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4 **The Species Problem in Myxomycetes Revisited**

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12 **Running title:** Species Problem in Myxomycetes

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14 **Species identification in the myxomycetes (plasmodial slime molds or myxogastriids) poses**
15 **particular challenges to researchers as a result of their morphological plasticity and**
16 **frequent alteration between sexual and asexual life strategies. Traditionally, myxomycete**
17 **morphology has been used as the primary method of species delimitation. However, with**
18 **the increasing availability of genetic information, traditional myxomycete taxonomy is**
19 **being increasingly challenged, and new hypotheses continue to emerge. Due to conflicts that**
20 **sometimes occur between traditional and more modern species concepts that are based**

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largely on molecular data, there is a pressing need to revisit the discussion surrounding the species concept used for myxomycetes. Biological diversity is being increasingly studied with molecular methods and data accumulates at ever-faster rates, making resolution of this matter urgent. In this review, currently used and potentially useful species concepts (biological, morphological, phylogenetic and ecological) are reviewed, and an integrated approach to resolve the myxomycete species problem is discussed.

Key words: Ecology; molecular taxonomy; myxomycete; phylogeny; slime mold; species concept.

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48 **Introduction**

49

50 “A particular kind of biological entity” is the definition of a species as proposed by Nixon and
51 Wheeler (1990), and this conforms to the general concept used by most modern biologists.
52 However, a debate has been ongoing since the time of Linnaeus as to the “best” definition of
53 what truly constitutes a species. Haldane (1956) defined a species as "a name given to a group of
54 organisms for convenience, and indeed of necessity." For practical reasons, the recognition of
55 species is necessary for communication and the general exchange of knowledge and, to be most
56 effective, a species should be defined in a way that is universally accepted yet subject to change
57 as additional data become available (Adl et al. 2007; Cavalier-Smith 1998). Defining a species is
58 of monumental importance because species are the fundamental units of ecology, carrying out
59 unique roles in their particular niches, communities and ecosystems (Cohan and Perry 2007).
60 Therefore, it is not surprising that reaching an agreement on just how to define a species has
61 proven to be so problematic. Over the years, this debate has led to the creation of more than two
62 dozen different species concepts (Wilkins 2006), ranging from the biological species concept
63 initially proposed by Mayr (1942) to more recent concepts based largely or completely on
64 molecular data (Vasilyeva and Stephenson 2010).

65 The definition of a species in the myxomycetes (plasmodial slime molds or
66 myxogastrids) has presented particular challenges. With a complex life cycle, several different

life strategies, varying levels of phenotypic plasticity and genetic variation, the myxomycetes challenge every major species concept that has been put forward. In this paper, the species concepts currently used for myxomycetes will be discussed. Other potential concepts that could be used, the problems associated with each, the implications of these problems on a larger scale and suggestions that might yield a possible solution also will be considered.

Background

The myxomycetes form a single well-supported clade within the supergroup Amoebozoa (Adl et al. 2012; Fiore-Donno et al. 2010a; Lahr et al. 2011a). Myxomycetes, together with the Dictyostelia (dictyostelids) and members of the genus *Ceratiomyxa*, form a larger clade referred to as the Macromycetozoa (Fiore-Donno et al. 2010a). Although the members of the Macromycetozoa are defined on the basis of their macroscopic fruiting bodies, this is not an apomorphic character, since the formation of fruiting bodies has many origins and is widespread throughout the eukaryotes (e.g., Adl et al. 2012; Brown et al. 2012; Shadwick et al. 2009). The myxomycetes, along with their sister group *Ceratiomyxa*, are generally defined by the presence of a flagellated stage in their life cycle and an often relatively large acellular plasmodial stage (Fiore-Donno et al. 2010a).

The myxomycetes are the most speciose group within the Macromycetozoa, with more than 900 described morphospecies (Lado 2005–2016, which is the source of the nomenclature used herein). Myxomycetes are traditionally recognized as predators of bacteria and (to a lesser extent) fungi that occur in association with decaying plant material (Stephenson 2011). As a

whole, myxomycetes are cosmopolitan organisms and have been found in virtually every major terrestrial habitat examined to date. However, recent work indicates that some groups of myxomycetes (Aguilar et al. 2013; Estrada-Torres et al. 2013), like many other protists (Foissner 2006), display a moderate level of endemism, and patterns in their biogeographic distributions are becoming ever more apparent. Myxomycetes commonly occur in all types of forests, where they have been isolated from all of the microhabitats found in forest ecosystems (e.g., the bark surface of living trees, coarse woody debris, ground litter and aerial litter). Myxomycetes also appear to be among the more abundant organisms in soil. In fact, recent evidence indicates that macromycetozoans are the most abundant amoeboid predators in some soils (Geisen et al. 2015; Urich et al. 2008), and their importance in that habitat is no longer questioned.

The generalized life cycle of a typical myxomycete, as first described by de Bary (1887) and recently illustrated in a number of monographs and papers (e.g., Everhart and Keller 2008; Stephenson and Stempen 1994), encompasses two strikingly different trophic stages. The first is a uninucleate amoeba with or without flagella (the term “amoebflagellate” encompasses both types) (Fig. 1, C1) and the second is a distinctive multinucleate structure, the plasmodium (Fig. 1, H1). Plasmodia are motile and range in size from a few micrometers to more than a meter across in some species. Plasmodia often contain many thousands of synchronously dividing nuclei. Given appropriate stimuli, the plasmodium gives rise to one or more (as many as several hundred or even a few thousand in some species) spore-containing fruiting bodies (also referred to as sporophores), most of which range between 0.5 and 4.0 mm in height (Fig. 1, A). Most of what is known about the myxomycete life cycle has been derived from studies of just two species (*Physarum polycephalum* and *Didymium iridis*), but current evidence suggests that most other species follow a similar general pattern.

Bacteria appear to represent the main food resource for both trophic stages (amoebflagellate cells and plasmodia) in the myxomycete life cycle, but plasmodia are also known to feed upon larger prey such as algae (Lazo 1961), yeasts and the spores, hyphae and fruiting bodies of filamentous fungi (Stephenson and Stempen 1994). Interestingly, plasmodia are also capable of engulfing amoebflagellates and small plasmodia of the same or other species of myxomycetes (Clark and Haskins 2012).

Under adverse conditions such as drying of the microhabitat, a drop in temperature and/or a lack of suitable food organisms, a plasmodium may convert into a hardened, resistant structure called a sclerotium (Fig. 1, G), which can quickly revert to the active stage upon the return of favorable conditions (Stephenson and Stempen 1994). Similarly, amoebflagellate cells² can undergo a reversible transformation to dormant structures called microcysts (Fig. 1, C1). All three of these resting stages, sclerotia, microcysts and spores, are capable of resisting relatively harsh conditions and remain viable for various periods of time. This is likely an important strategy for survival of myxomycetes in the often environmentally dynamic habitats (e.g. deserts) in which some species tend to occur.

