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Review

Blastocladian parasites of invertebrates

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ABSTRACT

Some species of zoosporic fungi in the Phylum Blastocladiomycota are obligate parasites of invertebrate animals. Various stages in the life history of the host can be infected, including eggs, larvae and adults. Some parasites, such as some species of *Coelomomyces*, alternate between two hosts; while others, such as some species of *Catenaria*, require only one host for reproduction. Infection generally begins after zoospores attach to the outer surface of the host body or to the gut wall. Host specificity and parasite virulence vary broadly among species. Some parasites such as *Coelomomyces* and *Catenaria* can naturally regulate dipteran insect or nematode populations. New roles for Blastocladian parasites in food web dynamics have recently been discovered. For example, the parasite *Polycaryum* can reduce the quantity and quality of *Daphnia* hosts as a food resource for planktivorous fish. We propose that zoosporic fungal parasites contribute significantly to the biodiversity and the complexity of the food webs in freshwater and soil ecosystems. Further research is needed to highlight the importance of zoosporic fungal parasites in aquatic ecosystems.

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1. Introduction

From a theoretical point of view, for a fungus to be considered a true parasite or pathogen, its taxonomic identity, its life cycle and the nature of the disease it causes in its host must be fully understood. Nevertheless, numerous reports in the literature refer to certain zoosporic fungi as parasites, when in fact very little evidence is presented to support these conclusions. Such reports may be based in part on the observation that many zoosporic fungi are commonly found growing as saprotrophs on sick, injured or dead animals or on incomplete data. In this review, we focus on common and clearly documented parasites and their hosts. Possible parasites that have been poorly described are not included.

The definitions of obligate and facultative parasitism have often been based on the criterion of culturability outside the host (Lucas, 1998). However, as more and more obligate parasites are grown on artificial media, these distinctions have become more subjective and arbitrary. Thus, the differences between obligately parasitic, facultatively parasitic and saprotrophic species of zoosporic fungi have often been confused. The terms biotroph, necrotroph and saprotroph, which are currently used for plant pathogens, can be useful in providing

investigators with more exacting criteria for describing the various parasitic strategies of all zoosporic fungi (Lucas, 1998).

Zoosporic fungi are a very diverse and species-rich group of microorganisms (Shearer et al., 2007). Many species in *Catenaria*, *Coelomomyces*, *Olpidium* and several other genera of zoosporic fungi are common parasites of small invertebrates in freshwater and soil ecosystems worldwide (Whisler, 1985; Powell, 1993; Dick, 2003; Barron, 2004). Almost any species of invertebrate which has eggs, larvae or adults in direct contact with freshwater or soil might be a target for parasitism by zoosporic fungi. Many invertebrate groups (*e.g.*, rotifers, nematodes, mollusks, crustaceans and hexapods) are found in aquatic environments at some stage in their life cycles. However, the numbers of genera and species of zoosporic fungal parasites of invertebrates which have been reported are comparatively small, possibly because of a lack of knowledge concerning the biology of potential hosts and appropriate sampling methods.

Although we have included major taxa of the Blastocladiomycota in our discussion of classification and life cycles, the present review is focused on those members that are biotrophic parasites (*i.e.*, incapable of growth outside the host) of invertebrate animals. We emphasize parasitic species of *Catenaria*, *Coelomomyces* and *Polycaryum* because these species have been studied intensively. *Callimastix*, *Coelomycidium* and *Sorochytrium* are briefly discussed as well. We begin with a discussion of the types of life cycles occurring in the Blastocladiomycota and more specifically in the major parasitic genera. Next, we consider the range of hosts infected by each parasite. Third, we consider the infection process. Finally, we discuss several important aspects of community ecology, particularly the roles of Blastocladian parasites in regulating host population sizes and influencing energy transfer in freshwater and terrestrial food webs.

2.   Classification of the zoosporic fungi

In recent higher order classifications, posteriorly uniflagellate fungi (or chytrids) have been placed into three phyla: Blastocladiomycota, Chytridiomycota and Neocallimastigomycota (James et al., 2000, 2006; Letcher et al., 2006, 2008; Hibbett et al., 2007). The appropriate placement of genera in the *Olpidium–Rozella* complex (*sensu* Karling, 1977) into phyla is still under investigation.

Zoosporic fungi are placed into the Phylum Blastocladiomycota based primarily on morphological and cytological characters, including a zoospore with a single whiplash flagellum, a nuclear cap, and side–body complex (microbody lipid–globule complex), as well as life cycles with alternation of gametophyte and sporophyte generations. Nutritional characters, such as nitrogen and sulfur requirements, and molecular characters, such as the mitochondrial genomes and sequences of 18S and 28S ribosomal genes have more recently been employed in systematic and phylogenetic studies of the phylum (Nolan, 1985; Whisler, 1985; James et al., 2006). The Phylum Blastocladiomycota presently contains one order (Blastocladiales) and five families (Blastocladiaceae, Catenariaceae, Coelomomycetaceae, Sorochytriaceae and Physodermataceae) (Sparrow, 1960; Karling, 1977;

Olson and Lange, 1978; Lange and Olson, 1980; Dewel and Dewel, 1990). The families Catenariaceae, Coelomomycetaceae and Sorochytriaceae contain many parasites of small invertebrates while the Physodermataceae contains plant parasites and the Blastocladiaceae contains saprotrophs. *Callimastix*, *Coelomycidium*, *Polycaryum* and the Blastocladian parasite of *Haematococcus* have not yet been placed into families (Johnson et al., 2006a; Hoffman et al., 2008).

