4 Rhizaria: Phytomyxea

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CONTENTS

I. Introduction

Phytomyxea comprises a group that historically was considered as fungi (Sparrow 1960; Waterhouse 1972) and for that reason is included here. Recent classifications place Phytomyxea in the protistan supergroup **Rhizaria** (Adl et al. 2005; Bass et al. 2009; Cavalier-Smith and Chao 2003), and molecular studies have led to the recognition of two orders within Phytomyxea (Bass et al. 2009; Cavalier-Smith and Chao 2003): **Plasmodiophorida** (the plasmodiophorids *sensu stricto*) and **Phagomyxida** (phagomyxids). Major reviews of the Phytomyxea, in addition to John Karling's monograph *The Plasmodiophorales* (1968), include Maire and Tison (1909), Cook (1933), Dylewski (1989), Dick (2001), and Neuhauser et al. (2010).

The most commonly recognized Phytomyxea are the plant pathogens *Plasmodiophora brassicae* Woronin, the causal agent of clubroot of cabbage and other brassicaceous crops worldwide (Cook and Schwartz 1930; Dixon 2009), and *Spongospora subterranea* (Wallroth) Lagerheim, the causal organism of powdery scab of potato (Kole 1954; Merz 2008; Merz and Falloon 2009). Also of economic significance are *Spongospora nasturtii* M. W. Dick, the causal agent of crook root in watercress (Tomlinson 1958), and *Polymyxa betae* Keskin, the vector for beet necrotic yellow vein virus (BNYVV), which causes rhizomania of sugar beet (McGrann et al. 2009). *Spongospora nasturtii*, *S. subterranea*, and *Polymyxa graminis* Ledingham also serve as vectors for plant-pathogenic viruses (Cooper and Asher 1988; Kanyuka et al. 2003; Rochon et al. 2004).

Karling (1981) proposed unified terminology to alleviate problems with nomenclature for stages in the life cycles of plasmodiophorids that had accumulated over the years because of contributions from researchers in a variety of disciplines. His major concern was the use of the term *cyst* for the single-celled resting structure that was not the result of a zoospore encysting on a substrate. By replacing the term **cyst** with **resting spore**, for consistency, the recommended term for the collection of resting spores would be ***sporosorus*** instead of ***cystosorus***. Other terms recommended by Karling included ***sporogenic***, when referring to developmental stages that lead to resting spores, and ***sporangial***, when referring to developmental stages that lead to **thin-walled sporangia** (zoosporangia) that contain zoospores. Sporogenic and sporangial phases of plasmodiophorid life cycles have also been referred to as secondary and primary, respectively.

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A unique type of nuclear division in Phytomyxea, **cruciform division**, was observed in *P. brassicae* as early as 1899 but was referred to as *promitosis* or *protomitosis* because it resembled some of the nuclear divisions in several protozoa (Cook 1933; Karling 1968). The currently used descriptive adjective *cruciform* was introduced by Blomfield and Schwartz (1910) because at metaphase the persistent nucleolus is elongated parallel to the spindle and perpendicular to the plate of chromatin, thus forming a crosslike (cruciform) configuration when viewed from the side (Figs. 4.1 and 4.2). Additional descriptive terms used for this type of nuclear division include *Saturn stage* as a synonym for cruciform and *double anchor* or *dumbbell stage* for mid to late anaphase (Blomfield and Schwartz 1910; Cook 1933) (Fig. 4.3). Features of cruciform divisions based on ultrastructural observations (Keskin 1971; Braselton et al. 1975; Dylewski et al. 1978; Garber and Aist 1979b; Dylewski and Miller 1983) (Figs. 4.1–4.3) include a persistent membrane of either nuclear envelope or endoplasmic reticulum origin, intranuclear spindle, centrioles at both poles, and a nucleolus that remains throughout nuclear division.

In addition to cruciform division, systematic features of plasmodiophorids include **multinucleate protoplasts** without walls (**plasmodia**) as growth forms (Fig. 4.1), zoospores with two anterior whiplash (lacking mastigonemes) flagella (undulipodia) of unequal lengths (Ledingham 1934; Kole and Gielink 1961), centrioles paired in an end-to-end fashion (Braselton and Miller 1973) (Fig. 4.4), environmentally resistant resting spores (Figs. 4.6 and 4.7), and intracellular, biotrophic growth forms (Dylewski 1989). Phagomyxida share these features with Plasmodiophorida, with the exception of environmentally resistant resting spores: resting spores have not been documented for *Maullinia* I. Maier, E. R. Parodi, R. Westermeier et D. G. Müller, or *Phagomyxa* Karling.

