

Some Zoosporic Fungi of New Zealand. VII.

Additional Monocentric Operculate Species

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With Plates XXIII und XXIV

In previous publications the author (1967 d) described some of the operculate intramatrical species of *Nephrochytrium* and *Endochytrium* which were studied in New Zealand. The present paper is concerned with additional operculate species of the genera *Chytriomycetes*, *Chytridium*, *Catenochytridium*, *Cylindrochytridium* and *Karlingia*. These include a new species and a variety of *Chytriomycetes*, a new species of *Chytridium*, and 16 other species which have been reported from other parts of the world.

Chytriomycetes cosmarii sp. nov.

Saprophyticus; sporangia luteo-rubra, levia, globosa, 12—42 μ diam., vel subovoidea corona lutea et sporis perdurantibus ejectis cincta. Operculo apicali, 6—10 μ diam., Apophysis ovoidea vel subglobosa, 6—9 μ diam. Zoosporae globosae, 2—2.6 μ diam., globulo refractivo luteo-rubro et flagello 11—13 μ longo praeditae. Sporae perdurantes globosae vel subglobosae, 8—12 μ diam., pariete levi; germinatio ignota.

Sporangia golden-orange, subspherical and slightly flattened at the base, spherical; 12—42 μ diam., filled with refractive golden-orange globules at maturity and surrounded by a slightly orange to yellow halo, 9—12 μ thick. Intramatrical apophysis ovoid to subspherical, 6—9 μ diam. Operculum shallow saucer-shaped, 6—10 μ diam., with a broad opaque to almost hyaline zone of substance or matrix underneath. Zoospores oozing out slowly in a globular mass, enveloped by a layer of matrix; after 3 to 5 minutes beginning to swarm in a vesicle which is continuous with the interior of the sporangium; spherical, 2—2.6 μ diam., with a brilliantly refractive yellowish-orange globule; flagellum 11—13 μ long. Resting spores formed as the sporangia, hyaline to light-yellow and surrounded by a slightly yellowish halo, 6—8 μ thick; subspherical to spherical, 8—12 μ diam., with a smooth wall; content coarsely granular with a large central globule; germination unknown.

Parasitic on *Cosmarium* spp. in soil (WT3) from a hillside sheep paddock above the Soil Bureau, Taita, Wellington Province.

This species is characterized primarily by its parasitic nature, golden-orange sporangia with a hyaline internal basal peg and surrounded by a yellowish halo, and minute zoospores. It differs in these respects from *C. aureus*, the only other known species of *Chytriomycetes* with golden-orange sporangia. So far it has been found to be a virulent parasite of two species of *Cosmarium*, and appears to be limited in host range. Species of *Spirogyra*, *Oedogonium*, *Closterium*, *Netrium* and several other unicellular algae developed in the soil culture, but none of these became infected by the parasite in the course of two months.

So far no conclusive evidence of rhizoids arising from the intramatrix apophysis has been found, and the thallus is frequently very similar to that of *Phlyctochytrium synchytrii* Köhler (1924: Karling, 1960) in this respect. In some host cells faint strands running from the apophysis to the clumped host protoplasm (fig. 1) were seen but even under the oil immersion lens they could not be identified conclusively as rhizoids.

Particularly characteristic of this species in the halo or corona around the sporangia and resting spores (fig. 1, 3, 7) and the presence of a cylindrical hyaline peg which projects up into the sporangium (fig. 4, 6). In some thalli it appeared to be continuous with the apophysis but this could not be determined with certainty. It appears fairly early in the incipient sporangia (fig. 1) and persists after the sporangium is empty (fig. 6). However, it was not found in the resting spores.

The developmental stages of the sporangia and resting spores are similar to those of other species of *Chytriomycetes* (fig. 1 to 7), except that during germination of the zoospores the tip of the germ tube enlarges (fig. 2) to form the incipient apophysis instead of branching. Also, the dehiscence of the sporangium (fig. 3) and the swarming of the zoospores in a vesicle, which is continuous with the interior of the sporangium (fig. 4), are similar to those of other species of *Chytriomycetes*. Accordingly, it is not necessary to describe them again.

Chytriomycetes hyalinus var. **granulatus** var. nov.