Most of the zoosporic fungi which parasitize invertebrates have been properly identified as members of the Phylum Blastocladiomycota. However, studies of zoospore ultrastructure and molecular phylogeny have focused more on saprotrophic members of the group or have been limited to a single representative of each genus. Therefore, additional data are needed to further clarify and resolve the taxonomic, morphological, cytological and systematic diversity within the phylum.

3.   Parasites and their hosts

Biodiversity

Many of the common zoosporic fungal parasites and their invertebrate hosts are listed in Table 1. The genera *Coelomomyces* and *Catenaria* include the greatest number of described species of parasites. The largest groups of hosts belong to the Crustacea, Hexapoda (Diptera) and Nematoda. Additional hosts include the other helminths, mites, rotifers and eggs of liver flukes (Couch, 1945; Tribe, 1977), but these reports are based on brief or incomplete observations and much more research is needed to properly understand the roles of zoosporic fungi in these infections. Also some parasites of the adult stages of rotifers have been inadequately identified (Barron, 2004). Studies using light microscopy have suggested that large numbers of both parasitic and saprotrophic zoosporic fungi are present in most freshwater and soil ecosystems (Sparrow, 1960; Shearer et al., 2007).

Parasitic strategies

Blastocladian parasites of invertebrates can reasonably be classified as biotrophs, hemibiotrophs or necrotrophs (Table 2). These terms were originally designed for plant pathogens based on parasitic or nutritional strategies. However, recent research on the mode-of-defence in the plant hosts is providing new insights into the relationships between parasites and their hosts (Oliver and Ipcho, 2004).

Life cycles

Three types of life cycle were first described in the genus *Allomyces* by Emerson (1941), and since that time these types have been observed in other members of the Blastocladiomycota (Couch, 1945; Sparrow, 1960; Karling, 1973). The "Euallomyces type" of life cycle, with an isomorphic alternation of diploid and haploid generations, has been carefully documented in some species of the saprophytic genera *Allomyces* (Emerson, 1941; Sparrow, 1960), *Blastocladiella* (Karling, 1973) and *Microallomyces* (Emerson and Robertson, 1974). This type of life cycle is present in several species of *Coelomomyces* (a parasitic

Table 1 – Zoosporic fungal parasites and their invertebrate hosts

Parasite			Host				References
Genus	Thallus	Phylum	Genus	Stage	Common name	Phylum/Class/Order	
Callimastix	M, En	B	*Cyclops* *Eucyclops* *Mesocyclops* *Macrocyclops*	A	Copepods	Arthropoda/Crustacea/ Copepoda	Vavra and Joyon, 1966
Catenaria	P, En	B	*Brachiomus* *Filinia* *Lecane* *Eosphora* *Sinantherina*	A	Rotifers	Rotifera	Gorbunov and Kosova, 2001; Barron, 2004
	P, En		*Anguina* *Aphelenchus* *Ditylenchus* *Globodera* *Helicotylenchus* *Hemicriconemoides* *Heterodera* *Hoploaimus* *Meloidogyne* *Panagrellus* *Romanomermus* *Scutellonema* *Seinura* *Tylenchorhynchus* *Xiphinema* *Other genera*	E L A	Nematodes	Nematoda	Tribe, 1977; Sterling and Platzer, 1978; Deacon and Saxena, 1997; Singh *et al.*, 1998, 2007; Barron, 2004
	P, En		*Chironomus* *Dicrotendipis* *Endochironomus* *Glyptotendipis*	E	Midges (Chirono-minae)	Arthropoda/Insecta/ Diptera	Martin, 1975, 1978, 1991
Coelomomyces	M, En	B	*Aedes* *Anopleles* *Culex* *Culiseta* *Opifex* Other genera	L	Mosquitoes (Culicidae)	Arthropoda/Insecta/ Diptera	Whisler, 1985; Lopez Lastra and Garcia, 1997; Scholte *et al.*, 2004; Whisler *et al.*, 2009
			Chironomus *Other genera*	E	Midges (Chirono-minae)	Arthropoda/Insecta/ Diptera	McCauley, 1976; Whisler, 1985
			Acanthocyclops *Cyclops* *Elaphoidella* *Eucyclops* *Harpacticus* *Mesocyclops* *Microcyclops* *Tigriopus* *Other genera*	A	Copepods	Arthropoda/Crustacea/ Copepoda	Federici, 1980; Whisler, 1985
			Heterocypris *Potamocypris* *Other genera*	A	Ostracoids	Arthropoda/Crustacea/ Ostracoda	Whisler, 1985; Whisler *et al.*, 2009
Coelomycidium		B	*Cnephia* *Simulium* *Stegopterna*	L	Black Flies (Simuliidae)	Arthropoda/Insecta/ Diptera	Weiser, 1964; McCreadie and Adler, 1999
Polycaryum		B	*Daphnia*		*Daphnia* (Cladocera)	Arthropoda/Crustacea/ Branchiopoda	Johnson *et al.*, 2006a,b
Sorochytrium		B	*Milnesium*		Tardigrades, Water Bears	Tardigrada	Dewel *et al.*, 1985

M = Monocentric, P = Polycentric, En = Endobiotic, Ep = Epibiotic, E = Egg, L = Larval, A = Adult.

genus) (Whisler, 1985) but is suspected to be present in a number of other genera and species as well (Emerson, 1950; Sparrow, 1960; Karling, 1973; Lange and Olson, 1980; Whisler *et al.*, 1974, 1975, 1983; Whisler, 1985). The

''Cystogenes type'' of life cycle, in which a cyst and gametes are the only haploid stages, is present in some species of *Allomyces*, *Blastocladiella* and *Catenaria allomycis* (Emerson, 1941; Karling, 1973; Couch, 1945; Sykes and Porter, 1981). Asexual

Table 2 – Parasitic or nutritional strategies of Blastocladian parasites of invertebrates

