

## Some Zoosporic Fungi of New Zealand. XII. Olpidiopsidaceae, Sirolpidiaceae and Lagenidiaceae

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With plates XXXII—XXXIII.

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In addition to the numerous chytrids, anisochytrids and plasmodiophorus species studied by the author (1965, 1967 a—1967 j) in New Zealand several members of the Olpidiopsidaceae, Sirolpidiaceae and Lagenidiaceae were observed as parasites of filamentous fungi and algae. Members of the first family were particularly abundant in species of *Pythium*, *Saprolegnia*, *Aphanomyces*, and *Achlya* which had been trapped on bits of hemp seed, corn leaves and snake skin in numerous soil samples. Eleven species of the genera *Olpidiopsis*, *Sirolpidium*, *Myzocyttium* and *Lagenidium* were identified, and all of these are commonly known species which appear to be worldwide in distribution. In addition, one species of *Rozellopsis* occurred quite frequently and is recorded here although it is not certain that it belongs in any of the three families mentioned above.

*Olpidiopsis saprolegniae* var. *saprolegniae* (Braun)

Cornu, 1872. Ann. Sci. Nat. Bot. Ser V 15: 145, pl. 3, fig. 10.

*Chytridium saprolegniae* Braun, 1855. Monatsber. Berlin. Akad. 1855: 384; 1856. Abhandl. Berlin Akad. 1855: 61, pl. 5, fig. 23.

*Diplophysa saprolegniae* (Cornu) Schroeter, 1885. Cohn Kryptogamenfl. Schlesiens 3 (1): 195.

*Pseudolpidium saprolegniae* Fischer, 1892. Rabenhorst Kryptogamenfl. 1 (4): 35, fig. 3 a—3 b.

*Olpidiopsis echinata* Petersen, 1909. Bot. Tidsskrift 29: 405, fig. 18 a; 1910, Ann. Mycol. 8: 540, fig. 18 a.

Parasitic in *Saprolegnia* sp. from soil sample AMA.

This species has been reported in a large number of saprolegniaceous hosts, but according to Shanor's (1940) extensive host range studies it is confined to species of *Saprolegnia* and *Isoachlya*. In this connection it may be noted that an *Achlya* growing on the same substratum with parasitized *Saprolegnia* hyphae was not infected.

*Olpidiopsis aphanomyces* Cornu, 1872. Ann. Sic. Nat. Bot. V, 15: 148, pl. 4, figs. 5—11.

*Pseudolpidium aphonomyces* (Cornu) Fischer, 1892. Rabenhorst Kryptogamen-Fl. 1 (4): 37.

Parasitic in *Aphanomyces laevis* from soil samples AMA. and WK2.

The sizes and shapes of the sporangia, zoospores and resting spores of the New Zealand specimens fall within the range of those described elsewhere in the world (see Karling, 1942 a).

*Olpidiopsis gracilis* (Butler) Karling, 1942. Simple holocarpic biflagellate Phycomycetes p. 47.

*Pseudolpidium gracile* Butler, 1907. Mem. Dept. Agric. India, Bot. Ser. 1: 131, pl. 7, figs. 1—8.

Parasitic in *Pythium* spp. from soil samples AMA, ADSIR, WT8, OGB, and ODL5.

The New Zealand specimens correspond closely to those described by Butler, and Whiffen (1942) and they are identified with Butler's species in *P. intermedium* in France. According to Whiffen, (1942) this species has a limited host range among members of *Pythium* and can be distinguished to some degree by this limitation. The author did not test the host range of the New Zealand parasite.

*Olpidiopsis pythii* (Butler) Karling, 1942. Simple biflagellate holocarpic Phycomycetes, p. 47.

*Pseudolpidium pythii* Butler, 1907. Mem. Dept. Agric. India, Bot. Ser. 1 (5): 127, pl. 7, figs. 9—16.

Parasitic in *Pythium* sp. from soil sample AOTH.

The New Zealand parasite caused the same reactions in the host as those described by several other authors elsewhere. In addition to sporangia and zoospores, parthenospores developed fairly abundantly, and these correspond in sizes and shapes to those described in the literature.

*Olpidiopsis achlyae* McLarty, 1941. Bull. Torrey Bot. Club 68: 62, figs. 1—26.

Parasitic in *Achlya flagellata* from soil samples AMA, WTI and OGB.