The above represents a very generalized model of the life cycle in a “typical” myxomycete (Fig. 1), but there are a number of important variations of this basic model that complicate things. Examples of sexual and asexual, heterothallic (possessing mating types) and non-heterothallic (not possessing mating types) life cycles are found (Table 1). In some

² The term "amoebflagellate" encompasses both amoeboid and flagellate forms. The two forms are reversible but it is unclear if the flagellated form is able to directly form a microcyst or if the flagellum must first be resorbed. However, flagellates can develop directly from microcysts.

instances, a single lineage has been characterized by more than one reproductive strategy, and can even switch from one strategy to another and then back again (e.g., Clark 1995). Clark and Haskins (2010) reported that 14 of the 51 morphospecies (largely overrepresented by members of the order Physarales) of myxomycetes in which the reproductive system had at that time been subjected to detailed examination were documented as displaying both heterothallism and non-heterothallism. Twenty-nine of the same 51 morphospecies investigated were found to display only a non-heterothallic reproductive system, thus suggesting that the 'typical' myxomycete life cycle is probably over simplistic.

Heterothallic myxomycetes generally follow the basic life cycle outlined above, with a single locus multi-allelic mating system controlling the fusion of two haploid amoebflagellate cells, first to form a diploid zygote which then matures into a coenocytic plasmodium. Meiosis is assumed to take place in the maturing spores, which then germinate to produce the next generation of uninucleate (haploid) amoebflagellate cells. Heterothallic lineages can possess as many as 16 mating types in a single morphospecies, as reported for *Physarum polycephalum* by Moriyama and Kawano (2010). Mating types may be considered to represent a situation similar to that of separate sexes, in that they block the fusion of “like” gametes (i.e. they are presumed to promote out-crossing). However, the application of the term “sexes” is best reserved for organisms with anisogamous life cycles. Therefore, many myxomycete morphospecies with a heterothallic mating system are composed of a number of biological sibling species. As a result, although they may be identical in morphology, they are unable to interbreed (due to mating type incompatibility) and are therefore on separate evolutionary trajectories (Clark 1995). Groups of such sibling species appear to be common and have been thoroughly investigated and documented in at least 10 morphospecies, including *Arcyria cinerea*, *Didymium iridis*, and *D.*

squamulosum (e.g., Betterley and Collins 1983; Clark and Collins 1976; Clark et al. 2002; El Hage et al. 2000). Identifying sibling species in natural populations of myxomycetes is more difficult, but with newly available molecular methods, evidence has been obtained to support the occurrence of sibling species in natural populations of *Trichia varia* in Germany (Feng and Schnittler 2015) and some species in the Stemonitales and Physarales associated with the snowbank habitat in northwestern Russia (Novozhilov et al. 2013b).

Myxomycetes with non-heterothallic modes of reproduction, which are not known to possess mating types, are largely apogamic (non-sexual, no ploidal change) (Fig. 1, C3) rather than homothallic (sexual, ploidal cycle) (e.g., Clark and Haskins 2010). These apogamic lines are able to convert directly into diploid or polyploid plasmodia without the need for fusion with other amoebflagellates (Clark 1997; Collins 1980; Therrien et al. 1977), allowing formation of essentially immortal clonal lines (Clark 1992). Although observed numerous times in laboratory cultures, the first evidence to suggest asexual clonal lineages of myxomycetes occurring in natural populations was only recently documented for several morphospecies within the genus *Lamproderma* (Fiore-Donno et al. 2011).

Further complicating things, a number of the aforementioned apogamic, diploid (non-heterothallic) isolates (all members of the Physarales or Stemonitales) have been reported to revert to heterothallism in culture (Collins and Therrien 1976; Collins 1980, Collins et al. 1983; Collins and Gong 1985; Collins and Tang 1988; Yemma et al. 1980). According to Clark and Haskins (2013) the most reasonable explanation for these revertant cultures is that they were the product a form of heterothallic reproduction called automixis, for which incomplete meiosis resulted in the production of genetically identical diploid descendants. This would explain the common overlap of heterothallic and non-heterothallic isolates in a single population and also

supports the concept that most myxomycete morphospecies are complexes composed of a sexually reproducing core of biological sibling species and a cluster of asexual, isolated clonal lines (Clark 2000). Presumably, apogamic isolates can more successfully colonize a new habitat than sexual isolates (Schnittler and Tesmer 2008). The former appear better adapted to efficiently exploit new and especially ephemeral habitats due to their smaller size and faster sporulation times (Betterley and Collins 1983; Clark and Haskins 2013). Exploiting new habitats in isolation could (and probably does) allow genetic and/or morphological divergence to take place due to niche specific adaptation, but the ability to revert back to heterothallism should maintain the cohesion of the species complex and be advantageous for the survivability of the species overall. However, it seems possible that these apogamic clonal lines could also accumulate mutations, ultimately causing them to lose the ability to revert back to heterothallism (Clark and Haskins 2010; Collins et al. 1983). With an inability to revert to heterothallism, the genetic isolation would presumably be permanent and lead toward continued divergence of the two lineages. Lending strength to this concept, morphologically distinct, reproductively isolated, non-heterothallic isolates are occasionally collected from ephemeral habitats such as aerial leaf litter and inflorescences. These collections conform to the general morphospecies description except for their miniature size, and when grown in culture, a number of these collections have been shown to retain their miniature form (e.g. *D. iridis* [Clark et al. 2001] and *P. pusillum* [Clark et al. 2004]).

Other variations on the reproductive cycle in myxomycetes include rarely seen “selfing” (Collins 1961), in which heterothallic amoebflagellates are able to produce haploid plasmodia without crossing. There also appear to be rare sterile reproductive systems that cannot form fruiting bodies at all, examples of which have been observed for some isolates of *Didymium*

201 *iridis* (Collins 1961), *Physarum flavicomum* (Henney 1967), *P. rigidum* (Henney and Henney
202 1968) and *Stemonitis flavogenita* (Collins et al. 1983). However, just because fruiting bodies are
203 not recorded for a particular isolate does not mean that the isolate in question cannot fruit, only
204 that fruiting has not been observed. The lack of fruiting body formation could be the result of
205 inappropriate growth conditions, the absence of genetically compatible amoebflagellates (in
206 heterothallic isolates, or a genetic mutation (Collins et al. 1983). Alternatively, it is possible that
207 these isolates have lost the ability to form fruiting bodies, suggesting that their life cycle could
208 consist entirely of the amoebflagellate³ stage (Fig. 1, C2). Consider for example the previously
209 recognized genus *Hyperamoeba*, all members of which have been shown to belong to the dark-
210 spored clade of myxomycetes (Fiore-Donno et al. 2010b). Isolates once assigned to this genus
211 have never been observed to form fruiting bodies, which is one reason they were not initially
212 regarded as myxomycetes. To more fully understand the lack of fruiting in some myxomycetes
213 will require further study.