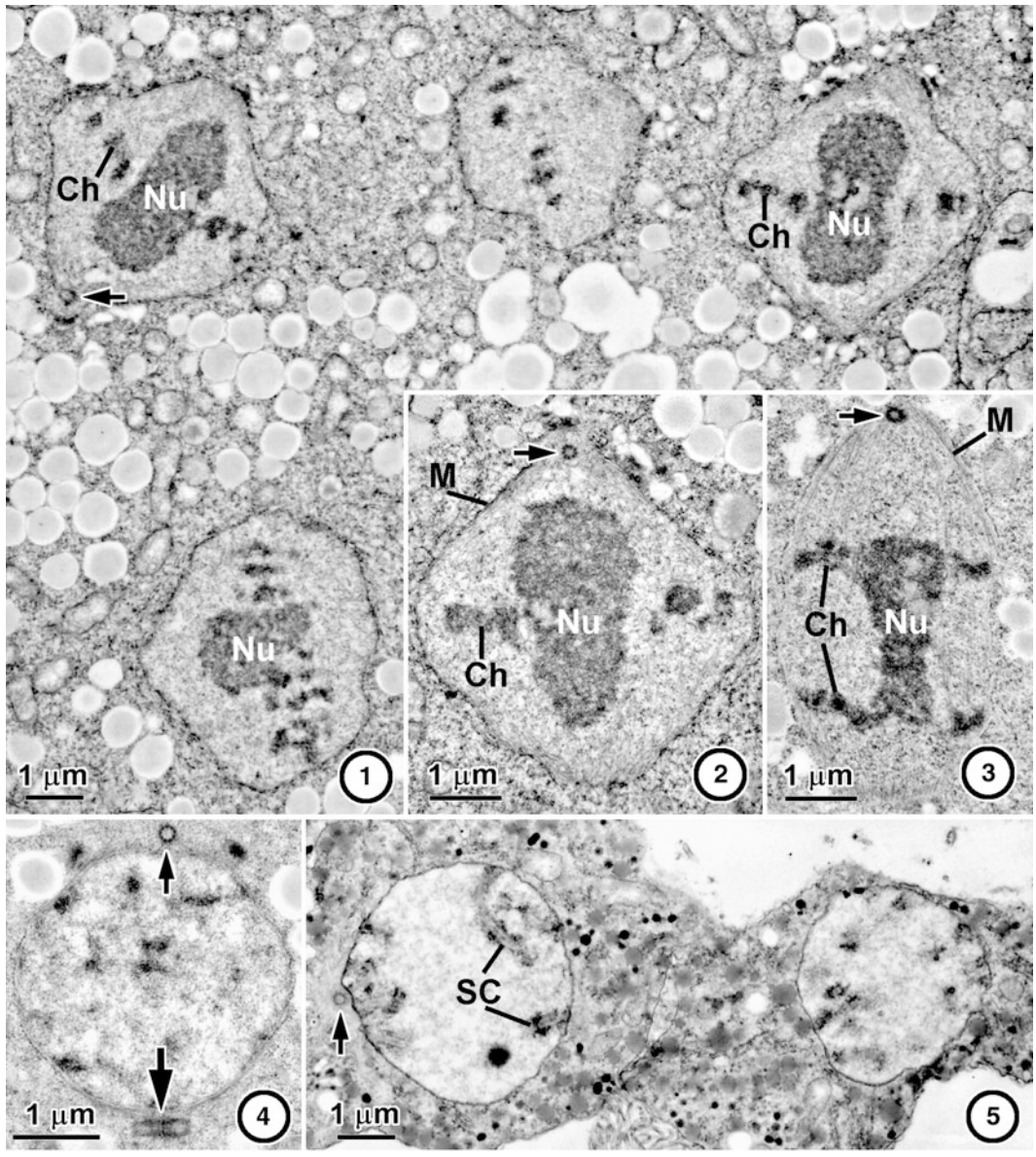
II. Life Cycle

Difficulties with describing phytomyxid life cycles arise in part because members of this group are obligate, intracellular biotrophs; no member has been shown conclusively to complete a life cycle in culture free of host cells. Dylewski's (1989) diagrammatic representation of the life cycle for members of the plasmodiophorids was in turn based on Karling's (1968) summary and serves as the basis for the life cycle presented here (Fig. 4.8). It should be emphasized that this generalized life cycle is the result of a compilation of observations made by various investigators and that variations in this scheme either have not been documented fully or are not currently understood.

Two major phases are recognized in the plasmodiophorid life cycle. The **sporogenic (secondary) phase**, which has not been observed in phagomyxids, culminates in the production of resting spores. The **sporangial (primary) phase** produces secondary zoospores within relatively thin-walled (zoo)sporangia.

In plasmodiophorids the life cycle arbitrarily may be considered to begin with a resting spore, a cell that contains a single nucleus and has an environmentally resistant cell wall. Resting spores may remain viable for several years, rendering infected soils unsuitable for susceptible hosts (Macfarlane 1952). The cell walls of *P. brassicae* (Yukawa and Tanaka 1979) and *S. subterranea* (Lahert and Kavanagh 1985) consist of three layers; *P. brassicae* cell walls contain chitin, lipids, and protein (Buczacki and Moxham 1983; Moxham and Buczacki 1983). The thickness of cell walls varies among members of the group (Figs. 4.6 and 4.7), but there has been no systematic treatment of the variations. Resting spores may occur singly, as in the genus *Plasmodiophora* Woronin, or in aggregations, sporosori, which remain the major morphological criterion for designating genera within Plasmodiophorida.