Saprophyticus. Sporangia hyalina, levia, globosa, 12—120 μ diam., Operculo apicali vel subapicali 7—19 μ diam. Zoosporae globosae vel ovoideae, 5—6 μ diam., complura granula continentes, flagellum 22—26 μ longum. Sporae perdurantes globosae, 8—18 μ diam., ovoideae 6—9 \times 10—16 μ , vel irregulares, globulum unum vel globulos complures centrales, globulis nonnullis minimis cinctos continentes et instar prosporangii germinantes; pariete levi pallide fusco.

Sporangia hyaline, smooth, apophysate, or usually non-apophysate, spherical, 12—120 μ diam., subspherical and slightly flattened at base; operculum apical or subapical, shallow saucer-shaped, 7—19 μ diam.

Rhizoids well-developed, main axes 6—16 μ diam., at base, extending for distances up to 400 μ . Zoospores spherical to slightly ovoid, 5—6 μ diam., with several small refractive granules; flagellum 22—26 μ long; emerging slowly and swarming in a vesicle before dispersing. Resting spores spherical, 8—18 μ diam., ovoid 6—9 \times 10—16 μ , or slightly irregular, with a smooth light-brown wall; containing one or more large central refractive globules surrounded by several smaller ones; functioning as a prosperangium in germination.

Saprophytic on bleached corn leaves, chitin, and snake skin in soil samples AME, AOTH, AWN, AT3, WT4, WT5, WT8, WK1, WW1, HBTF, OWL, MP, and CGB.

This variety was widely distributed in New Zealand and strikingly similar to *C. hyalinus*, except for its slightly larger non-guttulate zoospores. Also, its sporangia attained greater size and were usually non-apophysate. Otherwise, its morphology and development (figs. 8 to 15) were so similar to those of *C. hyalinus* that it is not essential to describe them in detail. While *C. hyalinus* has a predilection for chitinic substrata this variety occurred more commonly on cellulosic substrata such as corn leaves and anion skin, although it grew to some extent on snake skin and shrimp chitin.

Chytriomycetes hyalinus Karling, 1945. Amer. J. Bot. 32: 363, fig. 46—61.

Chytriomycetes nodulatus Haskins, 1946. Trans. Brit. Mycol. Soc. 29: 137, fig. 1—8.

Saprophytic on insect and shrimp chitin, corn leaves, fibrin film, and snake skin in soil samples AMA, AME, AK, AKT, ATK, ATPF, AOTH, AWN, HBTF, HBJF, WT1, WT2, WT3, WT5, WK1, WK3, WK4, WRFJ1, WW1, WW2, CGB, CLO2, OMS, OD1, OKF, OWL, OHT, ODLS, ODGB, and MP.

As indicated above this species occurred in a large number of soil samples and grew most abundantly on chitin. However, it occurred, also, on corn leaves, snake skin and fibrin film in less numbers and it is, obviously, not strictly chitinophilic, although it has a predilection for chitin.

Chytriomycetes aureus Karling, 1945. Amer. J. Bot. 32: 363, fig. 28—45.

Saprophytic on insect and shrimp chitin in soil samples WK3, WK4, CLO2, OHT, and OWL.

Chytriomycetes parasiticus Karling, 1947. Bull. Torrey Club 74: 334, fig. 1—15.

Parasitic on the mycelium of *Aphanomyces* sp. in soil sample OWL.

Chytriumyces appendiculatus Karling, 1947. Bull. Torrey Bot. Club, 74: 335, fig. 16—37, 43—48.

Saprophytic on shrimp chitin in soil sample ODL5.

***Chytridium proliferum* sp. nov.**

Saprophyticum. Sporangia hyalina, levia, sessilia vel stipite hyalino tenui 4—20 μ longo praedita, clavata vel piriformia, $23 \times 40 \mu$; pariete ad basim leniter incrassato; operculo plerumque apicali, scutelliformi, 7—10 μ diam.; zoosporae globosae vel subovoideae, 2—3 μ diam., globulo minutissimo, hyalino, splendido et flagello 16—20 μ longo praeditae; sporaе perdurantes ignotae.

Sporangia hyaline, smooth, sessile or attached to substratum or host by a hyaline stalk or filament, 4—20 μ long, narrowly pyriform, elongate, slightly irregular, clavate, up to 40 μ high by 23 μ in diameter, wall at base slightly thickening; proliferating 1 to 3 times; operculum usually apical, bowl-shaped, 7—10 μ diam. Zoospore spherical to slightly ovoid, 3—3.4 μ diam., with a small hyaline refractive globule; flagellum 16—20 μ long; emerging in a globular mass and dispersing. Resting spores unknown.