Econutritional modes	External resources	Subdivision	Definition	Examples
Biotrophy	Living cells	Biochemically obligate	Obligate parasites, incapable of growth outside the host	*Callimastix*, *Coelomyces*, *Coelomycidium* and *Polycaryum*
	Living cells	Ecologically obligate	Good growth on artificial media, but cannot compete with saprotrophs in the natural environment	Some species of *Catenaria*
Hemibiotrophy	Intermediate between biotrophy and necrotrophy		Saprophytic ability severely limited in the natural environment	
Necrotrophy	Living cells killed first by toxins and cytotoxic enzymes then used		Facultative parasites, but can live well as saprotrophs	*Sorochytrium*
Saprotrophy	Non-living organic substrata		No capacity for Parasitism	Some species of *Catenaria* and *Allomyces*

Lucas (1998) provides definitions of the terms "biotrophs, necrotrophs, saprotrophs, parasites, pathogens, obligate and facultative" and a good discussion of their use with plant pathogens.

life cycles with no sexual stages are found in some species of *Allomyces*, *Blastocladiella emersonii* and *Catenaria anguillulae* (Emerson, 1941; Olson and Reichle, 1978).

Whisler et al. (1975) discovered that successful infection of the mosquito *Culiseta inornata* in the laboratory by *Coelomyces psorophorae* required the presence of the copepod *Cyclops vernalis*. The coincidence of an alternation of ploidal generations with an alternation of hosts was soon discovered in several other species of *Coelomyces* (Whisler, 1985; Federici and Lucarotti, 1986; Apperson et al., 1992a). This is called the "full-life cycle" (Fig. 1). It is now known that two hosts are required for the reproduction of many species of *Coelomyces*, with the diploid or sporophyte phase in the mosquito (the primary host) and the haploid or gametophyte phase in a copepod or ostracod (the alternative host). New obligate alternative hosts are continuously being discovered. Recently the ostracod *Potamocyris unicaudata* was found to be the alternative host for *Coelomyces utahensis* (Whisler et al., 2009). Alternation of hosts through a complex life cycle is generally rare among parasites outside of select groups of protists, rust fungi, trematodes and cestodes. When it does occur in zoosporic fungi, two species are required for the reproduction and survival of the parasite. A variety of different primary and alternative hosts for *Coelomyces* are also listed in Table 1.

However, in the case of most infections of mosquitoes and chironomids with *Coelomyces*, copepods or ostracods serve as obligate alternate hosts. In fact, in an epizootic of *Coelomyces punctatus* in a larval population of *Anopheles quadrimaculatus*, the seasonal abundance of the copepod *Acanthocyclops robustus* was positively correlated with the rate of infection of the mosquito larvae (Apperson et al., 1992a). *Mesocyclops edax* was also a competent alternative host for this fungus and was found in the same pond as *A. robustus* (Apperson et al., 1992b).

In our opinion, the frequent use of the terms primary, secondary, alternative, intermediate and definitive hosts may be appropriate for parasitic protists, rust fungi, trematodes and cestodes but should not be used for *Coelomyces* and other species of zoosporic fungi. It is very difficult to

define which host is primary and for what reason. Instead we propose the use of **Type I host** for hosts infected with the diploid phase and **Type II host** for hosts infected with the haploid phase to avoid confusion in future research (Fig. 1).

In other zoosporic fungal parasites, such as *C. anguillulae*, only one host or several related host species can be infected, but alternation of hosts is not required for reproduction (Singh et al., 1996, 1998). The prevalence of alternation of hosts among species of zoosporic fungal parasites is not known, because the life cycles of many species have not been carefully studied. A complete understanding of the life cycle is often required for successful infection of healthy hosts as Whisler et al. (2009) demonstrated with *Coelomyces*. Although *Polycaryum*, an important parasite of *Daphnia* in lake ecosystems, has been intensively studied, its life cycle is still unclear. Possibly, for this reason, re-infection attempts have been unsuccessful (Johnson et al., 2006a).

4. Mechanisms of infection of hosts

Catenaria

Martin (1975) observed the penetration of zoospores of *Catenaria spinosa* from external environment into the matrix surrounding the eggs of the midge *Chironomus attenuatus*. Once within this gelatinous material, the amoeboid zoospores become elongated and move slowly toward the eggs by using filose pseudopodia while the flagella trail passively behind. Once the zoospores have reached the egg they attach to the outer shell, lose their flagella, encyst and produce slender penetration tubes. An obligate period of amoeboid motility as part of the infective process was later reported in other *Catenaria* species parasitic in midge eggs (Martin, 1978).