*Olpidiopsis brevispinosa* Whiffen, 1942. Amer. J. Bot. 29: 610, figs. 2, 14, 22, 27.

Parasitic in *Pythium* sp. from soil sample WT1.

The resting spores of the New Zealand specimens were dark-brown and bore relatively short straight spines like those described by Whiffen.

*Rozellopsis inflata* (Butler) Karling, 1942. Amer. J. Bot. 29: 34.

*Pseudolpidium inflatum* Butler, 1907. Mem. Dept. Agric. India, Bot. 1: 126, pl. 7, figs. 17—21.

Parasitic in *Pythium* sp. from soil samples AMA, WTI, WKI and WK2.

This species occurred in great abundance and caused swellings of the hyphal tips and incipient sporangia which were up to 80  $\mu$  in diameter. Unfortunately, no resting spores were developed by the New Zealand parasites. Until these are found it is impossible to classify this species. It is recorded and discussed here in relation to the Olpidiopsidaceae only because it produces biflagellate zoospores. Otherwise, the development of other species in relation to the host and the types of sporangia and resting spores produced are similar to those of *Rozella* in the chytrid family Olpidiaceae.

*Siroldidium bryopsidis* (de Bruyne) Petersen, 1905. Oversigt Kgl. Danske Vidensk. Selskabs. Forhandl. 1905: 480, fig. 9.

*Olpidium bryopsidis* de Bruyne, 1890. Arch. de Biol. 10: 85, pl. 5, figs. 1—15.

Parasitic (?) in *Bryopsis plumosa* and *Cladophora* sp., at The University of Otago Marine Biology Station, Portobello, Dunedin.

This species occurred in great abundance within a few days after the hosts were brought into the laboratory. The general morphology and development of the New Zealand specimens conformed closely to those described in the literature. In old and dying filaments of the hosts the thalli of the fungus became greatly elongate and hypha-like.

? *Myzocyttium microsporum* (Karling) Sparrow, 1960. Aquatic Phycomyces, p. 980.

*Lagenidium microsporum* Karling, 1944, Lloydia 7: 328, figs. 1—34.

Parasitic (?) in pollen grains of *Phyllocladus trichomanoides* from soil sample HBW, Hawkes Bay Province.

It is not certain that this is the correct identification of the New Zealand species, and although it resembles *M. microsporum* from Brazil it exhibits some noteworthy differences. In the first place it occurred in pollen grains and was not found in rotifers which were present in the same culture. Secondly, its zoospores and oospores are slightly smaller, and its oosporic thalli are sometimes 4-celled. Possibly, it might be considered as a variety of *M. microsporum*. In view of the differences noted above as well as some other minor ones, the New Zealand species is described and illustrated here in detail to emphasize its variations and differences.

The pollen grains of *Phyllocladus trichomanoides* are more favorable than those of *Pinus* for study of such an intramatrical species because they have small wings (fig. 5) and are almost transparent. Infection and development of the thallus and sporangia as well as sporogenesis can, therefore, be observed more clearly. The sporangial thalli are unicellular, and as many as 5 have been found in a pollen grain. These begin as a swelling at the end of the germ or infection tube (figs. 2, 3), and during a period of 3 to 5 days (figs. 6, 7) enlarge to become spherical, 8.8—12  $\mu$  diam., or broadly ovoid, 6—8  $\times$  9—11  $\mu$  incipient sporangia

(figs. 8—9). In the meantime, the zoospore cyst and germ tube apparently disintegrate. At least, persistent remnants of them have not been seen after 2 days. At this point it is pertinent to note that no infection stages and persistent zoospore cysts and germ tubes were observed in the Brazilian material, and in view of this the author (1944) conjectured that the zoospores may have been engulfed by feeding rotifers and germinated within the host.

The incipient sporangium develops an exit tube (fig. 8) of varying diameters and length (figs. 9—13). As it elongates and comes into contact with the host cell wall its elongation displaces or “pushes” the sporangium deeper into host cell so that later lies in the base of the pollen grain (fig. 9). At the same time the tip of the tube inflates slightly inside of the wall before passing through it (figs. 9—13). Such inflated canals were not observed in adults rotifers in the Brazilian material. The tubes formed in the pollen of *P. trichomanoides* vary from 7.7 to 11  $\mu$  in length and 3—4  $\mu$  in diameter, and project 2—4  $\mu$  beyond the surface of the wall.

