to *E. radiatum* in the characteristic number of germ cells and the size of epispore processes, has a smaller epispore body (16.0 x 11.5 versus 25.0 x 16.5), smaller polar capsules (3.9 x 2.4 versus 5.5 x 3.0), and obvious polar bodies.

Echinactinomyxon 'E'

(Fig. 6)

Mature spores composed of a style-less epispore with 3 straight, equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes enclosed posterior part of epispore body; single ovoid nucleus close to base of each process; length of processes 90.0 (85.0-95.0), and width of bases about 5.0-6.5; epispore cavity subspherical, contained sporoplasmic mass; epispore body 12.0 (11.5-12.5) in length and 9.0 (8.0-10.0) in width; 3 pyriform polar capsules, equal in size, 3.8 (3.5-4.0) in length and 2.7 (2.5-3.0) in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filament coiled 3-4 times; style absent; sporoplasmic mass in freshly released spores contains 8 germ cells, 16 germ cells in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 2 of 6550 worms (0.03%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0041).

Remarks

This form, although similar to echinactinomyxon 'A' and 'B' in the characteristic number of germ cells and the size of the epispore body, differs from echinactinomyxon 'A' in having no obvious polar bodies and smaller polar capsules (3.8 x 2.7 versus 5.0 x 3.5), and from echinactinomyxon 'B' in having longer epispore processes (90.0 versus 50.0).

Neoactinomyxum

(Figs. 7, 11, 15)

Mature spores composed of a style-less epispore with 3 processes of equal size, rounded and extended slightly downwards; bases of processes completely enclosed epispore body; single ovoid nucleus located randomly in each process; length of processes 24.0 (22.0-26.0), and width 23.0 (21.0-25.0); epispore cavity subspherical, contained sporoplasmic mass; epispore body 12.0 (11.3-13.2) in length and 10.0 (9.3-10.5) in width; 3 pyriform polar capsules, equal in size, 3.0 (2.8-3.2) in length and 2.0 (1.8-2.2) in width, situated at anterior end of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filaments coiled 3-5 times; style absent; sporoplasmic mass in freshly released spores contained 16 germ cells, 32 germ cells in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 2 of 6550 worms (0.03%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue

No. CMNPA1998-0043).

Remarks

This form, although similar to *Neoactinomyxum globosum* Granata, 1922 (see Marques, 1984) and *Neoactinomyxum eiseniellae* (Ormieres and Frezil 1969) in the spore shape and the size of the epispore body, has larger epispore processes (21.0 to 26.0 versus 12.0 to 16.0 in diameter), and 16 germ cells for freshly released spores and 32 for spores released 2 days or longer, whereas *N. globosum* has 16 and *N. eiseniellae*, 32.

Aurantiactinomyxon

(Figs. 8, 12)

Mature spores composed of a style-less epispore with 3 equal-sized, leaf-like processes which are pointed and curved downwards; bases of processes enclosed most of epispore body; single ovoid nucleus located randomly in each process; length of processes 24.0 (21.0-26.0), and width about 13.0-16.0; epispore cavity subspherical, contained sporoplasmic mass; epispore body 12.0 (11.5-13.8) in length and 11.0 (10.0-12.5) in width; 3 pyriform polar capsules, equal in size, 3.0 (2.7-3.4) in length and 1.5 (1.4-1.7) in width, situated at top of epispore and slightly inclined outward; nuclei of polar capsules small, kidney-shaped, close to bottom of capsules; polar filaments coiled 3-4 times; style absent; sporoplasmic mass with 64 to 128 germ cells; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Limnodrilus hoffmeisteri* Claparede, 1862.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 1 of 6550 worms (0.02%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the

Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0044).

Remarks

Although this form is morphologically similar to the aurantiactinomyxon stage of *Hoferellus carassii* Achmerov, 1960 (see El-Matbouli et al., 1992), further comparisons could not be made due to insufficient information in the original description. It also resembles *Aurantiactinomyxon raabeiiunioris* Janiszewska, 1952 in the size of epispore processes, and *Aurantiactinomyxon pavinsis* Marques, 1984 in the size of the epispore body, but differs from them in having 64 to 128 germ cells, whereas *A. raabeiiunioris* and *A. pavinsis* have 16 germ cells.

Guyenotia

(Figs. 9, 13)

Mature spores composed of a style-less epispore with 3 equal-sized, straight, finger-like processes with rounded free ends; bases of processes enclosed posterior half of epispore body; single ovoid nucleus located randomly in each process; length of processes 21.0 (16.0-25.0), and width about 4.5-6.5; epispore cavity almost globular, contained sporoplasmic mass; epispore body 9.5 (9.0-10.5) in length and 8.8 (8.0-9.5) in width; 3 pyriform polar capsules, equal in size, 3.0 (2.8-3.3) in length and 2.0 (1.8-2.2) in width, situated at top of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filaments coiled 3-4 times; style absent; sporoplasmic mass occupied epispore cavity completely; sporoplasmic mass in freshly released spores contained 8 germ cells, 16 germ cells in spores released 3 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores.

Taxonomic summary

Host: *Lumbriculus variegatus* Muller, 1744.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 2 of 1160 worms (0.17%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0045).

Remarks

This form, although morphologically similar to *Guyenotia sphaerulosa* Naville 1930 (also see Marques, 1984), the only known species of this conventional genus, has fewer germ cells (8 for freshly released spores and 16 for spores released 3 days or longer versus 32), a smaller epispore body (9.5 x 8.8 versus 15.0 x 15.0), and shorter epispore processes (21.0 versus 40.0).

Synactinomyxon

(Fig. 14)

Mature spores composed of a style-less epispore with 3 straight, equal-sized processes which gradually tapered towards their pointed tips; epispore processes resembled spines or rays; bases of processes enclosed posterior half of epispore body; single ovoid nucleus located randomly in each process; length of processes 138.0 (125.0-150.0), and width of bases about 12.0-14.0; epispore cavity slightly elongated ovoid, contained sporoplasmic mass; epispore body 31.0 (28.0-35.0) in length and 17.5 (16.0-18.5) in width; 3 pyriform polar capsules, equal in size, 7.5 (7.0-8.0) in length and 4.0 (3.8-4.2) in width, situated at top of epispore and slightly inclined outward; nuclei of polar capsules small, ovoid, close to bottom of capsules; polar filament coiled 3-4 times; style absent; sporoplasmic mass occupied epispore cavity completely; sporoplasmic mass in freshly released spores contained 16 germ cells, about 32 germ cells in spores released 2 days or longer; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores, which upon release, connected through a process with processes of other

spores close to the free pointed ends.

Taxonomic summary

Host: *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 5 of 1430 worms (0.35%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate Collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0046).

Remarks

This form, although similar to *Synactinomyxon longicauda* Marques and Ormieres, 1982 in morphology of 8-spore unit, has a longer epispore body (31.0 versus 22.0), longer spore processes (138.0 versus 80.0), and more germ cells (16 for freshly released spores and 32 for spores released 2 days or longer versus 16). It also differs from *Synactinomyxon tubificis* Stolc, 1899, the other known 'species' of this conventional genus, in the shape of the spore and processes. Since the configuration of the 8-spore unit of this form was not elucidated in this study, it is tentatively included in the collective group, synactinomyxon, based on the morphological similarities to those of *S. longicauda*.

Antonactinomyxon

(Figs. 10, 16, 17)

Mature spores composed of a style-less epispore with three equal-sized, straight, pointed processes; epispore processes resembled spines or rays; bases of spore processes surround posterior half of epispore cavity; ovoid nuclei of spore processes close to their bases; length of spore processes 112.0 (105.0-120.0), and width of bases about 11.0 (10.0-12.0); epispore ovoid, 27.0 (26.0-28.0) in length and 17.0 (16.0-18.0) in width; 3 equal-sized pyriform polar capsules, 7.5 (6.5-8.5) in length

and 4.0 (3.7-4.2) in width, situated at top of epispore with apex slightly inclined outward; nuclei of polar capsules tiny, kidney-shaped, close to bottom of polar capsules; polar filament coiled 4-6 times; sporoplasmic mass ovoid, occupied epispore cavity completely and contained 16 germ cells during the entire spore stage; pansporocysts developed in the intestinal epithelial cells of the worms; each pansporocyst with 8 spores, which upon release, connected through processes with processes of 3 other spores close to the distal free pointed ends (Fig. 17).

Taxonomic summary

Host: *Tubifex tubifex* Muller, 1774.

Site of infection: Intestinal epithelium.

Locality: Lake Sasajewun (45°35'N, 78°30'W), Algonquin Park, Ontario.

Prevalence of infection: 2 of 1430 worms (0.14%).

Type specimens: Diff-Quik® stained, air-dried smear of spores deposited in the Canadian Museum of Nature, Invertebrate collection, Ottawa, Ontario (Catalogue No. CMNPA1998-0047).

Remarks

This form resembles *Antonactinomyxon antonii* (Janiszewska, 1954), the only member of this conventional genus, with style-less epispore and 3-dimensional reticulum formed by 8 spores, but they differ in the shape of epispore processes (pointed ends versus rounded ends), and the way in which these processes unite. However, since the corresponding myxosporean stage is unknown, and there is no need to create a new actinosporean collective group (Kent et al., 1994; Lom et al., 1997b; Xiao and Desser, 1998), this form, therefore, has been assigned to the nearest collective group, antonactinomyxon.

DISCUSSION

Descriptions of actinosporean stages have been based on the morphology of pansporocysts, the number of germ cells, and the dimensions of the sporoplasmic mass and the waterborne spores. Successive observations of the waterborne spores of actinosporeans described in previous report (Xiao and Desser, 1998) and in this study revealed that the sporoplasmic mass of spores with a style-less ovoid epispore body was released through the anterior end of the epispore, and that 13 of the 25 forms from Lake Sasajewun exhibited a doubling in the number of germ cells in free floating spores, which usually occurred during the first 3 days after spores were released from the host. Documentation of the number of germ cells based on a single random observation of the spore stage is therefore incomplete. Observations of freshly released waterborne spores for at least 3 days are required to determine the characteristic number of germ cells of a form.

Only 2 'species' of the conventional genus *Echinactinomyxon* Janiszewska, 1957 have been described from worms of the genus *Tubifex* (see Marques, 1984). Of the 6 forms of echinactinomyxon described in this paper, 5 were found in *Limnodrilus hoffmeisteri* and 1 in *Tubifex tubifex*. Whereas the host range of echinactinomyxon was extended to species of *Limnodrilus*, a single form of this group was never found in both *Tubifex* and *Limnodrilus*, suggesting host specificity.

The conventional genus *Neoactinomyxum*, established by Granata in 1922, consists of 3 'species' (see Marques, 1984). The single form of neoactinomyxum found in Lake Sasajewun, although morphologically similar to *N. eiseniellae*, a 'species' originally described as *Aurantiactinomyxon eiseniellae* by Ormieres and Frezil (1969), which upon reexamination was subsequently transferred to *Neoactinomyxum* by Marques and Ormieres (1982), has much larger epispore processes.

Janiszewska (1957) established the genus *Aurantiactinomyxon* to accommodate

A. raabeiiunioris. Marques (1984) described *A. pavinsis* from *Stylodrilus heringianus,* and *A. stellans* and *A. trifolium* from tubificid worms. El-Matbouli et al. (1992) and Trouillier et al. (1996) reported 2 different forms of *Aurantiactinomyxon* as the alternate stage of *Hoferellus carassii* from an unidentified tubificid worm and a species of *Nais,* respectively. However, 2 members of this group, *A. trifolium* Marques, 1984 and an aurantiactinomyxon stage of *H. carassii* (see Trouillier et al., 1996), have finger-shaped processes with rounded ends rather than leaf-shaped processes with pointed ends, and therefore, should be transferred to the collective group, guyenotia. The form of aurantiactinomyxon from Lake Sasajewun resembles the aurantiactinomyxon stage of *H. carassii* (see El-Matbouli et al., 1992), *A. raabeiiunioris* and *A. pavinsis.* But, all the known forms of aurantiactinomyxon have 32 or less germ cells in their sporoplasmic mass, whereas the form described in this study has 64 to 128.

The collective group guyenotia, consists of *G. sphaerulosa* Naville, 1930, and, as previously discussed, *G. trifolium* (Marques, 1984) and a guyenotia stage of *H. carassii* (see Trouillier et al., 1996). The form of guyenotia described in this paper differs morphometrically from all other described forms of guyenotia.

Eight interconnected waterborne spores were first recorded in *Synactinomyxon tubificis* by Stolc (1899). The conventional family Siedleckiellidae Janiszewska, 1957, consisting of the conventional genera *Synactinomyxon* Stolc 1899, *Siedleckiella* Janiszewska 1955, and *Antonactinomyxon* Janiszewska 1957, was established to accommodate parasites exhibiting this arrangement of spores. Whereas spores of the conventional genera *Synactinomyxon* and *Antonactinomyxon* resemble those of the conventional genus *Echinactinomyxon,* having a style-less epispore and spine-like processes, only water-borne spores of *Synactinomyxon* and *Antonactinomyxon* form 8-spore units. The form of synactinomyxon and that of antonactinomyxon found in Lake Sasajewun differ morphometrically from the

known forms of these 2 groups, respectively. The adaptive advantage of parasites forming these reticular units is unclear.

In the conventional classification, about 1200 species of myxosporeans have been described (Lom and Dykova, 1992), in contrast to only about 39 described species of actinosporeans (Marques, 1984). This large discrepancy in species numbers was one of several reasons why the remarkable and controversial proposal of alternation between myxosporean and actinosporean parasites suggested by Wolf and Markiw (1984) was at first not widely accepted (Lom, 1987). Studies of fish and oligochaete parasites in Lake Sasajewun revealed that there are 56 species belonging to 8 genera of myxosporeans (Gowen, 1983; Li and Desser, 1985; Lom et al., 1989; Xiao and Desser, 1997) and 25 forms belonging to 8 conventional genera of actinosporeans (Xiao and Desser, 1998; this study). Although the numbers of myxosporean and actinosporean forms in Lake Sasajewun differ, the difference in their numbers is much smaller than the data, available previous to this study, suggested. Although our data do not support equal numbers of myxosporeans and actinosporeans, there is a close correspondence between the number of 'genera' of these parasites in the lake. This discrepancy in the numbers of myxosporeans and actinosporeans may be due to several factors. If myxosporeans exhibit polymorphism when infecting different species of fish hosts, the traditional practice of species designation would result in an overestimation of their diversity. Because the natural prevalence of actinosporeans is so low, a thorough survey requires an extremely large sample size. Finally, although alternation of actinosporean and myxosporean stages has been reported for about 14 myxosporeans (see Kent et al., 1994), the possibility of some myxosporeans undergoing a direct life cycle should not be excluded.

To date, echinactinomyxon stages have been associated with a species of *Zschokkella* (see Kent et al., 1994), neoactinomyxum stages with *Hoferellus carassii* (see

Yokoyama et al., 1993), aurantiactinomyxon stages with the PGD myxosporean (Styer et al., 1991), *H. carassii* (see El-Matbouli et al., 1992), *Myxidium giardi* (see Benajiba and Marques, 1993,) and *Thelohanellus hovorkai* (see Yokoyama, 1997), and aurantiactinomyxon or guyenotia stages with *Hoferellus cyprini* (see Großheider and Korting, 1992). Unlike their corresponding myxosporean stages in fish which undergo a variety of patterns of sporogenesis (Lom and Dykova, 1992), actinosporean sporogenesis is characterized by the production of 8 spores in each pansporoblast (Marques, 1984; Bartholomew et al., 1997; Xiao and Desser, 1998), suggesting that the myxozoan parasites were annelid parasites first which later evolved into parasites that alternated between annelid and fish hosts. Based on the current knowledge of the myxozoan life cycle, it is assumed that the actinosporean forms identified in this study have an alternate myxosporean stage in a fish host, although the corresponding myxosporean stages are presently unknown.

Figure 1. Line drawing of a freshly released spore of *Echinactinomyxon radiatum* Janiszewska. Scale bar = 50 µm.

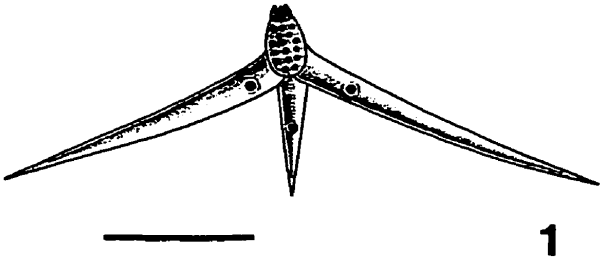
Figure 2. Line drawing of a freshly released spore of echinactinomyxon 'A'. Scale bar = 25 µm.

Figure 3. Line drawing of a freshly released spore of echinactinomyxon 'B'. Scale bar = 25 µm.

Figure 4. Line drawing of a 2 days old spore of echinactinomyxon 'C'. Scale bar = 25 µm.

Figure 5. Line drawing of a 2 days old spore of echinactinomyxon 'D'. Scale bar = 25 µm.

Figure 6. Line drawing of a freshly released spore of echinactinomyxon 'E'. Scale bar = 25 µm.



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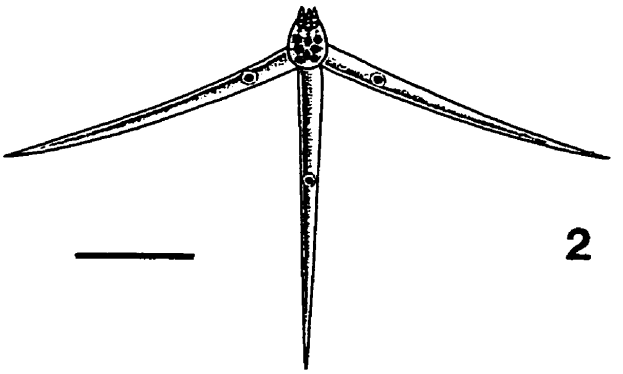

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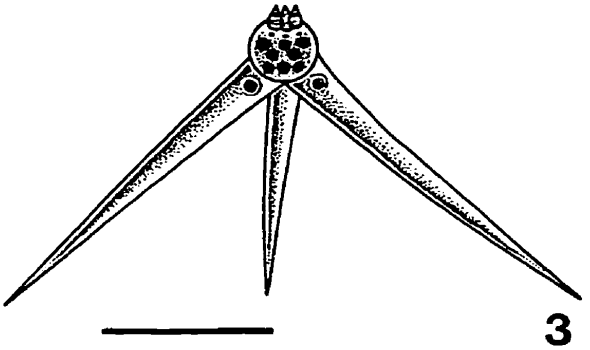

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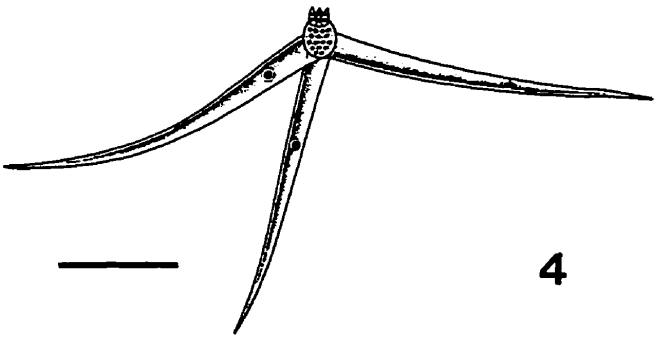

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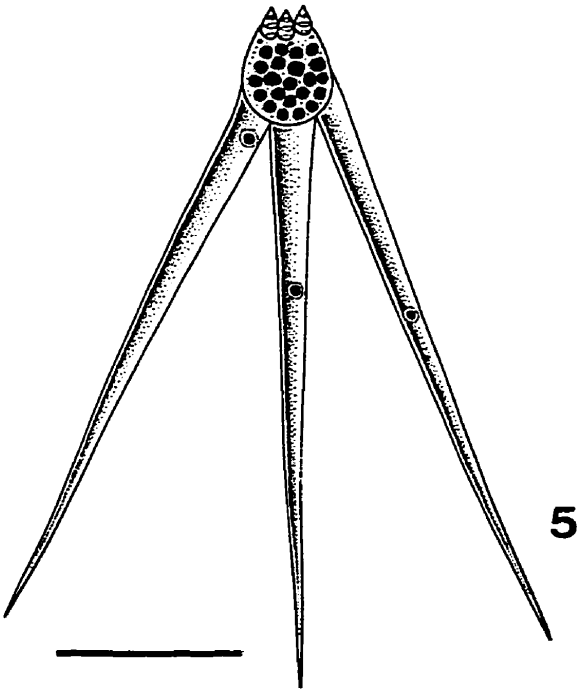

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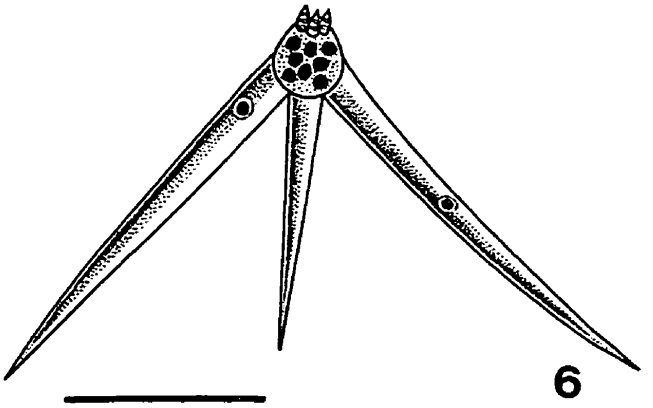

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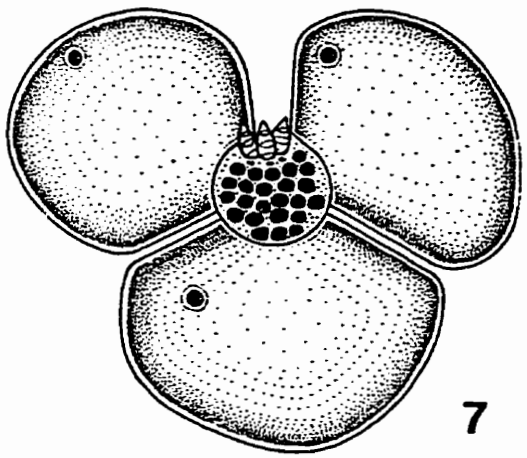
Figure 7. Line drawing of a 3 days old spore of a new form of neoactinomyxum. Scale bar = 20 μm.

Figure 8. Line drawing of a freshly released spore of a new form of aurantiactinomyxon. Scale bar = 20 μm.

Figure 9. Line drawing of a freshly released spore of a new form of guyenotia. Scale bar = 20 μm.



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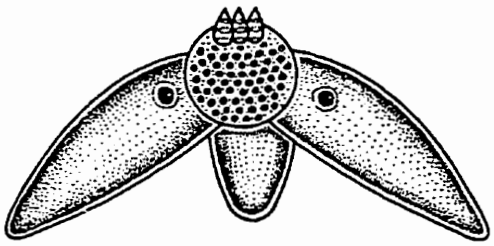

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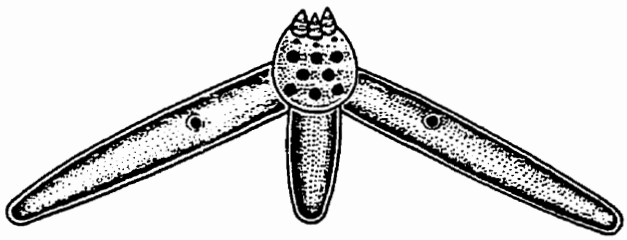

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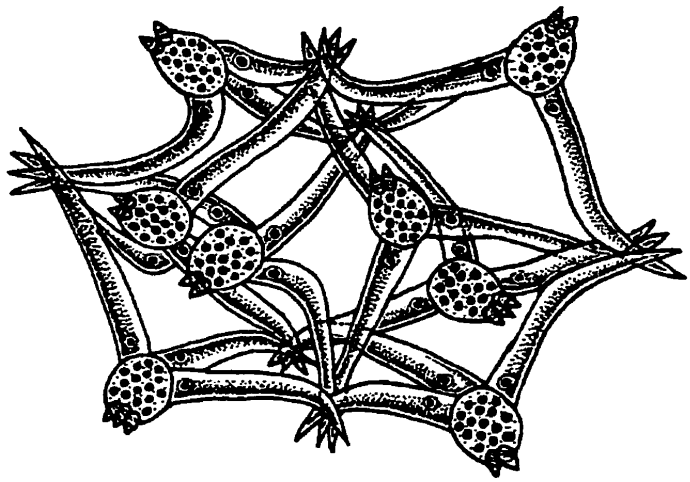
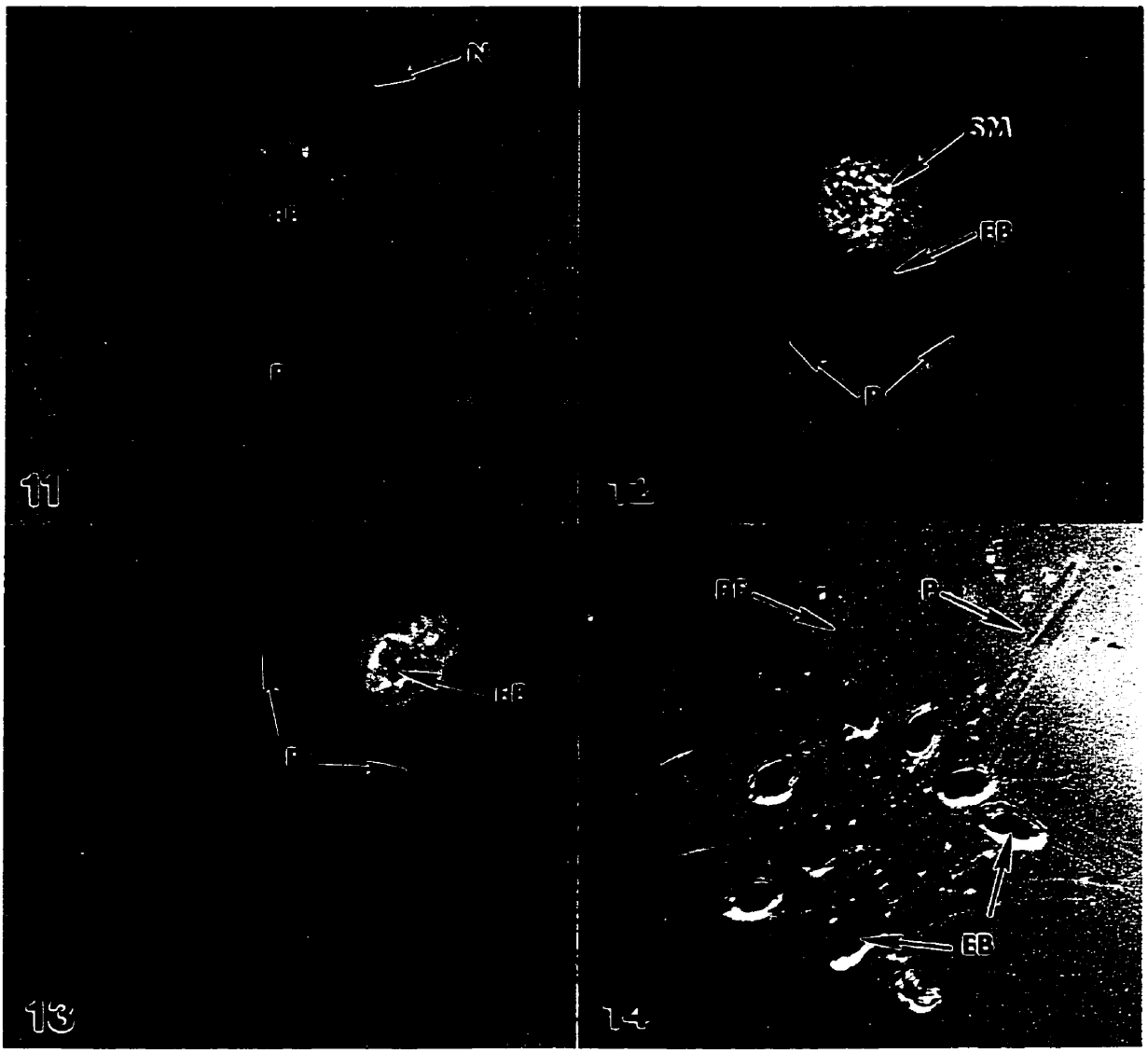
Figure 10. Line drawing of 8 fresh water-borne spores of a new form of antonactinomyxon, arranged in a typical reticulum. Scale bar = 50 μm.