Upon germination, a resting spore releases a single, heterokont, biflagellated, uninucleate, free-swimming, primary zoospore (Kole and Gielink 1962; Macfarlane 1970; Merz 1997). When a zoospore encounters the wall of a potential host cell, the zoospore encysts and retracts its flagella (Aist and Williams 1971; Claxton et al. 1996; Merz 1997). A dense, projectile-like structure (Stachel) is within a tubular cavity (Rohr), and together these pass with the majority of the zoospore's cytoplasm into



Figs. 4.1–4.5 TEMs of dividing nuclei of Phytomyxea. **Figs. 4.1–4.4** Sporangial plasmodia of *Spongospora nasturtii* on watercress. **Fig. 4.1** Survey TEM of young plasmodium with synchronous cruciform divisions. Nucleoli are elongated perpendicularly to the chromatin and centrioles are at the poles (*arrow*). **Fig. 4.2** TEM of metaphase ("Saturn stage") of cruciform nuclear division. **Fig. 4.3** TEM of anaphase of cruciform nuclear division ("double anchor stage"). **Fig. 4.4** TEM of transitional nucleus, with one pair of centrioles in the end-to-end orientation characteristic for the group shown in longitudinal view (*larger arrow*) and the other centriolar pair in transverse view (*smaller arrow*). **Fig. 4.5** *Tetramyxa parasitica* on *Ruppia maritima*. TEM of transitional sporogenic plasmodium with profiles of synaptonemal complexes in the nuclei and one centriole shown at a pole. Centriole (*arrow*), chromatin (Ch), nucleolus/ i (Nu), persistent membrane (M), synaptonemal complex (SC), and transmission electron micrograph (TEM)

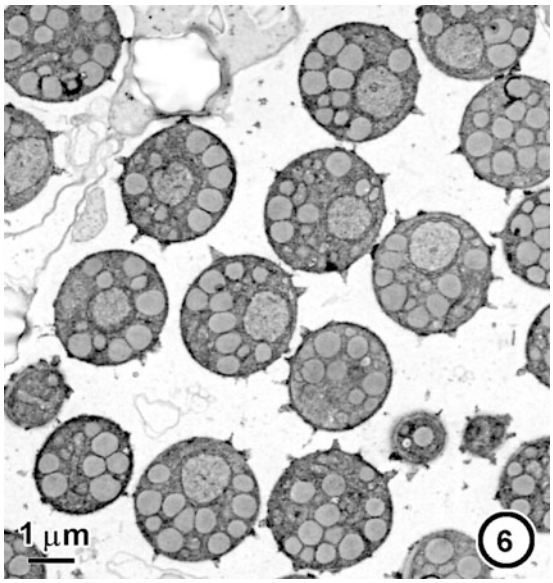


Fig. 4.6 *Plasmodiophora brassicae*. TEM of resting spores in root cell of Chinese cabbage

an outgrowth (adhesorium) from the main body of the encysted zoospore (Keskin and Fuchs 1969; Aist and Williams 1971; Claxton et al. 1996). Encystment of the zoospore with the formation of Stachel and Rohr takes approximately 2 h, formation of the adhesorium approximately 1 min, and the injection of zoospore contents through the host cell wall and plasma membrane into the host cytoplasm approximately 1 s (Aist and Williams 1971; Williams 1973).

Once within the host cell, the contents of the zoospore begin to grow by cruciform divisions (Fig. 4.1). The boundary between the plasmodium and host cytoplasm may be either a single, unit membrane for some members of the group (Braselton and Miller 1975) or a boundary thicker than a single membrane consisting of several layers for others (Williams and McNabola 1970).

What determines the path of development a plasmodium at this stage will take is not understood. For some phytomyxids, e.g., members of the genera *Polymyxa* Ledingham and *Ligniera* Maire & Tison, sporangial and sporogenic plasmodia may occur within adjacent cells of the same host tissue (Miller 1959). For others, such as *P. brassicae* and *S. subterranea*, sporangial plasmodia generally occur in root epidermal cells, particularly root hairs, whereas sporogenic plasmodia occur in cortical cells. For *Sorosphaera veronicae* Schröter,[1] sporogenic development is confined to shoots, whereas sporangial development occurs only in the roots (Miller 1958).

Conditions of the host growth medium may influence the development of the phytomyxid. For example, when *Woronina pythii* Goldie-Smith infects a *Pythium* sp. that has been growing in medium for less than a few days, the *W. pythii* will follow sporangial development. If, however, the host has been growing in medium for several days, and the medium is "stale," the *W. pythii* will follow sporogenic development (Miller and Dylewski 1983).