214 Another intriguing potential departure from the ‘typical’ myxomycete life cycle is a
215 possible form of asexual reproduction via the formation of amoeboid cells directly from a
216 plasmodium (Fig. 1, H2). Indira (1964 and 1969) first described this phenomenon with detailed
217 drawings of what she observed in cultures of *Stemonitis herbatica* and *Arcyria cinerea*. Later, the
218 phenomenon was also described and presented along with photographic evidence in a species of
219 *Physarum* (Ross and Cummings 1967) and *S. flavogenita* (Haskins 1991). Repeatedly, under
220 controlled conditions, tiny pseudopods were first observed to swell up and then have a

³ Division of amoebflagellates occurs only in the non-flagellated form. Because these forms are reversible, this means the flagellum must first be resorbed before the cell undergoes division.

constriction form behind them; the constriction continued to deepen until a uninucleate amoeboid mass was “cut off” from the larger multi-nucleate plasmodium. It is possible that these amoeboid masses are only an abnormal response to the culture environment and that this phenomenon may not be common in nature, if it occurs at all. These amoeboid masses could be plasmodial fragments formed to allow a plasmodium to increase in size to maximize the use of available resources and increase spore production (Haskins 1991) or they could be a means of releasing unwanted materials, neither of which would have genetic implications. However, a more compelling hypothesis is that these amoeboid cells are true amoebflagellate cells capable of propagation, thus representing a novel form of asexual reproduction. In *A. cinerea*, Indira (1969) reported that the newly independent cells became flagellated within just a few minutes and swam away, although the ultimate fate of those cells was unknown. The formation of flagella on such cells has not been witnessed in any other species nor is there photographic evidence of this occurring. Although intriguing and seemingly possible, more work is needed to assess just how common this phenomenon is in myxomycetes, to verify whether or not it ever occurs in nature and to determine what these cells are actually capable of doing (i.e. fusing with other amoebflagellates or directly producing new plasmodia).

Currently Used and Potential Species Concepts

Understanding the complexities of the myxomycete life cycle and the various modes of reproduction as described above, is crucial for understanding the problems associated with delimiting species in the myxomycetes. These life cycle complexities and variations have

bearing on all of the commonly used species concepts, the four most common of which (biological, morphological, phylogenetic and ecological) are described in the sections below, followed by a discussion of their use (or potential use) in myxomycetes.

The Biological Species Concept

The biological species as proposed by Ernst Mayr (1942) defines a species as a group of “actually or potentially interbreeding natural populations which are reproductively isolated from all other such groups.” Based on the information outlined above, the biological species concept is clearly difficult to apply to myxomycetes, particularly with respect to lineages that may be exclusively asexual. This important flaw in the concept, particularly when applied to protists, was first pointed out more than a half century ago by Sonneborn (1957), a prominent protistologist working with *Paramecium* (another asexual protist). Nonetheless, attempts to apply this species concept to protists persist.

In order to discuss the potential application of a biological species concept to myxomycetes, it is important to first define what is meant by use of the term sex. Herein we use the definition of sex put forth by Lahr et al. (2011b), which involves the presence of both meiotic reduction of the genome *and* subsequent fusion of nuclei during karyogamy. As discussed previously, a single myxomycete morphospecies often contains multiple biological sibling species that are reproductively isolated from one another, as well as a swarm of asexual clonal lines (Clark 1995). In addition, many taxa demonstrate multiple modes of reproduction, often within the same lineage, as is the case for *Echinostelium minutum*, *Fuligo septica* and *Physarum pusillum* (Clark and Haskins 2010 and references cited therein) to name a few, and it is possible

that strictly asexual lineages exist (Clark and Haskins 2011). Hence, the variation that exists within the myxomycete life cycle limits the ability to use a biological species concept outright.

Another problem with the use of the biological species concept in the myxomycetes is the need for direct evidence of sex, which is available only through isolation and laboratory culture. Currently, only about 10% of all known myxomycete morphospecies can be cultured, placing a severe constraint on taxon sampling in reproductive studies (Haskins and Wrigley de Basanta 2008). In addition, some reliance on morphology is still required initially to identify possible mating pairs to test. However, morphology is not always correlated with reproductive isolation; morphologically distinct populations sometimes can interbreed, while other morphologically similar populations cannot. If the biological species concept is to be used to test a hypothesis, then many biological species could be overlooked or over identified. To avoid these obvious constraints, most protistologists (including myxomycetologists) have historically relied on a morphological species concept (Clark 2000; Finlay 2004).

The Morphological Species Concept

Traditionally, as already noted, myxomycete taxonomy has delimited species using morphological features of the fruiting body. The characters used include the general structure and appearance of the fruiting body (Fig. 2) and its component parts (e.g. stalk, peridium, capillitium, etc.), the presence or absence of lime, and the color and ornamentation of the spores (e.g., Martin and Alexopoulos 1969). This morphological species concept assumes that a speciation event is always accompanied by a morphological change, when in fact this may not always be the case (Andersen 1998). Therefore, a single morphospecies may represent a range of distinct genotypes which may or may not be able to interbreed. This may explain why protist

morphology seems to remain relatively constant for very long periods of time. Indeed, the few myxomycete fossils that have been found (e.g. species of *Arcyria* and *Stemonitis*) from Baltic amber dating from the Eocene (Dörfelt and Schmidt 2006) show no apparent differences from modern forms (Keller 2012; Stephenson 2011). The morphological species concept also assumes that distinct morphological features reflect shared evolutionary history and isolation from other groups. However, molecular analyses have revealed that discordance between morphology and evolutionary relationships are common in microbial lineages throughout the tree of life (Lahr et al. 2014), and the myxomycetes are no exception. Numerous examples exist of convergent morphologies in the myxomycetes. Examples include the repeated loss and gain of stalk formation across the entire myxomycete clade (Fiore-Donno et al. 2012; Shadwick et al. 2009) or the repeated evolution of fruiting bodies bearing compound fruiting bodies such as in the distantly related genera of *Lycogala* (bright-spored clade) and *Brefeldia* (dark-spored clade) (Schnittler and Tesmer 2008). It should be noted that even for myxomycetes which appear to share most other characters, those species with compound fruiting bodies have almost invariably been placed in different genera from those with solitary fruiting bodies. Such a dramatic change in morphology is likely to be under the control of a very small set of genes, but this has yet to be investigated to any real extent.

Another assumption of the morphological species concept is that the characters used to distinguish different taxa show only slight variation within a species (Clark 2000). However, the characters used to identify myxomycetes, like many protists, can at times be extremely variable, blurring the lines between morphospecies and even genera. There are several examples of species that consistently bridge gaps between genera, containing features characteristic of more than one genus such as *Hemitrichia leiocarpa*, which could just as easily be assigned to the

genus *Arcyria*, which was the case in earlier monographic treatments of the myxomycetes (e.g., Martin and Alexopoulos 1969). Moreover, the morphological species concept assumes that the characteristics used for identification are stable. However, the conditions under which myxomycetes form fruiting bodies can greatly affect the morphology of the mature fruiting body (Schnittler and Mitchell 2000). For example, in moist chamber culture, fruiting bodies that form upside down on the lid of the Petri dish (a common occurrence) regularly have reduced or even absent stalks, which can conceal important morphological features (Novozhilov et al. 2013a). In nature too, variation among fruiting bodies is common and readily observed by collectors working across a range of habitats or even a range of microhabitats or substrata within one locality. Although such plasticity is widely acknowledged, its full extent is unknown for the vast majority of myxomycetes. In order to quantify the level of variation that normally can be expected for a particular taxon requires spore-to-spore culture under controlled laboratory conditions (Keller 1996). As one example, in their description of *Didymium annulisporum*, Keller and Schoknecht (1989) established cultures on four different substrata (sterile and unsterile dung and two types of agar) and recorded substrate specific fruiting body morphology in terms of lime content, overall size and general shape, number of fruiting bodies and variations in the peridium. Quantification of morphological variation in this manner is a valuable supplement to any new species description.