Deacon and Saxena (1997) studied another isolate of *C. anguillulae* which is a facultative endoparasite of nematodes. Amoeboid zoospores crawl on the surface of nematode hosts. While crawling, the zoospores produce pseudopods and the flagella are immobile. The pseudopods retract and the
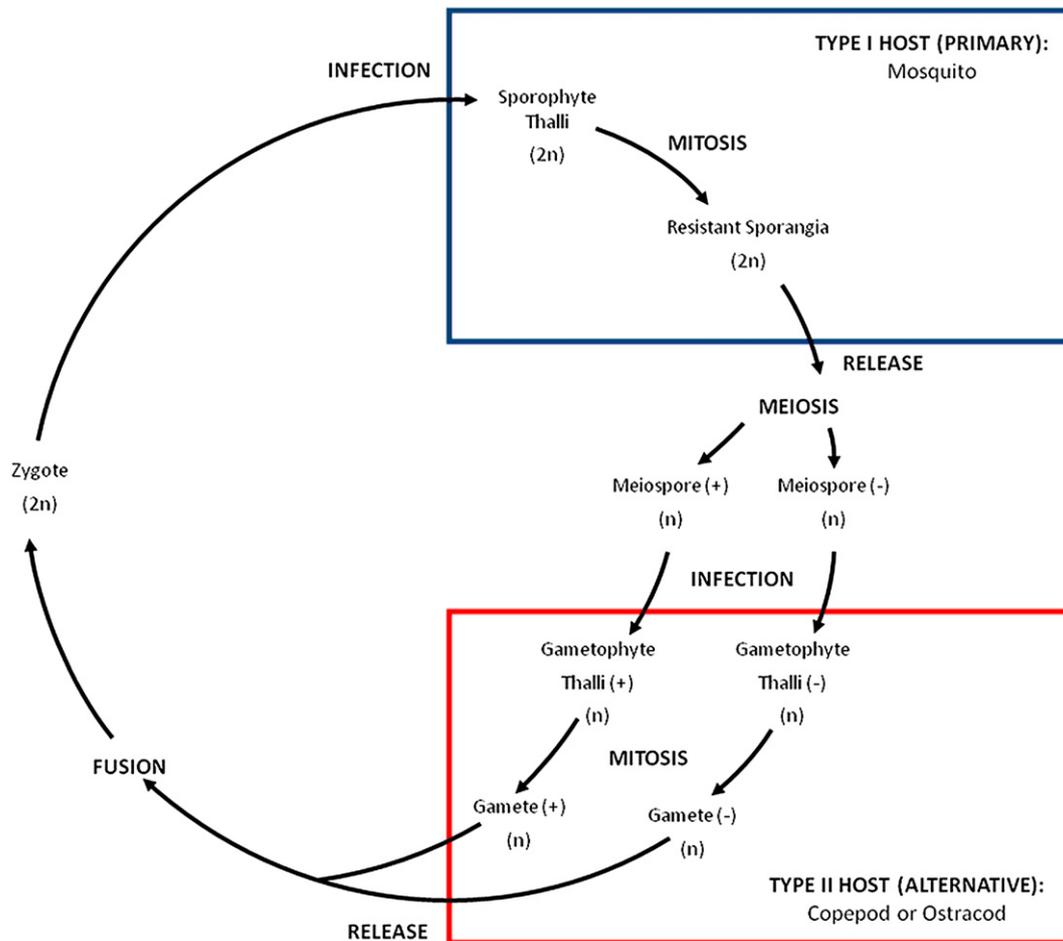
Fig. 1 – Life cycle of *Coelomomyces* redrawn from Whisler (1985).

zoospore assumes an oval shape before swimming again. Contact with surfaces appears to initiate crawling. One possible role of pseudopods may be the recognition of surface markers on the outside of the host by the cell membrane surrounding the zoospore (Deacon and Saxena, 1997). Zoospores are attracted to and encyst near the mouth, excretory pore and anus on second-stage juveniles where they accumulate (Singh et al., 1993). Then prior to encystment the zoospores adhere to the cuticle by a layer of extracellular protein which functions as an adhesive substance (Tunlid et al., 1991). Finally they encyst on the surface of the host. The cysts germinate forming a narrow germ tube. After growing a short distance the germ tube produces an intercalary vesicle from which the rhizoidal system emerges. Penetration usually occurs directly through the cuticle but can occur rarely through natural openings (Jaffee, 1986).

Coelomomyces

The ultrastructure and development of the different phases in the life cycle of the genus *Coelomomyces* have been discussed by Travland (1979) and by Bland and Couch (1985). The zygote of the parasite is produced after the fusion of gametes. Mosquito larvae (primary or Type I hosts) are infected by the

zygote which is a motile biflagellated stage. After the zygote stops swimming, it retracts its flagella, becomes amoeboid in shape and produces pseudopodia. It then crawls along the surface of the larva, and encysts near the intersegmental membranes. An adhesive substance is secreted from adhesion vesicles within the cytoplasm of the zygote which attaches the zygote to the surface (Travland, 1979). A thin-walled penetration tube extends from the appressorium and penetrates the cuticle of the mosquito host. The cytoplasm from the zygote cyst takes the form of rounded hyphagens which circulate within the hemolymph and give rise to vegetative hyphae. Resting sporangia are produced at maturity and are released following the death of the larva.

Outside the mosquito larvae the resting sporangia produce posteriorly uniflagellate meiospores with plus or minus mating types which infect the alternate hosts. The copepod (alternate or Type II host) is infected by a free-swimming meiospore in a manner similar to infection of the mosquito by a zygote. The meiospore stops swimming when it encounters the alternate host. In *Cyclops* (a copepod) encysted meiospores accumulate on the surface of the body near the intersegmental membranes. However, the ostracods are infected by a different mechanism. The ostracod ingests non-motile resting sporangia in the water column. After

release of meiospores inside the gut, infection occurs through the gut wall. One level of host specificity may be related to the process of attachment since zygotes do not attach to resistant hosts (Zebold *et al.*, 1979). The death of the microcrustacean host (copepod or ostracod) is required for the release of gametes. Timing of the life cycle is controlled by environmental triggers including redox potential, temperature and photoperiod (Federici, 1983; Whisler, 1985). For example, the release of gametes is controlled by photoperiod (Federici, 1983, 1985). Whisler *et al.* (2009) designed a good method for *in vivo* culture of *Coelomomyces* which has potential for further use in research.

Sorochytrium

Round or occasionally amoeboid zoospores attach to the cuticle in the dorsal, posterior portion of the tardigrade host (Dewel *et al.*, 1985). Then the zoospores encyst, an appressorium forms and finally a penetration tube is produced which penetrates the tardigrade. Dewel *et al.* (1985) noted differences in the penetration process in live and dead hosts. This fungus can be considered either a parasite or a saprobe.