When a plasmodium, whether sporangial or sporogenic, reaches a stage of maturity where growth ceases, cruciform divisions no longer occur, and the nuclei become difficult to see in paraffin-sectioned specimens. The nuclei in part are difficult to recognize because the nucleoli either are reduced in size to below the resolution of optical microscopy or have disappeared altogether. Terms for this stage used by earlier microscopists included *akaryotic stage*, *enucleate stage*, *chromidial stage*, and *transitional stage*. Because nuclei are now known to be present during this stage of development (Fig. 4.5), *transitional stage* is the most appropriate term because this stage marks a change in the development of the plasmodium from a period of growth to a period of differentiation. Nuclei in this stage may be referred to as transitional nuclei.

[1] *Sorosphaera* has been used throughout this review because historically *Sorosphaera* was the name used in the literature for the genus. Neuhauser and Kirchmair (2011) noted, however, that since both Phytomyxea and Foraminifera are now recognized as members of the supergroup Rhizaria (Archibald and Keeling 2004; Bass et al. 2009; Burki et al. 2010), based on the International Code of Zoological Nomenclature (ICZN), a homonomy exists between the plasmodiophorid *Sorosphaera* J. Schröter and the foraminiferan *Sorosphaera* Brady. To resolve the homonomy, Neuhauser and Kirchmair (2011) proposed that *Sorosphaerula* nom. n. replace *Sorosphaera* J. Schröter for this genus.

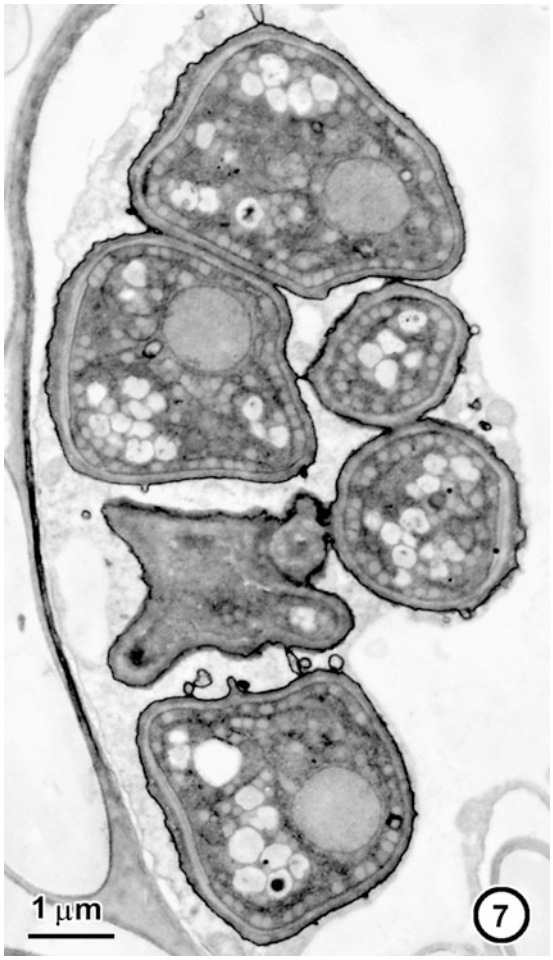


Fig. 4.7 *Polymyxa betae*. TEM of resting spores in a sporosorus in root cell of sugar beet

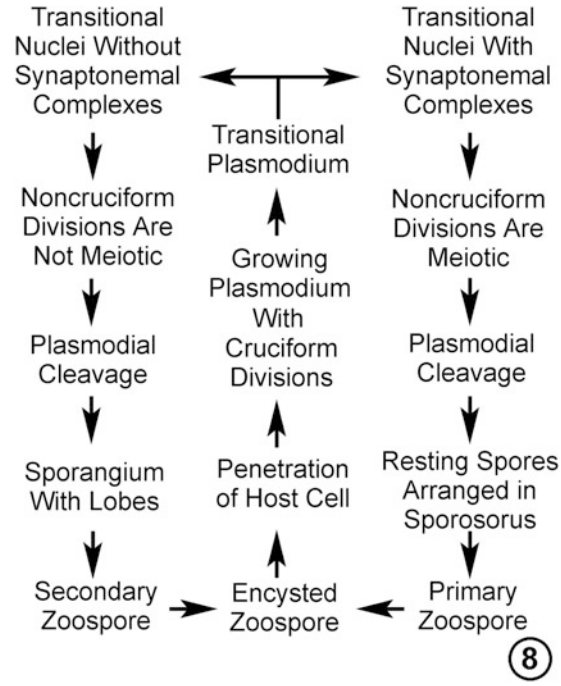


Fig. 4.8 Summary diagram of generalized life cycle for members of Plasmodiophorida

Up to the transitional stage, there are no obvious morphological distinctions between sporogenic or sporangial plasmodia (Miller 1959); the only time it is possible to determine definitively what type of plasmodium is present is in those situations where the two types of development occur in different host tissues as in *P. brassicae* and *S. subterranea*. Miller and Dylewski (1983) noted, however, that sporogenic plasmodia of *W. pythii* contained more lipoidal globules than sporangial plasmodia at the time cleavage is initiated. Nuclear divisions that occur in either sporogenic or sporangial transitional plasmodia are not of the cruciform type and, therefore, are referred to as *noncruciform divisions*. Although noncruciform divisions in both sporangial and sporogenic plasmodia appear similar at the level of optical microscopy, their prophases and, consequently, the type of division, may be distinguished by ultrastructural criteria.