One final problem with use of the morphological species concept in myxomycetes, is the requirement of mature, intact fruiting bodies available for study. Morphological identifications in myxomycetes cannot be made using any of the other life stages such as the amoebflagellate cells or plasmodia (Clark 2000; Lado 2005–2016; Stephenson and Stempen 1994). Therefore, when fruiting body material is limited or nonexistent, such as when spores or amoebae are

isolated from environmental samples and cannot be cultured (or if fruiting body formation does not result from culture), a strictly morphological identification is not possible.

Since 1965, the number of newly described myxomycete taxa has skyrocketed, in part due to increased sampling from diverse new habitats and localities, along with an increasing trend toward splitting genera and species into smaller groups (Keller and Everhart 2008). With this increase in the number of species of myxomycetes being described, there is also an increasing number of new taxa named on the basis of limited information. Lado (2001) estimated that monotypic myxomycete genera (i.e. those containing only a single species) account for 14 of 59 (or 24%) of all genera. Meanwhile Schnittler and Mitchell (2000) compiled a database containing 1012 subgeneric taxa, of which only 866 were classified at the species level and 305 taxa were described from only a single type locality. The amount of variation that can be observed in a single, localized, clonal population clearly indicates caution should be used in defining new taxa based on just one or even a few type specimens that differ in only minor respects from already described species. Instead, multiple specimens from multiple geographic locations should be thoroughly examined before a decision is made to recognize a species as new to science.

Although the morphological species concept has been the taxonomic standard for centuries, it is now clear that this approach to myxomycete species identification is in need of revision. One particularly noteworthy example in which the morphological species concept is not reliable for species identification is in the *Didymium iridis* species complex, the first myxomycete species complex (or group of sibling species) to be investigated extensively (Betterley and Collins 1983; Clark and Stephenson 1990; Clark et al. 1991; Clark and Landolt 1993; Collins 1976). This work showed that *D. iridis* has at least three non-interbreeding mating

types in the heterothallic lines and also contains multiple non-heterothallic, apogamic lines. Because the three heterothallic mating types are reproductively isolated from one another, and the non-heterothallic lines are also reproductively isolated, at least nine biological sibling species comprise this one morphospecies. In addition, detailed observations have shown that there is considerable overlap in the morphology of *D. iridis* and other stalked species in the same genus. Examples include *D. bahiense*, *D. nigripes*, *D. pertusum*, *D. ovoideum*, *D. melanospermum*, *D. megalosporum* and *D. verrucosporum* (Clark et al. 2001; El Hage et al. 2000). In fact, Lado (2001) suggested that there are actually as many as 30 morphospecies contained within the *D. iridis* complex, all of which may overlap with respect to the columella and/or stalk coloration, diameter of the sporotheca, total height of the fruiting body, color of the capillitium or plasmodium, the amount of lime present, and general shape (Fig. X). As such, the *D. iridis* complex, one of the best studied of all morphospecies of myxomycetes, is particularly in need of detailed taxonomic revision.

The Phylogenetic Species Concept

Phylogeny, particularly when based upon molecular sequences, is a powerful tool that can be used to predict species boundaries as well as to test biological and morphological species concepts (Andersen 1998, Caron 2013). A phylogenetic species concept relies on the use of phylogenetic trees, where the least inclusive cluster is considered a species (or phylospecies) (Mishler and Theriot 2000). Ideally, this also corresponds to the smallest biologically important unit deemed worthy of recognition (e.g. Cracraft 1983; Mishler 2010; Nixon and Wheeler 1990). In lieu of whole genome sequencing, which is still not feasible for most taxa, a phylogenetic species concept therefore requires the selection of an appropriate gene(s) with which to construct

the phylogeny. The most widely used gene for phylogenetic reconstruction in amoebozoans in general (Nassonova et al. 2010) and for myxomycetes in particular, is the ribosomal small subunit RNA gene [SSU], and a number of new species have already been identified and renamed based in large part on SSU sequences (Fiore-Donno et al. 2010b).

Although extremely slowly evolving across many major taxa, SSU can be a highly and sporadically variable phylogenetic marker in other groups, particularly in the myxomycetes, which also contain a high load of introns of variable lengths (Fiore-Donno et al. 2010a, 2012; Nandipati et al. 2012; Pawlowski et al. 2012; Shadwick et al. 2009). In lieu of full-length SSU sequences, which can be a challenge to obtain due to the aforementioned introns and sequence variation, Fiore-Donno et al. (2011) identified an intron-free region of ca. 700bp in the 5' SSU that can be amplified with a single pair of PCR primers that has proven powerful in the context of both phylogenetic and ecological studies (e.g. Fiore-Donno et al. 2012, 2016; Novozhilov et al. 2013b). Intraspecific SSU sequence variation can vary significantly between clades and even between multiple isolates of a single myxomycete morphospecies from a single population. When assessing myxomycete diversity with SSU sequence data parallel to morphology, one could find identical SSU sequences for multiple collections of a single morphospecies (Fiore-Donno et al. 2011) or alternatively one may find that SSU sequence diversity is twice as great as morphological diversity (Novozhilov et al. 2013b). Therefore, it is valuable to examine multiple molecular markers when constructing phylogenetic trees, particularly in the absence of other supporting data (e.g., Parfrey et al. 2006; Taylor et al. 2000).

