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Current Status of the Plasmodiophorids

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ABSTRACT: Plasmodiophorids are a monophyletic group with uncertain systematic affinities. Features of the group include cruciform nuclear division; obligate, intracellular parasitism; biflagellated, heterocont zoospores; and environmentally resistant resting spores. Economically significant members of the group include *Plasmodiophora brassicae,* the causative agent of clubroot of cabbage; *Spongospora subterranea,* the causative agent of powdery scab of potato; and two members of the genus *Polymyxa,* vectors for several plant pathogenic viruses.

KEY WORDS: clubroot, cruciform division, *Plasmodiophora,* Plasmodiophoromycetes, Plasmodiophoromycota, *Polymyxa,* powdery scab, *Spongospora.*

I. INTRODUCTION*

Because some plasmodiophorids continue to be significant plant pathogens and/or vectors for serious plant pathogenic viruses, and their terminology and systematics have become confused, the present review was initiated to focus on misunderstandings that have accumulated over the years and to update the terminology for the group. Not every paper published about the plasmodiophorids has been cited, but rather selected articles that the author considers to be important to a specific topic and/or excellent starting points for literature on the topic are included. For detailed historical treatments of the plasmodiophorids, reviews by Cook,[1] Karling,[2] and Dylewski[3] are recommended. Historical surveys of members of the group that are plant pathogens are included in Karling.[2]

Since their recognition as family Plasmodiophoraceae by Zopf,[4] the plasmodiophorids have been a systematic problem. Zopf originally included the Plasmodiophoraceae in the Monadinae in the myxomycetes, but they also have been considered as either fungi[5,6] or protoctists.[7,8] More recently, Barr[9] recommended that Phylum Plasmodiophoromycota (Plasmodiophoroa) be included in the Kingdom Protozoa, as did Lee et al.[10] This author prefers to use the informal term "plasmodiophorids" in this review as there are several formal names for phylum, class, order, and family, depending on where the group is classified. The two most commonly used recent names for the group are Plasmodiophorales and Plasmodiophoromycetes.[2,3]

Regardless of where they are classified, the plasmodiophorids are a discrete taxonomic unit, and may be considered as a monophyletic group: all members share the derived character state "cruciform nuclear division" (Figures 1 to 3). Cook[1] was the first to recognize that this unusual type of nuclear division, also called "promitosis" in the early literature, was the defining charac-

* All figures for this article follow the text.

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ter for the taxon. Other salient features of plasmodiophorids include (1) zoospores with two, anterior, whiplash flagella of unequal lengths (Figure 21); (2) multinucleated protoplasts (generally referred to as plasmodia) (Figures 4, 7, and 10); (3) environmentally resistant resting spores (cysts) (Figures 11 to 19); and (4) obligate, intracellular parasitism.

Several plasmodiophorids cause hypertrophy and/or hyperplasia of host tissues, producing either distortion of the infected organ or galls. Most attention in the literature has been paid to the economically significant members: *Plasmodiophora brassicae* Woronin, the causative agent of club root of cabbage and other brassicaceous crops,[11] and *Spongospora subterranea* (Wallroth) Langerheim f. sp. *subterranea* Tomlinson, the causative agent of powdery scab of potato[12] and the vector for potato mop top virus.[13,14] Other economically important plasmodiophorids include *S. subterranea* (Wallroth) Lager. f. sp. *nasturii* Tomlinson,[15] the causative agent of crook root of watercress; *Polymyxa betae* Keskin,[16] which, along with a virus, is associated with rhizomania of sugar beet and also is the vector for soil-borne viruses of sugar beet;[13,14] and *P. graminis* Ledingham, the vector for several soil-borne, pathogenic viruses of crops, including barley, oats, rice, and wheat.[13,14]

II. TERMINOLOGY

Terminology for the group has become confused because of contributions from a variety of disciplines. Karling,[17] with this confusion in mind, proposed a standard set of terms, which is the terminology used in the present review, with common synonyms included within parentheses. Terms recommended by Karling[17] include **resting spore** (cyst), **sporosorus** (cystosorus), **sporogenic** (cystogenous, secondary), and **sporangial** (sporangiogenous, primary).