Figures 11-14. Photographs of new forms of neoactinomyxum, aurantiactinomyxon, guyenotia, and synactinomyxon. Fig. 11, a Diff-Quik stained freshly released spore of neoactinomyxum, showing the nearly spherical epispore body (EB) and single nucleus (N) in each rounded process (P), x 1400; Fig. 12, a fresh 5-day old spore of aurantiactinomyxon, showing the release of its sporoplasmic mass (SM) through the anterior end of the epispore body (EB) and 2 of the 3 leaf-shaped processes (P) in this plane of focus, x 900; Fig. 13, a fresh spore of guyenotia, showing the epispore body (EB) and 2 of the 3 finger-like processes (P) in this plane of focus, x 1900; Fig. 14, a Diff-Quik stained 8-spore unit of synactinomyxon, showing the morphology of the unit, the discharged polar filaments (PF), the style-less epispore body (EB), and the spine-like processes (P), x 210.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12
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BB
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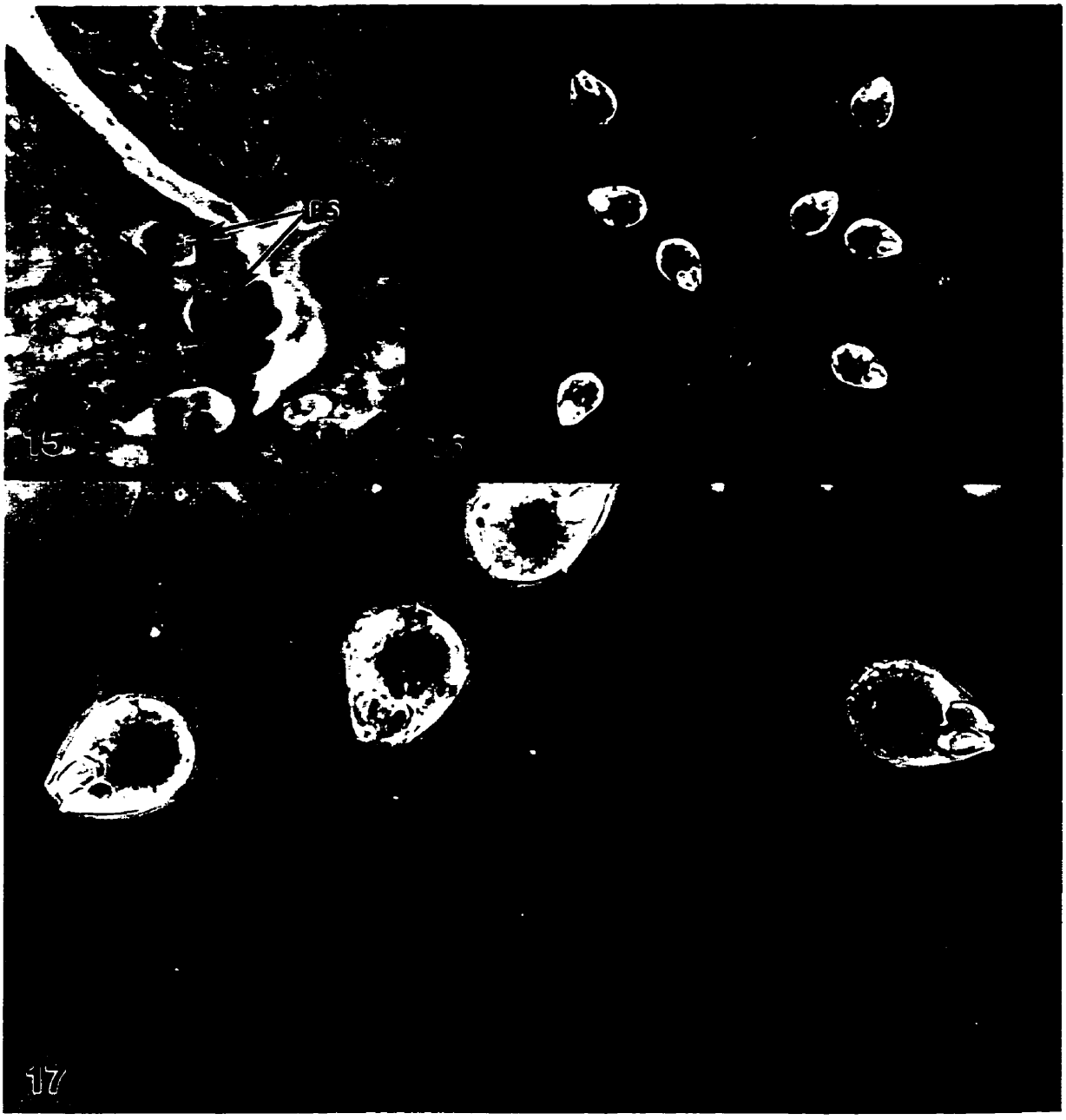
SM

Figures 15-17. Photographs of new forms of neoactinomyxum and antonactinomyxon. Fig. 15, cross-section of *Limnodrilus hoffmeisteri* infected with the form of neoactinomyxum, showing the pansporocysts (PS) developed in the intestinal epithelial cells of the worm, x 410; Fig. 16, a 3-dimensional reticulum (phase contrast) of the form of antonactinomyxon, formed by 8 spores, x 420; Fig. 17, a portion of a 3-dimensional reticulum (DIC) of the form of antonactinomyxon, showing the free pointed ends and the way in which the 4 processes are joined, x 880.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CHAPTER 6

The Longevity of Actinosporean Spores and their Reaction to Fish Mucus

ABSTRACT

During the study of biological characteristics of actinosporeans, longevity of the spores of 7 forms of actinosporeans and the spore reaction to fish mucus of 6 forms of actinosporeans from oligochaetes of Lake Sasajewun, Algonquin Park were investigated. The maximum longevity of actinosporean spores kept at ambient temperatures was 14 days. Spore longevity differed from 11 to 14 days among actinosporeans. Reaction to fish mucus of spores varied among actinosporeans. Triactinomyxon 'F' only reacted to the mucus of the common shiner, whereas the aurantiactinomyxon form and raabeia 'B' reacted readily to mucus of all fish species tested. The differences in reaction to fish mucus among actinosporeans may indicate their different host range. The results suggested that the short-lived actinosporean spores find their hosts by the chemodetection and that their portal of entry is the outer surface of their hosts.

INTRODUCTION

Since the discovery of actinosporeans by Stolc (1899), only 40 species (Marques, 1984; Wolf & Markiw, 1984) have been documented based on the conventional classification scheme. Wolf and Markiw's hypothesis (1984), that the actinosporeans of oligochaetes and the myxosporeans of fish are alternating stages of the same organisms, renewed interest in these parasites. However, most of the recent studies have concentrated on the completion of the myxozoan life cycle under laboratory conditions (see Kent et al., 1994; Uspenskaya, 1995; Yokoyama, 1997), with the exceptions of Markiw (1992) who investigated the longevity of the infective triactinomyxon stage of *Myxobolus cerebralis*, and Yokoyama et al. (1993, 1995) who studied some of the biological characteristics of actinosporeans of the oligochaete, *Branchiura sowerbyi*. In this chapter, the longevity of actinosporean spores and their reaction to the mucus of fish from Algonquin Park, Ontario were investigated in order to gain a better understanding of their transmission mechanism and the host specificity of their corresponding stages.

MATERIALS AND METHODS

Collection of actinosporean spores

A total of 14,100 worms from 19 species of oligochaetes collected from various sites in Lake Sasajewun were examined for actinosporean parasites from 1995 to 1997 (for details, see Xiao & Desser, 1998). Oligochaetes were checked on alternate days using the cell-well plate method (Yokoyama et al., 1991) over a 4-week period. When oligochaetes were found releasing actinosporean spores under a dissecting microscope, these worms were transferred to separate cell wells. Freshly released spores were collected daily and spores that were 1 day old or younger were used in the following investigations:

Longevity of water-borne spores

Preliminary observations revealed that all actinosporean spores lose their sporoplasms within 3 weeks after being released into water. The absence or presence of a sporoplasm was determined by phase-contrast microscopy, and spores containing sporoplasms were defined as viable. Freshly released spores of the neoactinomyxum form, aurantiactinomyxon form, antonactinomyxon form, triactinomyxon 'D' and 'F', raabeia "B', and echinactinomyxon 'C' were transferred to separate cell wells with lake water and kept at ambient temperatures which ranged from 10 to 22 °C. More than 100 spores of each form were examined daily to determine their viability.

Reaction to fish mucus

Fresh mucus was collected from the skin of the brown bullhead, *Ameiurus nebulosus*, yellow perch, *Perca flavescens*, pumpkinseed, *Lepomis gibbosus*, creek chub, *Semotilus atromaculatus*, golden shiner, *Notemigonus crysoleucas*, common shiner, *Luxilus cornutus*, white sucker, *Catostomus commersoni*, from Lake Sasajewun, and fathead minnow, *Pimephales promelas*, from Swan Lake. Each sample of fresh mucus was applied onto a clean glass slide and mixed with a drop of lake water. Suspensions of freshly released spores of the neoactinomyxum form, aurantiactinomyxon form, triactinomyxon 'C' and 'F', raabeia 'B', and echinactinomyxon 'C' were added to each of the different mucus preparations and examined by light microscopy to determine their reaction. Spore suspensions in lake water without fish mucus served as a control. One hundred spores of each form were examined and the number of spores that released their sporoplasms in reaction to the fish mucus after 10 minutes was given as a percentage and referred to as the measure of the intensity of the reaction.

RESULTS

Longevity

Seven days after being released into water, approximately 50% of the actinosporean spores lost their sporoplasm. The longevity differed slightly among species (Table 1). For example, echinactinomyxon 'C' remained viable for 11 days, in contrast to the neoactinomyxum form which persisted for 14 days.

Reaction to fish mucus

Spores that reacted to the mucus of fish extruded their polar filaments and released their sporoplasmic mass in approximately 1 minute. The released sporoplasm exhibited ameboid movement in the mucus solution. When spores of the aurantiactinomyxon form and raabeia 'B' came into contact with the mucus of all fish species tested, there was an instantaneous release of their sporoplasmic mass. In contrast, other actinosporeans examined did not react to the mucus of all fish species tested. For example, triactinomyxon 'F' only reacted to the mucus of the common shiner (Table 2).

The intensity of spore reactions to the same fish mucus varied among the actinosporeans examined. All of the actinosporeans examined reacted to the mucus of the golden shiner, with intensities of reaction greater than 90% observed for the neoactinomyxum form, aurantiactinomyxon form, triactinomyxon 'C', and raabeia 'B', 62% for echinactinomyxon 'C', and 12% for triactinomyxon 'F'. Reaction intensities of the same actinosporean form to mucus from different fish species also varied. For example, spores of raabeia 'B' exhibited an intensity of reaction greater than 90% when exposed to the mucus of the fathead minnow, golden shiner, and common shiner, while it was only 70% for yellow perch, pumpkinseed, and white sucker, and 51% for creek chub and brown bullhead (Table 2).

DISCUSSION

From this study, it was concluded that the actinosporean spores released into the external environment were a short-lived stage that remained viable for approximately 7 to 10 days under ambient temperatures, in contrast to their counterpart myxosporean spores which may remain viable for several months (Hoffman & Putz, 1969; El-Matbouli & Hoffmann, 1991). The longevity of the released actinosporean spores in this study was consistent with the findings of Ratliff (1983), who showed that water containing some unknown infective agent of *Ceratomyxa shasta* kept its infectivity to fish for 7 days, and Markiw (1992), who demonstrated that the actinosporean stage of *Myxobolus cerebralis* persisted for 5 days at 12.5 °C. The longevity of actinosporean spores in this study was slightly shorter, probably due to the maintenance of the spores at their normal temperatures which ranged from 10 to 22 °C, in contrast to the results of the study by Yokoyama et al. (1993) where spores were kept at a constant temperature.

Longevity was defined as the period of time from the release of the spores into the water until they released their sporoplasms (Yokoyama et al., 1993). Therefore, the period of infectivity of the released actinosporean spores to fish may be overestimated if it is assumed to be comparable to their longevity in the true sense of the term. The infectivity of the released actinosporean spores remains to be studied.

It is believed that susceptible fish are infected by myxozoans either through making contact with waterborne actinosporean spores or by ingestion of oligochaetes harboring actinosporeans (Wolf & Markiw, 1984; El-Matbouli & Hoffmann, 1989). In this study, the exhibited response to fish mucus of actinosporean spores supports the notion that chemodetection may play a role in the host-finding mechanism of actinosporean spores, and that their portal of entry is the

outer surface of fish (Markiw, 1989; Yokoyama et al., 1993; Uspenskaya, 1995; El-Matbouli et al., 1995).

The differences observed in the reaction intensities to the mucus of the same species of fish among actinosporeans indicate that the susceptibility of a species of fish probably differs with different actinosporeans. Although the reaction of an actinosporean form to the mucus of a species of fish does not guarantee the establishment of infection in that host, the results of the reaction to the mucus of fish may indicate the potential host range of an actinosporean form. This potential host range will facilitate the host selection for future experimental transmission of actinosporeans to fish.

In conclusion, data from this study have shown that actinosporean spores released into water are short-lived, and that they respond to certain chemical substance(s) in the fish mucus. When spores come into contact with this stimulus, polar filaments are extruded to anchor the spore to the host. This is followed by the release of their sporoplasms, and subsequently the invasion of the fish host by the sporoplasms.

Table 1. The longevity of actinosporean spores (percentage of viable spores after their release)

Actinosporeans	Days post-release 1	2	4	6	8	10	12
neoactinomyxum form	97	95	90	68	37	12	2
aurantiactinomyxon form	96	92	85	65	29	8	0
antonactinomyxon form	96	92	87	69	40	9	1
triactinomyxon 'D'	97	93	86	66	32	9	1
triactinomyxon 'F'	98	90	85	69	27	10	0
raabeia 'B'	97	91	86	65	33	7	0
echinactinomyxon 'C'	97	91	84	63	30	5	0

Table 2. Reaction of actinosporean spores to mucus from different species of fish (percentage of spores with sporoplasmic mass discharged)

	Fish species								
Actinosporeans	Ctl	YP	CC	FM	PS	GS	CS	BB	WS
neoactinomyxum form	3	10	59	92	100	92	85	84	88
aurantiactinomyxon form	4	97	95	92	95	94	98	78	93
triactinomyxon 'C'	2	2	12	93	7	95	92	3	2
triactinomyxon 'F'	2	8	2	6	4	12	77	2	2
raabeia 'B'	3	69	52	91	70	91	91	51	70
echinactinomyxon 'C'	3	51	11	8	99	62	10	86	78

Abbreviations: Ctl: control; BB: brown bullhead, *Ameiurus nebulosus*; YP: yellow perch, *Perca flavescens*; PS: pumpkinseed, *Lepomis gibbosus*; CC: creek chub, *Semotilus atromaculatus*; GS: golden shiner, *Notemigonus crysoleucas*; CS: common shiner, *Luxilus cornutus*; WS: white sucker, *Catostomus commersoni*; FM: fathead minnow, *Pimephales promelas*.

CHAPTER 7

The Oligochaetes and their Actinosporean Parasites in Lake Sasajewun, Algonquin Park, Ontario

(Adapted from Xiao, C. & Desser, S.S. 1998. The oligochaetes and their actinosporean parasites in Lake Sasajewun, Algonquin Park, Ontario. J. Parasitol., **84**, 1020-1026).

ABSTRACT

There is little ecological information on actinosporean parasites and their oligochaete hosts. Between 1995-1997, about 14,100 oligochaetes belonging to 19 species were collected from Lake Sasajewun. The oligochaete fauna consisted of 5 tubificid, 10 naidid, 3 enchytraeid, and 1 lumbriculid species. The most widely distributed species in the lake were *Limnodrilus hoffmeisteri*, *Dero nivea*, and *Stylaria lacustris*, with *L. hoffmeisteri* being the most prevalent. The diversity and abundance of worms decreased with increased water depth. Four species, *L. hoffmeisteri*, *Tubifex tubifex*, *Rhyacodrilus coccineus*, and *Lumbriculus variegatus*, harbored actinosporean parasites and were distributed along shallow areas of the lake shore where the sediment was comprised mainly of clay-mud, with the shoregrass, *Littorella americana*. The actinosporean parasites of the oligochaetes belong to 25 forms of 8 collective groups. Triactinomyxon 'F' was the most prevalent form, whereas triactinomyxon, raabeia, and echinactinomyxon were the most speciose groups. The overall prevalence of actinosporeans was about 1%. Water-borne spores of different actinosporean forms showed distinct temporal patterns. Spores of triactinomyxon 'F' occurred throughout the sampling season, whereas spores of other forms occurred only at certain times. The presence of water-borne spores peaked from late June to August when water temperatures ranged from 18 to 24 C, and coincided with the feeding and growing season of larval fish.

INTRODUCTION

Actinosporean parasites of annelids have been known since 1899, when Stolc described the first species from a tubificid oligochaete. Until recently, the Actinosporea has been conventionally recognized as 1 of 2 classes of the phylum Myxozoa, members of which are small multicellular, spore-forming parasites in the kingdom Protista (Corliss, 1984). Although direct life cycles of several actinosporeans of the genus *Triactinomyxon* have been described (Mackinnon and Adam, 1924; Janiszewska, 1955, 1957), direct infectivity from worm to worm has never been demonstrated unequivocally. The other class of the Myxozoa, Myxosporea, which are mainly parasites of fish, has been equally controversial as the purported fish-to-fish transmission has not been substantiated (Lom and Dykova, 1992; Kent et al., 1993) except in a recent report by Diamant (1997).

Wolf and Markiw (1984) demonstrated that *Myxobolus cerebralis* of salmonid fishes requires transformation of the myxosporean spore into an actinosporean stage, resembling members of the genus *Triactinomyxon* in the oligochaete *Tubifex tubifex*. This cycle linking the 2 classes of Myxozoa was confirmed by El-Matbouli and Hoffmann (1989). About 15 species of myxosporeans have since been shown to undergo this alternation with an actinosporean stage in an aquatic oligochaete (see Kent et al., 1994; Uspenskaya, 1995; Andree et al., 1997) or a polychaete (Bartholomew et al., 1997). To date, with the exception of a few field observations (Burtle et al., 1991; Styer et al., 1991; Yokoyama et al., 1991, 1993), there have been no studies focused on the ecological associations of these parasites and their hosts.

Several forms of actinosporeans of the oligochaetes from Lake Sasajewun, Algonquin Park, Ontario, were recently described (Xiao and Desser, 1998a, 1998b). In this chapter, the associations among these parasites, their oligochaete hosts and habitats are presented.

MATERIALS AND METHODS

Study site

Lake Sasajewun (45°35'N, 78°30'W), an artificial lake with a surface area of 0.44 km^2 and a maximum depth of about 9 m, was originally a natural valley with the Madawaska River running through it (Fig. 1). The lake was established by the construction of a dam which raised the water level of the river and flooded the valley. For the purpose of this study, the lake was artificially delineated into 4 zones based on depth and aquatic vegetation: in-shore (A), shallow (B), intermediate (C), and deep (D) (Fig. 1; Table 1).

Sampling

Sediment samples from Lake Sasajewun were collected using an Ekman dredge (250 cm^2), a trowel or Hester Dendy Plates at least twice a wk from May to September over a 3-yr-period (1995 to 1997). Sediment samples were sieved through a 0.3 mm mesh, hand-sorted, and examined for oligochaetes. In the first yr, 102 sediment samples were collected by random sampling to define the distribution of oligochaetes. In the second and third yr, in addition to random sampling, intensive and repetitive samples were collected at a fixed sampling area that had yielded the highest numbers of oligochaetes during the first yr since more oligochaetes were required for the study of actinosporean parasites (Fig. 1; Table 2).

Mature worms were sorted with a dissecting microscope, and at least 3 to 5 mature worms of each taxon were preserved in 70% alcohol, coverslipped with CMCP mountant (Masters Chemical Company, Inc., Illinois, USA), identified (Brinkhurst, 1986), and used as references for the identification of live worms. Immature oligochaetes were identified to the generic level based on the characters of the chaetae and assigned to species corresponding to the prevalent mature individuals in the sample.

The water temperature of the lake was recorded daily at a depth of 0.8 m during the mo when sediment samples were taken. Samples of aquatic plants were collected and identified (Newmaster et al., 1997), and a survey of their distribution in the lake was subsequently conducted (Table 1). Samples of sediment types in the lake were also collected and classified.

Observation of actinosporean spores

Oligochaetes were kept in 24 x 2 ml cell-well plates (Yokoyama et al., 1991) with 2 worms of the same species in each well filled with lake water at ambient temperatures. The plates were examined microscopically every other day over a 4-wk-period, and when actinosporean spores were found in a well, the 2 worms were separated into individual cell-wells and examined daily. Spores were identified on the basis of successive observations of fresh wet mounts using differential interference contrast microscopy and Diff-Quik® stained smears of spore suspensions. Samples of infected oligochaetes were fixed in Bouin's solution for histological examination (for detailed methodology, see Xiao and Desser, 1998a).

The prevalence of infection was defined as the percentage of oligochaetes releasing mature spores into the water. The prevalence of infection in its strict sense (Margolis et al., 1982) could not be determined because immature stages infecting the oligochaetes could not be counted by the cell-well plate method (Yokoyama et al., 1991; Xiao and Desser, 1998a).

The temporal occurrence of water-borne spores was determined by the time spores appeared in the water of the cell-well plates. The water temperature in the plates corresponded closely with that of the lake.

RESULTS

Oligochaete faunal composition and distribution

During the study periods, 14,100 worms of 19 species belonging to 4 families, the Tubificidae, Naididae, Enchytraeidae, and Lumbriculidae, were collected (Table 1). The Tubificidae was represented by 5 species which were the most prevalent oligochaetes in the lake, and of these, *Limnodrilus hoffmeisteri* was the most prevalent over the entire lake, especially in the in-shore and shallow muddy areas (Zones A and B). *Rhyacodrilus coccineus* was also widespread from the in-shore areas to those of intermediate depth (Zones A to C). *Ilyodrilus templetoni* and *Rhyacodrilus* sp. were found only in the in-shore muddy areas (Zone A). *Tubifex tubifex* was also found here and in shallow muddy areas (Zone B).

The Naididae, represented by 10 species, was more speciose than the Tubificidae, but less prevalent at every sample site. The majority of these species of Naididae was found in the in-shore and shallow areas (Zones A and B). They were more prevalent in the shallow muddy and sandy areas (Zone B) than in the in-shore areas (Zone A). *Dero nivea* and *Stylaria lacustris* were found throughout the lake, whereas *Nais variabilis*, *Nais communis*, and *Arcteonais lomondi* were found in the in-shore and shallow areas, and areas of intermediate depth (Zones A to C).

The Enchytraeidae was represented by 3 species and found only in the in-shore areas (Zone A), especially in the areas close to the shoreline. The Lumbriculidae was represented by a single species, *Lumbriculus variegatus*, which was found only in the in-shore and shallow areas (Zones A and B).

A gradual reduction, not only in the number of species, but also in the relative abundance of the oligochaetes, was observed with increased water depth (Table 1). Seventeen species were found in the in-shore areas (Zone A), 14 species in the shallow areas (Zone B), 7 species in areas of intermediate depth (Zone C), and

only 3 species in the deep areas (Zone D).

Actinosporean fauna

Twenty-five forms of actinosporean parasites belonging to 8 collective groups were found in 4 of the 19 species of oligochaetes (Table 3). Three collective groups, triactinomyxon, raabeia, and echinactinomyxon, were the most speciose, whereas other groups were each represented by a single form. Among the 25 forms of actinosporeans in the lake, triactinomyxon 'F' was the most common species.

Actinosporean prevalence, host, and tissue specificity

During the 3-yr study, only 146 worms (15 in 1995, 110 in 1996, and 21 in 1997), of the 14,100 (1.04%) examined, were observed to release actinosporean spores. With respect to each sampling yr, the prevalences of 1995, 1996, and 1997 were 0.50%, 1.31%, and 0.98%, respectively. Of these 146 infected worms, there were 125 *L. hoffmeisteri*, 15 *T. tubifex*, 4 *R. coccineus*, and 2 *Lu. variegatus*. Two *L. hoffmeisteri* were found with mixed infections of triactinomyxon 'F' and echinactinomyxon 'C', and triactinomyxon 'F' and raabeia 'F', respectively. The prevalences of actinosporeans in *L. hoffmeisteri*, *T. tubifex*, *R. coccineus*, and *Lu. variegatus* were 1.9%, 1.1%, 0.5%, and 0.2%, respectively. The prevalence of individual actinosporean forms ranged from 0.02% to 0.67%. Triactinomyxon 'F' was the most prevalent, whereas *Triactinomyxon ignotum* and an form of aurantiactinomyxon were the least (Table 3). Among the 25 actinosporean forms recorded in the lake, 22 exhibited host specificity occurring in only 1 of the 4 infected oligochaete host species (Table 3). All forms parasitized only the intestinal epithelium.

Temporal fluctuation of oligochaetes and actinosporeans

The oligochaetes did not display apparent temporal fluctuations in the prevalence and the number of species during the sampling period of a single yr but there were differences in the prevalence over the 3-yr study period (Table 4). During the study period, 3,500 worms were collected from 102 sediment samples in

the first yr, 8,500 worms from 86 sediment samples in the second yr, and 2,100 worms from 35 sediment samples in the third yr. However, the prevalence and the occurrence of water-borne spores of different actinosporean forms varied from May to September with respect to the water temperature (Tables 3 and 5). In May, when the average water temperature was 12 C, spores belonging to 5 forms of actinosporeans were released from only 7 worms; in June, when the average water temperature was 20 C, spores belonging to 9 forms of actinosporeans were released from 18 worms; and in July and August, when the water temperatures averaged 23 C, spores peaked both in prevalence and number of forms. In September, when the average water temperature dropped to 16 C, spores decreased both in prevalence and number of forms (Tables 3, 5 and 6). Spores of all forms of actinosporeans occurred only at certain times with the exception of triactinomyxon 'F' which was found in *L. hoffmeisteri* throughout the sampling periods (Table 3), but which peaked in July (Table 7).