At this stage the sporangia have a large irregular central vacuole (figs. 8, 9) surrounded by the more viscid, greyish granular cytoplasm in which lie a few refractive globules. The vacuole is usually stellate in appearance with furrows extending almost to the periphery and part way up into the exit canal. Cleavage into zoospore initials often appears to have occurred at this stage, but this may be an illusion. The subsequent physical changes in the protoplasm are quite striking. The central vacuole expands markedly until only a thin parietal layer of the more viscid and granular protoplasm is visible (fig. 10). Then, very quickly the vacuole seems to disappear, and the protoplasm in the exit canal flows back into the sporangium (fig. 11) as the tube becomes empty of the granular protoplasm (fig. 12). Quite probably it is filled with the less visibly differentiated cytoplasm. In the sporangium proper no vacuoles or furrows are visible at this stage, but within a few minutes a visible differentiation occurs. The exit canal becomes filled with granular protoplasm again, and faint cleavage lines reappear (fig. 13).

Shortly thereafter the tip of the exit canal deliquesces and the zoospore initials flow out. These form a globular cluster at the exit orifice (fig. 14), and within 30 minutes begin to move about and wiggle slowly. At this stage the slowly waving flagella become visible (fig. 15). The movement of the zoospores increases, and within a few minutes they begin to swarm actively in a confined vesicular area (fig. 16). The swarming continues for 3 to 5 minutes, after which the zoospores dart away. A vesicular membrane is not discernible around the zoospore initials after they have emerged nor latter during the swarming stage, but its presence is suggested by the confinement of the zoospores to a globular region at the exit orifice during this stage. In contrast to this behavior the zoospore initials in the Brazilian material emerged indi-

vidually and formed a loose mass of initials which gradually floated apart, elongated, developed flagella, and wiggled about slowly before becoming actively motile. No swarming of the zoospores in a confined area occurred as in the Brazilian species. The fully formed zoospores of the New Zealand species are ovately to elongately reiform,  $2-2.3 \times 2.7-3.3 \mu$ , and heterocont with the longer flagellum extending backward (fig. 1). They are, thus, slightly smaller than those in the Brazilian species, which measured  $2.5-3.5 \mu$ . In neither collection were cystospores or evidence of diplanetism observed.

In contrast to the unicellular sporangial thalli described above, the oosporic or resting-spore thalli may be 2- or 4-celled, and they may be recognized fairly early by their shape and denser, more granular content (figs. 17, 18). Subsequently, septa are formed between the cells, and the granular and globular refractive component of the protoplasm aggregates towards the center (fig. 19). At this stage one or two of the cells are slightly smaller, and these are the ones whose content later flows into the larger cells. Thus, so-called "male" and "female" gametangia may be recognized by differences in size and behavior of their content. However, they apparently possess the potentiality of functioning as sporangia. This is indicated by the fact that in a few 2-celled thalli both cells began to develop exit canals (fig. 24) as in the sporangia, but later the contents began to contract and that of one flowed into the other cell to form an oospore (fig. 25).

The process of fusion in the New Zealand species (figs. 20-22) is basically the same as described in the Brazilian material and results in the development of small, spherical to subspherical,  $7.8-10.5 \mu$  diam., oospores with hyaline walls and coarsely granular content in which lies a central vacuole (figs. 23, 25). As noted above, the gametangic or oosporic thalli may be 2- or 4-celled, but in the Brazilian species only 2-celled thalli were observed.

Thus, as described above the new Zealand species resembles *M. microsporium* from Brazil but exhibits some notable differences, and it remains to be seen whether the species are identical or one is a variety of the other.

*Myzocyttium proliferum* Schenk, 1858. Über das Vorkommen contractiler Zellen im Pflanzenreich, p. 10. Würzburg.

*Pythium proliferum* Schenk, 1859. Verhandl. Phys.-Med. Gesell. Würzburg, A. F., 9: 27, pl. 1, figs. 30-41.

*Pythium globosum* Schenk, 1859, *op. cit.* 9: 27, pl. 1, figs. 42-47.

*Pythium globosum* Walz, 1870 (pro parte) Bot. Zeit. 28: 553, pl. 9, figs. 13-15.

*Lagenidium globosum* Linsdstedt, 1872. Synopsis der Saprolegnieen, p. 54, Berlin.

*Myzocyttium globosum* (Schenk) Cornu, 1872. Ann. Sci. Nat. Bot. V, 15: 21.