5. Occurrence and ecological importance of the host–parasite interactions

Infection by zoosporic fungi can greatly affect the growth and reproduction of invertebrate hosts and will sometimes lead to rapid death of the host, especially when transmission depends on death of the host. In such cases, these parasites employ a 'parasitoid' life history strategy (*e.g.*, Lafferty and Kuris, 2002). There is growing evidence that zoosporic fungal parasites play major roles in regulating and/or controlling the population levels of herbivorous macro-invertebrates (various *Catenaria* species in eggs of Chironomidae) and zooplankton (*Polycaryum laeve* in Cladocera) in freshwater lakes and streams (Martin, 1981, 1984; Johnson *et al.*, 2006a). The ecological significance of other parasitic genera (*Callimastix*, *Coelomycidium* and *Sorochytrium*) is not yet known as there has been little mention of their occurrence in the literature and, as previously stated for *Polycaryum*, their complete life cycles are unknown at this time.

Improved culture techniques and accurate bioassays need to be developed for the measurement of infection and mortality rates so that the effects of biological and physical factors on the host–parasite relationships can be studied (Bland, 1985; Singh *et al.*, 1998).

Callimastix

Vavra and Joyon (1966) found *Callimastix cyclops* in the body cavities of four genera of copepods and describe the stages in the development of the disease. Unlike other zoosporic fungal parasites, the zoospores of *Callimastix* are multiflagellate. They suggest that this parasite is present in copepod populations throughout the year although it is more frequent in early spring. Unfortunately no other more recent reports have been published.

Catenaria

Although initially considered a low virulence parasite of nematodes, *Catenaria* is now known to cause varying degrees of pathology within its hosts. Sayre and Keeley (1969) tested zoospores of *C. anguillulae* for virulence in two genera of nematodes, *Panagrellus* (free-living) and *Ditylenchus* (plant parasitic), and developed a bioassay. They observed a strong correlation between the number of zoospores present and the infection prevalence. Tribe (1977) first documented that *Catenaria auxiliaris* is a common parasite in populations of the sugar beet cyst-nematode and the cereal cyst-nematode. Singh *et al.* (1996, 1998) developed techniques for testing the virulence of *C. anguillulae* and found that this fungus is a virulent pathogen of many genera of both free-living and plant parasitic nematodes. Singh *et al.* (1996) found that non-motile juveniles of *Anguina trici*, *Seinura* sp. and *Xiphinema basiri* and females of *Meloidogyne javanica* and *Heterodera cajani* were highly susceptible, while the parasite grew poorly in six other species of nematode without causing death. Singh *et al.* (1993, 1998) developed a method for rapid virulence testing using second-stage juveniles of *H. cajani*, *Meloidogyne incognita* and *A. trici*.

It is interesting to note that in *C. anguillulae* infection rates of nematodes were highest at pH 9 (Sayre and Keeley, 1969) and pH values less than 5 prevented progression of the disease (Sterling and Platzer, 1978). The pH values for maximum growth rates for *C. anguillulae* in culture are between 8 and 9 (Nolan, 1985).

Singh *et al.* (2007) observed that *C. anguillulae* parasitized and killed the eggs and the second-stage juveniles of *Meloidogyne graminicola*. This nematode causes root knot disease of rice, and *C. anguillulae* appears to be an efficient and effective agent for controlling populations of *M. graminicola* in rice fields (Singh *et al.*, 2007). Singh *et al.* (2007) also noted that *C. anguillulae* causes a sharp decline in populations of *Heterodera sorghi*, a cyst-nematode, and another important plant pathogen.

Martin (1984) investigated the natural regulation of midge populations in lakes, rivers, streams and swamps in Virginia by a community of parasites including zoosporic fungi and stramenopiles. In the study at Mechumps Creek, the dependent variables were percentages of infection and mortality of midge eggs, and the independent variables were seasonal patterns of temperature, rainfall, light and velocity of running water (physical factors) and species of parasites and hosts present (biological factors). Martin (1984) concluded that population size of midges was controlled largely by the population of parasites, particularly *Catenaria*, and other factors in littoral communities.

Coelomomyces

Infection prevalences in mosquito populations vary considerably among host and parasite species and with seasons and many other environmental factors (Apperson *et al.*, 1992b). Apperson *et al.* (1992b) observed that the seasonal abundance of the copepod (alternative host) was the most important variable correlated with infection rate in the mosquito *A. quadrimaculatus*. The abundance of late instar larvae was also an important variable. There can be differences in susceptibility

to infection in the different stages of development of the mosquito host. For example, in one study the fourth instar larvae of *A. quadrimaculatus* had a higher infection rate than lesser instars (Umphlett, 1969). Also there can be differences in susceptibility to infection in the different stages of development of the alternate host. The susceptibility of the nauplii stage of *C. vernalis* decreased as the copepod developed (Federici, 1980).

The range of susceptible hosts also varies with the species of the host and the parasite. For example, *C. psorophorae* infects a relatively broad range of mosquito species in the genus *Culiseta* in the presence of the alternate host *C. vernalis* (Zebold et al., 1979). Seven species of mosquitoes were susceptible and five species were resistant. *C. utahensis* can infect species in three genera of mosquitoes: *Aedes*, *Culex* and *Culiseta* (Whisler et al., 2009). Several alternative hosts may also be infected by the same parasite. For example, *C. utahensis* can infect both *Potamocypris unicaudata* and *Potamocypris smaragdina* (ostracods) (Whisler et al., 2009). Although studies on the ecology of many species of mosquito (primary hosts) have provided some useful information, our knowledge of the ecology of the parasites and their alternative hosts is still inadequate.