A. Sporogenic (Secondary) Plasmodia

Transmission electron microscopy of transitional nuclei in plasmodia known to be of the sporogenic type revealed **synaptonemal complexes** (Fig. 4.5), indicators of prophase I of meiosis (Garber and Aist 1979a; Braselton 1995). The noncruciform divisions that occur either immediately preceding or during cleavage of the protoplasm leading to the formation of incipient resting spores are therefore interpreted as being meiotic, as had been suspected by others (Cook 1933; Webb 1935; Heim 1955). Cleavage of cytoplasm into uninucleate cells leads to the formation of resting spores as cell walls are deposited.

B. Sporangial (Primary) Plasmodia

Synaptonemal complexes have not been observed in the transitional nuclei of sporangial plasmodia (Dylewski and Miller 1984), and the noncruciform divisions that occur during or immediately preceding cleavage of a plasmodium into sporangial lobes are therefore not interpreted as being meiotic. Cleavage of sporangial plasmodia results in the formation of lobes with relatively thin walls, each lobe containing four or more secondary zoospores; the walls of the lobes may partially disintegrate, leaving passages between the lobes (Ledingham 1935, 1939; Miller 1958; Clay and Walsh 1990). One or more of the lobes may develop a discharge papilla, through which zoospores pass freely from one lobe to another and eventually discharge into the surrounding environment (D'Ambra and Mutto 1977; Miller and Dylewski 1983; Clay and Walsh 1990). Some investigators refer to the collection of lobes as a *sporangium* (*zoosporangium*) because the collection presumably developed from one plasmodium or there are continuities between lobes once walls between them disintegrate [see Miller (1958) for a review of this terminology; Barr 1979]. Others use the term *sporangiosorus* for the collection of lobes, considering each lobe as a sporangium (Buczacki and Clay 1984).

C. Relationship of Life Cycle Phases

The relationship of the two life cycle phases is not completely understood. Dobson and Gabrielson (1983) reported that sporangial development is needed prior to sporogenic development in *P. brassicae*; sporogenic development is interpreted as being initiated by secondary zoospores produced from sporangia. Other observations for *S. subterranea* and *P. brassicae* respectively by Kole and Gielink (1963) and Mithen and Magrath (1992) have indicated that primary zoospores may give rise directly to sporogenic (secondary) infections and to sporangial infections. Secondary zoospores likewise may produce sporangial (primary) infections or, under some conditions, initiate sporogenic (secondary) infections (Kole and Gielink 1963; Mithen and Magrath 1992).

D. Karyogamy

The major unresolved aspect of phytomyxid life cycles is the location of **karyogamy**. Karling (1968) summarized the knowledge of sexuality in the group as "...largely indirect and presumptive," and the statement continues to be the best summary of our understanding of sexuality for Phytomyxea. After suggesting earlier that karyogamy possibly occurred in fused zoospores, Kole (1954) reviewed observations of fusion of zoospores of *S. subterranea* and noted that karyogamy could not be documented in fused zoospores. The idea that secondary zoospores fuse prior to initiating primary (sporogenic) infections in *P. brassicae* was presented by Ingram and Tommerup (1972) and Dobson and Gabrielson (1983). Tommerup and Ingram (1971) and Buczacki and Moxham (1980) suggested that karyogamy may occur later in sporogenic plasmodia immediately preceding meiotic divisions.

III. Classification

A. Phylogeny

Although many mycologists and plant pathologists have treated Phytomyxea as fungi (Sparrow 1960; Waterhouse 1972), others have grouped them with the protozoa (Barr 1992). Beginning with the sequencing of the *P. brassicae* ribosomal 18S gene (Castlebury and Domier 1998), DNA sequence phylogenies placed phytomyxids with a wide assemblage of protists in the Cercozoa (Cavalier-Smith and Chao 1997, 2003). Further evidence of a close relationship between phytomyxids and cercozoans came with confirmation that they shared a unique one- or two-amino-acid insertion between ubiquitin monomers (Archibald and

Keeling 2004). These insertions have been found in Cercozoa and Foraminifera but not in all other eukaryotes studied to date, including radiolarians (Archibald et al. 2003; Bass et al. 2005). Subsequently, Cercozoa was incorporated into a supergroup of diverse protists, the Rhizaria, which has been almost entirely circumscribed through molecular evidence (Bass et al. 2005; Moreira et al. 2007; Nikolaev et al. 2004) and which has an evolutionary closeness to two chromalveolate groups, stramenopiles and alveolates (Burki et al. 2007, 2008; Hackett et al. 2007; Rodriguez-Ezpeleta et al. 2007).