Habitat

There were 4 major types of sediments in Lake Sasajewun: fine (mainly silt and clay), detrital/woody debris (leaves, sticks, dead vegetation, rooted plants with some silt, clay and sand), sandy, and stony sediments. The first 2 contained 16 species of living vegetation (Table 1). The in-shore and shallow areas with fine silt and clay sediments were mainly occupied by the shoregrass, *Littorella americana*, 1 of 16 species of aquatic plants in the lake, and the burrowing species of the Tubificidae. *Limnodrilus hoffmeisteri* and *R. coccineus* were the most prevalent in this habitat. The in-shore and shallow areas with detrital/woody debris sediments were dominated by the Lumbriculidae, however, species of Enchytraeidae, Tubificidae, and Naididae also occurred in these habitats, but were rare. Only a few species of Tubificidae and Naididae occurred in the intermediate and deep areas with detrital/woody debris sediments with no one particular species being dominant. Species of Enchytraeidae,

Lumbriculidae, and Tubificidae also occurred in semi-aquatic areas along the shoreline with detrital/woody debris sediments containing leaves and rooted plants. These habitats were dominated by the Enchytraeidae, whereas species of Lumbriculidae and Tubificidae were rare. Although species of Naididae, Tubificidae and Lumbriculidae occurred rarely in areas with sandy and stony sediments in the in-shore and shallow zones, these habitats were dominated by Naididae.

Most infected oligochaetes were collected from the in-shore areas (Zone A) with silt and clay sediments, and the shoregrass, *L. americana*, where schools of larval minnows (mainly *Luxilus cornutus*, *Notemigonus crysoleucas*, and *Semotilus atromaculatus*), perch (*Perca flavescens*), and sunfish (*Lepomis gibbosus*) were often seen feeding.

DISCUSSION

Oligochaete composition and distribution

In this study 19 species of oligochaetes from Lake Sasajewun were identified, which belong to 15 genera (Table 1). The number of species and the overall prevalence declined with increased water depth. Seventeen species were present at a depth less than 0.5 m, 14 species at depths between 0.5 m and 1.5 m, and 7 species at depths between 1.5 m and 4.5 m, whereas only 3 species, *L. hoffmeisteri*, *D. nivea*, and *S. lacustris*, were present at depths greater than 4.5 m, albeit rare. This trend has also been observed in deep lakes (Bonomi, 1974; Probst, 1987; Casellato and Caneva, 1994), and may be attributed to the low oxygen concentration.

The number of species and prevalence of oligochaetes were also strongly influenced by sediment characteristics even in the same sampling zone, and changes in sediment parameters appeared to result in differences in the species composition

and prevalence. For example, all 4 types of sediments present in Zone A harbored worms which differed both in species composition and prevalence. Similar worm-sediment relations have also been observed in other lakes as well as in marine waters (Cook, 1971; Brinkhurst, 1974; Coates and Erseus, 1985; Diaz et al., 1987).

The modest density of oligochaetes in Lake Sasajewun (Table 4), as compared to natural lakes (Casellato and Caneva, 1994), may be attributed in part to the lack of an ideal substrate due to the presence of submerged bushes and trees rather than to the sedimentation of mineral particles (Lenat et al., 1981; Di Giovanni et al., 1996), as well as to the low planktonic productivity in artificial lakes (Bonacina et al., 1991).

Whereas there was no evidence for temporal changes in oligochaete species composition and prevalence during the sampling period of a single yr, there were large differences in prevalence amongst the 3-yr study (Table 4). These differences could not have been due completely to the intensive and repetitive collecting in the fixed sampling area, particularly for the 2 shallow zones, as these may have been due to the frequent artificial raising and lowering of the water level in the lake for flood protection.

Actinosporean composition, prevalence, and temporal fluctuation

Recent studies revealed a rich actinosporean fauna consisting of 25 forms in Lake Sasajewun (Xiao and Desser, 1998a, 1998b), when compared to the total of 39 previously described 'species' (reviewed by Marques, 1984; Wolf and Markiw, 1984). However, in our studies, the number of forms was low in all collective groups except for triactinomyxon, raabeia, and echinactinomyxon (Table 3). The number of actinosporean forms in Lake Sasajewun was less than half of the 56 recorded species of myxosporeans belonging to 8 genera from the same lake (Gowen, 1983; Li and Desser, 1985; Lom et al., 1989; Xiao and Desser, 1997). Despite this striking discrepancy between the numbers of actinosporeans and myxosporeans, the difference is much smaller than previous data had suggested. This may be due to

several factors including a direct life cycle for some myxosporeans (Diamant, 1997), polymorphism of myxosporeans in different fish hosts, and the extremely large sample size that would be required for a thorough survey of actinosporeans (Xiao and Desser, 1998b).

The prevalence of actinosporeans in oligochaetes from Lake Sasajewun corresponds with other data recorded for these parasites, which ranged from 0.1% to 4% (see Yokoyama et al., 1991). Although the prevalence of each form in the lake was low (0.02% to 0.66%) as determined by our methodology, the natural prevalence was probably higher as some infected worms may not have released spores during the time they were being observed in the cell-well plates.

The prevalence (Table 5) and the occurrence of water-borne spores (Table 6) of different forms of actinosporeans of oligochaetes from the lake showed similar temporal patterns, i. e., prevalence and number of forms of water-borne spores were low in the spring and early summer when the water temperature was low, and high during mid- to late-summer when the water temperature was high. However, with respect to each form, the temporal pattern of water-borne spores varied. Water-borne spores of triactinomyxon 'E' and a form of guyenotia occurred only in relatively cool water, whereas spores of other species were found only in warm water. Spores of triactinomyxon 'F' were found throughout the sampling periods, with their numbers peaking in warm water during mid-summer. These observations may suggest that each form has its optimal range of water temperature and that most develop to the mature spore stage when water temperature is high, which coincides with the feeding and growing season of larval minnows in the lake (Scott and Crossman, 1973). A similar synchronization of the peak of water-borne actinosporean spores of *Myxobolus cerebralis* and the emergence of larval rainbow trout was observed by Vincent (see Potera, 1997). Therefore, the synchronization between the feeding and growing season of susceptible larval fish and the

maturation of spores of most forms of actinosporeans may be the result of selective pressures on both phases of the myxozoan parasites.

Host, parasite, and habitat associations

The oligochaetes and aquatic plants showed a similar distribution pattern which is influenced by the water depth, the types of sediments, as well as other environmental factors. The shoregrass, *L. americana*, served as a reliable indicator of fine silt and clay sediments in the in-shore area, and also of a high prevalence of burrowing tubificid oligochaetes. The same areas served as prime feeding sites for larval fish, which probably become infected by either ingesting worms harboring actinosporeans or contacting water-borne actinosporean spores. These complex relationships within the habitat reflect the integrated results of the co-evolved responses by the parasites, their host, and their environments.

Like other actinosporean parasites (Marques, 1984; El-Matbouli and Hoffmann, 1989; Kent et al., 1993), those in Lake Sasajewun exhibited host and tissue specificity, with sporogonic development occurring exclusively in the intestinal epithelium of their annelid hosts. Actinosporean spores are thought to be released into the gut lumen and excreted into the water. Exceptional situations reported recently, include sporogenesis of the actinosporean stage of *Ceratomyxa shasta* in the epidermal layer of its polychaete host (Bartholomew et al., 1997), and sporogenesis of *Sphaeractinomyxon ersei* in the coelom of its oligochaete host (Hallett et al., 1998).

The planktonic nature of water-borne spores of actinosporeans permits a greater dispersal capacity by currents, which would result in their distribution throughout a water body. The fragility and short life of water-borne spores (Marques and Ormieres, 1982; Yokoyama et al., 1993) are counteracted by the production of numerous spores by each infected worm (Yokoyama et al., 1991) and the synchronization between the maturation of actinosporean spores and the feeding and growing season of larval fish.

Figure 1. Map of Lake Sasajewun showing the 4 zones, A, B, C, and D, and the fixed sampling area.

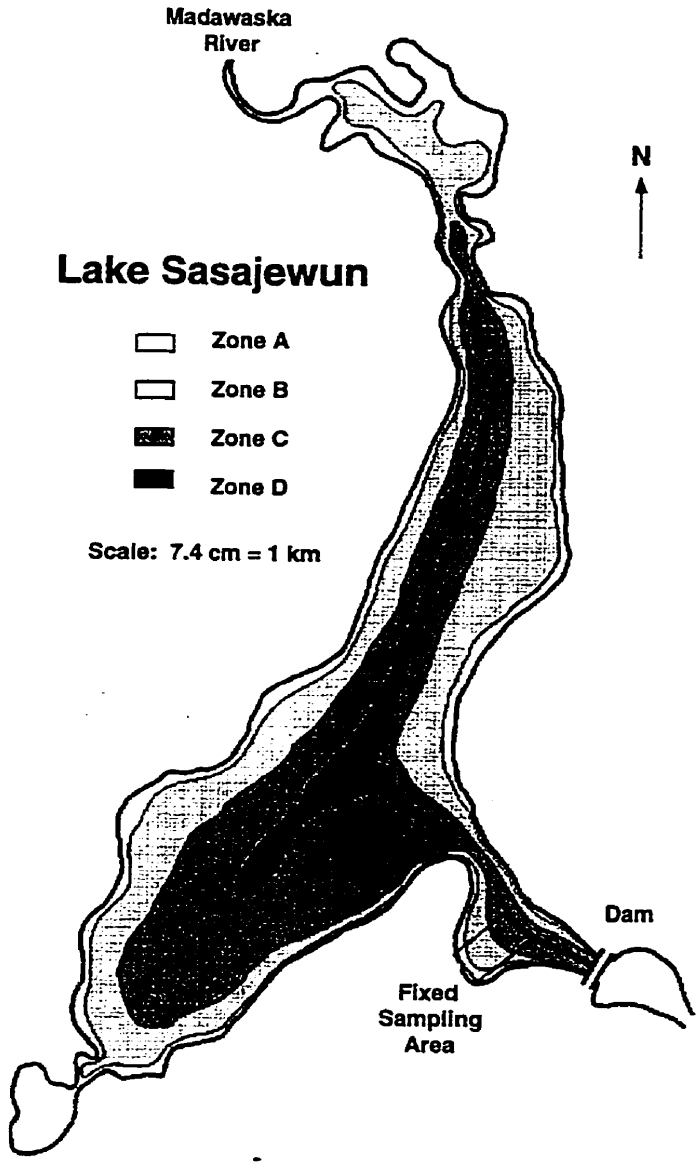

Madawaska
River
N
Lake Sasajewun
Zone A
Zone B
Zone C
Zone D
Scale: 7.4 cm = 1 km
Dam
Fixed
Sampling
Area

Table 1. Distribution of oligochaetes and aquatic plants in 4 zones of Lake Sasajewun

	Zone A	Zone B	Zone C	Zone D	
Water Depth (m)	0-0.5	0.5-1.5	1.5-4.5	4.5-9.0	Water Depth (m)
Oligochaetes					**Aquatic plants**
Family Tubificidae					
Rhyacodrilus coccineus	+, x	+, x	+		*Glyceria borealis*
Rhyacodrilus sp.	+, x				*Carex utriculata*
Limnodrilus hoffmeisteri	+, x	+	+	+	*Dulichium arundinaceum*
Tubifex tubifex	+, x	+, x			*Scirpus subterminalis*
Ilyodrilus templetoni	+, x				*Littorella americana*
Family Naididae					
Uncinais uncinata	+, x	+, x			*Sagittaria latifolia*
Dero digitata	+, x	+, x			*Sparganium emersum*
D. nivea	+, x	+, x	+	+	*S. fluctuans*
Vejdovskyella comata	x	+, x			*Brasenia schreberi*
Nais variabilis	+, x	+	+		*Nuphar variegatum*
N. communis	+, x	+, x	+		*Nymphaea odorata*
Stylaria lacustris	+, x	+, x	+, x	+	*Potamogeton amplifolius*
Arcteonais lomondi	x	+, x	+, x		*P. gramineus*
Ripistes parasita	+, x	+, x			*P. epihydrus*
Slavina appendiculata	+, x	+, x	x		*Ceratophyllum demersum*
Family Enchytraeidae					
Cognettia glandulosa	+, x	x			*Utricularia vulgaris*
C. sphagnetorum	+				
Marionina riparia	+				
Family Lumbriculidae					
Lumbriculus variegatus	+	+			

+: presence of the oligochaete species; x: presence of the aquatic plant.

Table 2. Number of sediment samples from the 4 zones of Lake Sasajewun from 1995 to 1997

	Zone A	Zone B	Zone C	Zone D
1995	32 (12)	27 (7)	24 (4)	19 (3)
1996	32 (22)	24 (14)	16 (6)	14 (4)
1997	21 (19)	6 (4)	4 (2)	4 (2)

Total number of samples (number of samples taken from fixed sampling area).

Table 3. Prevalence and temporal occurrence of actinosporean parasites of oligochaetes from Lake Sasajewun

Forms of actinosporeans	Worm hosts	Prevalence (%)	Temporal occurrence of water-borne spores
triactinomyxon 'A' Xiao and Desser, 1998	*Limnodrilus hoffmeisteri*	0.06	July, August
triactinomyxon 'B' Xiao and Desser, 1998	*L. hoffmeisteri*, *Tubifex tubifex*	0.03	July
triactinomyxon 'C' Xiao and Desser, 1998	*L. hoffmeisteri*	0.09	June to August
Triactinomyxon ignotum Stolc, 1899	*L. hoffmeisteri*	0.02	June
triactinomyxon 'D' Xiao and Desser, 1998	*L. hoffmeisteri*	0.06	July, August
triactinomyxon 'E' Xiao and Desser, 1998	*L. hoffmeisteri*, *T. tubifex*	0.03	May
Triactinomyxon dubium Granata, 1924	*T. tubifex*	0.14	May, August
triactinomyxon 'F' Xiao and Desser, 1998	*L. hoffmeisteri*, *Rhyacodrilus coccineus*	0.66	May to September
raabeia 'A' Xiao and Desser, 1998	*L. hoffmeisteri*	0.05	July, August
raabeia 'B' Xiao and Desser, 1998	*L. hoffmeisteri*	0.05	August, September
raabeia 'C' Xiao and Desser, 1998	*L. hoffmeisteri*	0.03	June, July
raabeia 'D' Xiao and Desser, 1998	*T. tubifex*	0.14	July, August
raabeia 'E' Xiao and Desser, 1998	*T. tubifex*	0.07	July
raabeia 'F' Xiao and Desser, 1998	*L. hoffmeisteri*	0.05	July

Echinactinomyxon radiatum Janiszewska, 1957	*T. tubifex*	0.07	June
echinactinomyxon 'A' Xiao and Desser, 1998	*L. hoffmeisteri*	0.12	May to August
echinactinomyxon 'B' Xiao and Desser, 1998	*L. hoffmeisteri*	0.03	July, August
echinactinomyxon 'C' Xiao and Desser, 1998	*L. hoffmeisteri*	0.34	June to August
echinactinomyxon 'D' Xiao and Desser, 1998	*L. hoffmeisteri*	0.24	July, August
echinactinomyxon 'E' Xiao and Desser, 1998	*L. hoffmeisteri*	0.03	August
neoactinomyxum form Xiao and Desser, 1998	*L. hoffmeisteri*	0.03	August, September
aurantiactinomyxon form Xiao and Desser, 1998	*L. hoffmeisteri*	0.02	July
guyenotia form Xiao and Desser, 1998	*Lumbriculus variegatus*	0.17	May, August
synactinomyxon form Xiao and Desser, 1998	*T. tubifex*	0.35	June, July
antonactinomyxon form Xiao and Desser, 1998	*T. tubifex*	0.14	June

Table 4. Temporal density of oligochaetes (individuals/m^2) and total number of worms harvested per year from Lake Sasajewun, between 1995 and 1997

	May	June	July	August	September	TW
1995	1,627	1,713	1,525	1,694	1,585	3,500
1996	2,591	2,264	2,438	2,342	NA	8,500
1997	1,749	1,826	1,658	1,733	NA	2,100

Based on an average of pooled density of fine sediment samples taken by Ekman dredge from Zones A and B. TW: total worms harvested. NA: data not available.

Table 5. Temporal overall prevalence (%) of actinosporeans of oligochaetes from Lake Sasajewun from 1995 to 1997

	May	June	July	August	September
1995	0.22	0.47	1.01	0.33	0.43
1996	0.64	0.90	1.62	1.23	NA
1997	0.13	1.17	2.82	0.87	NA
AT (°C)	12.7	20.9	21.6	21.7	16.7
NW	7	18	76	42	3

NA: data not available; AT: average water temperature; NW: number of infected worms.

Table 6. Temporal occurrence of water-borne actinosporean spores of oligochaetes from Lake Sasajewun from 1995 to 1997

	May	June	July	August	September
1995	triactinomyxon 'F'	triactinomyxon 'F', *T.ignotum*; raabeia 'C'	triactinomyxon 'A', 'B' and 'F'; echinactino-myxon 'C'	triactinomyxon 'A', 'D' and 'F'	Triactinomyxon 'F'; raabeia 'B'; neoactinomy-xum
1996	triactinomyxon 'E' and 'F', *T. dubium*; guyenotia; echinactino-myxon 'A'	triactinomyxon 'C', 'E' and 'F'; echinactino-myxon 'A' and 'C', *E. radiatum*; synactinomyxon; antonactino-myxon	triactinomyxon 'A', 'B', 'C', 'D' and 'F'; raabeia 'A', 'C', 'D', 'E' and 'F'; echin-actinomyxon 'A', 'B', 'C' and 'D'; synactinomyxon; aurantiactino-myxon	triactinomyxon 'C', 'D' and 'F'; raabeia 'A', 'B' and 'D'; echinactino-myxon 'A', 'B', 'C', 'D' and 'E'; neoactino-myxum	NA
1997	triactinomyxon 'F'	triactinomyxon 'C' and 'F'	triactinomyxon 'A', 'C', 'D' and 'F'; echinactino-myxon 'A' and 'C'	triactinomyxon 'F', *T. dubium*; echinactino-myxon 'D'; guyenotia	NA

NA: data not available.

Table 7. Temporal occurrence of water-borne spores of triactinomyxon 'F' from *Limnodrilus hoffmeisteri* of Lake Sasajewun

	Prevalence (%)				
	May	June	July	August	September
1995	0.33	0.34	0.64	0.34	0.29
1996	0.30	0.65	1.04	0.21	NA
1997	0.22	1.43	3.13	1.00	NA
Average	0.28	0.66	1.16	0.38	0.29

NA: data not available.

CHAPTER 8

Molecular Characterization of Myxozoan Parasites by Riboprinting

ABSTRACT

The SSU-rRNA genes of 18 myxozoans from Lake Sasajewun, Algonquin Park were amplified and digested with restriction endonucleases for riboprinting analysis. Identical riboprints were not found between the myxosporeans and the actinosporeans involved. The distinct riboprinting patterns observed among these myxozoans indicate considerable genetic diversity within this group. Identical riboprints were found for the myxosporeans, *Myxobolus pendula* and *Myxobolus pellicides*, and for the actinosporeans triactinomyxon 'C' and triactinomyxon ignotum, suggesting a close evolutionary relationship between them. Parsimony analysis of the riboprint data demonstrated that the myxosporeans and the actinosporeans have not formed monophyletic clades, but together formed a single monophyletic group. Some species of *Myxobolus* appear to be more closely related to forms of triactinomyxon, echinactinomyxon or raabeia than to other *Myxobolus* species. These results are consistent with the hypothesis that myxosporeans and actinosporeans are alternating stages of the same organisms.

INTRODUCTION

Since the pioneering work of Wolf and Markiw (1984), there has been growing evidence that myxosporeans and actinosporeans represent different developmental stages of myxozoans (see Kent et al., 1994; Uspenskaya, 1995; Trouillier et al., 1996; Yokoyama, 1997). This relationship has also been supported by recent molecular analyses (Andree et al., 1997; Bartholomew et al., 1997). However, each of the studies on the matching of myxosporeans and actinosporeans has focused on the completion of the life cycle of a single myxozoan species by experimental transmission under laboratory condotions. Although this approach provides accurate matching between the myxosporean and actinosporean stages of a single species, it is not practical for studying many myxozoan species in a complex ecological setting. One approach to circumvent this problem, albeit expensive and labor intensive, has been the use of the sequences of small subunit ribosomal DNA (SSU-rDNA) and other genes of myxosporeans and actinosporeans.

"Riboprinting", a term coined by Clark (1992, 1997) to describe his modification of a DNA fingerprinting technique using PCR-amplified ribosomal DNA, has been used to study many protistan parasites (Clark & Diamond, 1991, 1992, 1997; De Jonckheere, 1994; Brown & De Jonckheere, 1994; Clark et al, 1995; Pomport-Castillon et al., 1997) and fungi (Molina, Inoue & Jong, 1992; Molina, Shen & Jong, 1992). This technique allows for the rapid examination of numerous species by indirect sampling of 10-12% of a SSU-rDNA sequence. Riboprints have been shown to be diagnostic for the species being studied (Clark, 1997).

Myxozoan parasites in Lake Sasajewun, Algonquin Park have been documented from their fish and oligochaete hosts. Fifty-six species of myxosporeans have been described from fish (Gowen, 1983; Li & Desser, 1985; Lom et al., 1989; Xiao & Desser, 1997), and 25 forms of actinosporeans from oligochaetes

in the lake (Xiao & Desser, 1998a, 1998b). In this chapter, 9 myxosporeans from 3 genera and 9 actinosporeans from 6 collective groups (Kent et al., 1994; Lom et al., 1997) in Lake Sasajewun, Algonquin Park were examined using the riboprinting technique.

MATERIALS AND METHODS

Isolation and Purification of Spores

Spores of the myxosporean stage from fish and the actinosporean stage from oligochaetes were obtained from Lake Sasajewun, Algonquin Park (Table 1). Cysts of myxosporean species, containing mature spores, were excised from their fish hosts. Spore aggregates of non-cyst-forming histozoic species were isolated from the host tissues. Spores of the coelozoic species, *Myxidium* sp. and *Chloromyxum trijugum*, were collected from infected gall bladders. These spores were separated from fish tissues and preserved in 70% ethanol before DNA extraction. For the actinosporean stages, spore-releasing worms were rinsed with distilled water several times to reduce contamination prior to placing them into clean wells filled with distilled water. Water-borne spores of each species were collected using the cell-well plate method (Yokoyama et al., 1991), concentrated by centrifugation, and either processed immediately for DNA extraction or preserved in 70% ethanol.

Genomic DNA Extraction

Ethanol preserved spores of the myxosporean stages were washed twice with 1X PBS and resuspended in STE buffer with 1% SDS. These spores were boiled 10 min and plunged into liquid nitrogen 3 times in order to release their contents, digested using proteinase K at 37 °C for 2-3 hr, and extracted using phenol : chloroform.

Spores of the actinosporean stages were processed similarly to those of the myxosporean stages with slight modifications. The spores were boiled 10 min and plunged into liquid nitrogen only once. Then, they were either digested with proteinase K for 4-5 hr and extracted with phenol : chloroform, or processed using the IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, WA, USA).

PCR Amplification

The SSU-rDNA of the myxozoans was amplified using forward primer 5'-CCGAATTCCAGCTGCAGATCTGGTTGATCCTGCCAGT-3' and reverse primer 5'-CCAAGCTTCCGCTGCAGGATCCTTCCGCAGGTTCACCT-3'. This primer pair was modified from the universal 18S rDNA primers (Medlin et al., 1988; Clark & Diamond, 1991). PCR reactions were performed in a 100 µl volume consisting of 2 µl of DNA sample (about 150 ng genomic DNA/µl), 4 µl of each primer (5 µM/µl), 8 µl of 25 mM dNTP solution, 10 µl of 10X PCR reaction buffer (containing 500 mM KCl, 15 mM $MgCl_2$, and 100 mM tris-HCl. Pharmacia Biotech, Quebec, Canada), 0.5 µl of 5 units/µl Taq polymerase (Pharmacia Biotech, Quebec, Canada), and 71.5 µl ddH_2O. Amplification conditions were as follows: 7 min initial melt at 94 °C, then cycled 1 min at 94 °C, 1 min at 56 °C and 2 min at 72 °C for 35 to 40 cycles.

Purification of PCR Products and Riboprinting

The PCR products were run on 1.5% agarose gels in 1X TAE, and the SSU-rDNA fragments of myxozoans (about 2.1-2.2 kb) were purified using the Geneclean II Kit (Bio 101 Incorporated, La Jolla, California, USA). Restriction endonuclease analysis of the purified myxozoan SSU-rDNA were performed according to the instructions of the supplier. Nine arbitrarily chosen endonucleases were used: Alu I, Dde I, Eco RI, Hha I, Hind III, Hinf I, Rsa I, Sau 3A I, and Taq I. The digested SSU-rDNA were separated in 2% agarose gels using 1X TBE, and stained with ethidium bromide. Riboprint gels were photographed and fragment sizes were estimated

using molecular size markers run on the same agarose gels. To verify fragment comigration, purified SSU-rDNA from at least 9 different species were digested with the same restriction endonuclease and separated in adjacent lanes on the same gel.

Riboprint analysis

Riboprints of the myxozoans studied, with each fragment being considered a character, were used for parsimony analysis using: 1) the MIX program (parsimony) of the PHYLIP package (Felsenstein, 1993, version 3.5c) with its default settings except for the randomizing input order of species where the random number seed was 17 and the number of randomizations was 100, and 2) PAUP3.1.1 (Swofford, 1993) with the branch-and-bound search option. Uncut purified SSU-rDNA amplification products were not considered as comigrating fragments. Likewise, fragments measuring less than 100 basepairs (bp) in length were not included as they were not always visible on all riboprints. Although fragment comigration analysis makes the assumption that comigrating fragments are homologous, this is not always the case. This inherent error should not significantly affect the results as it occurs rarely and randomly within the most conserved region of the ribosomal gene (Clark & Diamond, 1997). Since the relationship among the taxa involved is unknown, none of the taxa could be used as the outgroup with certainty, so the analyses were performed using the default outgroup setting. The generated topologies were unrooted trees.

RESULTS

PCR amplified SSU-rDNA fragments of similar length were obtained from the different myxozoan species (Fig. 1). The riboprints of these purified PCR-amplified SSU-rDNA showed many differences except between *Myxobolus pendula* and *Myxobolus pellicides*, and between triactinomyxon 'C' and triactinomyxon

ignotum (Figs. 2, 3). The riboprints of *Myxobolus pendula* were identical to those of *Myxobolus pellicides* with the endonucleases that were utilized. Also, all riboprints of triactinomyxon 'C' were identical to those of triactinomyxon ignotum. Identical riboprints were not found between the myxosporeans and the actinosporeans.

Analysis of the riboprints (Fig. 3; Appendix I) yielded a single most parsimonious tree (Fig. 4) by using either the MIX program of the PHYLIP package or PAUP3.1.1. The most parsimonious tree had a consistency index of 0.384 (excluding uninformative characters), a retention index of 0.413, and a length of 401 steps. The results of these analyses suggest that species of *Myxobolus* are closely related to forms of triactinomyxon, echinactinomyxon and raabeia, and that neither the myxosporeans nor the actinosporeans form a monophyletic group.