Saprophytic on pollen of *Pinus sylvestris* and *Phyllocladus trichomanoides*, bleached corn leaves, snake skin, fibrin film, and weakly parasitic on moribund sporangia of *Chytriumyces hyalinus* and the aeciospores of a rust in acid, pH 4.7, soil sample ATK.

As indicated above, this is a ubiquitous species that occurred in great abundance on various substrata as well as on a chytrid and the aeciospores of a rust, No attempt was made to grow it on other substrata, but its abundance on those noted above suggests that it might be transferred to others as well.

The sporangia were sessile (fig. 20, 23, 25, 26) and bore a sparingly branched tuft of rhizoids, or stalked (fig. 21, 22, 24). On snake skin, in particular, the tip branched bluntly two or three times in the substratum (fig. 21, 24). The base of the sporangium wall, was slightly thickened in most of the thalli observed (fig. 20—23, 25, 26), and proliferation occurred frequently (fig. 25, 26). Unlike that of *Chytridium parasiticum* Willoughby (1956) which parasitizes several chytrids, the sporangium wall did not collapse but remained fairly rigid. As noted below Willoughby's species was found as parasite on *Chytriumyces hyalinus*, but is was quite distinct from *C. proliferum*.

Chytridium lagenaria Schenk, pro parte, 1858. Ueber das Vorkommen contractiler Zellen im Pflanzenreich, p. 5, fig. 1—13, Würzburg.

Rhizidium lagenaria (Schenk) Dangeard, 1889. Le Bot. 1: 64, pl. 3, fig. 23.

Rhizidium westii Masee, 1891. British Fungi, p. 155, pl. 2, fig. 36—37.

Weakly parasitic on *Nitella* sp., in a pond at the Soil Bureau, Taitia, Wellington Province, and *Oedogonium* sp. in Lake Ohau, Canterbury Province, and in a pond, Eglinton Valley, Otago Province.

Chytridium oedogonii Couch, 1938. J. Elish Mitchell Sci. Soc. 54: 256, pl. 24.

Parasitic on *Oedogonium* sp., Lake Ohau, Canterbury Province.

Chytridium parasiticum Willoughby, 1956. Trans. Brit. Mycol. Soc. 39: 132, fig. 5—7.

Parasitic on *Chytrium hyalinus* in soil sample AKT.

Catenochytridium carolineanum Berdan. 1939. Amer. J. Bot. 26: 461, fig. 1.

Saprophytic in bleached corn leaves in soil samples ATK and AERKF.

Catenochytridium laterale Hanson, 1944. Torreyia 44: 32; 1946, Amer. J. Bot. 33: 732—739, 49 fig,

Saprophytic in bleached corn leaves in soil samples ATH, WK1 and WW2.

Cylindrochytridium johnstonii Karling, 1941. Bull. Torrey Bot. Club 68: 383, fig. 1—16.

Saprophytic in bleached corn leaves in soil sample OKF.

So far as is known the genus *Karlingia* is represented by 7 species in New Zealand which conform fairly closely to those described from other parts of the world. This genus includes *Rhizophlyctis*-like species with endo- and exooperculate sporangia which differ in this respect from those of *Rhizophlyctis*. Sparrow (1960) excluded the endooperculate species from this genus, classified them as inoperculate species of *Rhizophlyctis*, and erected a new genus, *Karlingiomyces*, for the exooperculate members on the grounds of his belief that endooperculate species are inoperculate. However, careful studies on dehiscence and discharge of zoospores in endooperculate species by Johanson (1940), Willoughby (1958), and the author (1964, 1966) have shown that opercula are quickly pushed out as the zoospore emerge, and on this basis there are no grounds for including such species in *Rhizophlyctis*.

Karlingia chitinophila Karling, 1949. Mycologia 41: 506, fig. 1—8.

Rhizophlyctis chitinophila (Karling) Sparrow, 1960. Aquatic Phycomycetes, p. 444.

Saprophytic on shrimp chitin in soil samples AGB1, AME, AKT, ADSIR, WAV, WT4, WT5, MP, OWL, OHS, and OMS.