Although Phytomyxea is well settled in the Rhizaria, the position of the phytomyxids with respect to other rhizarians is not established. Ribosomal 18S sequences show the parasitic Phytomyxea and Ascetosporea, along with reticulose protists, solidly grouped in the subphylum Endomyxa (Bass et al. 2005, 2009; Cavalier-Smith 2003). The first phylogenomic study to include large numbers of phytomyxid gene sequences placed Phytomyxea with *Gromia* Dujardin and a clade of Acantherea and Foraminifera separate from the core Cercozoa (Burki et al. 2010). Increased density of 18S sequences from cultivated protists and anonymous sequences from environmental sources indicate that the terrestrial/freshwater Vampyrellidae in the Proteomyxidea are the closest known relatives of Phytomyxea (Bass et al. 2009). If confirmed, this will show that parasitism has arisen twice, independently of free-living ancestors in the Phytomyxea and Ascetosporea (Bass et al. 2009).

B. Genera and Species

Genera and species are based on morphological criteria; the biological species concept is not applicable for this group because sexuality has not been observed. Ten genera are recognized in the order Plasmodiophorida (Braselton 1995; Dylewski 1989; Karling 1968): *Ligniera*; *Membranosorus* Ostenfeld & Petersen; *Octomyxa* Couch, Leitner & Whiffen; *Plasmodiophora*; *Polymyxa*; *Sorodiscus* Lagerheim & Winge; *Sorosphaera*; *Spongospora* Brunchorst; *Tetramyxa* Goebel, and *Woronina*. Two genera are currently recognized in the Phagomyxida: *Maullinia* (Maier et al. 2000) and *Phagomyxa* (Schnepf 1994; Schnepf et al. 2000). Karling (1968) listed 35 recognized species in his consideration of Plasmodiophorales.

The genera of plasmodiophorids are based on the morphologies of sporosori as seen through compound optical microscopy. For several genera, sporosoral morphologies are incorporated into their generic names, such as *Tetramyxa* (four resting spores per sporosorus), *Octomyxa* (eight resting spores per sporosorus), *Membranosorus* (sporosorus consisting of resting spores primarily in a single layer), *Sorodiscus* (resting spores arranged in a disk-shaped sporosorus), *Sorosphaera* (resting spores arranged in a sphere), and *Spongospora* (resting spores arranged in a spongy-looking sporosorus). Although Palm and Burk (1933), and subsequently some reviewers of the group (e.g., Olive 1975), questioned the reliability of using sporosoral morphology, it has continued to be the main criterion for delimiting genera of plasmodiophorids. Species within genera are generally based on what hosts are infected by the given organism, with specific epithets reflecting the host name. Examples include *pythii*, *callitrichis*, *betae*, *graminis*, *subterranea*, *nasturtii*, *brassicae*, *heterantherae*, and *veronicae*.

At this time we are on the verge of a better understanding of Phytomyxea speciation based on molecular phylogenetics. While confirming the expected close relationship between the *Polymyxa* spp. and *S. veronicae*, comparisons of ribosomal DNA sequences have shown that there is considerable phylogenetic distance between *S. subterranea* and *S. nasturtii* (Bulman et al. 2001), which supported the renaming of these two members of the genus from their previously recognized formae speciales (Dick 2001).

Misidentification of some genera and species or incomplete studies have led to confusion as to whether all of the currently recognized genera are valid. Palm and Burk (1933) concluded that the presently recognized genera *Ligniera*, *Membranosorus*, and *Sorodiscus* should be considered as synonyms of *Sorosphaera*. It should be emphasized that their conclusion was based on observations of one plant of *Veronica* sp. infected

with *S. veronicae*. Analyses of chromosomal numbers through serial sections of synaptonemal complexes showed that ultrastructural karyotypes of the recognized genera differ, supporting the retention of the ten recognized genera of Plasmodiophorida as valid taxa (Braselton 1995).

A paper that has led to confusion about two genera was by Wernham (1935) in which *Membranosorus heterantherae* Ostenfeld & Petersen (Ostenfeld and Petersen 1930) was renamed *Sorodiscus heterantherae*. Wernham's misidentification created some doubt as to the validity of the genus *Membranosorus* (Karling 1968; Olive 1975), which apparently has led to its exclusion from other systematic reviews (Cavalier-Smith 1993). Ultrastructural and karyotypic studies (Braselton 1983, 1989b) supported the view that *Membranosorus* is a valid genus.