DISCUSSION

The distinct riboprinting patterns observed among the myxozoan species in this study indicate considerable genetic diversity in these parasites. The heterogeneity in the SSU-rDNA as found in this study has also been noted in other taxa of myxozoans by SSU-rDNA sequence comparisons (Schlegel et al., 1996; Hervio et al., 1997). With marked differences in the SSU-rDNA region, myxozoans were referred as "fast clock" organisms with respect to the evolution of the ribosomal gene (see Kent et al., 1996).

Among members of the conventional myxosporeans, the riboprints of the SSU-rDNA of *M. pendula* (Guilford, 1967) and *M. pellicides* Li and Desser, 1985 were identical. Although these species form cysts in the mucous membrane of the gill arches of the creek chub, *Semotilus atromaculatus*, their spores exhibit distinct morphological differences. Riboprints of the SSU-rDNA of triactinomyxon 'C' Xiao and Desser, 1998 and triactinomyxon ignotum Stolc, 1899 were also found to be

identical. There are also morphological differences between their spores. Since the SSU-rDNA is the most evolutionarily conserved region of the ribosomal gene and closely related species often do not show differences in this region (Hillis and Dixon, 1991), consequently, *M. pendula* and *M. pellicides*, and triactinomyxon 'C' and triactinomyxon ignotum may be closely related species, respectively. Since only 10-12% of the SSU-rDNA was indirectly sampled by riboprinting, more extensive studies, such as experimental transmissions and sequencing of the SSU-rDNA, are necessary before any definitive conclusions can be drawn.

Riboprints were not identical between any myxosporeans and actinosporeans suggesting that none of the 9 actinosporeans in this study corresponded with any of the 9 myxosporean species. The absence of a match may be due to the limited number of myxosporeans and actinosporeans available for this study.

Analysis of the riboprints using the MIX program and PAUP3.1.1 showed that some species of *Myxobolus* were more closely related to either triactinomyxon, echinactinomyxon or raabeia than to other *Myxobolus* species. These results are consistent with the current life cycle data that species of *Myxobolus* alternate with triactinomyxon and raabeia (see Kent et al., 1994). However, all the species of *Myxobolus* and forms of triactinomyxon, echinactinomyxon and raabeia did not form a monophyletic group. This could be due to the introduction of homoplasy as being homologous which is assumed when fragment comigration analysis is performed (Clark, 1997).

The parsimony analyses revealed that neither the myxosporeans nor the actinosporeans formed a monophyletic group. These results are consistent with the 2-host life cycle hypothesis of myxozoans and indicate that riboprinting studies involving more myxosporeans and actinosporeans from the same ecological setting may reveal corresponding stages between them. Recently, SSU-rDNA sequences have been used for the identification and matching of myxosporeans and

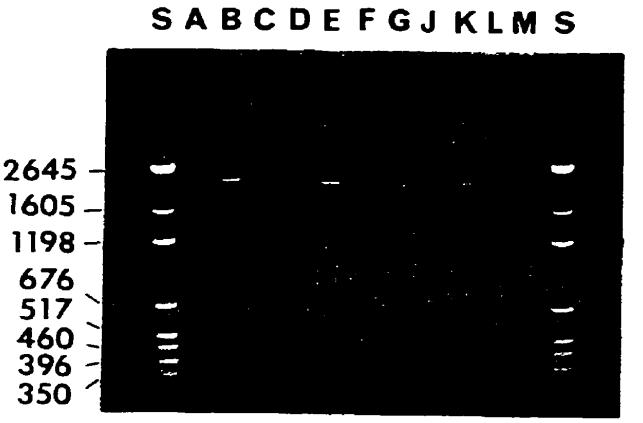
actinosporeans in a relatively simple ecological setting (Lin et al., 1998). While sequencing provides more detailed information on the relationship of myxosporeans and actinosporeans, riboprinting may be more suitable for larger scale studies of complex ecological settings.

Table 1. Myxozoans used for riboprinting and their hosts from Lake Sasajewun, Algonquin Park

Myxozoan species	Host species	Infection site
Myxidium sp.	*Catostomus commersoni*	gall bladder
Chloromyxum trijugum	*Lepomis gibbosus*	gall bladder
Myxobolus neurophilous	*Perca flavescens*	brain
Myxobolus pendula	*Semotilus atromaculatus*	gill arch
Myxobolus sp1.	*Luxilus cornutus*	gill
Myxobolus sp2.	*Luxilus cornutus*	brain
Myxobolus osburni	*Lepomis gibbosus*	muscle
Myxobolus pellicides	*Semotilus atromaculatus*	gill arch
Myxobolus bibullatus	*Catostomus commersoni*	gill
Neoactinomyxum form	*Limnodrilus hoffmeisteri*	intestine
Aurantiactinomyxon form	*Limnodrilus hoffmeisteri*	intestine
Echinactinomyxon 'D'	*Limnodrilus hoffmeisteri*	intestine
Antonactinomyxon form	*Tubifex tubifex*	intestine
Synactinomyxon form	*Tubifex tubifex*	intestine
Raabeia 'B'	*Limnodrilus hoffmeisteri*	intestine
Triactinomyxon 'F'	*Rhyacodrilus coccineus* & *Limnodrilus hoffmeisteri*	intestine
Triactinomyxon 'C'	*Limnodrilus hoffmeisteri*	intestine
Triactinomyxon ignotum	*Limnodrilus hoffmeisteri*	intestine

Figure 1. PCR amplified SSU-rDNA products from 12 myxozoans on 1.5% agarose gel. S, DNA size marker; A, *Myxidium* sp.; B, *Chloromyxum trijugum*; C, *Myxobolus* sp2; D, *Myxobolus neurophilous*; E, *Myxobolus pendula*; F, *Myxobolus* sp1.; G, *Myxobolus osburni*; J, neoactinomyxum form; K, aurantiactinomyxon form; L, echinactinomyxon 'D'; M, antonactinomyxon form.

Figure 2. Riboprint patterns of 18 myxozoan species using the restriction endonuclease Taq I. S, DNA size marker; A, *Myxidium* sp.; B, *Chloromyxum trijugum*; C, *Myxobolus* sp2; D, *Myxobolus neurophilous*; E, *Myxobolus pendula*; F, *Myxobolus* sp1.; G, *Myxobolus osburni*; H, *Myxobolus pellicides*; I, *Myxobolus bibullatus*; J, neoactinomyxum form; K, aurantiactinomyxon form; L, echinactinomyxon 'D'; M, antonactinomyxon form; N, synactinomyxon form; O, raabeia 'B'; P, triactinomyxon 'F'; Q, triactinomyxon 'C'; R, triactinomyxon ignotum. The patterns obtained for *Myxobolus pendula* (lane E) and *Myxobolus pellicides* (lane H), and for triactinomyxon 'C' (lane Q) and triactinomyxon ignotum (lane R), were identical with all endonucleases, respectively.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1605
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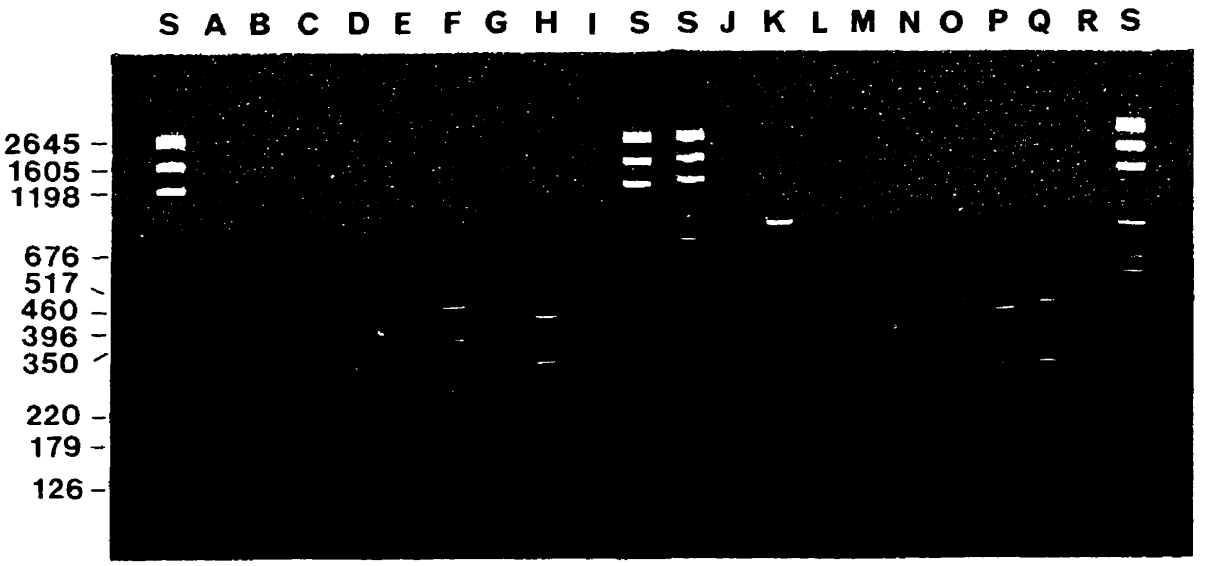
1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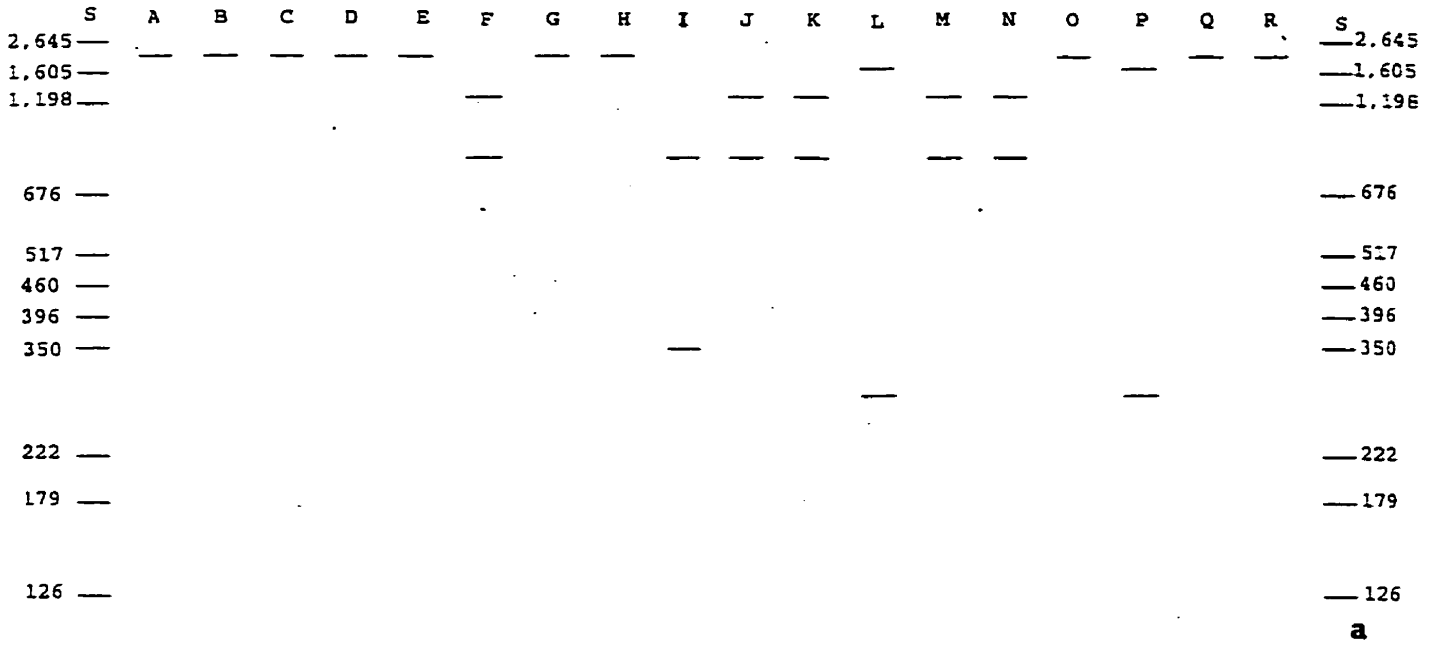
2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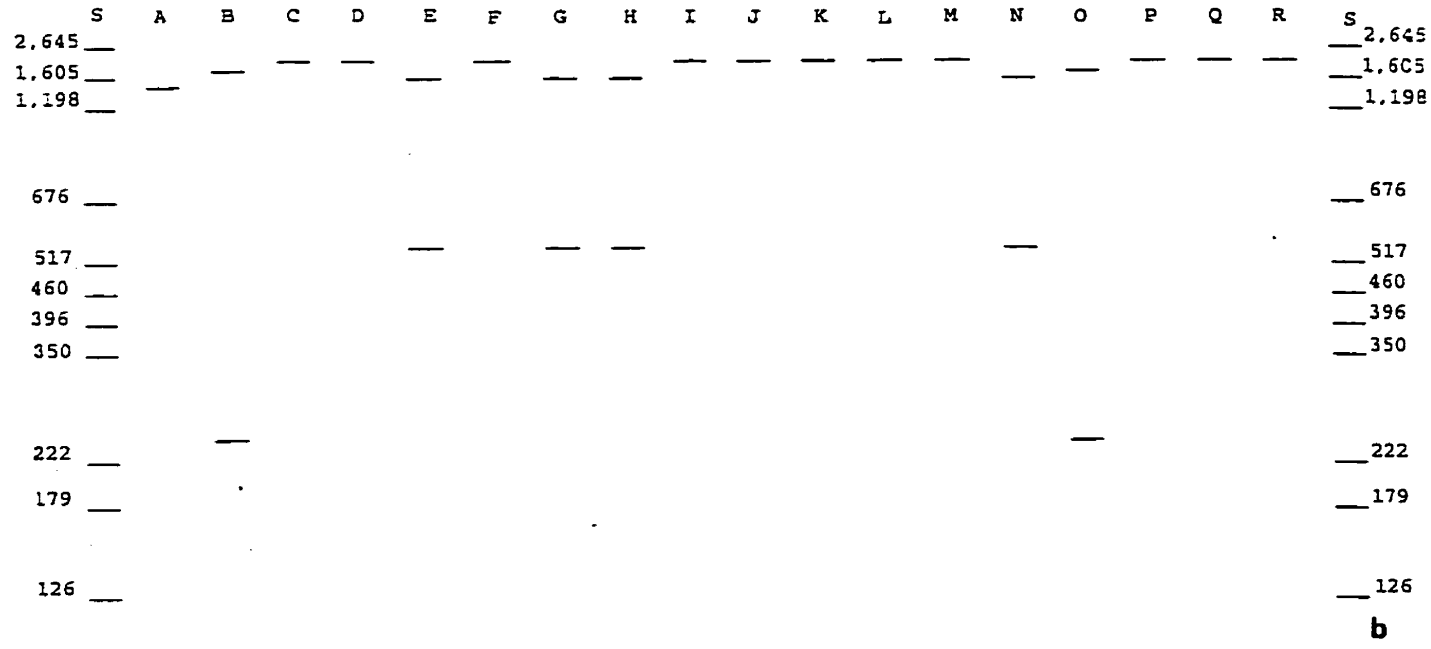
a


S A B C D E F G H I J K L M N O P Q R S
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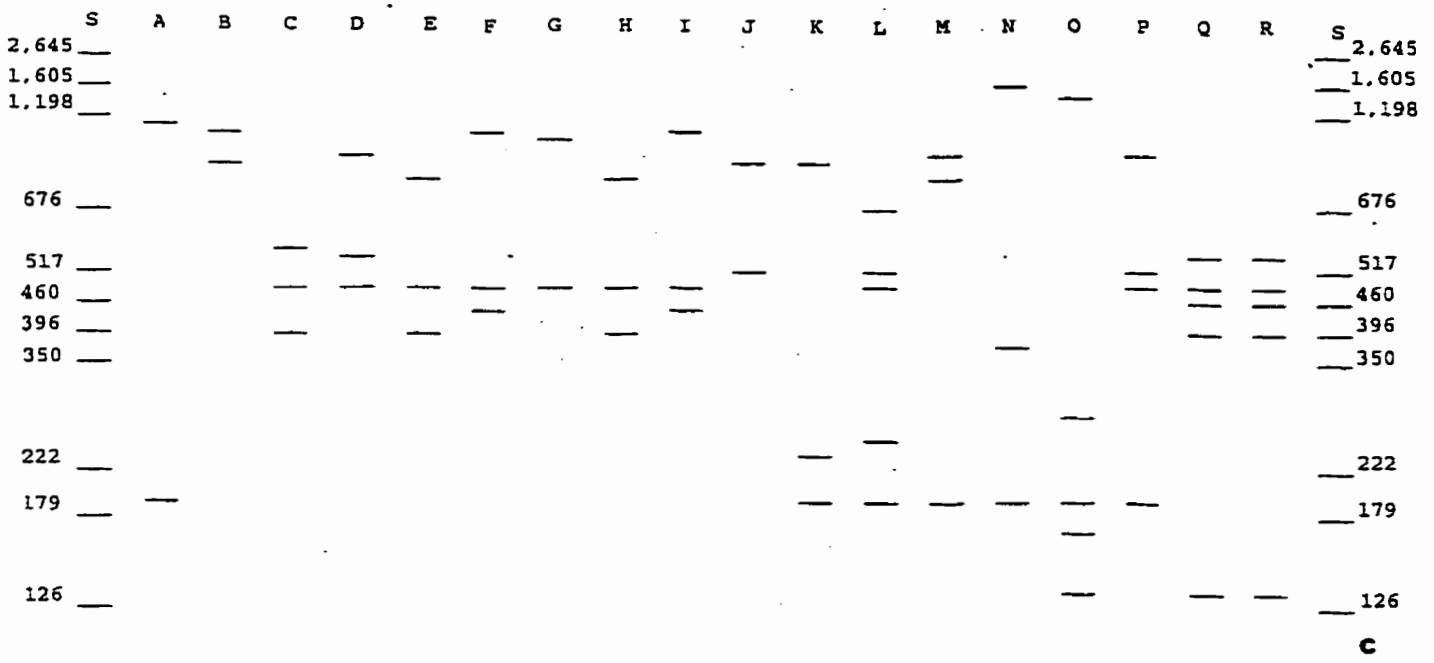
b