Parasitic in *Spirogyra* sp. in a pond at the Soil Bureau, Taita, Wellington, Province.

*Lagenidium* sp.

Thalli usually multicellular, consisting of a few or up to 30 segments, constricted slightly at septa and sometimes forming a few short branches; rarely uni- or bicellular. Individual segments barrel-shaped, oblong, 4.5–6  $\mu$  diam., by 5.5–8.8  $\mu$  long, or ovoid to subspherical or elongate and cylindrical; transformed into sporangia and usually forming a short, narrow, barely-perceptible exit papilla or a short narrow tube, 1.5–2  $\mu$  diam., by 3.8–4.2  $\mu$  long, which do not extend beyond the surface of the host cell. Zoospores elongately reniform, 3.2–3.6  $\mu$  long by 2–2.4  $\mu$  wide, heterocont, longer flagellum extending backward; forming spherical cystospores, 3–3.8  $\mu$  diam., near exit orifice which may germinate by a tube; diplanetism not observed. Oospores or resting spores unknown.

Parasitic in sporelings of *Oedogonium* sp. from a small brook on the Rangimaire Farm, Judgeford District, Wellington Province.

This species is incompletely known so far as the basic generic distinctions are known, and it is difficult to classify it at present. Its thalli are somewhat bead-like and slightly constricted at the septa as in some species of *Myzocytium*, but it rarely produces short branches as in *Lagenidium*. For the latter reason it is tentatively placed in *Lagenidium*, but further studies may show that it has a *Myzocytium* type of sexual reproduction and oospore development. Nonetheless, it is distinguishable from the other species of these two genera which parasitize *Oedogonium* by its small zoospores.

So far only one species of *Myzocytium*, *M. proliferum* Schenk (1858), and 5 of *Lagenidium*, including *L. rabenhorstii* Zopf (1878), *L. syncytiorum* Klebahn (1892), *L. zopfii* de Wildeman (1889–1890), *L. Marchalianum* de Wildeman (1897) and *L. oedogonii* Scherffel (1925) have been reported in *Oedogonium* species. In addition to these, other unidentified and incompletely known specimens, *Lagenidium* sp. Deckenback (1903), and *Lagenidium* sp. Couch (1935), have been recorded as parasites of this alga. Among the identified species zoospore size is known only in *M. proliferum* (3.6–6  $\times$  5.4–10  $\mu$ ), *L. rabenhorstii* (6  $\times$  8.5  $\mu$ ), *L. oedogonii* (approx. 6  $\mu$  long) and *Lagenidium* sp. Couch (4  $\times$  5.5  $\mu$ ), and it is evident on the basis of zoospore size that the present fungus is not identical with any of these species. *Lagenidium syncytiorum* is known only from preserved material, but its thalli as figured by Klebahn resemble somewhat those of the New Zealand species. *Lagenidium zopfii*, also, is incompletely known, and Cook (1935) listed it as a synonym of *L. syncytiorum*. In *L. marchalianum* the sporangia are cylindrical and filamentous, 30–60  $\mu$  long, with exit tubes which extend beyond the surface of the host cell. In these respects it, too, differs from the present species.

To better record the occurrence of the New Zealand species and its known morphological and developmental characteristics for comparative purposes it is illustrated and described here in some detail. It occurred fairly often in sporelings attached to the under surface of floating strips of cellophane and killed the host cell within 5 to 12 days. The chloroplasts and remainder of the host protoplasm became clumped around the parasite and frequently obscured the details of structure and development. For the sake of clarity the surrounding and degenerating protoplasm been purposely omitted in figures 26 to 43. In this host the size and structure of the thallus vary considerably. It is usually multicellular and consists of a linear series of segments (figs. 30, 34, 35, 41, 42) with occasional short branches (figs. 30, 34). Rarely did uni-, bi- and tricellular thalli occur (figs. 31, 32, 33), and in the case of the unicellular ones they were usually elongate (fig. 31). In several instances the sporelings were almost completely filled with series of segments (fig. 35), and where these were short and tightly packed together they gave the whole mass the appearance of a sporangiosorus.