C. Molecular Applications

Molecular investigations of Phytomyxea lag behind those for other microbial groups of comparable economic significance. *P. brassicae* has been the most extensively studied phytomyxid; the progression of molecular studies in this organism was summarized by Siemens et al. (2009). A consistent driver of molecular studies for phytomyxids has been the need for rapid and accurate detection of the important plant pathogens and viral vectors. This need has led to progress toward rDNA-targeted, quantitative-PCR assays for *P. brassicae* [reviewed in Faggian and Strelkov (2009)], *S. subterranea* (Lees et al. 2008; van de Graaf et al. 2003), and *Polymyxa* spp. (Vaïanopoulos et al. 2007; Ward et al. 2005).

From the earliest studies (Buhariwalla and Mithen 1995; Buhariwalla et al. 1995; Ito et al. 1994; Möller and Harling 1996), molecular techniques have been used for detecting genetic diversity within species. Molecular techniques for differentiating the highly variable *P. brassicae* accessions remain at an exploratory phase (e.g., Manzanares-Dauleux et al. 2001), but examinations of ribosomal sequences have been successful in delimiting new variations in the genus *Polymyxa* (Legrève et al. 2002).

Large-scale genomic studies have not been completed for any phytomyxid. This is in part because of the need to sort plant from phytomyxid sequences (Burki et al. 2010). There has been progress, however, in revealing the structure of several genes from *P. brassicae* (Siemens et al. 2009) and constructing a pilot-scale DNA library for *S. subterranea* (Bulman et al. 2011). Brodmann et al. (2002) attributed an increase in trehalose in roots and hypocotyls of *Arabidopsis thaliana* (L.) Heynh. infected with *P. brassicae* to the expression of a putative trehalose synthase gene from *P. brassicae*. An in-depth characterization of a phytomyxid gene was completed for a putatively secreted proteolytic enzyme from *P. brassicae* (Feng et al. 2010). Given the plummeting cost of generating new DNA sequences, complete phytomyxid genomes are undoubtedly accessible, although correct assembly plus a full and detailed annotation of such genomic data will be more time consuming.

IV. Occurrence, Distribution, Maintenance, and Culture

Depending primarily on their respective hosts, members of the Phytomyxea occur in a variety of habitats, including terrestrial, marine, and freshwater. Hosts range from vascular plants to algae and water molds.

The commonly recognized plant pathogens *P. brassicae* and *S. subterranea* and viral vectors *P. graminis* and *P. betae* are observed on a yearly basis on crops in various parts of the world and may be obtained from crop plants grown in infected soils (Colhoun 1957).

Most investigations for maintaining Phytomyxea in the laboratory or in glasshouse conditions concern *P. brassicae* and *S. subterranea*. Clubbed roots can be stored at –20 °C and used for inoculum of *P. brassicae* for several years. *Plasmodiophora brassicae* is maintained on various *Brassica* L. (Brassicaceae) species grown in soil in the greenhouse or growth chambers by inoculating seedlings with purified resting spores or slices of infected roots (Castlebury and Glawe 1993). Root galls are visible 3–7 weeks after inoculation. Castlebury et al. (1994) described how to purify resting spores from root galls, and several reports detailed methods for initiating infections from

single resting spores (Buczacki 1977; Jones et al. 1982; Scott 1985; Tinggal and Webster 1981; Voorrips 1996). Both phases of the life cycle of *P. brassicae* may be expressed on *Brassica* seedlings grown in defined, liquid, nutrient media (Crute et al. 1981; Macfarlane 1958; Williams et al. 1971). Methods for maintaining *S. subterranea* in the greenhouse on potatoes and tomatoes follow protocols similar to those used for *P. brassicae* (Kole 1954).

Polymyxa graminis may be grown on wheat in sand inoculated with infected soil samples (Barr 1987) and *P. betae* by growing sugar beet under similar conditions with sand inoculated with soils from sugar-beet-growing regions (Barr and Asher 1992). Neither *Polymyxa* species causes hypertrophy of host tissues, so localization of portions of roots that are infected must be made with optical microscopy. Both sporangial and sporogenic stages are observable in young, intact roots viewed with brightfield optical microscopy.

Collection of infected hosts from nature is the method of choice for obtaining representatives of *Ligniera*, *Membranosorus*, several species of *Plasmodiophora* other than *P. brassicae*, *Sorodiscus*, *Sorosphaera*, and *Tetramyxa*. With the exception of *Ligniera*, these parasites cause galls, which are easily identified with the unaided eye on host shoots or roots, depending on the particular host and parasite.