Figure 3. Schematic representation of riboprint patterns of 18 myxozoan species obtained with restriction endonucleases: a, Hind III; b, Eco RI; c, Rsa I; d, Sau3A I; e, Hha I; f, Dde I; g, Hinf I; h, Alu I. See Figure 2 for abbreviations.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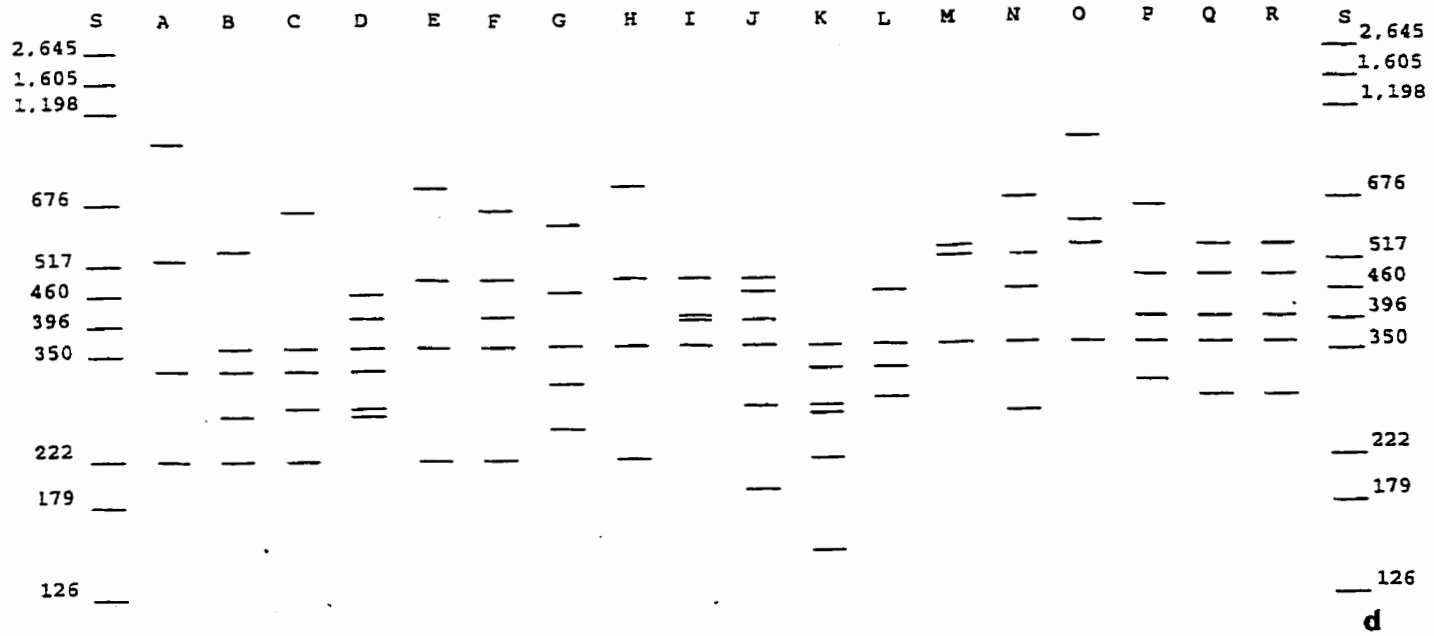
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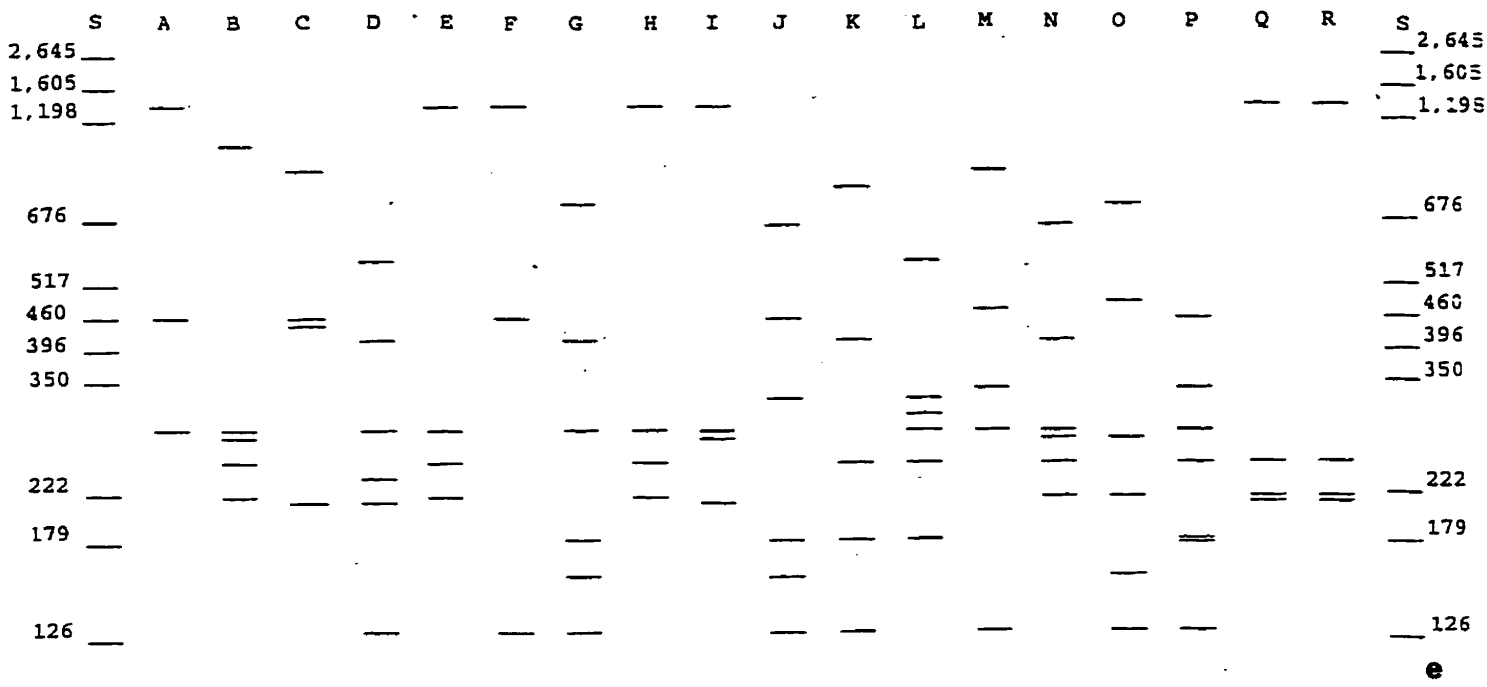
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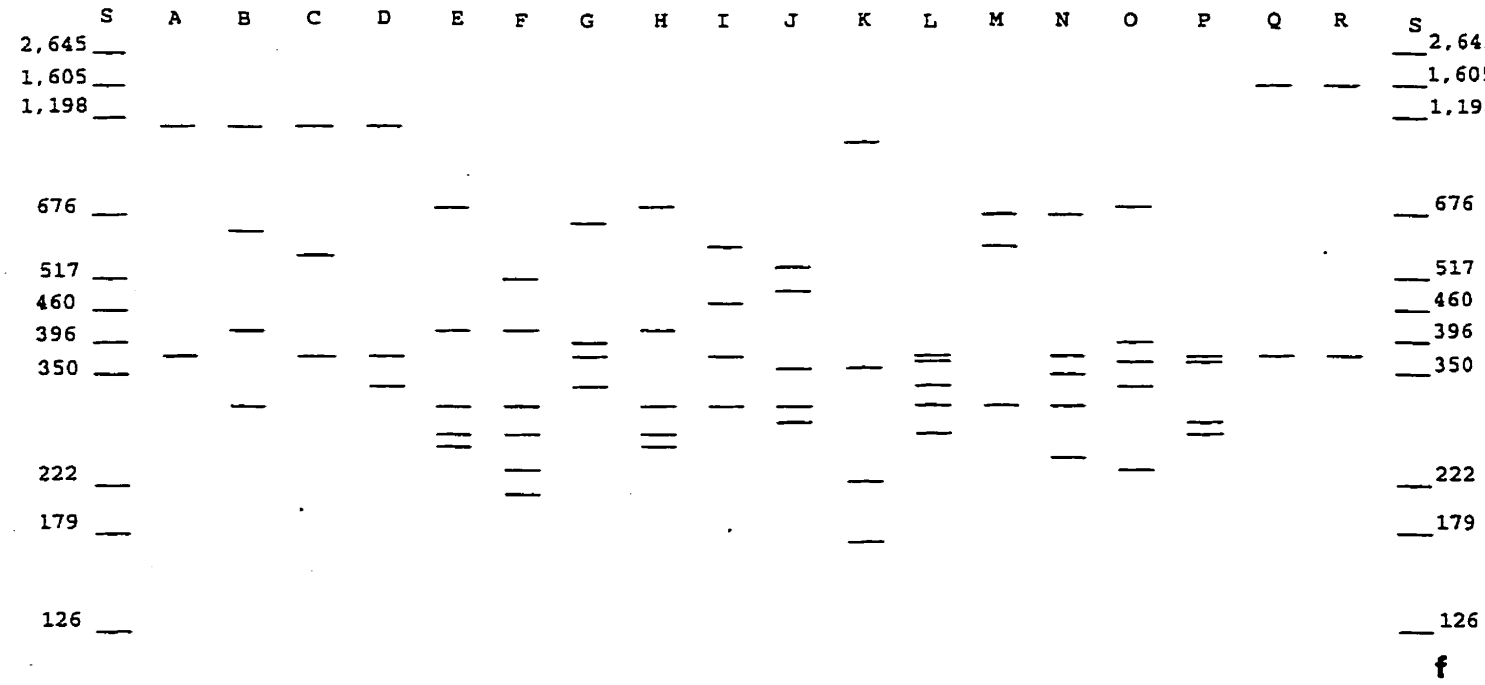
e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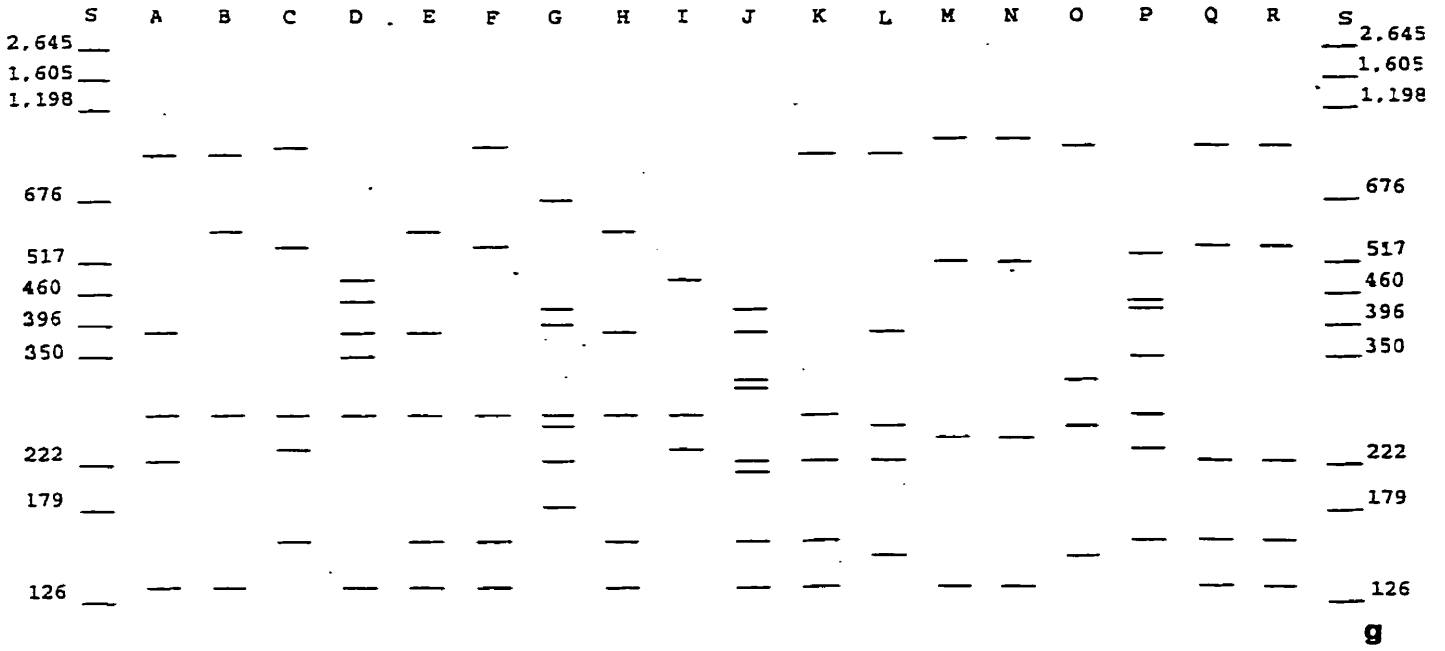
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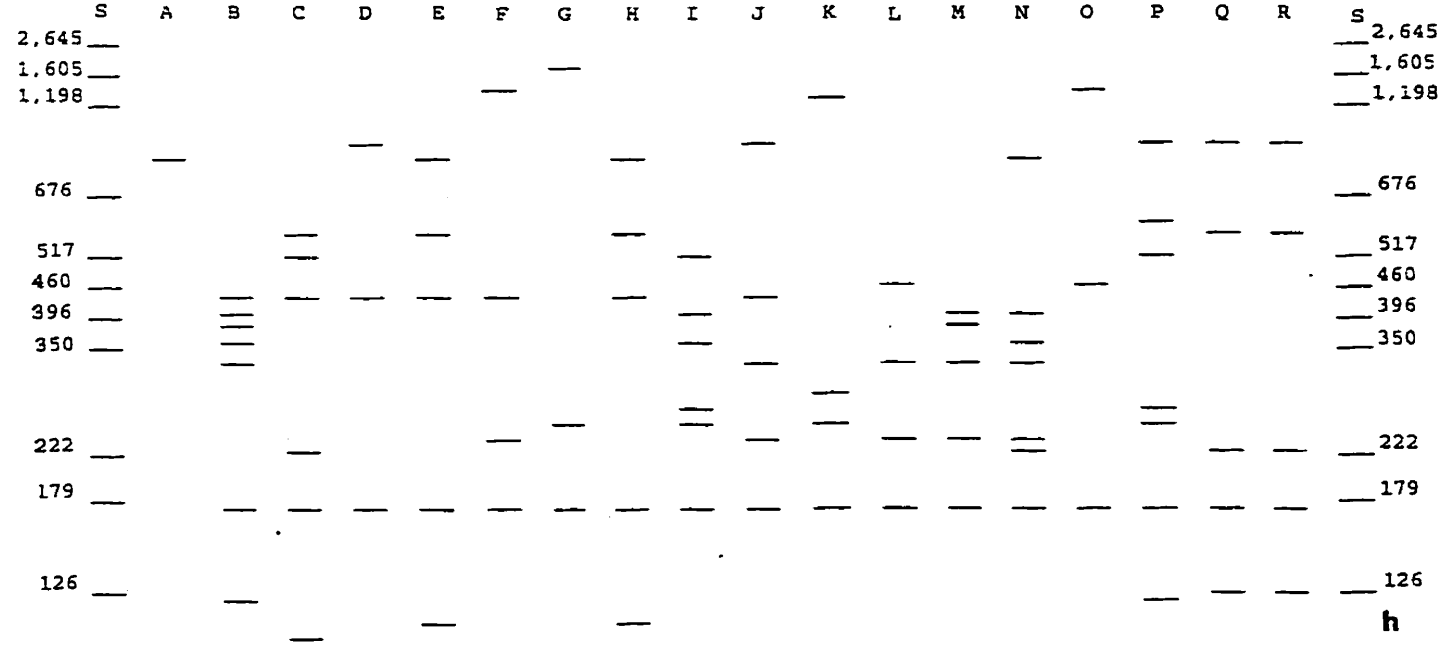
g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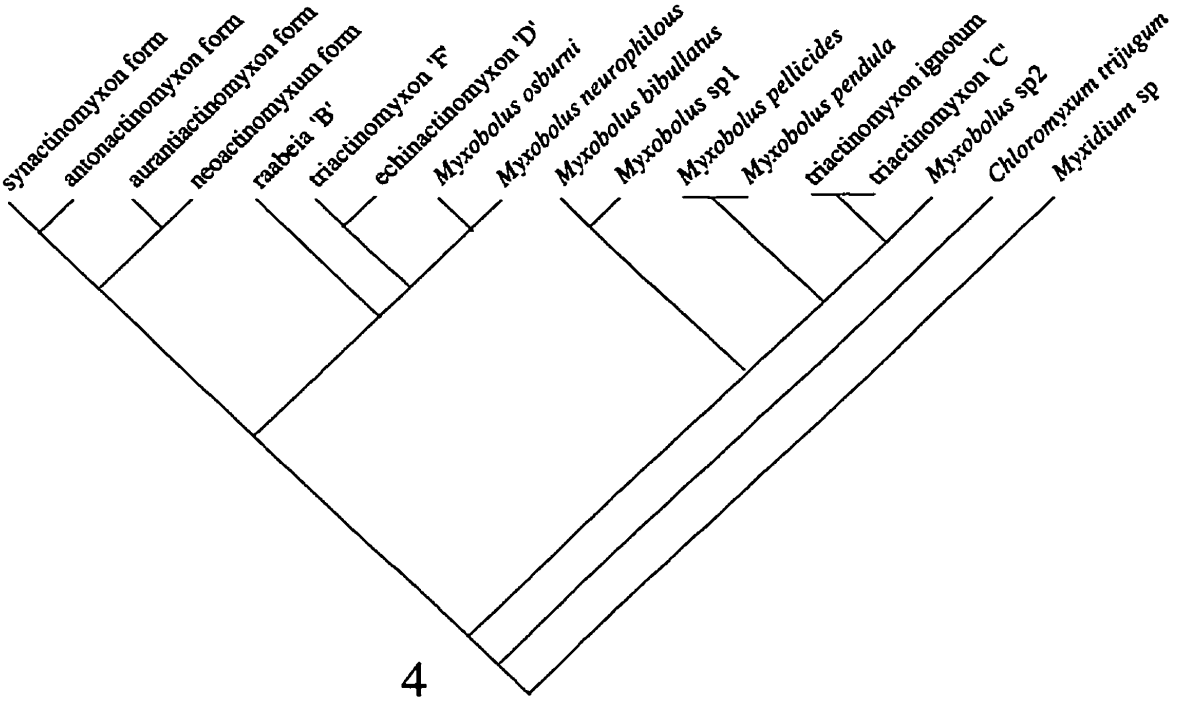
h

Figure 4. Unrooted Phylogenetic tree based on the myxozoan SSU-rDNA riboprints using the MIX program and PAUP3.1.1.


synactinomyxon form
antonactinomyxon form
aurantiactinomyxon form
neoactinomyxum form
raabeia 'B'
triactinomyxon 'F'
echinactinomyxon 'D'
Myxobolus osburni
Myxobolus neurophilous
Myxobolus bibullatus
Myxobolus sp1
Myxobolus pellicides
Myxobolus pendula
triactinomyxon ignotum
triactinomyxon 'C'
Myxobolus sp2
Chloromyxum trijugum
Myxidium sp

4

CHAPTER 9

Sequence Analysis of the 18S rRNA Genes of Six Myxozoans from Lake Sasajewun, Algonquin Park

ABSTRACT

In an earlier study, identical riboprint patterns were found between *Myxobolus pendula* and *Myxobolus pellicides*, and between triactinomyxon 'C' and triactinomyxon ignotum, but their actual sequence similarities remain unknown. In this study, sequences of the 18S rRNA genes of *M. pendula*, *M. pellicides*, triactinomyxon 'C', triactinomyxon ignotum, raabeia 'B', and the aurantiactinomyxon form were investigated. Sequence comparisons revealed that *M. pendula* and *M. pellicides* were 98.8% similar, and triactinomyxon 'C' and triactinomyxon ignotum, 97.3% similar, which are consistent with the earlier findings of riboprint analysis. Phylogenetic analysis of the 18S rDNA sequences from this study with sequences of *Myxobolus cerebralis*, *Henneguya salminicola*, *Henneguya doori*, *Myxidium lieberkuehni*, and *Polypodium hydriforme* from GenBank showed that the conventional genera *Myxobolus* and *Henneguya* are not monophyletic. Results from this study also support the hypothesis that myxosporeans and actinosporeans are alternating stages of the same organisms.

INTRODUCTION

The life cycles of most myxozoan species have not been elucidated, and morphological features of a single stage from their life cycle do not provide sufficient information to determine their phylogenetic relationships. Sequence data from ribosomal RNA genes have been used to investigate the phylogenetic relationships of a wide variety of organisms (Hillis & Dixon, 1991). Based on sequence analysis of the 18S rRNA genes of several myxozoans, Smothers et al. (1994), Siddall et al. (1995), and Schlegel et al. (1996) confirmed the metazoan affinities for Myxozoa, but these authors differed on the phylogenetic position of Myxozoa within the Metazoa. Recently, Hervio et al. (1997) hypothesized the taxonomic affinities among species of *Kudoa* using the small-subunit ribosomal DNA sequence. In order to clarify the phylogenetic position of Myxozoa and to investigate the phylogenetic relationships among myxozoans, the rDNA data base will have to be enlarged to encompass members of different representative groups.

In this chapter, the partial sequence of the genomic DNA encoding the 18S rRNA gene of 6 myxozoans from Lake Sasajewun, Algonquin park was determined, and the relationship among these myxozoans was investigated.

MATERIALS AND METHODS

Isolation and Purification of Spores

Spores of 6 myxozoans, *Myxobolus pendula* and *Myxobolus pellicides* from the creek chub, *Semotilus atromaculatus*, the aurantiactinomyxon form, raabeia 'B', triactinomyxon 'C', and triactinomyxon ignotum, from *Limnodrilus hoffmeisteri*, were obtained from fish and oligochaetes of Lake Sasajewun, Algonquin Park. Cysts of *M. pendula* and *M. pellicides*, containing mature spores, were excised from their fish

hosts. These spores were separated from fish tissues and preserved in 70% ethanol before DNA extraction. For the aurantiactinomyxon form, raabeia 'B', triactinmyxon 'C', and triactinomyxon ignotum, spore-releasing worms were rinsed with distilled water several times to reduce contamination prior to placing them into clean wells filled with distilled water. Water-borne spores of each form were collected using the cell-well plate method (Yokoyama et al., 1991). The actinosporean spores were concentrated by centrifugation and processed either immediately for DNA extraction or preserved in 70% ethanol.

Genomic DNA Extraction

Ethanol preserved spores of the myxosporean stages were washed twice with 1X PBS and resuspended in STE buffer with 1% SDS. These spores were boiled 10 min and plunged into liquid nitrogen 3 times in order to release their contents, digested using proteinase K at 37 °C for 2-3 hr, and extracted using the phenol : chloroform method.

Spores of the actinosporean stages were processed similarly to those of the myxosporean stages with slight modifications. The spores were boiled 10 min and plunged into liquid nitrogen only once. Then, they were either digested with proteinase K for 4-5 hr and extracted with phenol : chloroform, or processed using the IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, WA, USA).

PCR Amplification

The SSU-rDNA of the myxozoans was amplified using forward primer 5'-CCGAATTCCAGCTGCAGATCTGGTTGATCCTGCCAGT-3' and reverse primer 5'-CCAAGCTTCCGCTGCAGGATCCTTCCGCAGGTTCACCT-3'. This primer pair was modified from the universal 18S rDNA primers (Medlin et al., 1988; Clark & Diamond, 1991). PCR reactions were performed in a 100 µl volume consisting of 2 µl of DNA sample (about 150 ng genomic DNA/µl), 4 µl of each primer (5 µM/µl), 8 µl

of 25 mM dNTP solution, 10 µl of 10X PCR reaction buffer (containing 500 mM KCl, 15 mM $MgCl_2$, and 100 mM tris-HCl. Pharmacia Biotech, Quebec, Canada), 0.5 µl of 5 units/µl Taq polymerase (Pharmacia Biotech, Quebec, Canada), and 71.5 µl ddH_2O. Amplification conditions were as follows: 7 min initial melt at 94 °C, then cycled 1 min at 94 °C, 1 min at 56 °C and 2 min at 72 °C for 35 to 40 cycles.

Purification of PCR Products and Sequencing

The PCR products were run on 1.5% agarose gels in 1X TAE, and the SSU-rDNA fragments of myxozoans (about 2.1-2.2 kb) were purified using the Geneclean II Kit (Bio 101 Incorporated, La Jolla, California, USA). Gene-cleaned fragments generated by PCR amplification were sequenced in both strands with the PCR primers and universal internal primers for the 18S rRNA gene (White et al., 1990) using an ABI Prism 377 DNA sequencer.

Sequence Alignment and Phylogenetic Analysis

The 18S rDNA sequences obtained for 6 myxozoans in this study were initially aligned with *Myxobolus cerebralis* (Accession No. U96492), *Henneguya salminicola* (Accession No. AF031411), *Henneguya doori* (Accession No. U37549), *Myxidium lieberkuehni* (Accession No. X76639), and *Polypodium hydriforme* (Accession No. U37526) sequences from GenBank, using the CLUSTAL W software, version 1.5. (Thompson et al., 1994) with the default gap penalty setting (Appendix 1). This alignment was modified visually and regions of ambiguity were removed. Only informative sites were included in the phylogenetic analysis.

Phylogenetic inferences were determined using algorithms provided in PAUPSTAR, version 4.0b1 (Swofford, 1998) with *Polypodium hydriforme* as an outgroup (Siddall et al., 1995). To test the sensitivity of the data, 2 ratios of transition to transversion (ts to tv as 2:1 and 5:1) were introduced in the analysis. Statistical estimates of branch point validity were presented as bootstrap confidence

levels of 1,000 bootstrap replicates.

RESULTS

The nucleotide sequences of the 18S rRNA genes generated for the 6 myxozoans were between 2,003 and 2,073 base pairs (bp) (Appendix II). The 18S rRNA gene sequence of *M. pendula* exhibited 98.8% similarity to *M. pellicides*. Triactinomyxon 'C' was 97.3% similar to triactinomyxon ignotum. These results were consistent with the findings in Chapter 8. Identities between any 2 of these 6 myxozoans ranged from 61 to 98.8%.

Sequences of the 6 myxozoans in this study were aligned with *M. cerebralis*, *H. salminicola* , *H. doori* , *M. lieberkuehni*, and *P. hydriforme* from GenBank. The alignment, after exclusion of ambiguous regions, yielded 1699 aligned nucleotide sites with 752 informative characters. Analysis of the data with *Polypodium hydriforme* as an outgroup and a transition to transversion ratio of 2:1 resulted in 2 most parsimonious trees (Figs. 1 and 2). These 2 equally parsimonious trees had a consistency index of 0.669 (excluding uninformative characters), a retention index of 0.677, and a length of 1932. If analysis on the data used a transition to transversion ratio of 5:1, a single most parsimonious tree with the same topology as Figure 1 was yielded, which had a consistency index of 0.649 (excluding uninformative characters), a retention index of 0.665, and a length of 3810. These analyses revealed that the conventional genera *Myxobolus* and *Henneguya* are not monophyletic groups.

Bootstrap confidence levels reached 100 for the following clades: 1) triactinomyxon 'C' and triactinomyxon ignotum; 2) *M. pendula* and *M. pellicides*; 3) clade 1) and 2); 4) clade 3), *M. cerebralis* and *H. salminicola*; 5) *H. doori* and the aurantiactinomyxon form. Low confidence levels obtained for other clades.

DISCUSSION

Sequence comparisons revealed that *M. pendula* and *M. pellicides* were 98.8% similar, and that triactinomyxon 'C' was 97.3% similar to triactinomyxon ignotum, whereas each of the 2 pairs showed identical riboprinting patterns in the previous study (Chapter 8). These results indicated that riboprinting missed some variation due to either a small percentage of the gene sequence being sampled or a size difference between the fragments being too small to be noticed. Thus, sequence differences of the 18S rDNA with high similarity might not be detected by riboprinting.

Comparison among sequences of 18S rRNA genes of the 6 myxozoans in this study revealed considerable genetic diversity, which is in agreement with the distinct riboprinting patterns among *M. pendula/M. pellicides*, triactinomyxon 'C'/triactinomyxon ignotum, raabeia'B', and the aurantiactinomyxon form of the previous study (Chapter 8). These results are also consistent with the heterogeneity found in the 18S rDNA of other myxozoans based on sequence comparisons by Schlegel et al. (1996) and Hervio et al. (1997).

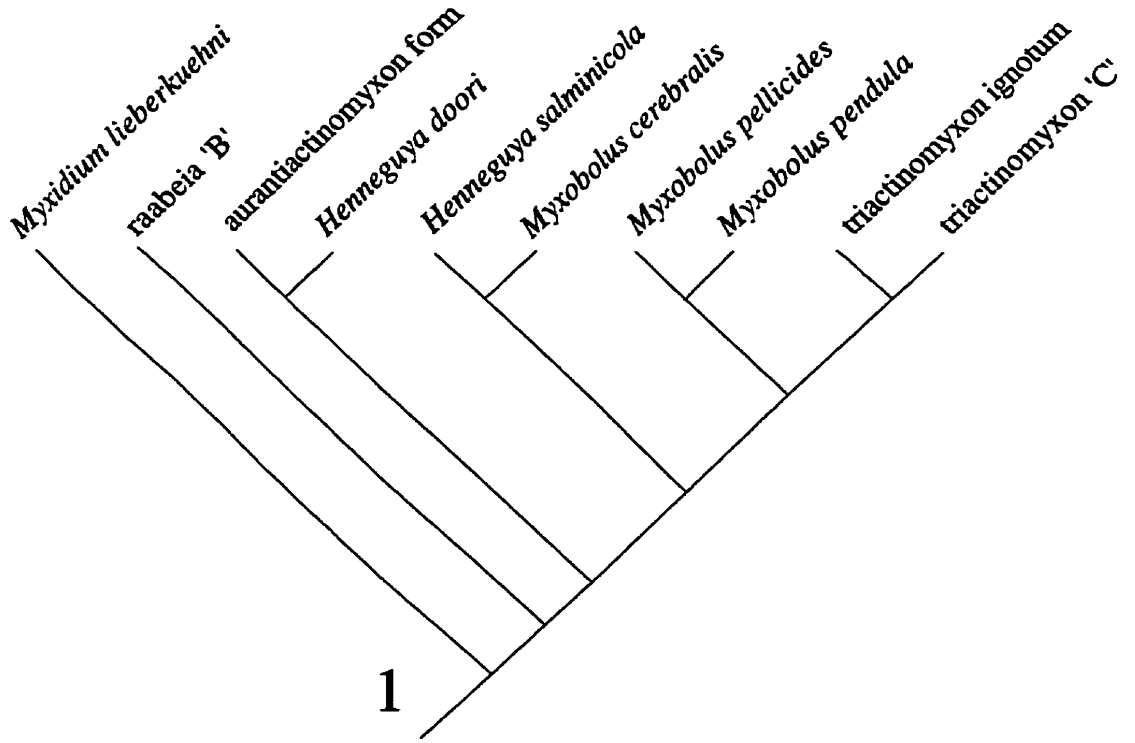
The monophyly of the myxozoan genera *Myxobolus* and *Henneguya* has often been questioned (Mitchell, 1989; Cone and Overstreet, 1997). Smothers et al. (1994) showed that certain species of *Myxobolus* are apparently more closely related to species of *Henneguya* than to other species of *Myxobolus* based on rDNA sequence studies. Phylogenetic analysis of the 18S rDNA data in this study with other species of the genera *Myxobolus* and *Henneguya*, as well as actinosporeans, showed that neither species of *Myxobolus* nor species of *Henneguya* formed a monophyletic group. These results confirm the relationship between *Myxobolus* and *Henneguya* hypothesized by Smothers et al. (1994) and suggest that a systematic revision of

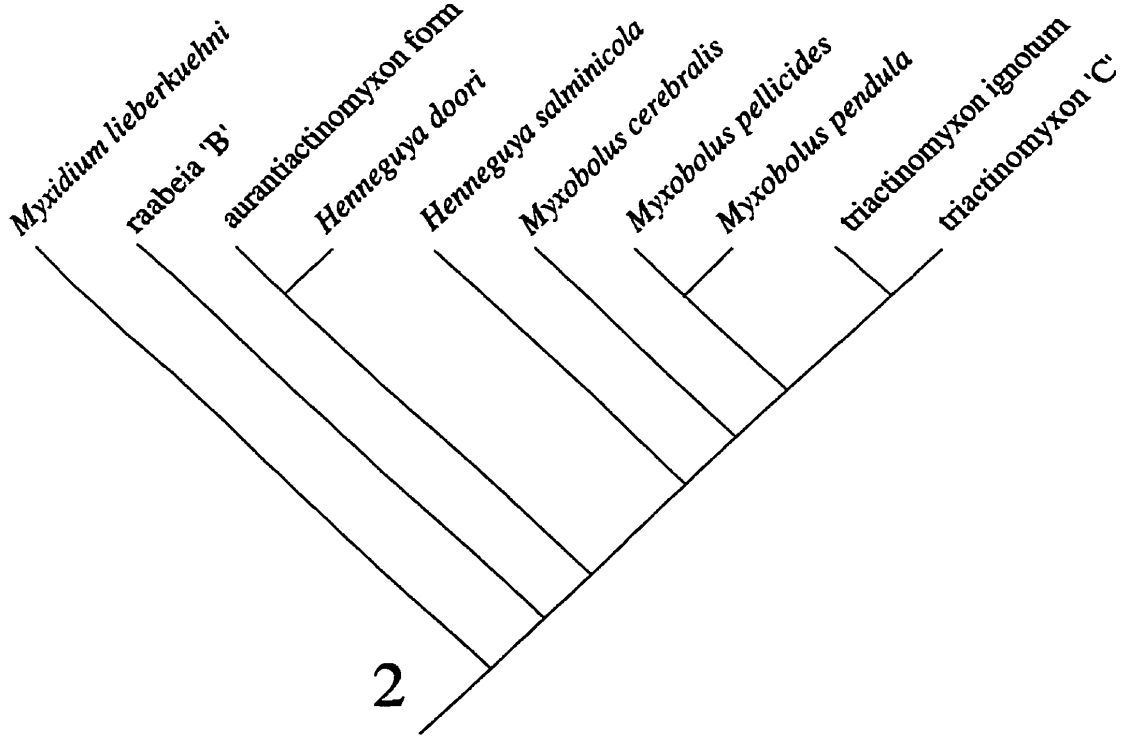
these parasites is necessary in order to reflect their phylogenetic relatoinships.

The phylogenetic analysis of the sequence data from this study together with sequences of *M. cerebralis, H. salminicola, H. doori, M. lieberkuehni,* and *P. hydriforme* from GenBank also revealed that neither the myxosporeans nor the actinosporeans formed a monophyletic group. Species of *Myxobolus* are closely related to members of the collective group triactinomyxon. These results are consistent with the parsimony analysis based on riboprinting data in the previous chapter (Chapter 8) and support the 2-host life cycle hypothesis of myxozoans.

Further molecular phylogenetic analyses of myxozoans are needed to determine the within group relationships of these parasites since there are only a few species with known life cycles. In order to do this, it is necessary to enlarge the ribosomal DNA data base with members from both myxosporeans and actinosporeans. This will also be useful for studying the corresponding relationships between myxosporeans and actinosporeans.

Figures 1 and 2. Two most parsimonious trees generated by PAUPSTAR using the 18S rDNA with ts to tv ratio as 2:1.