The major recommendation by Karling in the terminology is replacement of the term "cyst" with "resting spore." Because zoospores encyst on the host prior to infection, he felt that using the term "cyst" in another part of the life cycle for a thick-walled, single-celled, resting structure was inconsistent and confusing. By replacing "cyst" with the more appropriate term "resting spore," for consistency terms associated with resting spores (cysts) are changed to **sporosorus** (cystosorus) for the aggregation of resting spores, and **sporogenic** (cystogenous), the adjective used in reference to the phase of the life cycle that produces resting spores.

III. LIFE CYCLES

When one compares reported life cycles[2] for the genera within the plasmodiophorids, there appears to be much variation. Although there undoubtedly are some deviants from the basic life cycle, many of the variations illustrated by Karling[2] probably are due to differing interpretations of fixed material for light microscopy. Transmission electron microscopy has helped our understanding of some of the major events in the life cycles and has revealed that there is consistency among genera with regard to where meiosis occurs, cruciform nuclear division, zoospore structure, and the infection process. There are two major phases in plasmodiophorid life cycles (Figure 23): sporangial (sporangiogenous or primary) and sporogenic (cystogenous or secondary). Each phase is initiated when a single, uninucleate zoospore infects a host cell.

How plasmodiophorids infect host cells is an important feature of the group: plasmodiophorids are truly intracellular. This is accomplished through a unique infection mechanism[18,19] in which an encysted zoospore produces a tubular structure (**Rohr**) that contains a dense, projectile-like struc-

ture (**Stachel**). Zoospore contents, including Rohr and Stachel, pass into an outgrowth of the main body of the zoospore (the **adhesorium**). Encystment with formation of Rohr and Stachel occurs over about 2 h, and the formation of adhesorium takes approximately 1 min. In approximately 1 s, the zoospore contents are injected through the host cell wall and plasma membrane into host cytoplasm.[19]

After entering the host cell, the zoospore contents begin to grow, which is characterized by synchronous cruciform nuclear divisions (Figures 1 and 2), ultimately forming a multinucleate plasmodium (Figure 4). Transmission electron microscopy has allowed us to characterize cruciform nuclear division (Figures 1 and 3) as a type of mitotic division in which a persistent nucleolus is elongated perpendicularly to the metaphase plate of chromatin, and end-to-end centriolar pairs occur at each pole.[20–23] An envelope remains intact around the nucleus through metaphase: it has been interpreted as either the original nuclear envelope in *Sorosphaera veronicae*[20,21] or the perinuclear endoplasmic reticulum in *P. brassicae.*[22]

Boundaries between plasmodia and hosts may be thicker than a unit membrane, sometimes consisting of several layers,[24] or one single, unit membrane (cf., Figures 5 and 6).[25] Even those species with host-parasite boundaries of several layers for young, developing plasmodia have their boundaries change to a single, unit membrane as the parasite matures.[24,26]

The conditions that determine whether an infection will become sporogenic or sporangial are not known. With some species, such as those in *Polymyxa*[27] and *Ligniera,*[28] sporangial and sporogenic development may occur within the same host root in cells very close to each other. For *P. brassicae* and *Spongospora subterranea,* sporangial development occurs in epidermal cells of host roots, including root hairs, whereas sporogenic development occurs in other tissues. Dobson and Gabrielson[29] showed that secondary zoospores from sporangial stages of *P. brassicae* are what initiated infections in cortical cells of brassicaceous roots; infections in cortical cells lead to sporogenic development, which causes hypertrophy and hyperplasia of infected host tissues, the basis for the pathological condition known as club root. *S. subterranea* sporogenic development occurs in either potato root cortical cells or in epidermal and subepidermal cells of potato tubers, the latter of which produces the pathological condition "powdery scab."

In the case of *Woronina pythii,*[30] conditions in the culture media for the host, *Pythium* sp., affect the phase of the parasite's life cycle. If the medium is fresh, that is, has not had *Pythium* growing in it for more than a few days, the parasite will follow sporangial development, resulting in continued production of zoospores, which, after infection of new host cells, also will develop into sporangial plasmodia. Use of so-called "stale" media, however, will result in production of sporogenic plasmodia and subsequent production of resting spores.