Natural regulation of mosquito populations by *Coelomomyces* is more complicated because two hosts are involved. Apperson et al. (1992b) pointed out the complex relationships between populations of the primary host, the alternate host and the parasite. Although many factors are involved, natural regulation of both mosquito and copepod populations by *Coelomomyces* does occur. Umphlett (1969) suggested two mechanisms for control of mosquito populations: *Coelomomyces* may cause death of the larvae or it may delay the development of the larvae for extended periods of time thus subjecting them to other destructive forces in the environment. Since *Coelomomyces* is a highly specific parasite of mosquito larvae and the rate of infection is high under experimental conditions (Chapman, 1985), this fungus potentially appeared to be an ideal agent for biological control of mosquitoes.

Coelomycidium

Coelomycidium is a common parasite of black flies (Simuliidae) (Weiser, 1964; McCreadie and Adler, 1999). For example, mid- to late instar larvae were examined from 115 stream sites in South Carolina for the presence of *Coelomycidium simulii*. Although the prevalence was generally low (<1 %), infection was detected in 17 host species (40 %) (McCreadie and Adler, 1999).

Polycaryum

Johnson et al. (2006a,b, 2009) studied the regulation of host population dynamics in *Daphnia pulicaria*, a keystone zooplankter in lake ecosystems. Infection by *P. laeve*, which was largely specific to *D. pulicaria* but could sometimes be noted in other cladocerans (e.g., *Daphnia mendotae* and *Holopedium gibberum*), causing up to 99 % declines in host population densities during epizootics. Infection prevalence varied with season. It was highest during late winter and early spring (up to 80 %) and lowest in late summer (1 %). Infected

individuals produced no eggs, molted less frequently and died sooner than uninfected individuals. Because of their altered appearance, infected *Daphnia* were also more susceptible to visual predators such as fish, indicating both direct and indirect effects of infection on host survival (Johnson et al., 2006b).

After compiling a 15-y data set on *Polycaryum* infection in *Daphnia* populations, Johnson et al. (2009) used autoregressive time-series analyses to evaluate the importance of biological and physical factors (independent variables) in controlling infection prevalence (dependent variable). These factors included host density, host food availability, water temperature, dissolved oxygen and lake mixing. The authors found that patterns of infection were driven primarily by changes in lake mixing, which influenced spatial interactions between host and parasite, and to a lesser extent by overall changes in host density. Infections were estimated to decrease host populations by 11–50 %. The dependent variables included measures of reproductive rates, infection prevalence and host population size. As one of the few long-term studies of zoosporic fungal disease, this study has given greater insight into the mechanisms of population regulation in *Daphnia* and the potential importance of parasitism in lake food webs.

6. Roles of zoosporic parasites in food webs

Food webs are essential to our general understanding of the diversity, complexity and functioning of ecosystems (Lafferty et al., 2008). Parasites may influence the structure, connectedness, patterns of the flow of matter and energy, and stability of ecosystems. Parasites can also create or facilitate additional trophic interactions. Because of differences in consumer and resource body sizes, the outcome of a given interaction (e.g., death *versus* morbidity) and its *per capita* impact differ substantially between parasitism and predation (Lafferty and Kuris, 2002). Unfortunately, however, the small size and cryptic nature of many parasites have hindered their inclusion into conventional food web analyses. Thus, while parasitism is one of the most common consumer strategies among organisms, it is rarely included in food webs (Lafferty et al., 2008).

Recently Gleason et al. (2008) reviewed available knowledge of the roles of zoosporic fungi in aquatic food webs. Previously, little attention has been devoted to the roles of zoosporic fungi including parasites in the dynamics of matter and energy transfer in food webs. However, zoosporic fungi can affect energy transfer in at least two different trophic levels. As emphasized by Kagami et al. (2007), actively swimming zoospores are efficiently grazed by crustaceans, such as *Daphnia*, and possibly other filter feeders. Consequently, zoospores from saprobes (primary consumers) transfer matter and energy to higher trophic levels in the food webs (Kagami et al., 2007; Gleason et al., 2008). Similarly, zoospores released from parasites (secondary or tertiary consumers) also transfer matter and energy from their hosts to higher trophic levels. In some cases, grazers could even feed on the zoospores produced *via* parasitism of the grazer species itself, creating particularly tight looping within the food web. Given the number of sporangia in a given host population and their

rate of zoospore production, the biomass of zoospores available in the environment likely represent a significant food resource. According to Zebold *et al.* (1979), degenerated zygotes from the parasite *Coelomomyces* have been found in the digestive tract of mosquito larvae of resistant species and possible may provide a food source for these larvae.

The role of *P. laeve* in the control of population size in *Daphnia* was discussed previously. Infected *Daphnia* are frequently consumed by planktivorous fishes while *Polycaryum* zoospores are likely eaten by other grazing zooplankton. Interestingly, however, infections by *Polycaryum* reveal additional complexities in understanding the role of zoosporic fungi in trophic interactions. While infected *Daphnia* are more conspicuous and therefore preferentially consumed by visual fish predators, the thick-walled sporangia of *Polycaryum* are relatively undigestible to fish, such that most sporangia are excreted from fish in a viable state. This suggests that a significant portion of the infected host's biomass is unavailable to predators, lessening the quantity of the apparent prey while possibly accelerating transmission of the parasite. In addition, the quality of infected hosts as a food resource is reduced. Forshay *et al.* (2008) found that infected *Daphnia* have a lower content of nitrogen, phosphorus and fatty acids than uninfected individuals. More importantly, the content of docosahexaenoic acid, an essential fatty acid for fish growth, is also reduced in infected *Daphnia* individuals.