Elongation factor-1 α [EF-1 α] is another commonly sequenced gene used for constructing myxomycete phylogenetic trees (e.g., Feng and Schnittler 2015; Fiore-Donno et al. 2005, 2010a). Due to its role in protein synthesis EF-1 α is highly conserved and abundant in actively growing

cells (Baldauf 1999). Furthermore, the nearly complete sequence can be amplified with a single pair of universal primers (Baldauf and Doolittle 1997). Two additional markers that have been used to construct myxomycete phylogenetic trees are the more rapidly evolving ribosomal internal transcribed spacer [ITS] and mitochondrial cytochrome c oxidase [COI] genes, although with varying levels of success. Unlike the successful use of ITS sequencing in fungi (Schoch et al. 2012; White et al. 1990) and some other groups, the power of ITS for phylogenetic reconstruction in the myxomycetes is rather limited. Myxomycete ITS sequences are extremely variable and in many cases cannot even be aligned (Fiore-Donno et al. 2011; Martin et al. 2003). The high level of sequence variation limits its ability to resolve evolutionary relationships in the myxomycetes although when used as a supplementary marker it can be valuable for differentiating between some species (Baba et al. 2015; Winsett and Stephenson 2008). Similarly, when COI is used as a supplemental tool it has proven successful for some amoebae (Nassanova et al. 2010), ciliates (Barth et al. 2006; Chantangsi et al. 2007), diatoms (Evans et al. 2007) and numerous other non-protistan groups (e.g. Hebert et al. 2004; Pawlowski et al. 2012). In myxomycetes however, success with use of the COI gene sequence has been limited due to the high levels of sporadic variation among lineages. Even with primers specifically designed to amplify the first half of the myxomycete COI, sequences can be difficult to obtain and provide varying results (e.g., Feng and Schnittler 2015; Walker et al. 2011). Therefore, both ITS and COI can be valuable as supplementary markers to distinguish between closely related species but are not reliable markers to use for reconstructing deeper evolutionary relationships among myxomycetes.

Integrating molecules with morphology. Although molecular sequences from a few laboratory-cultured species have been used to confidently place myxomycetes as a whole in the larger eukaryotic tree (e.g., Baldauf 1999; Cavalier-Smith et al. 1996), DNA had not been successfully recovered from field-collected material until 2003 (Martin et al. 2003). The ability to use field-collected myxomycetes as a source of DNA greatly increased opportunities in molecular phylogeny, due to the great abundance of available herbarium specimens and the limited number of species that can be cultured for DNA isolation (Clark 2000). This increased availability of molecular data has led to an increased awareness of the ecological roles of myxomycetes and an increasingly sophisticated understanding of morphological evolution in myxomycetes.

Fiore-Donno and colleagues have resolved a number of important questions relating to myxomycete phylogeny, primarily by building trees from concatenated SSU and EF-1 α sequence data (e.g., Fiore-Donno et al. 2005). Unlike the situation with the dictyostelids (Schaap et al. 2006), these and other molecular data have revealed some level of congruence between molecules and morphology in myxomycetes (Myxogastria). Molecular data have confirmed the monophyly of the myxomycetes and its division into two clearly defined groups commonly referred to as the dark-spored and bright-spored clades, more recently recognized as the superorders Columellidia and Lucisporidia, respectively (Cavalier-Smith 2013). As their names suggest, the dark-spored and bright-spored clades are historically distinguished on the basis of spore color, and the taxonomic distribution of this character is largely consistent with molecular phylogeny (Fiore-Donno et al. 2005). Meanwhile, within these two clades the traditionally recognized five orders—Echinosteliales, Physarales and Stemonitales in the dark-spored clade and Liceales and Trichiales in the bright-spored clade (e.g., Martin and Alexopoulos 1969, Olive 1970) do not seem to hold together. In fact, molecular phylogenetic data indicate that the current

taxonomy (based on morphology) does not accurately reflect the phylogenetic relationships that exist within both the dark-spored clade (Fiore-Donno et al. 2008, 2010) and the bright-spored clades (Fiore-Donno et al. 2013), and many of these relationships are currently being revised. Figure 3 reflects this newly emerging myxomycete phylogenetic tree, based upon currently available data.

Current molecular data indicate that within the dark-spored clade (Columellidia), the monophyletic order Echinosteliales is the earliest diverging clade and branches as a sister group to a taxonomic assemblage consisting of the orders Stemonitales and Physarales (Kretzschmar et al. 2016). The genus *Barbeyella*, with its capillitium adhering to peridial plates, may represent the transitional form between Echinosteliales and the rest of the dark-spored clade. Members of the Stemonitales and Physarales do not sort into two clearly distinct groups as current traditionally used taxonomic concepts have suggested. Instead, there are three main branches including (1) the "*Meriderma* group" (from the Stemonitales), (2) the traditional Physarales together with the "*Lamproderma* group" (also from the Stemonitales) and (3) the rest of the Stemonitales (except for the *Meriderma* and *Lamproderma* groups) (Fiore-Donno et al. 2010a, 2012). Within the bright-spored clade (Lucisporidia), there are two main branches, the first consisting of a monophyletic Cribrariaceae (from the Liceales) and the second composed of the Reticulariaceae (also from the Liceales) and three other clades that consist of various members of the orders Trichiales and Liceales (Clissmann et al. 2015; Fiore-Donno et al. 2013). The recently re-erected genus *Alwisia* (Liceales, Reticulariaceae) may represent the form, closest to the last common ancestor of the second clade referred to above (Leontyev et al. 2014b, c). It is readily apparent that the traditionally used morphological species concept is no longer appropriate for many of the taxa that have been considered to make up the Trichiales and Liceales (Fiore-Donno

et al. 2013; Leontyev et al. 2014a; Walker et al. 2015). As such, it would seem that there exist examples of incongruence between morphology and molecular data throughout myxomycete phylogeny, particularly at the ranks of genus and species.

At the genus level, let us first consider the genus *Lamproderma* in the dark-spored clade. Traditionally regarded as morphologically distinct, the monophyly of *Lamproderma* is rejected by molecular phylogenetic analyses, which indicate instead that “*Lamproderma*” appears to consist of several clades intermingled with species from three other genera (*Diacheopsis*, *Colloderma* and *Elaeomyxa*) (Fiore-Donno et al. 2012). Consequently, the stalk, columella, spotted (or not) peridium and the presence of “white splinters” in the peridium, which are characters previously used to identify members of genus, are shown by SSU phylogeny and further comparative morphology to be labile at the species level. Instead, a more reliable character for delimiting species of *Lamproderma* appears to be the presence of a shining peridium. The example mentioned above and numerous others exemplify how molecular data can guide morphological studies and lead to an increasingly sophisticated understanding of the mode and tempo of morphological evolution, which in turn will lead to a better understanding of the significance of various morphological characters used in myxomycete taxonomy. For example, the combination of molecular and morphological data indicate that the stalk of the myxomycete fruiting body is far less reliable as a taxonomic marker than has long been thought to be the case. A stalked fruiting body appears to be the ancestral state for myxomycetes because the earliest diverging groups in both the dark-spored (Echinosteliales) and bright-spored (Cribrariaceae) clades are almost always stalked. However, the stalked condition of fruiting bodies also appears to be readily lost and can encompass considerable variability in length, even within a single population (Martin and Alexopoulos 1969), which makes the stalk unreliable at

many taxonomic levels (e.g., Shadwick et al. 2009). Two other characters traditionally considered important for species delimitation in various myxomycete genera—spore color and ornamentation (Kalyanasundaram 2004)—have been revealed by molecular data to be unreliable in at least some cases, such as for *Lamproderma puncticulatum* and *L. columbinum*, which show a great deal of variation in spore morphology among isolates (Fiore-Donno et al. 2011; Schnittler et al. 2010).