Sporangial plasmodia produce thin-walled zoosporangia (Figures 20 and 22), which contain secondary zoospores. After cruciform divisions have ceased, noncruciform nuclear divisions occur in secondary plasmodia during cleavage of the plasmodia into sporangial lobes and subsequently into incipient secondary zoospores. Noncruciform divisions in sporangial plasmodia are not meiotic as are those in sporogenic plasmodia. Secondary zoospores (Figures 21 and 22) produced by sporangial plasmodia are released into the environment and after infection of host cells may develop into either sporangial or sporogenic plasmodia.

Sporogenic plasmodia develop into unicellular resting spores (cysts). After cruciform divisions have ceased, noncruciform divisions occur immediately prior to or during cleavage of mature sporogenic plasmo-

dia into resting spores. Noncruciform divisions in sporogenic plasmodia are considered to be meiosis as synaptonemal complexes (SCs) occur in prophases (Figures 7 to 10).[36,37] Resting spores may be grouped into sporosori (cystosori), the morphologies of which are the major taxonomic characters for the genera (Figures 11 to 19). During germination, each resting spore releases one primary zoospore, which, after infection of a host cell, develops into a sporangial plasmodium.

Although meiosis occurs during cleavage of sporogenic plasmodia into resting spores, convincing evidence for location of karyogamy in the life cycle is lacking. Electron micrographs of purported karyogamy[31] were of poorly fixed material, and the images of distorted membranes sticking together are often found in poorly fixed and embedded samples. Also, nuclei fuse during an elimination process, as described by Dylewski and Miller,[32] for *W. pythii,* but this is not karyogamy as part of a sexual cycle per se, and it would be possible to confuse this process for sexual karyogamy. Similarly, other reports of purported karyogamy did not convincingly demonstrate sexual fusion.[33,34] Until there is unequivocal documentation of karyogamy, one major, unresolved problem with plasmodiophorids remains: when and how are nuclei that are capable of undergoing meiosis formed?

IV. THE GENERA

We currently recognize 10 plasmodiophorid genera with a total of 35 species.[2] The genera are *Ligniera, Membranosorus, Octomyxa, Plasmodiophora, Polymyxa, Sorodiscus, Sorosphaera, Spongospora, Tetramyxa,* and *Woronina.* Some of the species have been described only once, and their validity may be in question because there are no type specimens.

There has been confusion regarding whether some of the currently recognized genera should be considered as synonyms. Palm and Burk[35] suggested that *Sorosphaera, Sorodiscus, Ligniera, Spongospora,* and *Membranosorus* be treated as one genus. The conclusion of Palm and Burk was based on the variations of sporosori they observed in one specimen of *Veronica* sp. infected with *Sorosphaera veronicae.* When one examines galls of *Veronica,* there are variations in the sporosori of *Sorosphaera,* but any serious, critical observations preclude confusing the slight variations in *Sorosphaera* sporosori with the sporosori and their variations of the other genera (cf., Figures 11 to 19). It is time to lay the Palm and Burk paper to rest: their conclusion was based on a very limited sample of *Veronica* and *Sorosphaera* and did not include accurate, comparative observations of sporosori of the other genera. Also, ultrastructural karyological evidence of chromosomal numbers[36,38] are consistent with the view that the genera are valid, separate taxa.

Another paper that has led to confusion about two genera was by Wernham.[39] Although Wernham acknowledged that the organism he described on *Heteranthera dubia* was similar to *Membranosorus heterantherae* Ostenfeld and Petersen,[40] he assigned it to the genus *Sorodiscus.* This has led others to regard *Membranosorus* as a doubtful genus,[6] or to even ignore it, considering the organism as a species of *Sorodiscus.*[8] The sporosori of the organism that infects *H. dubia* are mostly single layered as opposed to the double layers of *Sorodiscus* (cf., Figures 16 and 18), and the number of SCs (hence haploid chromosome numbers) for *M. heterantherae* and *S. callitrichis* differ.[41,42] The organism that infects *H. dubia* is distinct from *Sorodiscus* and should be recognized as *M. heterantherae.*[43]

Two genera that are difficult to distinguish by optical microscopy are *Polymyxa* and *Ligniera.* Barr[44] addressed this issue from

the standpoint of sporangial structure and concluded that the genera are valid. This conclusion was supported by Miller and coworkers in an ultrastructural developmental study of *L. verrucosa.*[45] Braselton's TEM analyses of SCs[46–48] also supported Barr's view of the two genera by reporting that the two species of *Polymyxa* had identical ultrastructural karyotypes, but that *L. verrucosa* had a karyotype that differed from *Polymyxa* in number and lengths of SCs.