The individual cells or segments vary markedly in size and shape as noted in the diagnosis and shown in figures 37 to 40. Each segment functions as a sporangium, and at maturity forms a short and narrow, barely-perceptible exit pappila which penetrates the host wall (fig. 33, 34, 42) but does not extend beyond it. However, deeper-lying or more centrally located sporangia may develop a narrow exit tube (fig. 36). The zoospore initials appear to be delimited within the sporangium and undergo maturation on the outside (fig. 43), but this has not been determined with certainty. The enveloping degenerated host protoplasm is usually so obscuring that the details of sporogenesis are difficult to see. Numerous cystospores may occur in the vicinity of the exit orifices (fig. 42) which indicates that the zoospores encyst shortly after emerging. Also, several empty cysts may be present, and their occurrence suggests that a second motile stage occurs, although this has not been observed. Among the groups of cystospores may be some with fairly long germ tubes (fig. 42).

As in other species of *Lagenidium*, the thallus begins as an enlargement of the end of the germ tube (figs. 26—28) which elongates and usually becomes septate (fig. 30). Rarely, as noted before, it remains unicellular and is transformed into one sporangium without dividing. In the meantime the empty cystospore case and germ tube persist for several days and are readily visible on and in the host cell (fig. 28).

*Lagenidium rabenhorstii* Zopf, 1878. Verhandl. Bot. Verein Prov. Brandenburg 20: 79; 1884, Nova Acta Ksl. Leop.-Carol Deut. Akad. Nat. 47: 145, pl. 1, figs. 1—28; pl. 2, figs. 1—9.

Parasitic in *Spirogyra* sp. in a pond at the Soil Bureau, Taita, Wellington Province.

*Lagenidium pygmaeum* Zopf, 1887. Abhandl. Nat. Gesell. Halle  
17: 97, pl. 1, figs. 21—39; pl. 2, figs. 1—12.

In pollen grains of *Pinus sylvestris* and *Phyllocladus trichomanoides*  
in soil samples HBGF1, HBW and WK1.

#### Summary

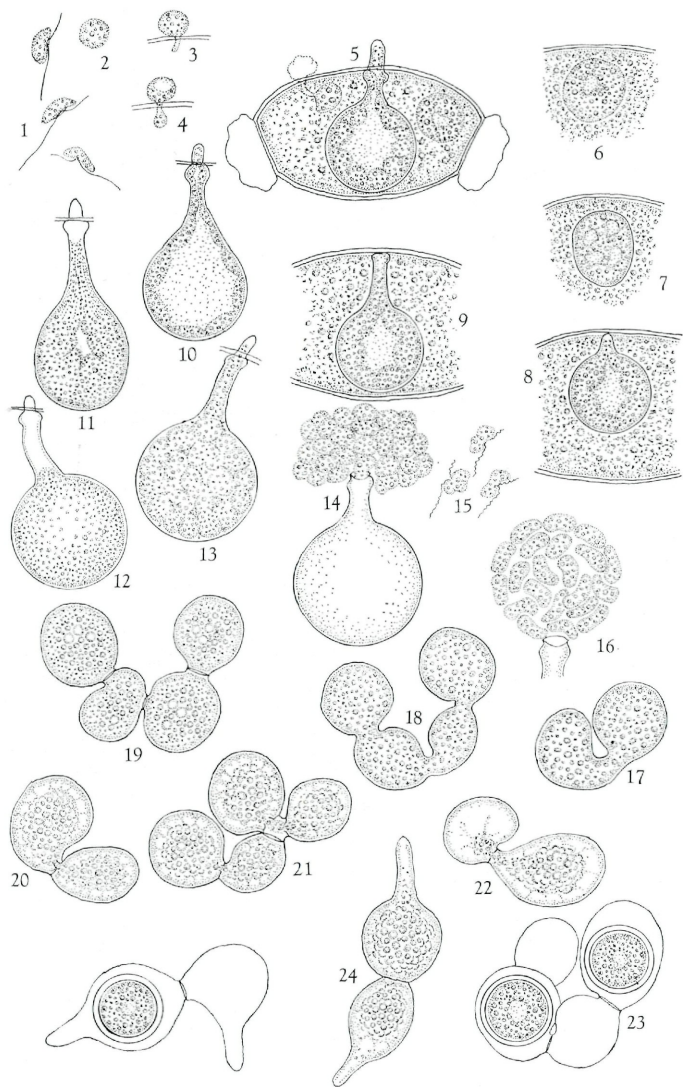
Twelve species of the families of the Olpidiopsidaceae, Siropidiaceae  
and Lagenidiaceae were identified as parasites of fungi, algae and in  
pollen grains. A variant or possibly a variety of *Myzocyttium microsporium*  
was found in pollen of *Phyllocladus trichomanoides* which differed in  
some details from specimens described previously from Brazil. Also, an  
unidentified species of *Lagenidium* was observed in sporelings of *Oedogonium*  
which is characterized by small zoospores. *Rozellopsis inflata*  
occurred fairly frequently a parasite of *Pythium*.