The parasites of invertebrates listed in Table 1 are likely involved in many missing trophic links, underscoring the importance of incorporating these parasites into freshwater and soil food webs. Possible modifications of the pattern of a food web by *Coelomomyces* are shown in Fig. 2. Blastocladian parasites moderate the population levels of both Type I and Type II hosts and therefore, the dynamics of food webs. In addition, meiospores and zygotes provide food resources for other grazing zooplankters.

7.   Sampling methods and techniques for biodiversity studies

Biodiversity studies in freshwater and terrestrial ecosystems have not accurately estimated the population sizes and densities of zoosporic fungal parasites of invertebrates. The sampling methods rarely provide random or quantitative samples for many of the invertebrate hosts, and the parasites themselves are typically overlooked. Although some parasitic species associated with invertebrates can be studied with classical microscopic techniques employed for saprotrophic species, it is essential to have at least a basic understanding of the life cycles of both the parasites and the hosts. Appropriate protocols depend on including the stage of the host (*i.e.* eggs, larvae or adults) likely to support infection. For example, some parasites are associated only with the larval stage of the invertebrate host, which may appear for a very short time during a single season each year. Furthermore, the fungi may be ephemeral in both somatic and sporulation phases of the life cycle. Thus, in most cases, sampling methods for the parasites must be targeted at the susceptible host. Hence, meaningful measurements of population sizes and densities of zoosporic fungal parasites of invertebrates are not currently available. With improvement of techniques designed specifically for studying Blastocladian parasites of invertebrate hosts, random and quantitatively gathered samples could be used to meaningfully assess species richness in freshwater and terrestrial ecosystems.

Molecular methods are increasingly being used for biodiversity studies in freshwater and soil ecosystems. For example, planktonic assemblages of small eukaryotes in freshwater lakes have recently been analyzed by environmental 18S ribosomal DNA surveys. Matching the sequences from environmental samples with data from GenBank has allowed many clones to be assigned to known species. Using appropriate primers some of these studies have revealed a relatively high diversity of zoosporic fungal parasites within these communities (Johnson *et al.*, 2006a,b; Lefèvre *et al.*, 2007).

Although PCR and DNA sequence analysis have become standard tools for identification and detection of fungi, the preparation of pure DNA is sometimes problematical due to the large number of species that are incapable of growth in the laboratory, the minute quantity of available infected material and the contamination by DNA from the host or other microorganisms in the environment (Richard *et al.*, 2004). The amplification of DNA samples from impure material could be subject to misinterpretation if more than one species is present. The use of techniques such as *in situ* PCR, that could be adapted for use on any sample containing mixed
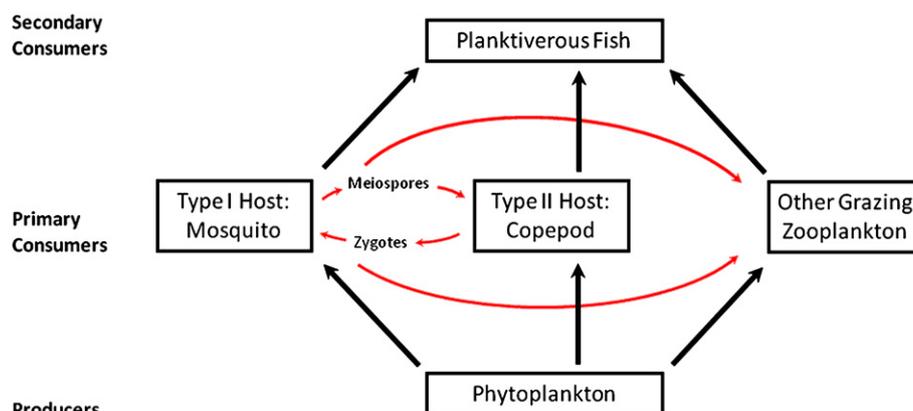

Fig. 2 – Possible additional links in food webs due to parasitism by *Coelomomyces*.

fungal species, may allow positive confirmation of the origin of genes cloned from obligate pathogenic fungi.

Conventional PCR is unable to provide quantitative data on fungal populations, but the development of real-time PCR has enabled important advances in molecular diagnostics (Atkins et al., 2003) and it has been used to successfully determine the population levels of a number of fungal species (Yu and Coosemans, 1998; Bohm et al., 1999; Bates et al., 2001). Although real-time PCR provides a quantitative estimate, enabling comparisons of fungal populations, it is not possible to directly relate the values obtained to the numbers of viable fungal propagules, because it amplifies DNA from both nonviable as well as viable propagules. Thus, new methods need to be designed specifically for quantitative analysis of populations of these fungi. Also many more sequences from parasitic species need to be added to GenBank.

Methods currently used for studies of parasitism in phytoplankton such as staining zoospores with suitable dye (i.e., calcofluor white) and the use of epifluorescence microscopy (Rasconi et al., 2009) have good potential for the detection and quantification of Blastocladian parasites. Appropriate methods for concentrating invertebrates should be used in water samples before staining. A defined number of invertebrates need to be inspected under an inverted epifluorescence microscope for the presence of infection. Two infectivity parameters that have been traditionally used for phytoplankton parasites should be calculated (Holfeld, 2000a,b; Alster and Zohary, 2007; Rasconi et al., 2009): (i) prevalence of infection (P_r): the proportion of individuals in a given invertebrate population infected by zoosporic fungi, expressed as P_r (%) [$(N_i/N) \times 100$], where N_i is the number of infected hosts, and N is the total number of susceptible hosts; and (ii) mean intensity of infection (I), calculated as $I = N_p/N_i$ where N_p is the number of zoosporic parasites, and N_i is the number of the infected individuals within a host population. Staining methods in combination with real-time PCR appear to be the most promising approaches for quantitative studies of Blastocladian parasites.