In addition to supporting the validity of the genera as based on sporosoral morphologies (one exception is considered in the following paragraph), ultrastructural studies of plasmodiophorids show that there are two groups of genera: one group exemplified by *Sorosphaera,* the other by *Plasmodiophora.*[36] The *Plasmodiophora* group differs from the *Sorosphaera* group by having SCs with a narrower central region (Figures 8 and 9), HP boundaries of several layers during early sporogenic development (Figures 5 and 6), and nuclei with volumes <14 μm^3 (Figures 7 and 10). Genera in the *Sorosphaera* group, in addition to *Sorosphaera,* are *Ligniera, Membranosorus, Polymyxa, Sorodiscus,* and *Spongospora.* The *Plasmodiophora* group consists of *Plasmodiophora* and *Woronina.* Although *Sorodiscus* is listed as a member of the *Sorosphaera* group, there is one member currently in the genus that fits into the *Plasmodiophora* group and eventually should be reclassified.

Ultrastructural studies of two members in the genus *Sorodiscus*[42] showed the only case known so far where a plasmodiophorid may have been placed in a genus incorrectly based on sporosoral morphology. *Sorodiscus callitrichis* differs significantly from *S. cokeri: S. callitrichis* is a member of the *Sorosphaera* group and should be kept as the type species for the genus *Sorodiscus. Sorodiscus cokeri,* however, is indistinguishable from *Woronina* in the *Plasmodiophora* group in all aspects except sporosoral morphology, and it is recommended that *S. cokeri* be reclassified as a member of the genus *Woronina.*[42]

V. SUMMARY AND CONCLUSIONS

There is a need for further research on several aspects of the plasmodiophorids. The most notable technical problem has been the inability of anyone to culture members of the group free of host tissues. Although there have been successes in maintaining infected host tissues in culture,[49] the conditions for maintaining pure cultures of plasmodiophorids are not known. Applications of tissue culture methods to obtain plasmodiophorids free of hosts could help in our understanding of the genes that control the phases of the life cycles, what controls germination of resting spores, and how zoospores recognize host plants.

Several major gaps in our knowledge of plasmodiophorids are based on limited observations of life cycles of members in the group. The sporangial phases of *Membranosorus heterantherae, Tetramyxa parasitica, Plasmodiophora diplantherae,* and *Sorodiscus callitrichis* have not been described: these taxa are known only from their sporogenic phases. The most obvious gap in our knowledge of life cycles for all plasmodiophorids is the lack of convincing evidence of where karyogamy occurs, or, if it does not occur, how nuclei become capable of undergoing meiosis. Because of the small sizes of plasmodiophorid nuclei, light microscopy of paraffin-embedded plasmodiphorids does not provide for the resolution needed for these organisms. Use of plastic-embedded materials for optical microscopy, in addition to electron microscopy and confocal microscopy, is essential for accurately visualizing nuclei and morphological events.

Poor understanding of plasmodiophorids also is caused by the lack of application of modern, molecular methods to problems associated with systematics and genetics of

members of the group. Molecular studies could be the beginning for locating genes associated with pathogenicity. In addition, molecular studies are essential for determining relatedness of plasmodiophorids to other groups.

Although where the plasmodiophorids should be classified remains unsolved, continuing to classify them as both protozoa and fungi creates unnecessary confusion. Because the true fungi are being more narrowly defined,[9,50] and because plasmodiophorids both historically and recently have been associated with protozoa,[9] this author proposes that we stop considering plasmodiophorids as fungi and treat them as protozoans until there are sufficient molecular and/or other data to warrant moving the group to another taxon.