In establishing this species the author pointed out that it is the chitinophilic counterpart of *Karlingia rosea* and whose sporangial thalli are so similar to those of the latter species that he did not think it necessary to illustrate them. Accordingly, he illustrated only the resting spores. This species occurs widely and in abundance in New Zealand, as indicated above, and since the thalli varied to some degree from those found in Maryland, U.S.A., and Liberia they are illustrated fully in figures 27 to 43. The chief differences noted in the New Zealand specimens was the presence of an appendage on most of the sporangia and resting spores and thinner-walled resting spores which lacked radial striations. Also, the zoospores were slightly larger but had the same shape and granular appearance.

Sometimes the thalli occurred free and away from the substratum and were attached to the strips of chitin only by the terminal branches of the rhizoids. In such cases, the broad and frequently branched rhizoids were, coiled, irregular or straight (fig. 34) and extended for distances up to 4 mm. in the water. The sporangia varied from spherical (fig. 34), subspherical, ovoid, pyriform, elongate, and clavate to irregular in shape as in the Maryland specimens and bore up to 20 exit papillae or short to fairly long tubes. Occasionally, the tubes were 13 to 47 μ long. In the apex of the papillae and tubes were plugs of hyaline to opaque material which were almost spherical in shape (fig. 34, 35). Underneath this material occurred the saucer-shaped operculum. The plugs usually deliquesced and disappeared before dehiscence so that the papillae or tubes appeared to be empty above the operculum (fig. 36). At the instant of dehiscence opercula are quickly pushed out of separate papillae, and unless one is watching a papilla at the instant of dehiscence the operculum may be overlooked. It usually becomes obscured quickly by the emerging mass of zoospores. After a large number of the zoospores have emerged, those within the sporangium swarm rapidly as in *K. rosea*. In the sudden release of zoospores from several papillae, some opercula in other papillae may be drawn back into the sporangium where they are whirled about by the swarming zoospores. This retraction of opercula appears to be due to a release of pressure within the sporangium as zoospores are discharged.

Two types of development of the sporangia and resting spores were observed in the New Zealand specimens. In one, the incipient sporangium or resting spores begin as an enlargement of the germ

tube (fig. 30, 31) and in such cases the zoospore cyst and germ tube remain attached as an appendage and develop into various shapes and sizes as their walls thicken (fig. 32, 33). But even in such cases, the zoospores did not expand equally in all directions, and the sporangia or resting rudiment developed at one side. The unexpanded portion of the zoospore remained attached as an adnate appendage as its wall thickened (fig. 34). Thus, there may be transitions from one type of development to the other. The appendages on the resting spores in particular varied remarkably in size and shape (fig. 40, 41). Some were distinctly vesicular, 3—4 μ , in diameter by 7—8 μ long, and bore rhizoids (fig. 40, 41). In germination the resting spore functioned as a pro-sporangium and produced a sporangium on its surface (fig. 43) as was shown previously for the Maryland specimens.

Whether the New Zealand specimens are identical with *K. chitinophila* or a variety of it remains to be seen. The differences noted may not prove to be significant, and for the time being the author is identifying this fungus as *K. chitiniphila*.

Karlingia spinosa Karling, 1947. Mycologia 39: 60, fig. 23—33.

Rhizophlyctis spinosa (Karling) Sparrow, 1960. Aquatic Phycomycetes, p. 443.

Saprophytic on bleached corn leaves in soil sample AAD.

Karlingia asterocystis Karling, 1949. Mycologia 41: 509, fig. 9—19.

Karlingiomyces asterocystis (Karling) Sparrow, 1960. Aquatic Phycomycetes, p. 560.

Saprophytic on shrimp chitin in soil samples AAD, WAV, and WFG.

The New Zealand specimens were identical to those isolated in Maryland, U.S.A., and occurred in such abundance that the substratum became yellowish to light-amber in color. It was not found on any but chitinic substrata, and this is explained by Murray and Lovett's (1966) discovery that it is an obligate chitinophile which displays an absolute requirement for chitin or preformed N-acetyl-D-glucosamine.

Karlingia curvispinosa Karling, 1949. Mycologia 49: 511, fig. 20—35.

Karlingiomyces curvispinosus (Karling) Sparrow, 1960. Aquatic Phycomycetes, p. 560.

Saprophytic on shrimp chitin in soil sample WAV.

Expect for the lack of relatively clear zone of material surrounding the spiny resting spores, the New Zealand specimens were identical with those found in America.

Karlingia marylandica Karling, 1949. Mycologia 41: 518, fig. 70—78.

Karlingiomyces marilandicus (Karling) Sparrow, 1960. Aquatic Phycomycetes, p. 562.