8. Future prospects and conclusions

Interactions between species

Many groups of true fungi, stramenopiles and protozoa parasitize invertebrates in freshwater and soil ecosystems (Dick, 2003; Barron, 2004). Zoosporic fungi are thus only one part of the whole microbial community, and they must interact with other saprobes and parasites as well as the available hosts. In community ecology the nature of interactions between species of parasites needs further exploration. Since many physical and biological factors are involved in the control of the population levels of invertebrate hosts in addition to the parasites present, experimental studies and multivariate statistical methods are necessary for analysis of the most significant factors. Adding parasites to food webs will highlight the importance of parasites in the flow of matter and energy.

Fig. 3 – *Daphnia pulcaria* heavily infected by *Polycaryum laeve*. The total length of the animal is 2.1 mm. The small dots are sporangia of the parasite. (Photograph by P. Johnson).
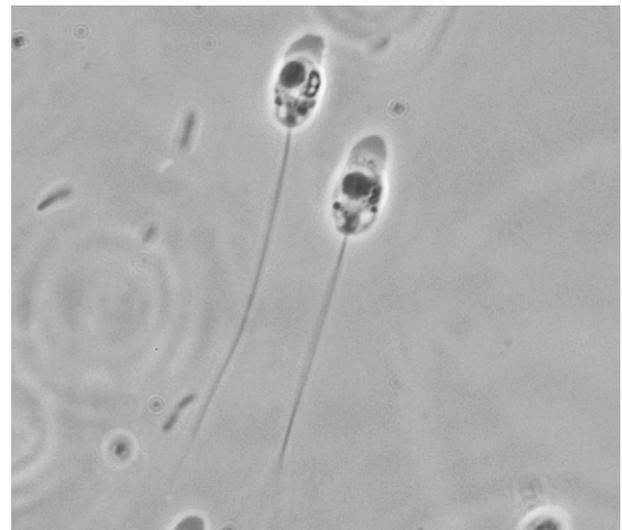
Fig. 4 – Two zoospores of *Catenaria uncinata* moving through the gelatinous matrix of an egg mass of *Glyptotendipes lobiferus*. The zoospore is propelled by the very small filose pseudopodia at the anterior end of the zoospore while the flagellum trails passively behind. The zoospore excluding the flagellum is approximately 3 mμ in length (phase-contrast photograph by W.W. Martin).

Biodiversity

We expect zoosporic fungal parasites to contribute significantly to biodiversity indices in freshwater and soil ecosystems. Also, since the population densities and the degrees of infection of many of the common host species (such as insects, crustaceans and nematodes) are relatively high, zoospores of these parasites should represent a significant proportion of the total biomass of small heterotrophic flagellate ($<5 \mu m$) populations in many ecosystems. Environmental 18S ribosomal DNA surveys, using techniques similar to those described by Lefèvre *et al.* (2007), will be useful for identifying the species and estimating population densities of zoosporic fungi present in the environment. With the steadily increasing amount of data on 18S sequences in GenBank it may become possible to identify many of the unknown sequences found in environmental samples.

Identification of parasites

The type of zoospore (based on its ultrastructure) is a key taxonomic characteristic which has been used to place parasites into the Blastocladiales (James *et al.*, 2006). The ultrastructure of zoospores in some isolates of parasites has been studied, but not in many other parasites. Furthermore, without information on ultrastructure and 18S sequence data, it is difficult to accurately identify these microorganisms. For example, many of the parasites putatively identified as *Olpidium* solely by morphological characters could belong to other genera, such as *Catenaria* (Barron and Szuarto, 1986).

Life cycles

The life cycles of a few *Coelomomyces* species have been documented in detail, but the life cycles of many other parasites are not known. The ecology of parasites cannot be fully understood without a thorough knowledge of their life cycles. The proclivity of the Blastocladiomycota toward retention of the ''Euallomyces'' or ''full-life cycle'' in its biotrophic species (*Coelomomyces* and the plant parasite *Physoderma*) may provide some direction in studies aimed at determining the life cycles of such genera as *Callimastix*, *Coelomycidium* and *Polycaryum*. An initial hypothesis that the latter genera may represent either the diploid phase in a Type I host or the haploid phase in a Type II host seems warranted.

The process of infection

In general, very little is known about the process of infection of invertebrate hosts. In *Coelomomyces* and *Catenaria* the mechanisms of attachment of zoospores to hosts have been studied and biological assays for virulence have been developed. Research on other genera lags far behind.

The potential impacts of parasitism on aquatic ecology

Quantitative studies need to be conducted in the field to accurately estimate the impact of Blastocladian parasites on host populations. Undoubtedly many more host–parasite relationships await discovery in freshwater ecosystems.

Many host animals can be heavily infected with parasites. We have included photographs of two examples in this review. An infection of one individual of *Daphnia pulcaria* by *P. laeve* is shown in Fig. 3. Frequently zoospores of *Catenaria* can be observed entering the gelatinous masses around midge eggs in samples collected from ponds and streams (Fig. 4).

Conclusion

We propose that zoosporic fungi, which are common parasites of small invertebrate animals, make significant contributions to the biomass and biodiversity of microbial communities as well as the complexity of food webs in both freshwater and soil ecosystems. At present there are no meaningful quantitative data available to support this hypothesis. This hypothesis is based primarily on the published descriptions of parasites and their hosts as observed in microbial communities and qualitative estimates of population densities. Accurate quantitative estimates of population densities of both parasites and their hosts and more knowledge of their roles in food web dynamics are needed for a greater understanding of ecological processes. We believe that more intensive research will highlight the importance of these poorly studied microorganisms in ecological processes.

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