Understanding the surprising evolutionary instability of such morphological characters may explain why the placement of a number of species currently do not seem to agree with traditional taxonomy. For example, the lack of a capillitium is the major character used to distinguish between the traditional orders Liceales and Trichiales in the bright-spored clade. However, the phylogenetic significance of this character has been under scrutiny for quite some time (Alexopoulos 1976) because the lack of a capillitium (or any other single character) is not universal among or uniquely restricted to the Liceales. Not only are there species within the Liceales that undeniably have a true capillitium, such as *Listerella paradoxa* (Eliasson 1977) or *Alwisia bombarda* (Leontyev et al. 2014a), but some species of *Perichaena* (Ramírez-Ortega et al. 2009 and references cited therein) in the Trichiales and even some members of the dark-spored clade of myxomycetes also lack a capillitium. An example of the latter is *Didymium eremophilum* (Blackwell and Gilbertson 1980). Although the dark-spored clade has been studied to a much greater depth and seems to be more clearly delimited, there are also morphological characters used in classical identifications within this clade that are in need of molecular analyses to verify their significance. For example, the color of lime found in fruiting bodies has been used to distinguish between several species pairs in the genus *Physarum* (e.g., *P. globuliferum* and *P. bilgramii*) (Aldrich 1982; Stephenson 2003). Another character that should be investigated in the

dark-spored clade is the form of the capillitium. Although the presence or absence of capillitial lime being used to separate the Didymeaceae from Physaraceae is supported by molecular data (Fiore-Donno et al. 2009; Nandipati et al. 2012), the use of capillitial structure (calcareous tubules versus nodules) being used as a character to separate the genera *Badhamia* and *Physarum* has not been supported by molecular phylogenetic data. However, the form of the capillitium or the overlap of other characters used to distinguish between these two genera may not be surprising, given those same molecular data indicate that the genus *Physarum* is polyphyletic, containing at least three clades interspersed with members of the genus *Badhamia* (Nandipati et al. 2012).

The first use of SSU phylogeny to reclassify a "species" of myxomycete was in 2010, when the genus *Hyperamoeba* (mentioned earlier) was shown to be invalid and the species formerly assigned to the genus were transferred to various genera in the dark-spored clade of myxomycetes (Fiore-Donno et al. 2010b). Species of *Hyperamoeba* traditionally had not been recognized as belonging to the Macromycetozoa because they never formed fruiting bodies and occupied habitats extraordinarily different from those of most the Macromycetozoa. For example, "hyperamoebae" have been found in human feces (Zaman et al. 1999), the coelomic cavity of sea urchins as endosymbionts (Dykova et al. 2007), freshwater sediments (Walker et al. 2003), physiotherapy water baths and water treatment plants (Walochnik et al. 2004). However, the "hyperamoeba" SSU sequences clearly cluster within the dark-spored clade of myxomycetes (e.g., Fiore-Donno et al. 2010b; Walochnik et al. 2004). Subsequently, a more rigorous morphological examination uncovered the overlooked second flagellum, a character that strongly places these taxa among the myxomycetes (Dykova et al. 2007; Fiore-Donno et al. 2010b; Walochnik et al. 2004).

Yet another example of the power of an integrative approach to taxonomy to modify the placement of particular taxa is the myxomycete *Kelleromyxa fimicola*. This taxon was described initially in 1929 as *Licea fimicola* due to its apparent resemblance to members of the genus *Licea* in the bright-spored clade (Dearness and Bisby 1929). However, upon re-evaluation of the type specimen and other collections consisting of numerous fully matured fruiting bodies, Eliasson et al. (1991) concluded that its assignment to the genus *Licea* could not be supported and that, in fact, this taxon shares more characters (e.g. dark spores, phaneroplasmodium, and the presence of a capillitium) in common with members of the Physarales in the dark-spored clade. Noting its similarities to members of the order Physarales, a new genus was erected within the order for its temporary placement, and the taxon was renamed *Kelleromyxa fimicola* (Eliasson et al. 1991). This was finally confirmed by Erastova et al. (2013) using an SSU phylogeny that clearly placed *Kelleromyxa fimicola* within the dark-spored myxomycete clade, confirming the significance of the Physarales-like morphological characteristics noted by Eliasson et al. (1991).

Morphology is not always able to distinguish molecular differences however. Therefore, in situations where a single gene molecular phylogeny delimits two groups but there is no corroborating evidence (e.g. additional gene markers, ecology or morphology) to support the existence of two groups, the researcher must consider whether or not there is an urgency to separate them. Over-naming taxa is a longstanding problem that can lead to undue confusion and misinformation with the potential to slow the scientific process (Dayrat 2005). To avoid this problem, until more data are available to support further delimitation, the two isolates potentially could be accepted as two haplotypes that represent variation within the morphospecies. Numerous examples exist in the literature of cases in which molecular data from single gene analyses suggests delimitation but the authors cannot justify the split without further

investigation. For example, during their revision of the *Tubifera ferruginosa* complex Leontyev et al. (2015) identified four out of their 10 18S sequences of *Lycogala epidendrum* that appeared to represent four new species. Inspection of the fruiting bodies however showed no morphological variation, so rather than describe these specimens as a new taxa based on partial SSU sequences, the authors instead identified the need to investigate more material and in greater detail. Similarly, Aguilar et al. (2013), who studied 125 specimens of *Badhamia melanospora* from 91 localities, identified two groups strongly distinguished by SSU sequence divergence, morphology and biogeography. However, none of the characters (morphology or geography) could a priori distinguish between the two groups, so the authors instead concluded at that time that they were likely to be cryptic species.

The Ecological Species Concept

The ecological species concept is grounded in Gause's theory of competitive exclusion (1934), in which organisms must differentiate in order to avoid having exactly the same role in the environment and thus occupying the same ecological niche. This differentiation of groups to exploit different resources requires each group to locally adapt and eventually leads to an irreversible separation into ecologically distinct groups or 'ecotypes'. Ecotypes are thus considered to equate to species in the traditional sense and are defined as populations of closely related organisms which share adaptations to the local niche and whose genetic divergence is capped by one or more mechanisms (e.g. reproductive isolation) (Cohan 2002).

Due to the nature of how they are formed, ecotypes should directly represent biologically significant taxa because each one is carrying out a unique ecological role, thereby making the ecological species concept an intuitive and natural way of delimiting taxa (Ward 1998). The

ecological species concept assumes that the characters which allow the occupation of a particular niche will be reflected in physiology, morphology or sequence divergence (Fenchel 2005; Kopac et al. 2014; Palys et al. 1997). However, just as with the concepts previously discussed, the thresholds used to delimit clades in this way (e.g. physiology, morphology or sequence divergence) do not always reflect distinct ecotypes (Cavalier-Smith 2010; Finlay 2004; Finlay et al. 2006; Knowles and Carstens 2007).