Membranosorus heterantherae occurs throughout the range of the host, *Heteranthera dubia* (Jacq.) MacMill. (Pontederiaceae), in freshwater lakes and rivers in the continental USA and southern Canada (Forest et al. 1986). *Sorosphaera veronicae* has been observed to cause shoot galls on various species of winter annuals in the genus *Veronica* (Plantaginaceae) in Athens, Ohio, USA (Harris et al. 1980); Chapel Hill, North Carolina, USA (Braselton and Miller 1973; Miller 1958); Sevenoaks, UK (Blomfield and Schwartz 1910); and near La Veta, Colorado, USA (Palm and Burk 1933). *Tetramyxa parasitica* Goebel is found on species of *Zannichellia* (Potamogetonaceae) and *Ruppia* (Ruppiaceae) in shallow, brackish water in Finland, Denmark, Sweden, Norway, UK, Germany, France, Italy, the USA (Luther 1949), and the Netherlands (den Hartog 1963). What was reported to be *T. parasitica* on *Halophila stipulacea* Asch. (Hydrocharitaceae) (Marziano et al. 1995) seems to be a species of *Plasmodiophora*. Two species of *Plasmodiophora* that deserve further study are widely distributed on their respective seagrass hosts, *P. diplantherae* on *Halodule* species (Cymodoceaceae) (den Hartog 1965; Walker and Campbell 2009) and *P. bicaudata* on species of *Zostera* (Zosteraceae) (den Hartog 1989). *Sorodiscus callitrichis* may be found on *Callitriche* (Plantaginaceae) species throughout Sweden in freshwater streams and ponds (Martinsson 1987).

Since species of *Ligniera* do not cause hypertrophy of host tissues, compound optical microscopy must be used to locate the various species by examining young, intact roots of hosts that have been collected from their native habitats. *Ligniera* spp. located in this manner include *L. junci* (Schwartz) Maire & Tison in roots of *Juncus triglumis* L. (Juncaceae) from englacial streams in Austria (Neuhauser and Kirchmair 2009); *L. verrucosa* Maire & Tison in roots of *Veronica* spp. collected from lawns on university campuses in Athens, Ohio, USA (Braselton 1989a; Miller et al. 1985) and Chapel Hill, North Carolina, USA (Miller 1959); and *L. pilorum* Fron & Gaillat in roots of various grasses in Ontario, Canada (Barr 1979).

Members of the genus *Woronina* are found worldwide and infect a variety of taxa of water molds and algae. *Woronina* may be located by "baiting" soil samples with hemp seeds in Emerson's (P/3) water for its hosts, primarily species of *Pythium* (Dylewski 1987; Miller and Dylewski 1983). Infected regions of hosts are enlarged and are detectable with brightfield, phase contrast, or differential interference contrast microscopy.

Location of phagomyxids has so far been largely a byproduct of research into their host species. Capture of *Phagomyxa bellerocheae* Schnepf and *P. odontellae* Kühn, Schnepf & Bulman requires close observation and expertise with phytoplankton from the Wadden Sea (Schnepf 1994; Schnepf et al. 2000). *Maullinia ectocarpii* I. Maier, E. R. Parodi, R. Westermeier et D. G. Müller has been identified as a parasite of economically important brown algae in Chile

(Maier et al. 2000). The size of infections and the culturability of its host (*Ectocarpus siliculosus* [Dillwyn] Lyngbye) mean that this phagomyxid represents the best chance for ongoing studies of these organisms.

No phytomyxid has been shown to complete a life cycle in culture free of host cells. There have been, however, successes in growing *P. brassicae* and *S. subterranea* in tissue culture with their respective plant hosts. These studies have used two approaches. First, *P. brassicae* and *S. subterranea* have been propagated successfully in hairy root cultures established by *Agrobacterium rhizogenes* (Asano et al. 2006; Qu and Christ 2007). Secondly, both *P.brassicae* and *S. subterranea* have been propagated for significant periods in plant callus cultures (Asano and Kageyama 2006; Bulman et al. 2011; Ingram 1969; Tommerup and Ingram 1971; Williams et al. 1969).

V. Conclusions and Future Prospects

Phytomyxea comprises a discrete taxonomic group that contains several members of economic importance. Despite the extensive applied literature on the control of the plant pathogens *P. brassicae* and *S. subterranea* and the viral vectors *P. graminis* and *P. betae*, several unresolved questions about the life cycles of members of the group remain. These include:

- Where in the life cycle does karyogamy occur?
- What determines when a resting spore germinates?
- How does a zoospore recognize a host cell?
- What determines whether a plasmodium will follow sporogenic or sporangial development?

It seems inevitable that Phytomyxea species are more abundant and widespread than is currently known (Neuhauser et al. 2011). Searches of potential hosts in other locations would be rewarding, and studies of environmental DNA samples may provide a new window into the group by determining the presence of undescribed species of Phytomyxea in terrestrial and aquatic environments. Further studies could include comprehensive Basic Local Alignment Search Tool searches of anonymous ribosomal RNA sequences in public databases for the presence of sequences of likely phytomyxean origin (Lesaulnier et al. 2008), multiple PCR-primer approaches (Stoeck et al. 2006), and the use of PCR primers biased toward the detection of phytomyxids (Neuhauser et al. 2011).

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