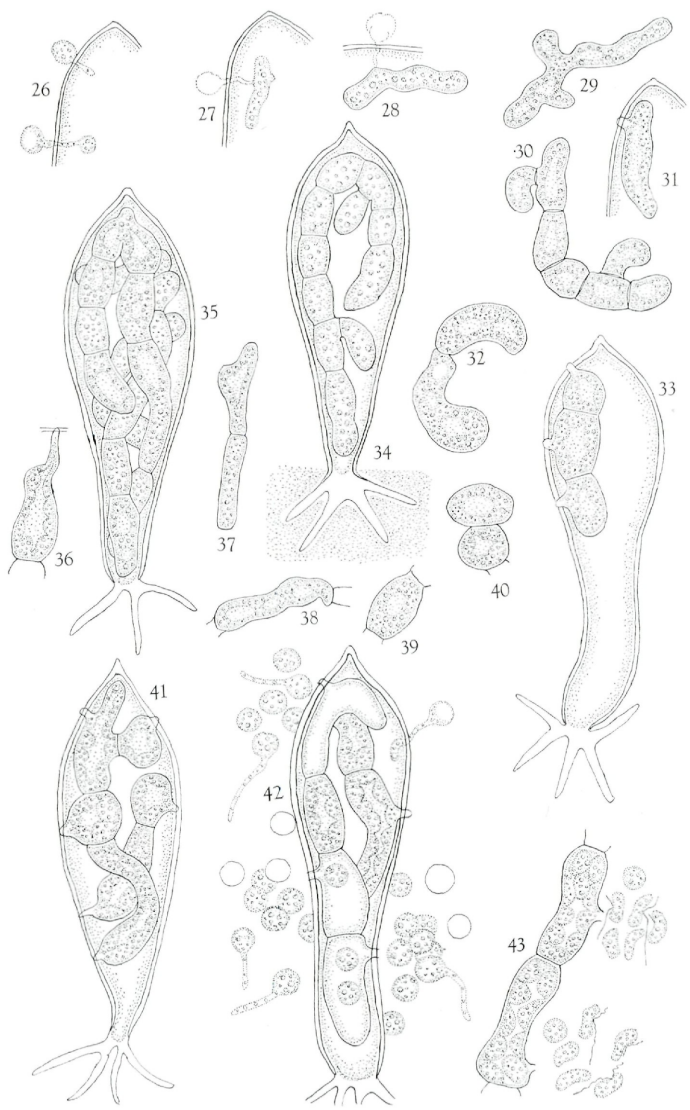
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#### Explanation of Figures.

Figs. 1—25. (?) *Myzocytium microsporum*. Fig. 1. Zoospores. Fig. 2. Zoospore after coming to rest. Figs. 3, 4. Infection stages. Fig. 5. Pollen grain of *Phyllocladus trichomanoides* with 2 thalli and a sporangium. Figs. 6, 7. Three- and 4-day old thalli, respectively. Fig. 8. Young vacuolate sporangium forming an exit canal. Fig. 9. Same sporangium 32 hrs. later; elongating exit tube has pushed sporangium to bottom of pollen grain. Figs. 10, 11, 12, 13. Sporangia enlarged showing expansion of vacuole, its disappearance, contraction of viscid granular protoplasm from exit canal, expansion of protoplasm, and cleavage lines. Fig. 14. Discharge of zoospores in a globular mass. Fig. 15. Individual zoospores from such a mass. Fig. 16. Zoospores swarming in a localized area at exit orifice. Figs. 17, 18. Incipient, potential 2- and 4-celled oosporic thalli, respectively. Fig. 19. Septation of a 4-celled thallus and concentration of refractive material towards the center of the cells. Figs. 20, 21, 22. Stages in the contraction and fusion of protoplasts in 2- and 4-celled oosporic thalli, respectively. Fig. 23. Four-celled thallus with 2 oospores. Fig. 24. Two-celled thallus, each cell of which had formed exit tubes; protoplasm contracting toward centers of cells. Fig. 25. Similar thallus following fusion of protoplast and formation of an oospore.

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