For the myxomycetes and many other groups of protists, an ecological species concept is not yet feasible, simply because their ecology remains poorly understood and cannot be linked reliably to physiology, morphology or genetics. Until we know more, ecological information is valuable only as a complement to other forms of data. A few hypotheses have been proposed which would link myxomycete ecology to some aspects of their morphology although not at the species level. For example, aphanoplasmodia, which are unique to the Stemonitales, lack a protective slime sheath such as that found in phaneroplasmodia (the plasmodial type typical of many other myxomycetes). This has led to the suggestion that members of the Stemonitales or any myxomycetes with aphanoplasmodia are unlikely to be found in particularly dry environments. In fact, most taxa with aphanoplasmodia are thought to spend the majority of their plasmodial existence within substrates such as woody tissues, where they are protected from desiccation (Clissmann et al. 2015; Stephenson and Stempen 1994). In line with this hypothesis, members of the Stemonitales are almost entirely lignicolous (associated with wood). However, there are some notable exceptions such as *Lamproderma scintillans* and *Stemonitis herbatica* which are almost invariably associated with non-woody substrates (Martin and Alexopoulos 1969). Another hypothesis that could relate ecology to morphology has been proposed for some coprophilous (inhabiting dung) myxomycetes. Although the occurrence of myxomycetes on

dung is not uncommon (more than 100 species have been reported to occur on dung), there are only 16 species that have either been reported to occur only on dung or that have been rarely reported on other substrates and therefore may be truly coprophilous (Eliasson 2013). Three of these possibly obligate coprophilous species (*Licea alexopouli* [Blackwell 1974], *Kelleromyxa fimicola* [Eliasson et al. 1991] and *Trichia brunnea* [Eliasson and Keller 1999]) display unique thick-walled spores that could represent an adaptation to passing through the digestive tract of herbivores. However, once again more research is needed to test this hypothesis.

Given the difficulties involved in working with protists such as myxomycetes, obtaining a more accurate picture of habitat preference or niche specialization will require the enhancement of traditional culture methods and the increased utilization of rapidly developing molecular tools such as metagenetics, metagenomics and single-cell genome sequencing. These and other emerging molecular methods should potentially allow greater depth and breadth in detection of myxomycete diversity in a given sample, thereby avoiding, at least in part, the inherent biases associated with finding, identifying and culturing myxomycetes. The use of these techniques in conjunction with detailed environmental studies and information on temporal, geographic and chemical gradients will allow a far greater understanding of the ecological roles of myxomycetes (and other protists) than previously possible, and this is likely to affect our understanding of their taxonomy.

Moving Towards a Solution

The problems associated with species delimitation are, of course, not limited to the protists or the myxomycetes but are widespread across the tree of life. The complications of rampant lateral gene transfer obscuring phylogenetic signals appear to be restricted largely to prokaryotes (Keeling and Palmer 2008) and thus is not considered in the present discussion. However, it will be exceedingly useful to integrate an understanding of other groups of eukaryotes in which similar challenges exist, such as guidelines proposed by protistologists and those studying some other amoebozoans (e.g., Adl et al. 2007; Finlay 2004; Smirnov et al. 2011).

To improve our understanding of the evolution and biology of the myxomycetes and other eukaryotic microbes, as well as identify a suitable way to delineate taxa, it is important that molecular data be considered in conjunction with traditional taxonomic methods especially since the latter represents the foundation for the classification used in all monographs of the group published to date. The combined use of multiple types of data (e.g. morphological, molecular, and ecological) provides a natural approach to species delimitation which has been referred to as an integrative taxonomic approach (Dayrat 2005; Will et al. 2005). Based on the limited number of studies in which an effort has been made to apply an integrative approach to myxomycete taxonomy (e.g., Feng and Schnittler 2015), developing an understanding of the entire subject of myxomycete taxonomy at the level of species and below (e.g. cryptic species and haplotypes) will require an enormous amount of research on the part of the individuals who work with these organisms.

Due to their morphological plasticity, genetic variability and the presence of several alternative reproductive strategies (sometimes within a single lineage), all of which complicate traditional species concepts, an integrated approach to myxomycete taxonomy should be particularly beneficial. This has led to the recommendation (e.g., Keller and Everhart 2008;

Schnittler and Mitchell 2000) that any description of a myxomycete species should, when feasible, include (1) investigation of multiple specimens from multiple localities and associated microhabitats, (2) detailed description of the collection locality, microhabitat or substrate, and associated abiotic conditions at the time of the collection, (3) detailed information on the comparative morphology of fruiting bodies, including microscopic images, (4) spore-to-spore culture to verify the consistency of morphological and physiological traits, and (5) molecular phylogenetic trees based on multiple independent markers. A few recent and noteworthy examples of an integrative approach to myxomycete species delimitation include the description of *Physarum pseudonotabile* by Novozhilov et al. (2013a) and the revision of the *Tubifera ferruginosa* complex by Leontyev et al. (2015). Of course, some of the recommended components of this “ideal” species description are not possible in every instance. For example, the revision of the *Tubifera ferruginosa* complex by Leontyev et al. (2015) did not involve spore-to-spore culture since *T. ferruginosa* has not yet been successfully cultured. Detailed descriptions of the collection locality, microhabitat and associated abiotic conditions at the time of collection may not always be available for every specimen. In other cases, fruiting body material may be limited, forcing the researcher to choose between the destruction of fruiting body material for DNA extraction or its preservation for microscopic and culture methods. In still other cases, obtaining high quality sequence data from specimens may not be possible, regardless of the amount of time and effort involved.

Conclusions

The myxomycetes challenge many of the currently recognized taxonomic systems, largely due to their morphological plasticity, genetic variability and the presence of several alternative reproductive strategies (sometimes within a single lineage). At present, not everyone who works with this group shares the same concepts for species recognition, even for some of the more common morphospecies that have long been recognized. Furthermore, myxomycetes face a problem that is common in protistology, that of an old and extensive set of literature (more than 3000 papers before 2001) focused solely on reporting species inventories from particular localities (Schnittler and Mitchell 2000). With such a wealth of available data, it is crucial that the research community reach some kind of agreement on an approach to species delimitation and taxonomy that can be used to simultaneously exploit the information contained in those data as well as build upon them without great complication.

Undoubtedly, the concept of a species for the myxomycetes as well as other groups of protists will remain the subject of debate well into the future. The review presented herein is certainly not intended to be the final word on the subject, and more detailed discussions concerning the species debate at broad are readily available in the published literature (e.g., Mishler 2010; Pigliucci 2003; Podani 2010). Instead, our objective was to examine the biological side of the debate as it relates to the myxomycetes as there are clearly different issues with respect to other groups of organisms.

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1075

1076

1077 **Glossary**

1078 **Amoeboflagellate** - A uninucleate cell that represents one of the two trophic stages in the life
 1079 cycle of a myxomycete. The term is used to describe both amoeboid and flagellate forms;
 1080 in myxomycetes, the two forms are reversible, although division occurs only in the
 1081 amoeboid form, meaning the flagellum must first be resorbed before undergoing division.