ACKNOWLEDGMENTS

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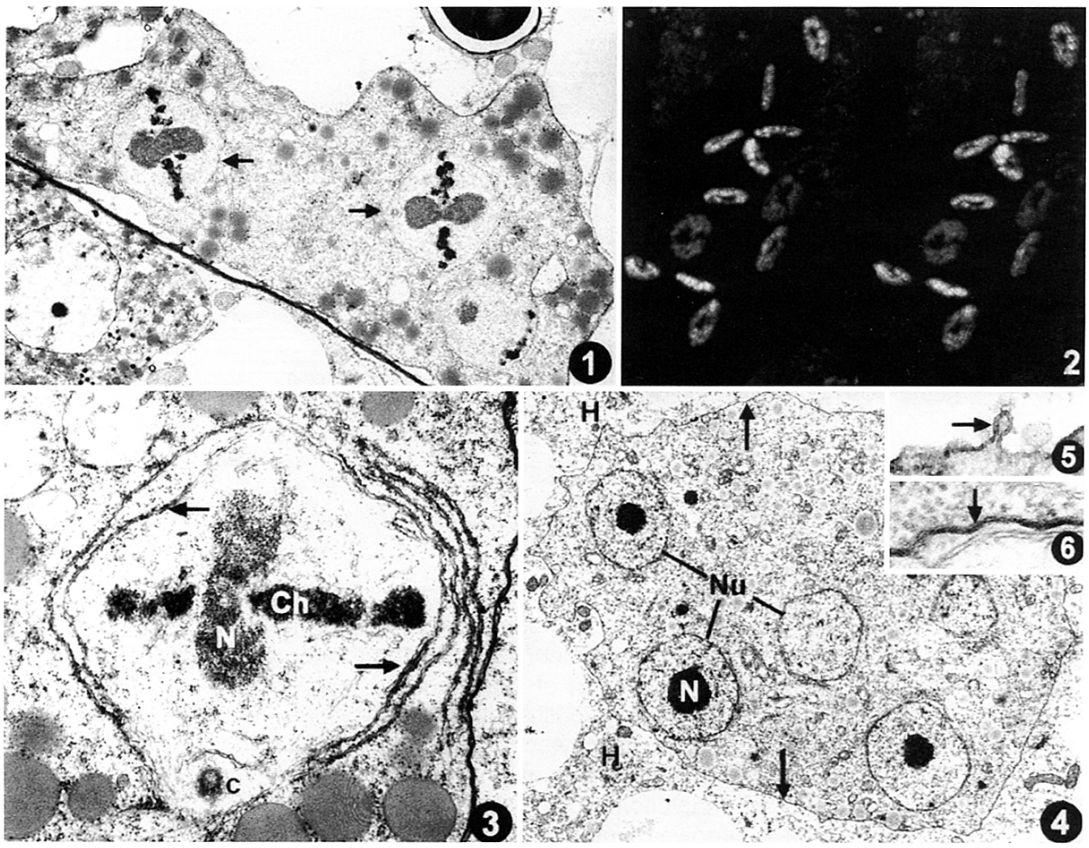

FIGURE 1. Survey transmission electron micrograph of *Tetramyxa parasitica* in shoot cell of *Ruppia maritima* showing two nuclei (arrows) at metaphase in cruciform division. Nucleoli are elongated perpendicularly to the metaphase plates of chromatin. (Magnification ×5000.) **FIGURE 2.** Stereo pair of confocal scanning laser micrograph of Feulgen-stained, LR White-embedded plasmodium of *Sorosphaera veronicae* with 12 metaphase nuclei. Because the Feulgen procedure stains chromatin but not nucleoli, each nucleus appears as a "doughnut," with the hole at the site of the nucleolus. Confocal microscopy allows for visualization of material in three-dimensions without distortions due to reconstruction from serial sections. (Magnification ×2000.) **FIGURE 3.** Transmission electron micrograph of near median longitudinal section of metaphase cruciform division of *Plasmodiophora brassicae.* One of the poles with a centriole (C) is in the plane of section, the elongated nucleolus (N) is perpendicular to the plane of the metaphase chromatin (Ch), and a persistent membrane (arrows) is at the periphery of the nucleus. (Magnification ×20,000.) **FIGURE 4.** Survey transmission electron micrograph of young plasmodium of *Sorosphaera veronicae* with interphase nuclei (Nu) that contain prominent, centrally located nucleoli (N). Parasite cytoplasm is separated from host cytoplasm (H) by a thin host-parasite boundary (arrows). (Magnification ×4500.) **FIGURE 5.** Host-parasite boundary (arrow) of the single, unit membrane type in *Tetramyxa parasitica.* (Magnification ×80,000.) **FIGURE 6.** Host-parasite boundary (arrow) of the relatively thick type in *Plasmodiophora brassicae* for comparison to boundary shown in Figure 5. (Magnification ×80,000.)