Myxidium lieberkuehni
raabeia 'B'
aurantiactinomyxon form
Henneguya doori
Henneguya salminicola
Myxobolus cerebralis
Myxobolus pellicides
Myxobolus pendula
triactinomyxon ignotum
triactinomyxon 'C'
1


Myxidium lieberkuehni
raabeia 'B'
aurantiactinomyxon form
Henneguya doori
Henneguya salminicola
Myxobolus cerebralis
Myxobolus pellicides
Myxobolus pendula
triactinomyxon ignotum
triactinomyxon 'C'
2

CHAPTER 10

Phylogenetic Analysis of Myxozoans with Known Alternating Life cycles

ABSTRACT

The phylogenetic relationships of myxozoan species with known alternating life cycles were investigated in order to provide insight into the puzzling matches between myxosporeans and actinosporeans of the myxozoan life cycle data. Phylogenetic analyses were performed using two partitioned data sets of life cycle stages and a combined data set. A cnidarian parasite of fish, *Polypodium hydriforme*, was used as the outgroup. The supraspecific level grouping in the conventional classification of actinosporeans was not supported in the analysis of the partitioned data from the actinosporean phase, which yielded two equally parsimonious trees. Analysis of the partitioned data from the myxosporean phase provided 24 equally parsimonious trees and did not support the current classification of myxosporeans. The analyses of the partitioned data of myxozoans by life cycle stage revealed a lack of taxonomic congruence between partitions. The suborder Variisporina of Myxozoa was not supported by the total evidence trees, while the monophyly of the species of *Myxobolus* and of Myxidiidae were supported. The cladograms from the combined data revealed that these myxozoan species formed four major monophyletic groups. Among them, two were supported by the partitioned data of the actinosporean phase. The phylogenetic signals and the better resolution reflected by the trees of combined data suggest that the phylogenetic total evidence approach should be employed in future studies of the systematics of myxozoans.

INTRODUCTION

Species in the phylum Myxozoa Grasse, 1970 are mainly parasites of aquatic annelids and fish. The myxozoans have complex life cycles that include multinuclear vegetative stages and multicellular spores with nematocyst-like polar capsules. Their systematics is based solely on the shape and structure of the spore and, conventionally, two classes have been recognized (Lom, 1990). The class Myxosporea Butschli, 1881 contains approximately 1200 species, and the class Actinosporea Noble, 1980, about 40 species. Since the pioneering work of Wolf and Markiw (1984), there is compelling evidence based on experimental transmission studies that 13 species of myxosporeans and their corresponding actinosporean phases do not constitute different classes, but different developmental stages of the same organism (see Kent et al., 1994; Uspenskaya, 1995; Yokoyama, 1997). This connection between myxosporeans and actinosporeans has also been confirmed by recent molecular analyses (Andree et al., 1997; Bartholomew et al., 1997). Although it is not yet clear whether all myxozoans have alternating life cycles and whether this feature is applicable to marine as well as freshwater myxozoans, alternating life cycles are now widely accepted (Lom et at., 1997).

Myxozoans are defined by a variety of observable synapomorphies including spores composed of several cells which form one to seven spore shell valves, one to two amoeboid infective cells (sporoplasms), two to seven nematocyst-like polar capsules with an extrudable filament, as well as morphological and functional specialization of cells. The myxozoans are unique among eukaryotes with respect to their ontogenetic development. Metazoan affinities for Myxozoa were suggested a century ago by Stolc (see Lom et al., 1997) and have been confirmed by recent molecular analyses (Smothers et al., 1994; Siddall et al., 1995; Schlegel et al., 1996; Anderson et al., 1998). In addition, Siddall et al. (1995) postulated the inclusion of

myxozoans in the cnidarians, as a sister group of fish-infecting narcomedusan *Polypodium hydriforme* Ussov, 1885.

High level taxonomic changes of Myxozoa based on the unification of the two former classes Myxosporea and Actinosporea were proposed by Kent et al. (1994). They recommended that the class Actinosporea be suppressed and its genera provisionally reduced to the status of collective groups, except for *Tetractinomyxon* Ikeda, 1912, which was tentatively shifted to the myxosporean order Multivalvulida Shulman, 1959 due to one character, the single binucleate sporoplasm, which is shared with the conventional myxosporeans. Thus, with the exception of the genus *Tetractinomyxon*, the revised systematics of myxozoans has been based solely on one of its two life cycle stages, namely the myxosporean phase from the fish host. This revised classification was not adopted by Hallett et al. (1998). In addition, recently Bartholomew et al. (1997) revealed that *Ceratomyxa shasta* Noble, 1950 alternates with *Tetractinomyxon* to complete its life cycle. However, despite the recent advances in our understanding of the Myxozoa, the traditional approach to unravelling their within-group relationships by utilizing only the overall similarity of the myxosporean stages, and ignoring the corresponding actinosporean stages, still persists. The reluctance thus far to adopt the phylogenetic approach to the systematics of the myxozoans has prevented their within-group relationships from being resolved.

In order to provide insight into the sometimes puzzling relationships between myxosporeans and actinosporeans, and to establish a more robust phylogenetic hypothesis of relationships among myxozoan species with known alternating life cycles, morphological and developmental data were analyzed using data sets partitioned by the life cycle stage and a combined data in a total evidence approach (Kluge and Wolf, 1993).

MATERIALS AND METHODS

Thirteen myxozoan species, the alternating life cycles of which have been confirmed by experimental transmission, were included in this study. One of them, *Hoferellus carassii* Akhmerov, 1960, has been associated with three different types of actinosporean spores and this discrepancy was explained by the possibility that each *H. carassii* with its specific corresponding actinosporean stage may be a different species (Yokoyama et al., 1993; see Kent et al., 1994). For the purpose of this study, each of the *H. carassii* with its specific actinosporean stage was treated as a different taxon. Consequently, 15 myxozoan taxa were included in the analysis. The taxonomic scheme of unified myxozoans proposed by Kent et al. (1994) was adopted in this study.

Character state information for the taxa in Table 1 was compiled from the following sources: for *Polypodium hydriforme* Ussov, 1885 - Raikova (1994); for *Myxobolus cerebralis* Hofer, 1903 - Lom and Dykova (1992), Wolf and Markiw (1984) and El-Matbouli et al. (1995); for *Myxobolus cotti* El-Matbouli and Hoffmann, 1987 - El-Matbouli and Hoffmann (1987, 1989); for *Myxobolus pavlovskii* Akhmerov, 1954 - Lom and Dykova (1992) and Ruidisch et al. (1991); for *Myxobolus carassii* Klokacheva, 1914 - Lom and Dykova (1992) and El-Matbouli and Hoffmann (1993); for *Myxobolus arcticus* Pugachev and Khokhlov, 1979 - Lom and Dykova (1992) and Kent et al. (1993); for *Myxobolus cultus* Yokoyama, Ogawa and Wakabayashi, 1995 - Yokoyama et al. (1991, 1995); for *Myxidium giardi* Cepede, 1906 - Lom and Dykova (1992) and Benajiba and Marques (1993); for *Zschokkella sp.* - Yokoyama (1993); for *Zschokkella nova* Klokacheva, 1914 - Lom and Dykova (1992) and Uspenskaya (1995); for *Hoferellus carassii* Akhmerov, 1960 with aurantiactinomyxon type 1 - Lom and Dykova (1992) and El-Matbouli et al. (1992); for *H. carassii* with aurantiactinomyxon type 2 - Lom and Dykova (1992) and Trouillier et al. (1996); for *H. carassii* with

neoactinomyxum - Lom and Dykova (1992) and Yokoyama et al. (1993); for PGD myxosporean = *Sphaerospora ictaluri* Hedrick, McDowell and Groff, 1990 - Hedrick et al. (1990), Lom and Dykova (1992), Burtle et al. (1991) and Styer et al. (1991); for *Ceratomyxa shasta* Noble, 1950 - Lom and Dykova (1992) and Bartholomew et al. (1997); for *Thelohanellus hovorkai* Akhmerov, 1960 - Lom and Dykova (1992) and Yokoyama (1997). In addition, certain character and character state information of the actinosporean collective groups in Table 1 were compiled from Marques (1984).

Phylogenetic Analysis

Characters were polarized or partly polarized using outgroup comparison (Watrous and Wheeler, 1981; Maddison et al., 1984; Wiley et al., 1991) with *Polypodium hydriforme*, a cnidarian parasite of fish, which was shown to be closely related to the myxozoans (Siddall et al., 1995). Only one outgroup was employed in this study, because the inclusion of other cnidarians or related metozoans did not improve the polarization of all characters. A total of 49 characters were selected for the analyses (Table 1), 23 from the actinosporean stage (characters 1-23) and 26 from the myxosporean stage (characters 24-49). A missing data code, "?", was used when the character state was unknown or when the terminal taxon was polymorphic for the character. Character states used in this analysis are defined as follows:

Characters of the actinosporean stage

1. Shell valves absent (0), present with three (1).
2. Polar capsules numerous (0), only three (1).
3. Sporoplasmic mass absent (0), present (1).
4. Valve processes absent (0), three (1), six (2).
5. Free spores absent (0), separated (1), united (2).
6. Sticky processes absent (0), present (1).

7. Leaf-like processes absent (0), present (1).

8. Leaf-like processes with pointed ends absent (0), present (1).

9. Leaf-like processes with rounded ends absent (0), present (1).

10. Downward curved leaf-like processes absent (0), present (1).

11. Straight leaf-like processes absent (0), present (1).

12. Lobed or spherical processes absent (0), present (1).

13. Spine-like process numbers (0), three (1), six (2).

14. Spine-like process absent (0), present and attenuated with pointed end (1), present and not attenuated with rounded end (2).

15. Upward curved spine-like processes absent (0), present (1).

16. Straight spine-like processes absent (0), present (1).

17. Spore body shape, absent (0), tetrahedral (1), spherical (2), cup-shaped (3), cylindrical (4) in lateral view.

18. Epispore with style absent (0), present (1).

19. Sporocyst with eight maturing spores absent (0), present (1).

20. Process bases absent (0), present, embracing its epispore completely (1), about half (2), only posterior end (3).

21. Sporoplasmic mass absent (0), present, triangular (1), spherical (2), cup-shaped (3), elongated ellipsoidal (4) in lateral view.

22. Germ cells in sporoplasmic mass absent (0), one (1), more than one (2).

23. Spore body absent (0), present, triangular (1), spherical (2) in apical view.

Characters of the myxosporean stage

24. Spore, absent (0), subspherical (1), elongated spindle-shaped (2), elongated mitre-like (3), ellipsoidal (4) in front view.

25. Spore, absent (0), arcuate (1), subspherical (2), elongated spindle-shaped (3), elongated mitre-like (4), fusiform (5) in sutural view.

26. Spore width (valvular view) to spore thickness (sutural view), absent (0), width < thickness (1), width = thickness (2), width > thickness (3).

27. Polar capsules numerous (0), two (1), one (2).

28. Sporoplasm, absent (0), present in the mid part of the spore (1), present not in the mid part of the spore (2).

29. Valve absent (0), present with smooth surface (1), present with ridged surface (2).

30. Spore absent (0), present without polarity (1), present with polarity (2).

31. Sporoplasm, absent (0), kidney-shaped (1), semi-spherical (2), cylindrical (3) in sutural view.

32. Sutural ridge absent (0), present as sutural line (1), present as elevated sutural ridge (2).

33. Sutural ridge, absent (0), straight (1), "s"-shaped (2).

34. Polar capsule position, random (0), only at anterior end of spore (1), at both ends of spore (2).

35. Well defined anterior polar capsules absent (0), present with single well defined polar capsule plane (1), present with multiple polar capsule planes (2).

36. Well defined anterior polar capsules absent (0), present with its single polar capsule plane perpendicular to the sutural plane (1) or overlapping with the sutural plane (2), present with many polar capsule planes (3).

37. Position of polar capsules in relation to valves, absent (0), equal number corresponded to each other at the anterior end (1), equal number located at the anterior end and not corresponded to each other (2), equal number located at both ends and not corresponded to each other (3), unequal number at anterior end (4).

38. Direction of polar filaments discharge, random (0), anterior (1), opposite in direction along the longitudinal spore axis (2), lateral (3).

39. Spore filament appendages absent (0), present (1).

40. Ridged valve surface, absent (0), present but not extended into filament

appendages (1), present and extended into filament appendages (2).

41. Sporogony absent (0), disporic (1), monosporic (2), polysporic (3).

42. Spore absent (0), present without mucus envelope (1), present with mucus envelope (2).

43. Size of polar capsules, equal (0), unequal (1).

44. Spore with two polar capsules at one end, absent (0), present without intercapsular process (1), present with intercapsular process (2).

45. Shape of polar capsules, subspherical (0), pyriform (1), elongated bulb-shaped (2).

46. Valve absent (0), present and conical in side view (1), present and not conical in side view (2).

47. Valve absent (0), present, with rounded anterior end (1), with pointed anterior end (2).

48. Valve absent (0), present, with rounded posterior end (1), with pointed posterior end (2), with truncate posterior end (3), with slightly inward curved posterior end (4).

49. Spore absent (0), present with the symmetry axis only along the longitudinal axis (1), present with the symmetry axis perpendicular to the longitudinal axis (2).

Analyses were conducted using Hennig86 (Farris, J.S., 1988, version 1.5) using the implicit enumeration option ("ie*") and PAUP3.1.1 (Swofford, D.L., 1993, version 3.1.1) using the branch-and-bound search option. All multistate characters in the data matrix were introduced into the analysis as unordered since the evolution of these characters could not be postulated with certainty. In order to determine the relative robustness of the analysis, bootstrapping tests were performed.

RESULTS

The partitioned data set from the actinosporean phase yielded two equally parsimonious trees (Figures. 1 & 2). These two equally parsimonious trees had a consistency index of 0.962 (excluding uninformative characters), a retention index of 0.980, a rescaled consistency index of 0.954 and a length of 38 steps. These two trees indicated a sister-group relationship of raabeia to triactinomyxon and hexactinomyxon as well as of echinactinomyxon to siedleckiella. These two trees also revealed that the conventional family Triactinomyxidae Kudo, 1931 was not a monophyletic group.

The partitioned data set from the myxosporean phase yielded 24 equally parsimonious trees. These equally parsimonious trees had a consistency index of 0.889 (excluding uninformative characters), a retention index of 0.917, a rescaled consistency index of 0.826 and a length of 71 steps. All the trees indicated paraphyly of the genus *Myxobolus*. The consensus tree for these equally parsimonious trees was computed (Figure 3). The suborder Variisporina Lom and Noble, 1984 was not supported as a monophyletic group by the consensus tree (Figure 3).

The analysis of combined actinosporean and myxosporean characters yielded two equally parsimonious trees (Figures 4 & 5). The two equally parsimonious trees had a consistency index of 0.771 (excluding uninformative characters), a retention index of 0.821, a rescaled consistency index of 0.663 and a length of 125 steps. These two trees indicated monophyly of species of the genus *Myxobolus*, a similar hypothesis to the analysis of the partitioned data from the actinosporean phase; and a sister-group relationship of the genus *Myxidium* to the genus *Zschokkella*, a better resolution than the analysis of the partitioned data from the myxosporean phase. These two trees also revealed that the suborder Variisporina was not a monophyletic group.

The bootstrap values for the cladograms in Figures 1, 3 and 4 were determined by 1000 replicates with the exclusion of uninformative characters. There were nine uninformative characters for the partitioned data from the actinosporean stage and four of them were stable plesiomorphic characters of myxozoans (characters 1, 2, 3, and 19), whereas there were four uninformative characters for the partitioned data from the myxosporean stage but none of them were stable plesiomorphic characters of myxozoans. The monophyly of *Myxobolus* species and of species of the family Myxiididae in the total evidence tree were supported with bootstrap values of 99 and 89, respectively, whereas monophyly of the suborder Variisporina was not supported in any of the 1000 bootstrap replicates.

DISCUSSION

Partitioned Data Analyses

Although the actinosporean data had fewer characters, it provided fewer trees than the myxosporean data set. The two equally parsimonious trees based upon the actinosporean data set did not support the supraspecific level grouping in the conventional classification of actinosporeans (Marques, 1984; Lom, 1990), except for the separation of tetractinomyxon from other actinosporeans. These two trees revealed a sister-group relationship of raabeia to triactinomyxon and hexactinomyxon, in contrast to the conventional classification in which raabeia and triactinomyxon were placed in one family and hexactinomyxon in another. A sister-group relationship of echinactinomyxon and siedleckiella to raabeia, triactinomyxon and hexactinomyxon was hypothesized in the analysis. This grouping contrasted with the conventional classification (Lom, 1990) in which siedleckiella and hexactinomyxon were placed in two different families, and echinactinomyxon, raabeia and triactinomyxon were included in the family Triactinomyxidae.

Although there was a tritomy near the base, the conventional placement of aurantiactinomyxon, echinactinomyxon, raabeia and triactinomyxon in the same family was not supported by the analysis.

The partitioned data set of the myxosporean stage yielded 24 equally parsimonious trees, and a consensus tree did not resolve the relationship among *Myxobolus*, *Thelohanellus*, *Hoferellus*, *Sphaerospora* and *Ceratomyxa*. The consensus tree also did not resolve the relationship among the members of the family Myxidiidae Thelohan, 1892, which was represented by species of the genera *Myxidium* and *Zschokkella* in this study. The consensus tree did not support the monophyly of the suborder Variisporina in the classification of myxosporeans (Lom, 1990; Lom and Dykova, 1992; Kent et al., 1994; Hervio et al., 1997). The consensus tree also revealed paraphyly of the genera *Myxobolus* and *Thelohanellus*, suggesting some *Myxobolus* species are more closely related to *Thelohanellus* species than to other *Myxobolus* species.

Analyses of the partitioned data revealed a major problem of partitioning data by life cycle stage, the lack of taxonomic congruence between partitions. Figures 1 to 3 illustrated this lack of congruence between data sets from the two life cycle stages. In fact, none of these cladograms had any major clades in common. The problem of incongruence between life stages has been observed only recently for myxozoans following the elucidation of life cycles of more than a dozen myxozoan species (Kent et al., 1994), while this phenomenon was observed as early as the 19th century and has been a recurring theme in attempts to classify insects (see Judd, 1998). There are two contrasting explanations for the lack of congruence between life stages. Michener (1977) and Roback and Moss (1978) revived the arguments of evolutionary taxonomists, that different life stages are subject to different environmental selection pressures, which account for the observed morphological differences that mask phylogenetic relationships. Therefore,

different classifications are to be expected from data partitioned by life stage. In contrast, Farris (1971) argued that congruent patterns presumably reflect shared common evolutionary history and incongruent patterns therefore reflect errors caused by incorrect methodological procedures and/or misinterpretation of homologies. Similarly, Brooks and McLennan (1991, 1993) argued that the entire ontogenetic program is a coherent and integrated sequence whose parts are correlated primarily with phylogenetic history and secondarily with selective pressures peculiar to portions of the ontogeny. Although incongruence is not a factor if a total evidence approach is used, patterns of incongruence observed in the partitioned data sets may provide information on the rates of evolution and homoplasy in characters from different life stages, and therefore provide useful information for character choice in future analyses for establishing a stable and predictive classification using the total evidence approach.

Combined Data Analysis

Combined data resulted in fewer trees and better resolution than the partitioned data of the myxosporean stage, and the same number of trees as, and better resolution than the partitioned data of, the actinosporean stage. The two equally parsimonious trees from the combined data supported the grouping of *Myxobolus* species, which was also hypothesized by the partitioned actinosporean data. These two trees also supported the grouping of *Myxidium* and *Zschokkella* species, which was remained unresolved by the partitioned myxosporean data set, and the grouping of *Sphaerospora* and *Hoferellus* species in the unified classification of myxozoans (Kent et al., 1994). However, these trees did not support the monophyly of the family Myxobolidae and the suborder Variisporina of the revised classification.

Although the combined data did not fully resolve the relationships of the taxa

considered, the two equally parsimonious trees contained four major monophyletic groups and revealed the following patterns: i) a monophyletic group of *Myxobolus* species and their corresponding triactinomyxon, hexactinomyxon and raabeia stages, where significant morphological modifications have occurred in the actinosporean stage but not in the myxosporean stage; ii) a monophyletic group of *Hoferellus*, *Sphaerospora*, *Thelohanellus* species and their corresponding aurantiactinomyxon stages, in contrast to the first group, few significant morphological modifications have occurred in the actinosporean stage; iii) a group consisting of *Ceratomyxa* species and their corresponding tetractinomyxon, which separated from other groups at a node near the base; and iv) the last group consisting of *Myxidium* and *Zschokkella* species, and their corresponding aurantiactinomyxon (similar to the second group, [ii]), echinactinomyxon and siedleckiella stages. In the latter group, the morphological modifications occurred in both the actinosporean and myxosporean stages, and parallel evolution of the actinosporean stages did not mask their relationship. The character evolution indicated by the two equally parsimonious trees of the combined data suggests that characters of the actinosporean stages from worm hosts are more conservative than characters of the myxosporean stages from fish hosts.

Systematics

Phylogenetic analyses of partitioned and combined data of myxozoans species in this study revealed that most groupings of the conventional classifications of actinosporeans (Marques, 1984; Lom, 1990) and myxosporeans (Lom, 1990; Lom and Dykova, 1992) are not monophyletic. The lack of congruence between the partitions of myxozoan life cycle stages indicated that the systematics of myxozoans based solely on one of its two life cycle stages may result in incorrect supraspecific level groupings. Our combined data analyses also did not support the grouping

proposed in a recent unified classification of myxozoans (Kent et al., 1994) except for the family Myxidiidae and the genus *Myxobolus*. Current classifications of myxozoans do not reflect phylogenetic signals and consequently caution should be taken when using these classification schemes.

Although the debate on whether characters from different data sets should be combined or analyzed separately continues in systematics (Kluge, 1989; Faith and Cranston, 1991; Bull et al., 1993; Kluge and Wolf, 1993; De Querioz et al., 1995; Huelsenbeck et al., 1996; Judd, 1998), the current trend in phylogenetic studies of combining characters from different life stages into a single analysis has been supported not only by theoretical arguments (Hennig, 1966; Brooks and McLennan, 1993), but also by selected clade analysis (Judd, 1998). Our study indicated that combined data not only retained phylogenetic information of each life stage and provided better resolution, but also revealed phylogenetic information missed by data partitioning and ensured that less noise was reflected in the cladograms. Therefore, a more stable and predictive classification of myxozoans should be based on combined total evidence of both life cycle stages.

Only discrete qualitative characters were considered in the present study because myxozoan parasites have multicellular spores which exhibit different shapes and structures as evidenced by the 49 characters in the data matrix. Quantitative characters such as spore and polar capsule measurements are sometimes the only useful criterion for distinguishing between closely related species. However, these were not taken into account because they were not relevant for distinguishing taxa at a supraspecific level. The main problem in conducting this study was the incomplete species descriptions in the literature, especially for the actinosporean stages. It was necessary to piece together information of the actinosporean stage from disparate sources to generate a usable data set.

Even though the phylogenetic relationships determined in this study were

inconsistent with current classifications of Myxozoa, no attempt was made to revise the systematics of the group to reflect the diverse range of life stages and significant morphological differences among groups of species due to its limited available life cycle data. However, the hypothesized relationships by the phylogenetic total evidence approach of myxozoan species with known life cycles should serve as a basis in future attempts to modify the systematics of myxozoans.

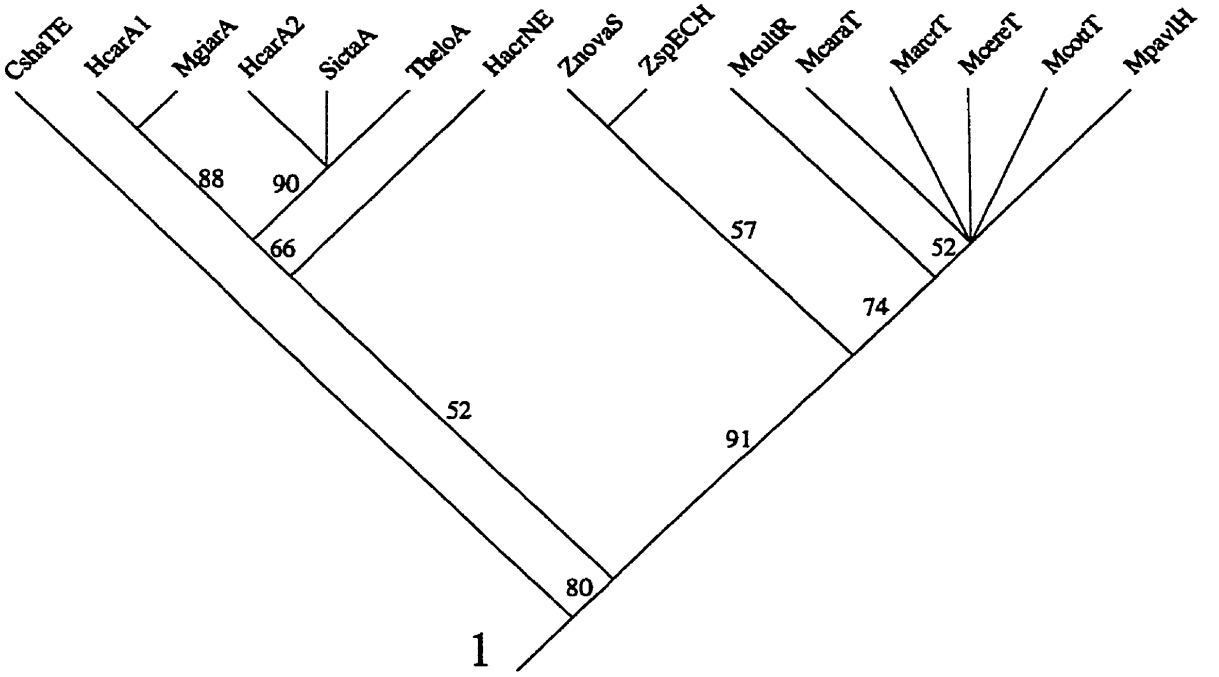
Table 1. Character matrix used in the analysis of myxozoan species with known life cycle

	Actinosporean Character	Myxosporean Chracter
	11111111112222	22222233333333334444444444
Taxa	12345678901234567890123	45678901234567890123456789
Phydri	00000000000000000000000	00000000000000000000000000
McereT	11111000000011104113422	45312112211122100320212111
McottT	11111000000011104113422	45312112211122100?10?12111
MpavlH	11121000000021104113422	45312112211122100311212111
McaraT	11111000000011104113422	45312112211122100310122211
MarctT	11111000000011104113422	45312112211122100310222211
McultR	11111000000011104013422	45312112211122100310212111
MgiarA	11111011010000002011222	23211223112003201310002222
ZspECH	11111000000011013012322	23211223122003301?10002112
ZnovaS	11112100000012014113222	23211223122003301310002112
HcarA1	11111011010000002011222	34112213211111112310112231
HcarA2	11111010101000002011222	34112213211111112310112231
HcarNE	11111000000100002011222	34112213211111112310112231
SictaA	11111010101000002011222	12112212211111101210102211
CshaTE	11101000000000001010111	11112111111111100110101141
TheloA	11111010101000002011222	45322112211234100320?012211

Abbreviations:

Phydri: *Polypodium hydriforme*
McereT: *Myxobolus cerebralis* + matching Triactinomyxon
McottT: *Myxobolus cotti* + matching Triactinomyxon
MpavlH: *Myxobolus pavlovskii* + matching Hexactinomyxon
McaraT: *Myxobolus carassii* + matching Triactinomyxon
MarctT: *Myxobolus arcticus* + matching Triactinomyxon
McultR: *Myxobolus cultus* + matching Raabeia
MgiarA: *Myxidium giardi* + matching Aurantiactinomyxon
ZspECH: *Zschokkella* sp. + matching Echinactinomyxon
ZnovaS: *Zschokkella nova* + matching Siedlekkiella
HcarA1: *Hoferellus carassii* + matching Aurantiactinomyxon type 1
HcarA2: *Hoferellus carassii* + matching Aurantiactinomyxon type 2
HcarNE: *Hoferellus carassii* + matching Neoactinomyxum
SictaA: *Sphaerospora ictaluri* + matching Aurantiactinomyxon
CshaTE: *Ceratomyxa shasta* + matching Tetractinomyxon
TheloA: *Thelohanellus hovorkai* + matching Aurantiactinomyxon

Figures 1 and 2. Two equally parsimonious trees of the partitioned data from the actinosporean stage. Bootstrap values were shown on Figure 1. See Table 1 for abbreviations.