1082

1083 **Aphanoplasmodium** - A type of plasmodium that is flat, transparent, lacks a protective slime
1084 sheath and is difficult to observe in nature; characteristic of the Stemonitales.
1085

1086 **Apogamic** - A form of non-heterothallic reproduction that does not involve the fusion of
1087 amoebflagellates (gametes) and lacks a haploid-diploid cycle.
1088

1089 **Automictic** - A form of heterothallic reproduction in which the fusion of haploid
1090 amoebflagellates (gametes) results in the production of a diploid plasmodium.
1091

1092 **Capillitium** - A system of sterile elements (takes numerous forms) found within the spore mass
1093 of many myxomycetes (Fig. 2D).
1094

1095 **Columella** - A structure that extends into the spore mass from below; in stalked fruiting bodies
1096 the columella appears to represent an extension of the stalk (Fig. 2E).
1097

1098 **Fruiting body** - A general term for the spore-producing structure or reproductive stage in the
1099 myxomycete life cycle; the structure within which (or in one case, on the surface of
1100 which) the spores are produced; also referred to as a fructification or sporocarp.
1101

1102 **Heterothallic** - Possessing mating types and having a haploid-diploid sexual reproductive cycle.
1103

1104 **Homothallic** - A form of non-heterothallic reproduction in which the fusion of haploid
1105 amoebflagellates (gametes) results in the production of a diploid plasmodium.

1106

1107 **Hypothallus** - Thin layer deposited by the plasmodium onto the substrate at the time of fruiting.

1108 The hypothallus when present has many forms and may connect multiple fruiting bodies.

1109 (Fig. 2A)

1110

1111 **Lignicolous** - Living on or within wood.

1112

1113 **Macromycetozoa** - The monophyletic group composed of the Dictyostelia, Myxogastria and

1114 *Ceratiomyxa* (Fiore-Donno et al. 2010a).

1115

1116 **Microcyst** - A dormant, resistant structure formed by an amoebflagellate cell under adverse

1117 conditions.

1118

1119 **Myxogastria** - The plasmodial slime molds which produce macroscopic fruiting bodies

1120 (although excluding the other slime molds, Dictyostelia, protostelids and *Ceratiomyxa*

1121 (Cavalier-Smith 2004).

1122

1123 **Non-heterothallic** - Not possessing mating types; non-heterothallic myxomycetes may be either

1124 homothallic (sexual, ploidal cycle) or apogamic (non-sexual, no ploidal cycle).

1125

1126 **Peridium** - The covering over the spore mass of a fruiting body (Fig. 2C).

1127

1128 **Plasmodium** - An acellular, multinucleate mass of protoplasm that represents one of the two
1129 trophic stages in the life cycle of a myxomycete.

1130

1131 **Phaneroplasmodium** - A type of plasmodium that may be relatively large and conspicuous
1132 enough to be observed in nature. Typically composed of a network of vein-like strands
1133 containing the streaming protoplasm within, protected by a slime sheath.

1134

1135 **Sclerotium** - A dormant, resistant structure formed by a plasmodium under adverse conditions.

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1137 **Stipe (or stalk)** - A structure supporting the spore mass; often elevated some distance above the
1138 substrate (Fig. 2F).

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Table 1. Mating systems in myxomycetes. Table does not include the complications of polyploidy or mutations.

	Heterothallic			Non-heterothallic	
Mating System	Sexual	Selfing^c	Automictic	Apogamic^a	Homothallic^b
Mating types	Yes	Yes	Yes	No	No
Syngamy required to produce plasmodia	Yes	No	Yes	No	Yes
Meiosis	Yes	No	Incomplete	No	Yes
Ploidy:					
Spores and amoebflagellates	1n	1n	1n	1n or 2n	1n
Zygote	2n	-	2n	-	2n
Plasmodia	2n	1n	2n	1n or 2n	2n
Resultant spores and amoebflagellates	1n	1n	2n	1n or 2n	1n
Mating system of resultant amoebflagellates	Sexual	Sexual, facultatively apogamic^d	Apogamic until reversion to sexual	Apogamic	Homothallic

^a Apogamic clonal lines can be produced by several different mechanisms, from heterothallic and non-heterothallic lines

^b True homothallism has not been confirmed in myxomycetes

^c Selfing occurs secondary to a normal sexual cycle; sexuality is not lost in the isolate

^d Amoebflagellates retain their sexuality but lack mating types; they can produce plasmodia with or without crossing

Figure 1. Life cycle (not drawn to scale) of a "typical and simplified" heterothallic myxomycete, with a few possible alternate cycles also indicated. Ploidy level is indicated only for the "typical" heterothallic life cycle and is placed in parentheses throughout this figure caption (for a list of alternative mating cycles and associated ploidy levels see Table 1). (A) Spores dispersing from a mature fruiting body. (B) Uninucleate amoebflagellate (haploid) emerging from a spore. (C1) Depending upon the environmental conditions, amoebflagellates may or may not have flagella; under adverse conditions, amoebflagellates may convert into a resistant structure called a microcyst. (C2) The non-flagellated form is able to undergo cellular division. The dotted line from the dividing cell (C2) back to the general amoebflagellate stage (C1) represents a hypothetical alternative life cycle in which a lineage no longer forms plasmodia or fruiting bodies. (C3) Representation of an apogamic life cycle in which the amoebflagellate is able to directly form a plasmodium without the need for fusion with other amoebflagellates. (D) Fusion of two compatible amoebflagellates (haploid) to form an amoeboid zygote (diploid). (E) The zygote (diploid) will feed and grow by undergoing multiple rounds of mitosis without cytokinesis, first to create a single large multinucleate cell (F) and then a plasmodium (diploid). (H1) Plasmodium displaying a vein-like network in which the cellular contents are shuttled throughout. Given appropriate stimuli, the plasmodium gives rise to mature fruiting bodies (A). During the formation of fruiting bodies, meiosis occurs to produce four spores (haploid). (H2) Another hypothetical alternate life cycle in which amoebflagellates are produced by the plasmodium and could potentially act as gametes. (G) Under adverse conditions, a plasmodium may convert into a hardened, resistant structure called a sclerotium. (Drawing courtesy of Angela Mele.)

Figure 2. Structural components of a typical fruiting body. (A) Hypothallus (B) Spore mass and capillitium (C) Peridium (D) Capillitium (E) Columella (F) Stalk (or stipe) (Drawing adapted from Stephenson [2003]).

Figure 3. Illustrated representation of myxomycete SSU phylogeny. Tree topology was derived from SSU phylogenies in the literature (e.g. Cavalier-Smith et al. 2013; Clissmann et al. 2015; Fiore-Donno et al. 2010a, 2012, 2013; Kretzschmar et al. 2016; Leontyev et al. 2014a, 2015; Walker et al. 2015). The tree is rooted by the last common ancestor (LCA). Because the SSU phylogeny does not completely correspond to traditionally recognized taxonomy for the group based largely on morphological data, each taxon named on the tree is followed by a letter in parentheses that corresponds to the order from which the taxon is currently ascribed [(E) Echinosteliales, (S) Stemonitales, (P) Physarales, (L) Liceales, (T) Trichiales]. (Drawing courtesy of Dmitry Leontyev.)

Figure 1

Figure 2

Figure 3