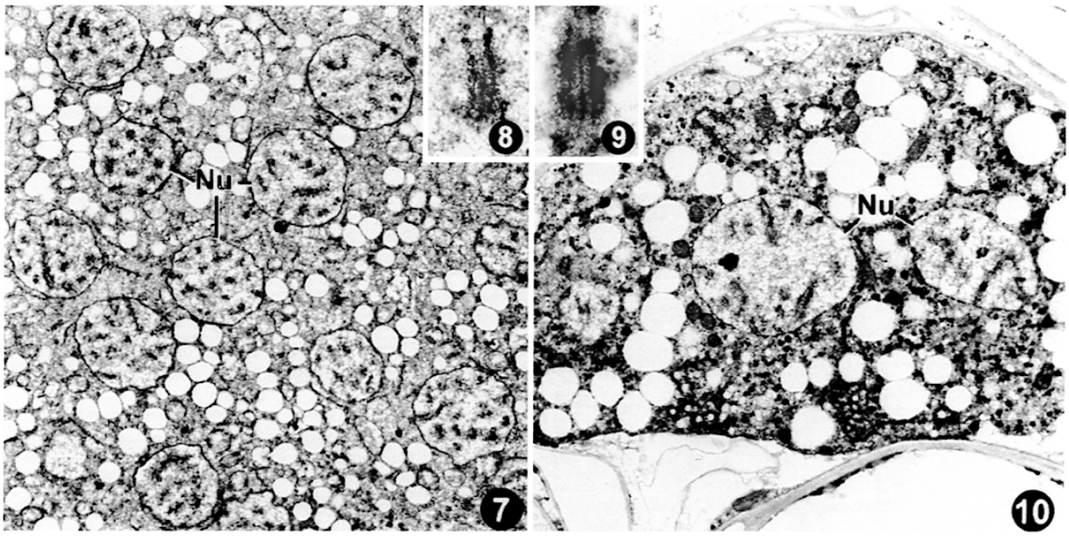

FIGURE 7. Survey transmission electron micrograph of transitional plasmodium of *Plasmodiophora brassicae* in Chinese cabbage root cell. Nuclei (Nu) are interpreted as being in pachynema of meiotic prophase I because of the presence of synaptonemal complexes (see Figure 8). This micrograph is for comparison to Figure 10 to illustrate that pachytene nuclei of members of the group *Plasmodiophora* are smaller than pachytene nuclei of members of the *Sorosphaera* group (Figure 10). (Magnification ×6000.) **FIGURE 8.** Synaptonemal complex of *Plasmodiophora brassicae* in Chinese cabbage root cell. Synaptonemal complexes of members of the *Plasmodiophora* group are not as defined and have narrower central regions than SCs of members of the *Sorosphaera* group. (See the synaptonemal complex in Figure 9.) (Magnification ×30,000.) **FIGURE 9.** Synaptonemal complex of *Polymyxa betae* in sugar beet root cell. Synaptonemal complexes of members of the *Sorosphaera* group have wider central regions and are more defined than SCs of members of the *Plasmodiophora* group. (See the synaptonemal complex in Figure 8.) (Magnification ×30,000.) **FIGURE 10.** Survey transmission electron micrograph of transitional plasmodium of *Polymyxa graminis* in wheat root cell. Nuclei (Nu) are interpreted as being in pachynema of meiotic prophase I because of the presence of synaptonemal complexes (see Figure 9). This micrograph is for comparison to Figure 7 to illustrate that pachytene nuclei of members of the *Sorosphaera* group are larger than pachytene nuclei of members of the *Plasmodiophora* group (Figure 7). (Magnification ×6000.)