CshaTE
HcarA1
MgiarA
HcarA2
SictaA
TheloA
HacrNE
ZnovaS
ZspECH
McultR
McaraT
MarcrT
MoereT
MoutT
MpavlH
88
90
66
52
57
52
74
91
80
1

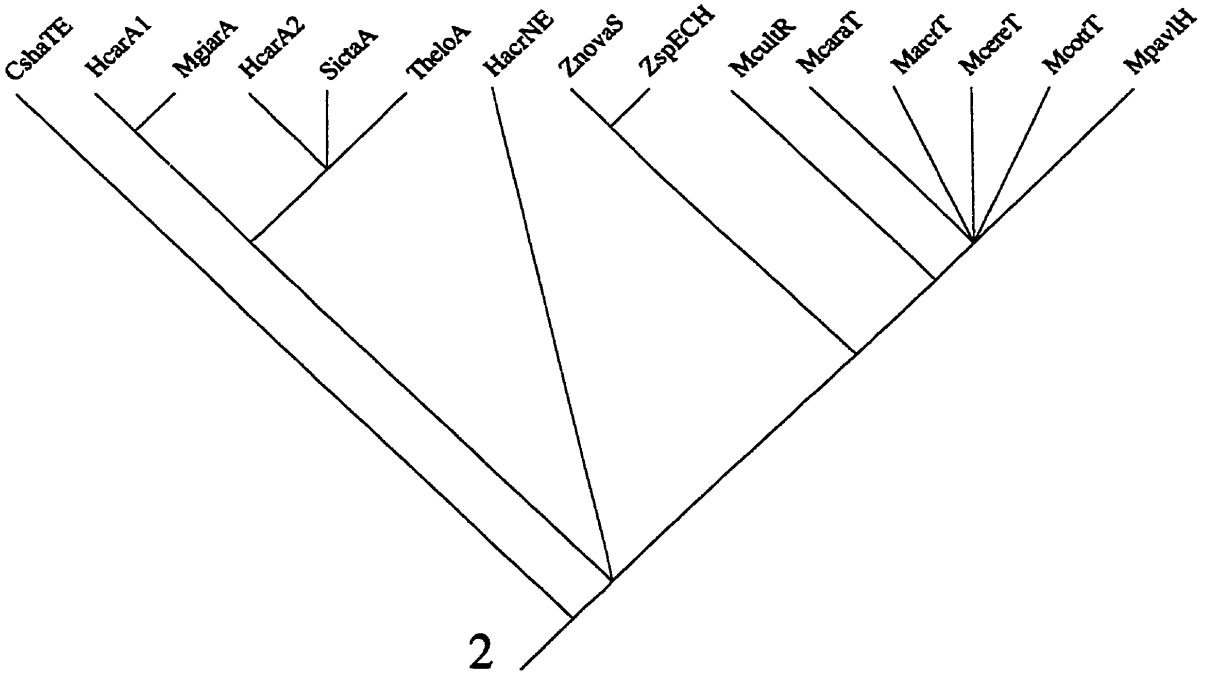

CshaTE
HcarA1
MgiarA
HcarA2
SictaA
TheloA
HacrNE
ZnovaS
ZspECH
McultR
McaraT
MarcrT
MoereT
MoutT
MpavlH
2

Figure 3. Strict consensus tree of the 24 equally parsimonious trees of the partitioned data from the myxosporean stage. Bootstrap values were shown on it. See Table 1 for abbreviations.

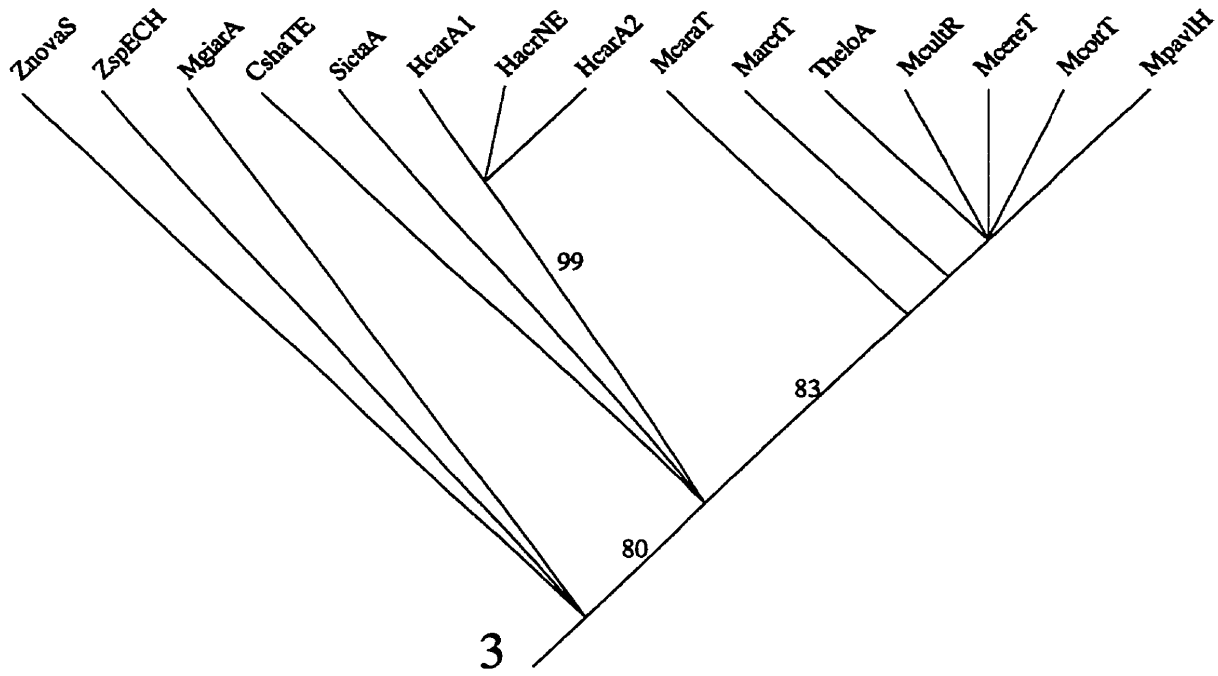
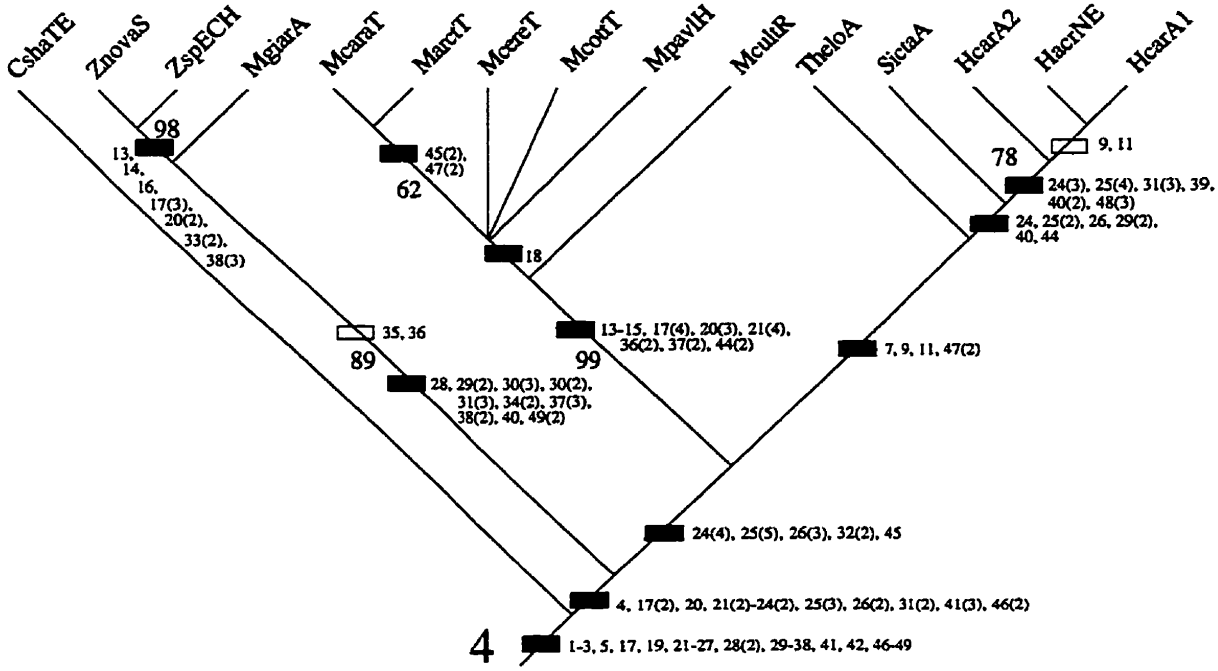
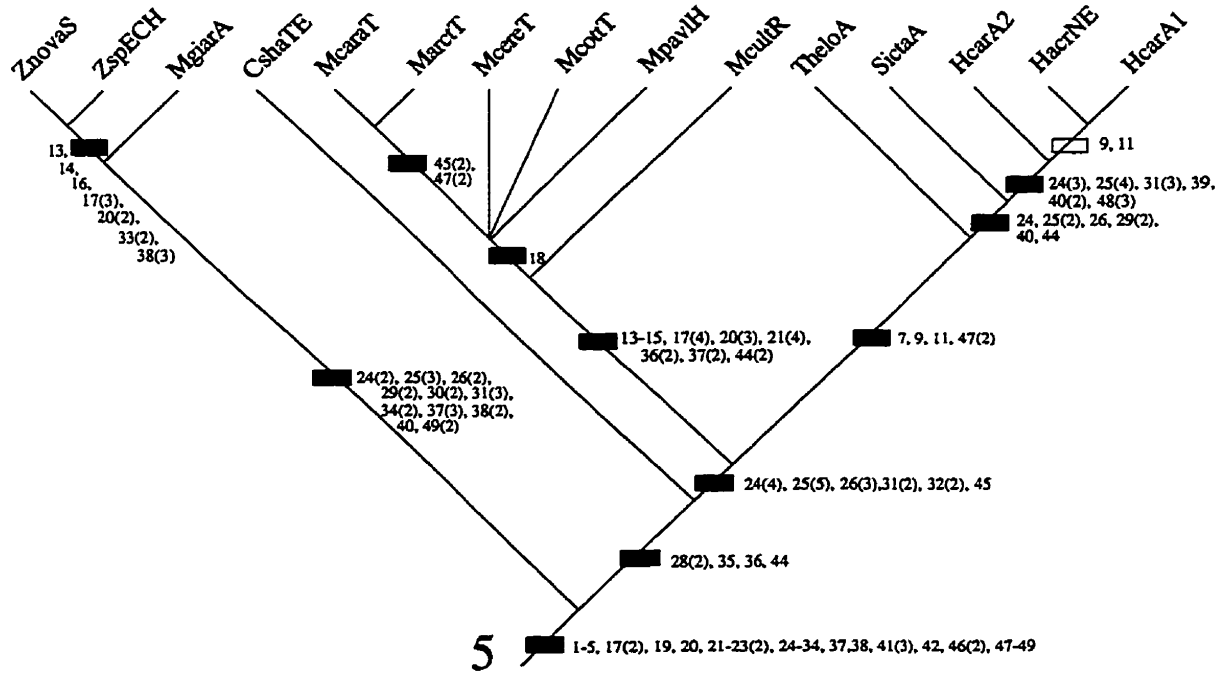

ZnovaS
ZspECH
MgiarA
CshaTE
SiciaA
HcarA1
HacrNE
HcarA2
McaraT
MarcT
TheloA
MculR
McreT
McouT
MpavlH
99
83
80
3

Figure 4 and 5. Two equally parsimonious trees of the total evidence of combined data with synapomorphy characters shown (solid boxes represent status changes, clear boxes represent reversal changes). Bootstrap values were shown on figure 4. See Table 1 for abbreviations.


CshaTE
ZnovaS
ZspECH
MgiarA
McaraT
MarctT
MceteT
McortT
MpavlH
MculR
TheloA
SictaA
HcarA2
HactNE
HcarA1
98
13, 14, 16, 17(3), 20(2), 33(2), 38(3)
45(2), 47(2)
62
18
78
9, 11
24(3), 25(4), 31(3), 39, 40(2), 48(3)
24, 25(2), 26, 29(2), 40, 44
35, 36
89
28, 29(2), 30(3), 30(2), 31(3), 34(2), 37(3), 38(2), 40, 49(2)
13-15, 17(4), 20(3), 21(4), 36(2), 37(2), 44(2)
99
7, 9, 11, 47(2)
24(4), 25(5), 26(3), 32(2), 45
4, 17(2), 20, 21(2)-24(2), 25(3), 26(2), 31(2), 41(3), 46(2)
4
1-3, 5, 17, 19, 21-27, 28(2), 29-38, 41, 42, 46-49


ZnovaS
ZspECH
MgiarA
CshaTE
McaraT
MarctT
MceteT
McortT
MpavlH
MculR
TheloA
SictaA
HcarA2
HactNE
HcarA1
13, 14, 16, 17(3), 20(2), 33(2), 38(3)
45(2), 47(2)
18
9, 11
24(3), 25(4), 31(3), 39, 40(2), 48(3)
24, 25(2), 26, 29(2), 40, 44
13-15, 17(4), 20(3), 21(4), 36(2), 37(2), 44(2)
7, 9, 11, 47(2)
24(2), 25(3), 26(2), 29(2), 30(2), 31(3), 34(2), 37(3), 38(2), 40, 49(2)
24(4), 25(5), 26(3),31(2), 32(2), 45
28(2), 35, 36, 44
5
1-5, 17(2), 19, 20, 21-23(2), 24-34, 37,38, 41(3), 42, 46(2), 47-49

CHAPTER 11

General Discussion

GENERAL DISCUSSION

Prior to the investigations detailed in this thesis, knowledge of the myxozoan parasites of fish and oligochaetes was limited mainly to the myxosporean phase of fish. Despite Wolf and Markiw's landmark paper of 1984 in which they hypothesized that myxosporeans and actinosporeans are alternating developmental stages of the same organisms, relatively few attempts have been made to study the actinosporean phase of these parasites (Yokoyama et al., 1991, 1993, 1995; Kent et al., 1993; Lom et al., 1997; McGeorge et al., 1997; El-Matbouli and Hoffmann, 1998; Hallett et al., 1998). Based on this hypothesis, each myxosporean has a corresponding actinosporean form. As it currently stands, however, the situation is somewhat perplexing because the myxosporeans greatly outnumber the actinosporeans (Lom, 1987). This is further complicated by the fact that the systematics of myxozoans continues to be based solely on the myxosporean stage, with the actinosporean stage being ignored due to limited 2-host life cycle data. The paucity of studies on actinosporeans may be attributed to several factors: 1) the natural prevalence of these parasites is only about 1% (see Yokoyama et al., 1991); 2) the difficulty in obtaining and maintaining large numbers of live annelid hosts of these parasites from nature; 3) the time-consuming detection of these parasites in their worm hosts; 4) the inability to culture the short-lived actinosporean spores; and 5) the difficulty in achieving experimental transmission of these parasites. If the gross imbalance between myxosporeans and actinosporeans has resulted from the paucity of studies on actinosporeans, then the comparison between the numbers of species of myxosporeans and actinosporeans based on the biased pooled data is meaningless, and if the actinosporean stage holds phylogenetic signals absent in the myxosporean stage, the current systematics of myxozoans is incomplete. Therefore, a comprehensive study of myxosporeans and actinosporeans in a single ecological

setting and a revision of the systematics of myxozoans adopting a total evidence approach are critical to understand the biology of myxozoans.

Biology and Diversity of Myxozoans

Data in this thesis have contributed substantially to the knowledge of the diversity and biological characteristics of myxozoans, in particular to the actinosporean phase of these parasites in their oligochaete hosts. Successive observations of freshly released actinosporean spores revealed that some actinosporeans exhibited an increase in number of germ cells and, for those with a style, an increase in size of the sporoplasmic mass in free floating spores. Although the number of germ cells and the size of the sporoplasmic mass and style are useful diagnostic characteristics (Marques, 1984; Lom et al., 1997), these features change as the germ cells divide and the sporoplasmic mass moves posteriorly within the style of free floating spores. Therefore, documentation of the number of germ cells and the size of the sporoplasmic mass and style based on single random observations of the spore stage is incomplete. Successive observations of the spores are necessary to accurately document these features.

Based on current life cycle data, actinosporeans were suppressed as collective groups and it was suggested that newly described actinosporeans with unknown corresponding myxosporean stages be placed into these recognized collective groups (Kent et al., 1994; Lom et al., 1997). Hallett et al. (1998) disagreed with the proposal for collective naming and argued that the establishment of new genera and assignment of species to actinosporeans would provide more phylogenetically useful information than a collective naming system, and that this would make synonymies easier and the relationships clearer when the corresponding myxosporean stages are eventually elucidated. Because Hallett's approach would produce 2 Linnaean systems for the same organisms, the collective naming system

was adopted for the documentation of the actinosporean stages in this thesis (Chapters 4 and 5). Actinosporeans that did not fit into the currently recognized collective group names were placed in a recognized collective group name based on their taxonomic similarities.

As a result of the investigations described in Chapters 4 and 5, 25 forms of actinosporeans have been documented from oligochaetes of Lake Sasajewun, Algonquin Park. This constitutes a rich actinosporean fauna in the lake in comparison to a total of approximately 40 previously described species (see Lom et al., 1997). However, 56 species of myxosporeans have been described from the same lake (Gowen, 1983; Li and Desser, 1985; Lom et al., 1989; Xiao and Desser, 1997). Despite this striking discrepancy between the numbers of actinosporeans and myxosporeans, the difference is much smaller than previous data suggested (Lom, 1987), lending further support to the 2-host life cycle hypothesis.

The potential fish host range of selected actinosporeans was also investigated by the fish mucus test, and some showed high specificity (Chapter 6). These results are similar to those of Yokoyama et al. (1993). Although the reaction of an actinosporean form to the mucus of a species of fish does not guarantee the establishment of infection, the potential fish host range generated from the mucus test should facilitate host selection for future experimental transmission of actinosporeans to fish.

Ecology of the Actinosporean Stage

Prior to this study, nothing was known regarding the seasonal changes in the appearance of actinosporean spores in Lake Sasajewun. The ecology of actinosporean parasites was investigated for the purpose of gaining a better understanding of the interactions among actinosporeans, their hosts and habitats as well as their mode of transmission. Several novel observations were made, the most

significant of which were the determination of the seasonal appearance and longevity of actinosporean spores at ambient temperatures (Chapters 6 and 7). Observations from these studies revealed that each form of actinosporean had its optimal range of water temperature and that most developed to their mature spore stage when the water temperature was high. This coincided with the feeding and growing season of larval fish in the lake. The actinosporean spores were shown to be shorted-lived at their ambient temperatures. In addition, the localization of the hosts of actinosporean parasites was found to overlap with areas that served as prime feeding sites for larval fish. Therefore, the synchronization between the feeding and growing season of susceptible larval fish and the maturation of spores of most forms of actinosporeans may be the result of selective pressures on both phases of the myxozoan parasites and provides evidence for the 2-host life cycle hypothesis from an ecological perspective.

Molecular Biological Characterization

Systematics has benefited greatly from the availability and incorporation of independent data sets because the information from these data sets are additive, although there is currently a debate on how these data should be analysed (Kluge, 1989; Faith and Cranston, 1991; Bull et al., 1993; Nixon and Carpenter, 1993; Friedlander et al., 1994; De Querioz et al., 1995; Huelsenbeck et al., 1996). Therefore, in addition to the morphological features, molecular biological features, in the form of data from riboprint and DNA sequence analyses of the 18S rRNA genes of selected actinosporeans and myxosporeans in this study, were investigated and incorporated into phylogenetic analyses of myxozoans (Chapter 8). Most taxa studied showed distinct riboprints, whereas identical riboprints were found between *M. pendula* and *M. pellicides*, and triactinomyxon 'C' and triactinomyxon ignotum. Some variations may be missed when using the riboprinting technique

due to the small sample size and the inability to differentiate small size differences among fragments. However, riboprinting allows for the rapid examination of numerous species and has been shown to be useful for distinguishing among species (Clark, 1992, 1997). Therefore, this technique is useful for screening potential corresponding stages between myxosporean and actinosporean phases in complex ecological settings.

The riboprint and sequence data of the 18S rRNA genes of the actinosporeans and myxosporeans from oligochaetes and fish of Lake Sasajewun were used for parsimony analyses (Chapters 8 and 9). The phylogeny of these parasites supports the 2-host life cycle hypothesis. In addition, results from the analysis of sequences generated in this study along with sequences data from GenBank confirmed that the genera *Myxobolus* and *Henneguya* are not monophyletic groups. Since the life cycle of most myxozoans have not yet been elucidated, generating molecular phylogenies based on the nucleotide sequence of relatively conserved 18S rRNA genes might be more useful for myxozoans.

Systematics and Phylogeny

Despite recent advances in our understanding of the biology of the Myxozoa (Wolf and Markiw, 1984; El-Matbouli and Hoffmann, 1987, 1989, 1993; Kent et al., 1993; Lom and Dykova, 1992, 1997; Lom et al., 1997; Yokoyama, 1997), the traditional approach of unravelling their within-group relationships by utilizing only the overall similarity of the myxosporean stages, and ignoring the corresponding actinosporean stages, still persists (Lom and Dykova, 1992; Kent et al., 1994). The phylogenetic total evidence approach was adopted for the first time to hypothesize the relationships among myxozoans with known alternating life cycles (Chapter 10). A total of 49 characters and 15 taxa were included in this analysis. The most significant observations from this study were that the combined

data resulted in fewer trees and better resolution than the partitioned myxosporean and actinosporean data sets, and that characters of the actinosporean stages from worm hosts were more conservative than characters of the myxosporean stages from fish hosts. Another novel observation was that the suborder Variisporina and the family Myxobolidae did not form monophyletic groups. This study indicated that combined data not only retained phylogenetic information of each life stage and provided better resolution, but also revealed phylogenetic information missed by data partitioning. Due to the limited available life cycle data, the systematics of Myxozoa was not revised. However, this study may serve as a basis in future attempts to modify the systematics of myxozoans using the phylogenetic total evidence approach.

Perspectives on Myxozoan Life Cycle Patterns

Substantial data detailed in this study supported the 2-host life cycle hypothesis of Wolf and Markiw (1984). The distribution of the majority of sympleisiomorphic characters of myxozoans in their actinosporean phase led me to hypothesize that the myxozoan parasites were annelid parasites first and later evolved into parasites that alternated between annelid and fish hosts (Chapter 5). This is in agreement with the recent findings that annelids are the definitive hosts and fish are the intermediate hosts (El-Matbouli and Hoffmann, 1998). However, Diamant's (1997) demonstration of direct transmission of a myxosporean parasite from fish to fish and the striking discrepancy between the numbers of species of myxosporeans and actinosporeans in Lake Sasajewun raised some interesting questions. Is it possible that not all myxozoans undergo a 2-host life cycle and that different life cycle patterns of myxozoans could coexist?

I propose the following evolutionary scenario: All myxozoans, with respect to a variety of observable synapomorphies and their unique ontogenetic

development, originated from a common ancestor. The retention of the majority of sympleisiomorphic characters of myxozoans in their actinosporean phase in worm hosts, and the appearance of many morphological variations in their myxosporean phase in fish hosts, suggests that these parasites had a longer association with the worm than with the fish, namely that they were parasites of worms first and evolved into parasites that exhibited a 2-host life cycle. The retention and modification of this type of life cycle might result in the coexistence of the following different life cycle patterns: 1) evolving into a 2-host life cycle as demonstrated by Wolf and Markiw (1984); and 2) evolving the ability to undergo an opportunistic direct life cycle between fish, or evolving into an obligatory direct life cycle between fish due to the loss of the actinosporean phase of worms as demonstrated by Diamant (1998). Other types of 2-host life cycles could also have originated by host switching when the worm hosts were not available. There is also the possibility that a reversal to the ancestral state of the 2-host life cycle pattern has occurred whereby the direct worm-to-worm life cycle pattern has been re-established. Further investigations of the life cycles of myxozoans are required to verify these hypothesized scenarios. In order to fully understand the true nature of these enigmatic parasites, the life cycle, morphology, and molecular and ecological data must be considered in an evolutionary framework.