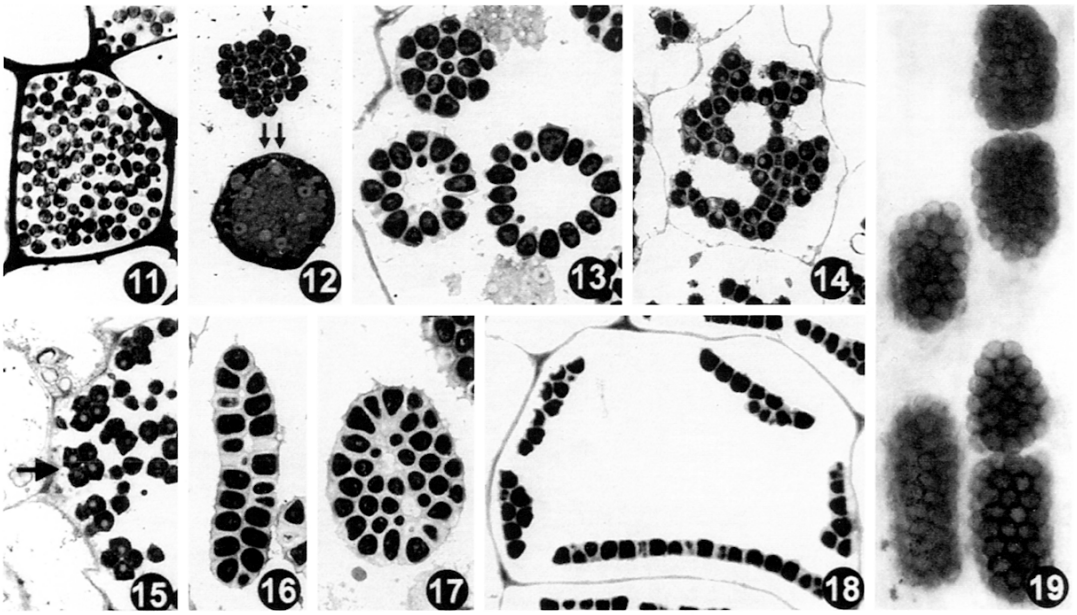

FIGURE 11. Light micrograph of resting spores of *Plasmodiophora brassicae* filling a root cortical cell of Chinese cabbage. (Magnification ×750.) **FIGURE 12.** Light micrograph of sporosorus of *Woronina pythii* (arrow) and young plasmodium (double arrows) in host, *Pythium* sp. (Magnification ×750.) **FIGURE 13.** Light micrograph of slices of different levels through three spherical sporosori of *Sorosphaera veronicae* in cell of *Veronica* sp. (Magnification ×750.) **FIGURE 14.** Light micrograph of "spongy" sporosorus of *Spongospora subterranea* in cortical cell of cultivated potato tuber. (Magnification ×750.) **FIGURE 15.** Light micrograph of resting spores arranged in tetrads (arrow) of *Tetramyxa parasitica* in shoot cell of *Ruppia maritima.* (Magnification ×750.) **FIGURE 16.** Light micrograph of longitudinal section of two-layered, disk-shaped sporosorus of *Sorodiscus callitrichis* in shoot cell of *Callitriche* sp. (Magnification ×750.) **FIGURE 17.** Light micrograph of frontal section of disk-shaped sporosorus of *Sorodiscus callitrichis* in shoot cell of *Callitriche* sp. (Magnification ×750.) **FIGURE 18.** Light micrograph of longitudinal sections of singled-layered, disk-shaped sporosori of *Membranosorus heterantherae* in root cell of *Heteranthera dubia* (water star grass). (Magnification ×750.) **FIGURE 19.** Light micrograph of sporosori of *Ligniera verrucosa* in an intact root of *Veronica* sp. stained with cotton blue in Amman's lactophenol. (Magnification ×750.)