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Appendix I

Character Matrix Used in the Parsimony Analysis of Myxozoans Based on Riboprinting Data

Appendix I. Character matrix used in the parsimony analysis of myxozoans based on riboprinting data

	Hind III	Eco RI	Dde I
		1	11111111122222222233333333333
Taxa	12345	67890	12345678901234567890123456789
Myxidium sp.	00000	00100	0100000000000001000000000000
Chloromyxum trijugum	00000	10001	0100001000000100000010000000
Myxobolus neurophilus	00000	00000	0100000000000001000100000000
Myxobolus pendula	00000	01010	0001000000000100000010110000
Myxobolus sp1	01100	00000	0000000000100100000010100101
Myxobolus sp2	00000	00000	0100000010000001000000000000
Myxobolus osburni	00000	01010	0000010000000011000100000000
Myxobolus pellicides	00000	01010	0001000000000100000010110000
Myxobolus bibullatus	00110	00000	0000000100001001000010000000
Neoactinomyxum form	01100	00000	0000000001010000010011000000
Aurantiactinomyxon form	01100	00000	0010000000000000010000000101
Echinactinomyxon 'D'	10001	00000	0000000000000001100110100000
Antonactinomyxon form	01100	00000	0000100100000000000010000000
Synactinomyxon form	01100	01010	0000100000000001001010010000
Raabeia 'B'	00000	10001	0001000000000010100100001000
Triactinomyxon 'F'	10001	00000	0000000000000001100001100000
Triactinomyxon 'C'	00000	00000	1000000000000001000000000000
Triactinomyxon ignotum	00000	00000	1000000000000001000000000000

	Taq I	Hha I
	44444444445555555555666666666	77777777778888888888999999
Taxa	01234567890123456789012345678	01234567890123456789012345
Myxidium sp.	0000000001000010100100000001	1000000001000001000000000
Chloromyxum trijugum	0000000001000110001001100000	0100000000000001110100000
Myxobolus neurophilus	0001000000000001100001011000	0000001000010001001010001
Myxobolus pendula	0001000100000001001010001000	1000000000000001010100000
Myxobolus sp1	0000010000001000011001011001	1000000001000000000000001
Myxobolus sp2	0000000110100000001000001000	0010000001100000000010000
Myxobolus osburni	0001001000000000010000110100	0000100000010001000001011
Myxobolus pellicides	0001000100000001001010001000	1000000000000001010100000
Myxobolus bibullatus	0000000100001000111001001000	1000000000000001100010000
Neoactinomyxum form	0000111000000010100101001010	0000010001000100000001011
Aurantiactinomyxon form	1000000000000000000101011010	0001000000010000010001001
Echinactinomyxon 'D'	0010000000000010001000001010	0000001000000111010001000
Antonactinomyxon form	0100000000010010101000001000	0010000010001001000000001
Synactinomyxon form	0100001000000000011001001010	0000010000010001110100000
Raabeia 'B'	0000000100000101001001001101	0000100100000000100100011
Triactinomyxon 'F'	0010000000100000001001010001	0000000001001001010001101
Triactinomyxon 'C'	0001000110000000001000001000	1000000000000000010110000
Triactinomyxon ignotum	0001000110000000001000001000	1000000000000000010110000

	Alu I	Rsa I
	1111111111111111111	11111111111111111111111
	99999000000000111111111	12222222223333333333344
Taxa	567890123456789012345678	901234567890123456789O1
Myxidium sp.	000010000000000000000000	001000000000000000000100
Chloromyxum trijugum	000000000111110000010000	000100100000000000000000
Myxobolus neurophilus	000100000100000000010000	000001000010100000000000
Myxobolus pendula	000010100100000000010010	000000010000100100000000
Myxobolus sp1	010000000100000001010000	000100000000101000000000
Myxobolus sp2	000000110100000000110101	000000000100100100000000
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Myxobolus pellicides	000010100100000000010010	000000010000100100000000
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Echinactinomyxon 'D'	000000001000010001010000	000000001001100000010100
Antonactinomyxon form	000000000011010001010000	000001010000000000000100
Synactinomyxon form	000010000010110001110000	100000000000000001000100
Raabeia 'B'	010000001000000000010000	010000000000000000100111
Triactinomyxon 'F'	000101010000000110010100	000001000001100000000100
Triactinomyxon 'C'	000100100000000000111000	000000000010110100000001
Triactinomyxon ignotum	000100100000000000111000	000000000010110100000001

	Sau 3A I	Hinf I
	11111111111111111111	11111111111111111111111111
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Chloromyxum trijugum	000001000001100010100	001010000000000001000000001
Myxobolus neurophilus	000000001101100110000	000000001100110010000000001
Myxobolus pendula	010000010001000000100	000010000000100010000000101
Myxobolus sp1	000100010101000000100	010001000000000001000000101
Myxobolus sp2	000100000001100100100	010001000000000001001000100
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Myxobolus pellicides	010000010001000000100	000010000000100010000000101
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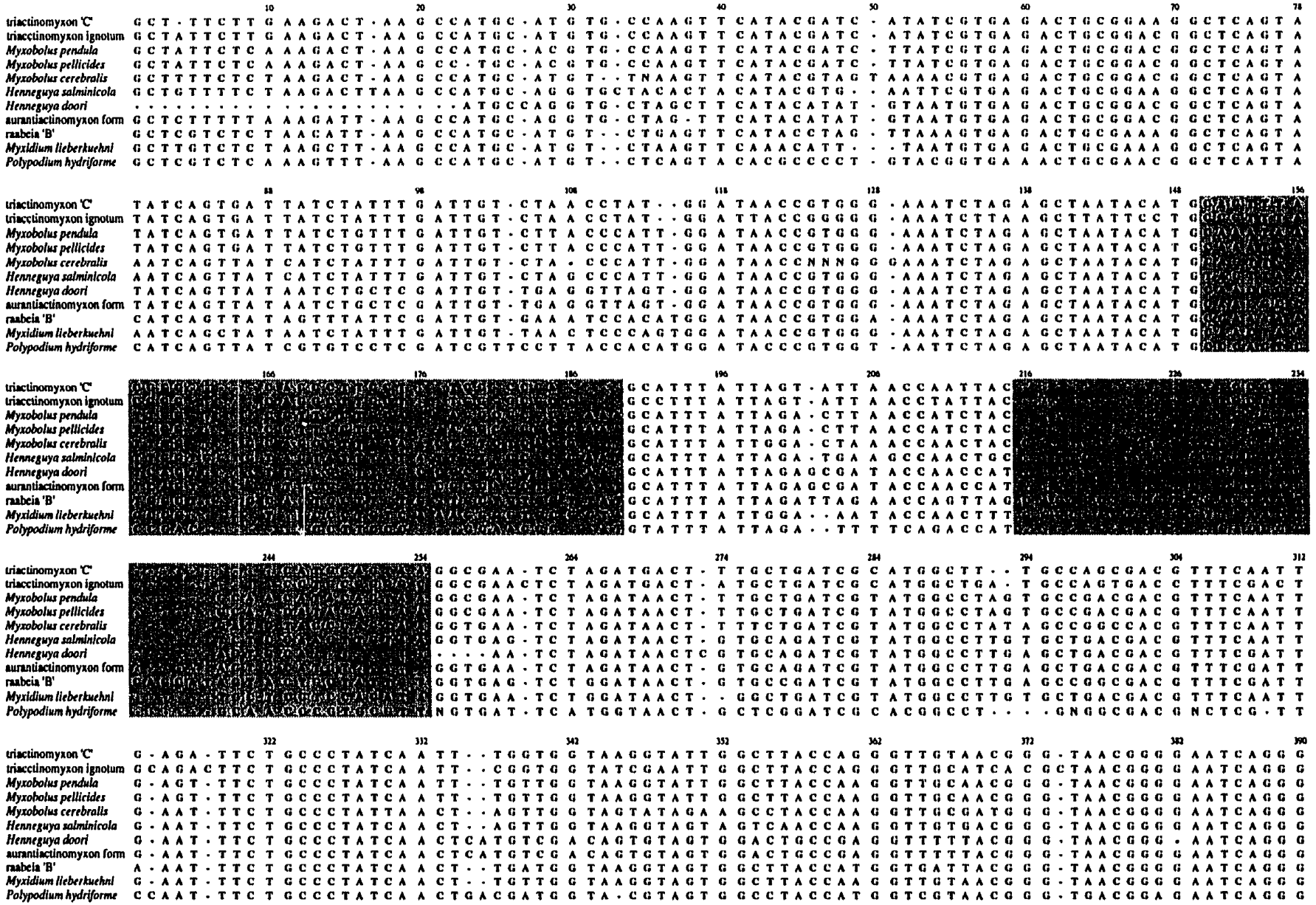
Echinactinomyxon 'D'	000000001001101000000	001000000000100001001000l0
Antonactinomyxon form	000001100001000000000	100000010000000000100000001
Synactinomyxon form	001000101001000010000	100000010000000000100000001
Raabeia 'B'	100011000001000000000	010000000000001001000000010
Triactinomyxon 'F'	000100010011010000000	000000100110010010010001O0
Triactinomyxon 'C'	000001010011001000000	010001000000000000000100101
Triactinomyxon ignotum	000001010011001000000	010001000000000000000100101

Appendix II

Sequence Alignment of 18S rRNA genes of Ten Myxozoans with *Ploypodium hydriforme* as Outgroup (shaded areas were excluded in the phylogenetic analysis)

APPENDIX II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Myxobolus pellicides
Myxobolus cerebralis
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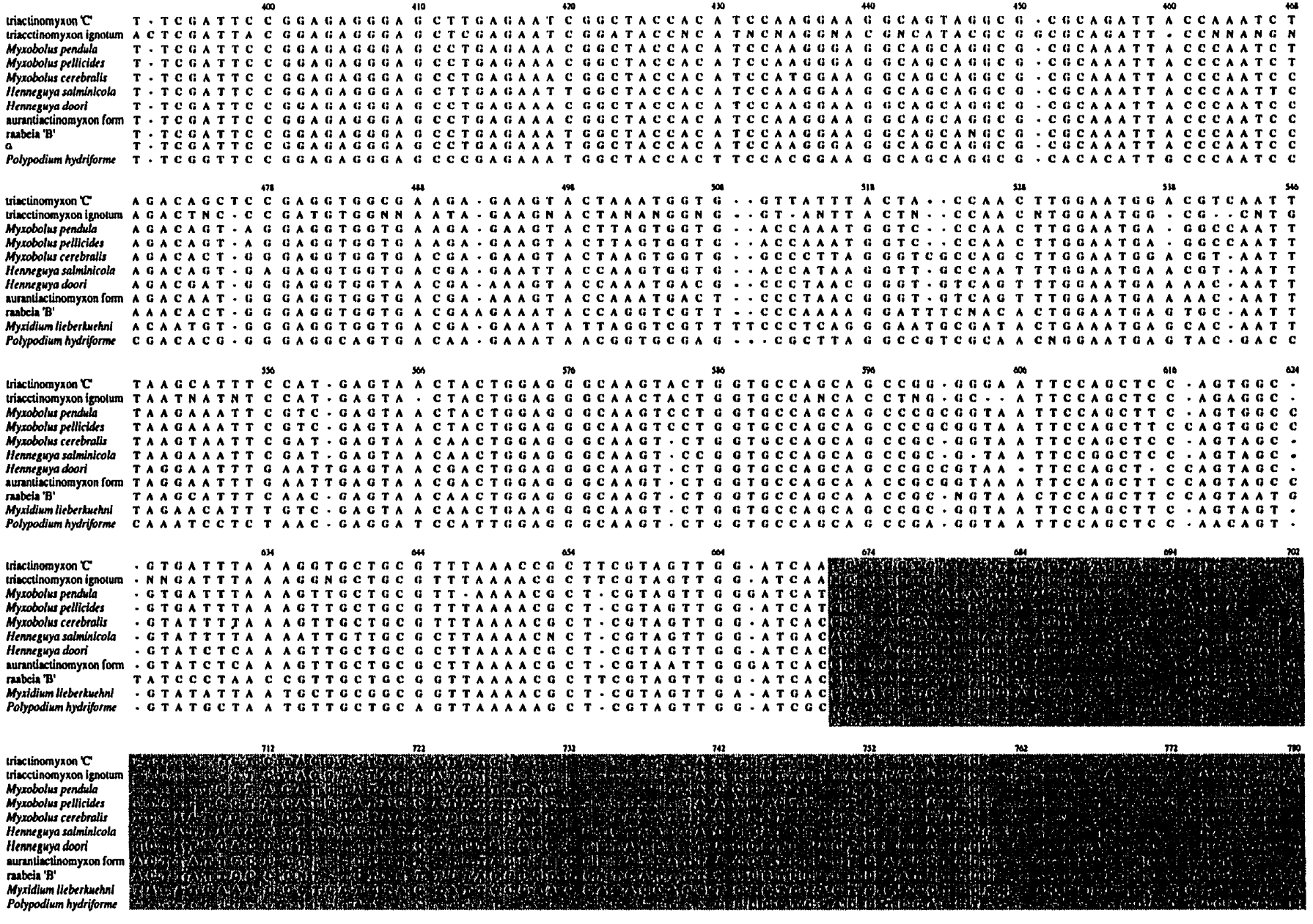
Henneguya doori
aurantiactinomyxon form
raabeia 'B'
Myxidium lieberkuehni
Polypodium hydriforme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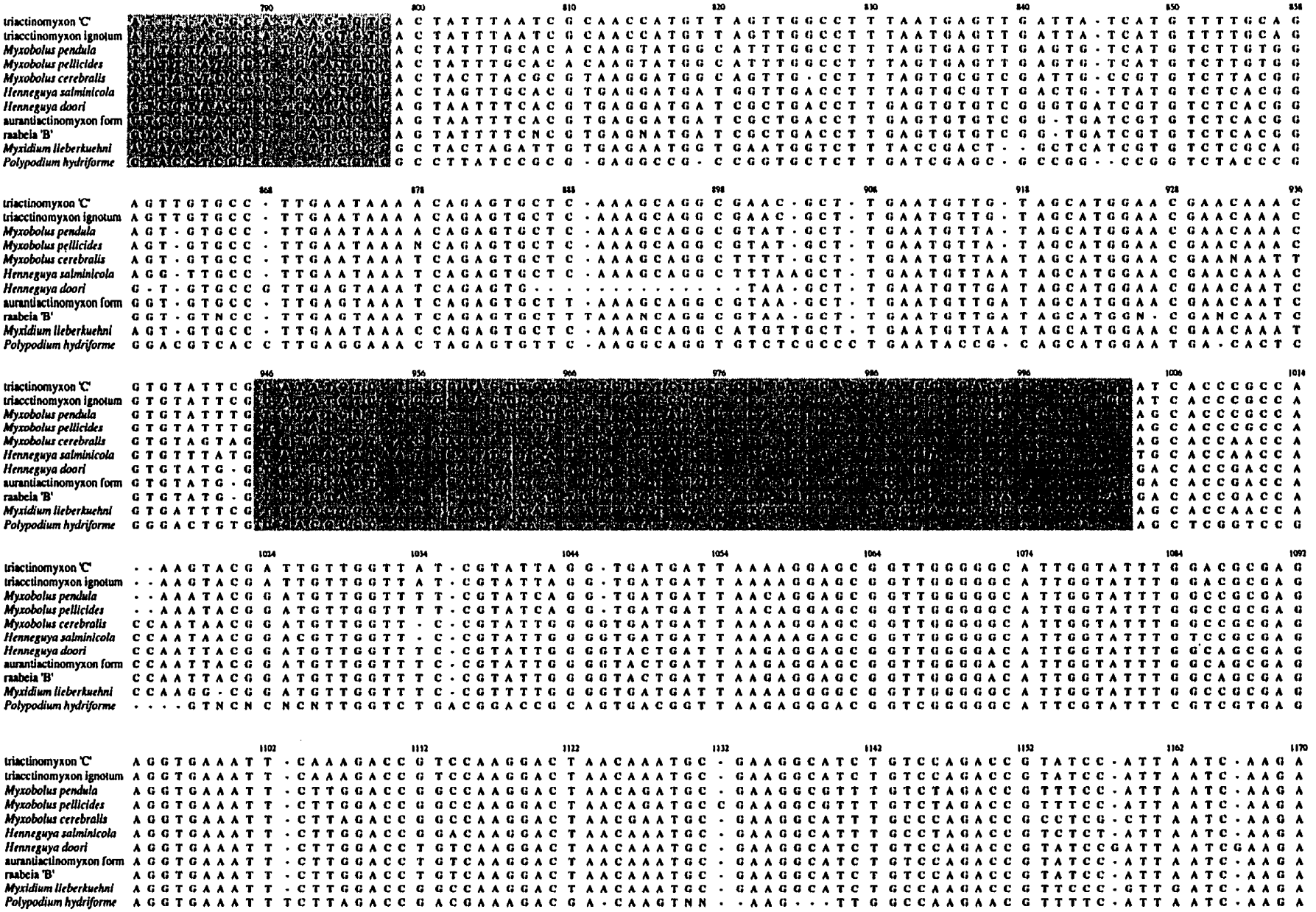
Polypodium hydriforme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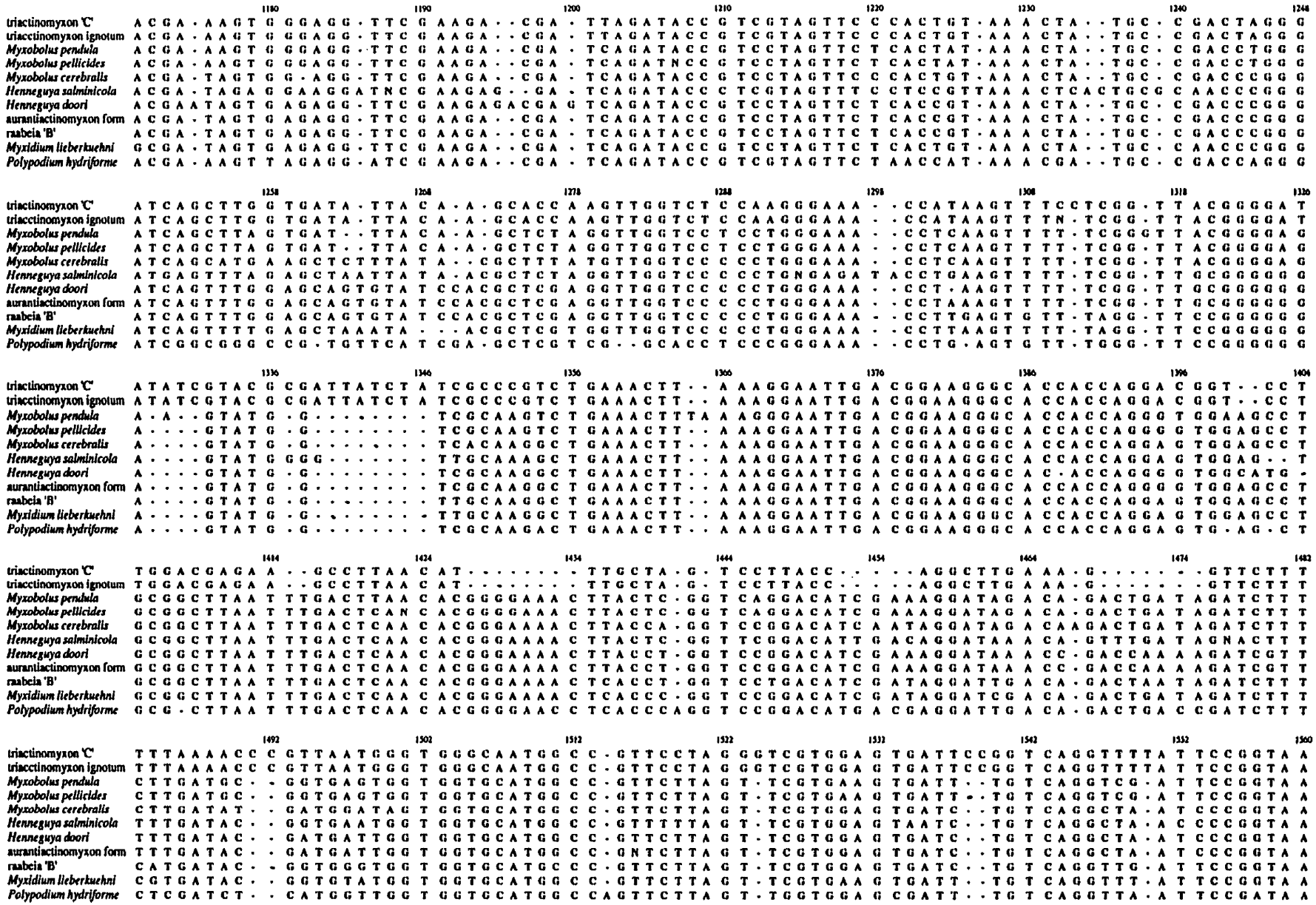
raabeia 'B'
Myxidium lieberkuehni
Polypodium hydriforme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Henneguya doori A T C A G T T T G G A G C A G T G T A T C C A C G C T C G A G G T T G G T C C C C C T G G G A A A - - C C T - A A G T T T T - T C G G - T T G C G G G G G G
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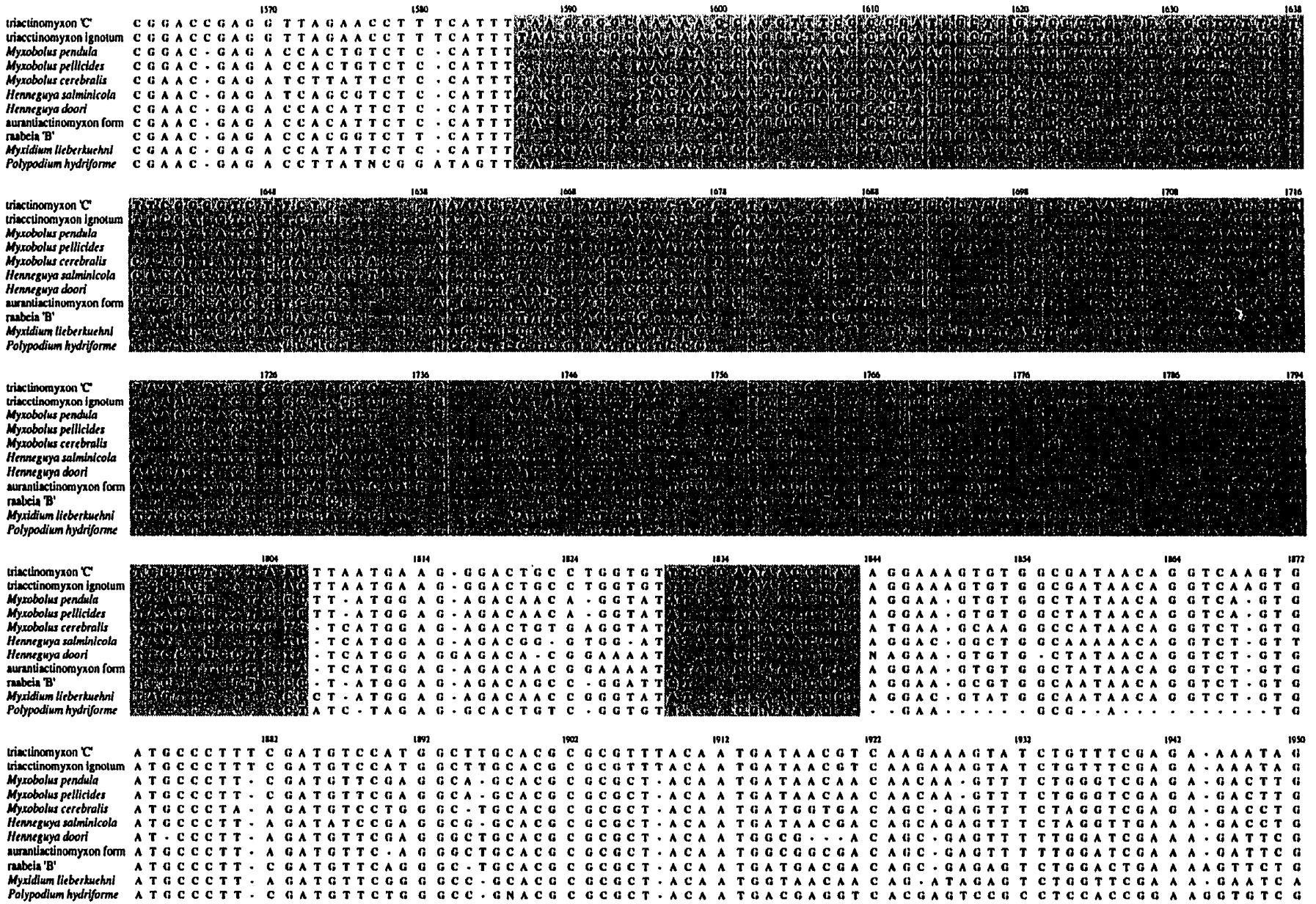
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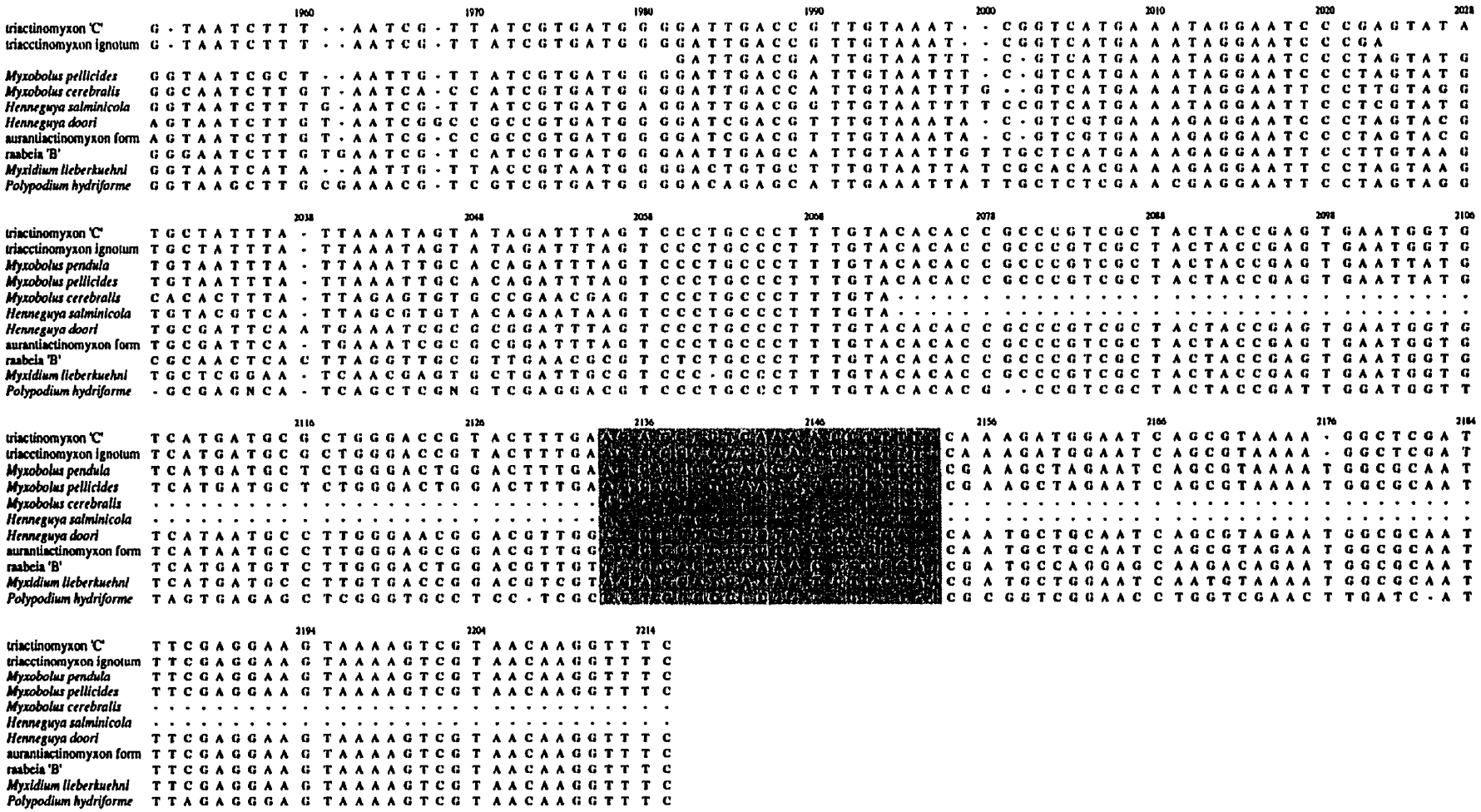
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Henneguya doori
aurantiactinomyxon form
raabcia 'B'
Myxidium liebertuehni
Polypodium hydriforme
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triacctinomyxon ignotum
Myxobolus pendula
Myxobolus pellicides
Myxobolus cerebralis
Henneguya salminicola
Henneguya doori
aurantiactinomyxon form
raabcia 'B'
Myxidium liebertuehni
Polypodium hydriforme
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triacctinomyxon ignotum
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Myxobolus cerebralis
Henneguya salminicola
Henneguya doori
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raabcia 'B'
Myxidium liebertuehni
Polypodium hydriforme
2194 2204 7214
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triacctinomyxon ignotum
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Henneguya salminicola
Henneguya doori
aurantiactinomyxon form
raabcia 'B'
Myxidium liebertuehni
Polypodium hydriforme