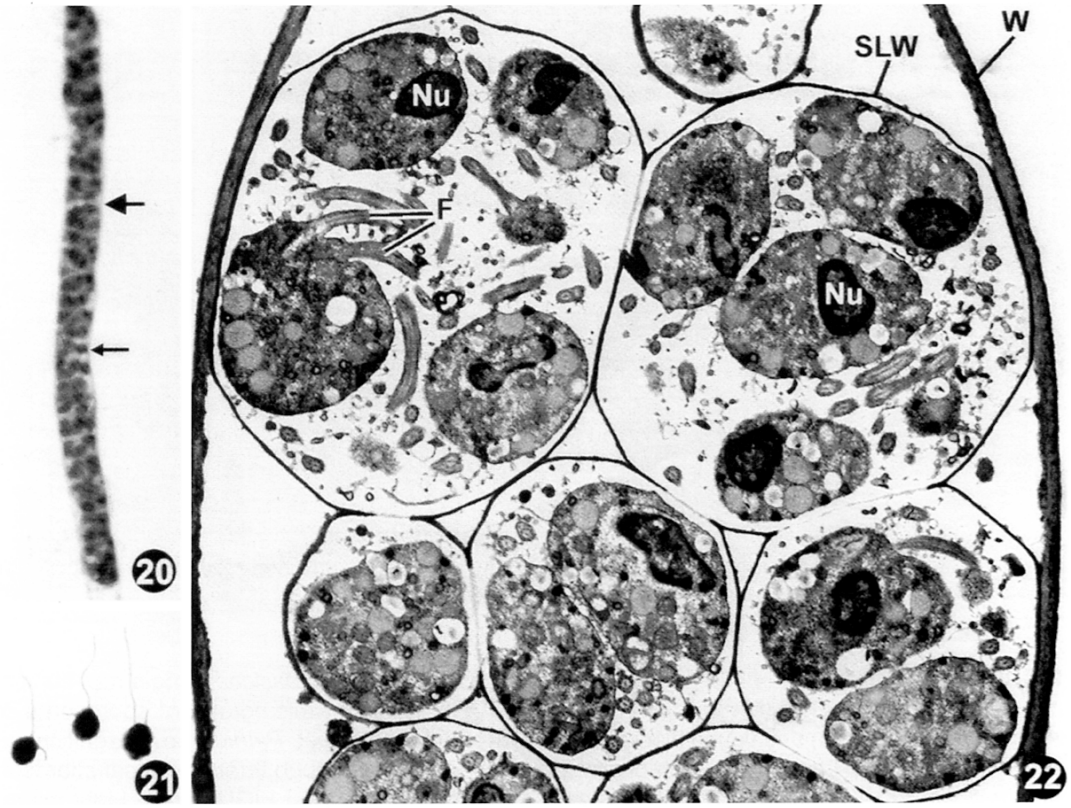

FIGURE 20. Light micrograph of sporangiosorus of *Spongospora subterranea* in root hair of cultivated potato stained with cotton blue in Amman's lactophenol. Boundaries between lobes of sporangia are light (large arrow), and individual zoospores are dark (small arrow). (Magnification ×750.) **FIGURE 21.** Secondary zoospores of *Spongospora subterranea* fixed with glutaraldehyde, air dried, and stained with Toluidine blue to show two flagella of unequal lengths. (Magnification ×1700.) **FIGURE 22.** Transmission electron micrograph of portion of Chinese cabbage root hair with secondary zoospores within sporangial lobes. Each zoospore has an electron opaque nucleus (Nu) and two flagella (F). Also labeled are wall of root hair (W) and thin walls of sporangial lobes (SLW). (Magnification ×10,500.)

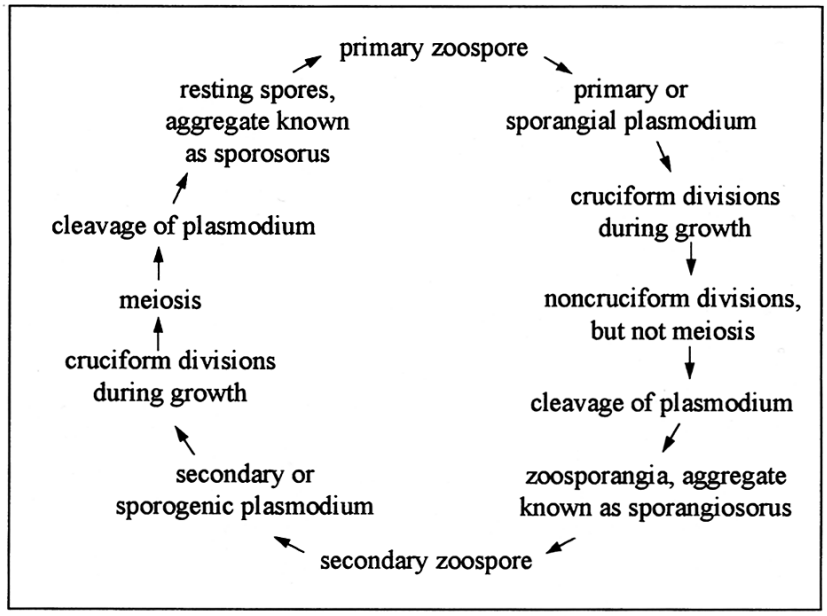

FIGURE 23. Summary of a generalized life cycle for plasmodiophorids.

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