Saprophytic in bleached corn leaves in soil samples WAVS and OHT.

Karlingia rosea (de Bary and Woronin) Johanson, 1944. Amer. J. Bot. 31: 399, fig. 1—37.

Chytridium roseum de Bary and Woronin, 1865. Ber. Verhandl. Naturf. Gesell., Freiburg 3 (2): 52, pl. 2, fig. 17—20.

Rhizophlyctis rosea (deBary and Woronin) Fischer, 1892. Rabenhorst Kryptog.-Fl. 1 (4): 122.

Saprophytic on bleached corn leaves and cellophane in all Provinces of New Zealand.

This species occurred in all but 9 of the soil collections listed in the initial paper of this series (Karling, 1966) and it was the most common chytrid found in New Zealand. Its abundance on corn leaves varied greatly in the soil samples, but, as Willoughby (1965) discovered in Australia it was more abundant in cultivated and fertilized soils.

Karlingia dubia Karling, 1949. Mycologia 41: 513, fig. 36—51.

Karlingiomyces dubius (Karling) Sparrow, 1960. Aquatic Phycomycetes, p. 561.

Saprophytic on shrimp chitin in soil sample ADSIR and AKT.

Summary

Eighteen species of the operculate genera *Chytriumyces*, *Catenochytridium*, *Cylindrochytridium* and *Karlingia* were isolated, studied and identified in New Zealand. Among these *Chytriumyces cosmaridis*, *Chytriumyces hyalinus* var. *granulatus* and *Chytridium proliferum* were identified and described as new.

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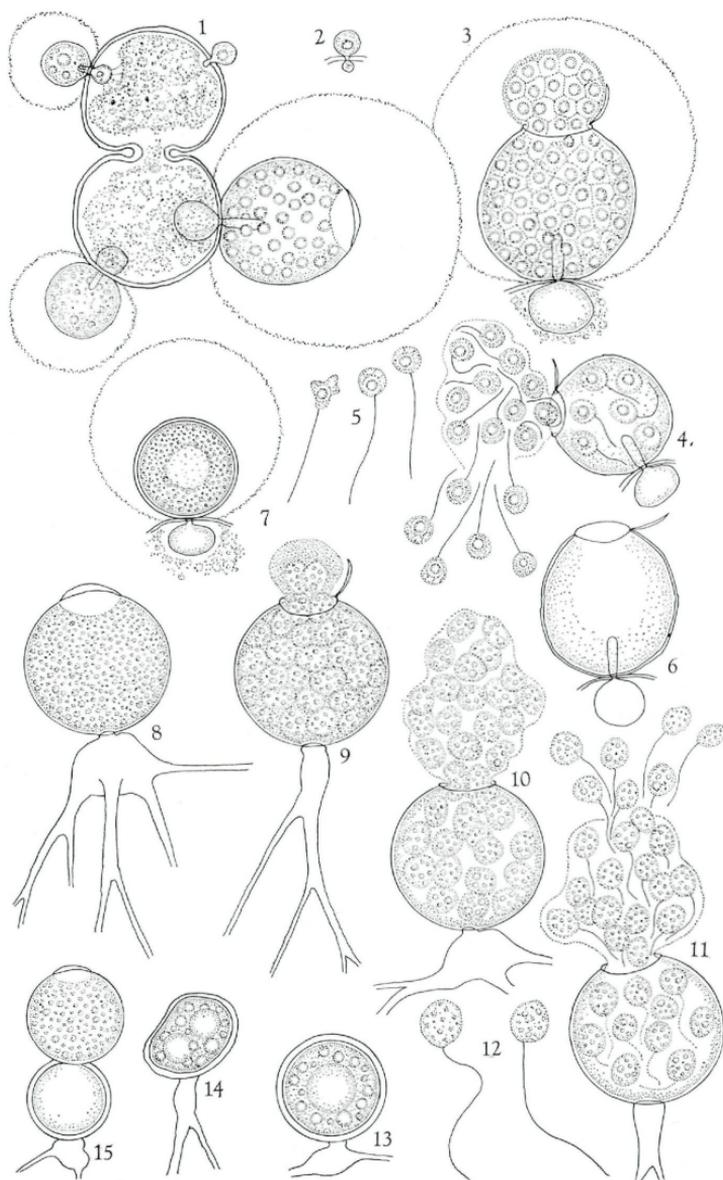
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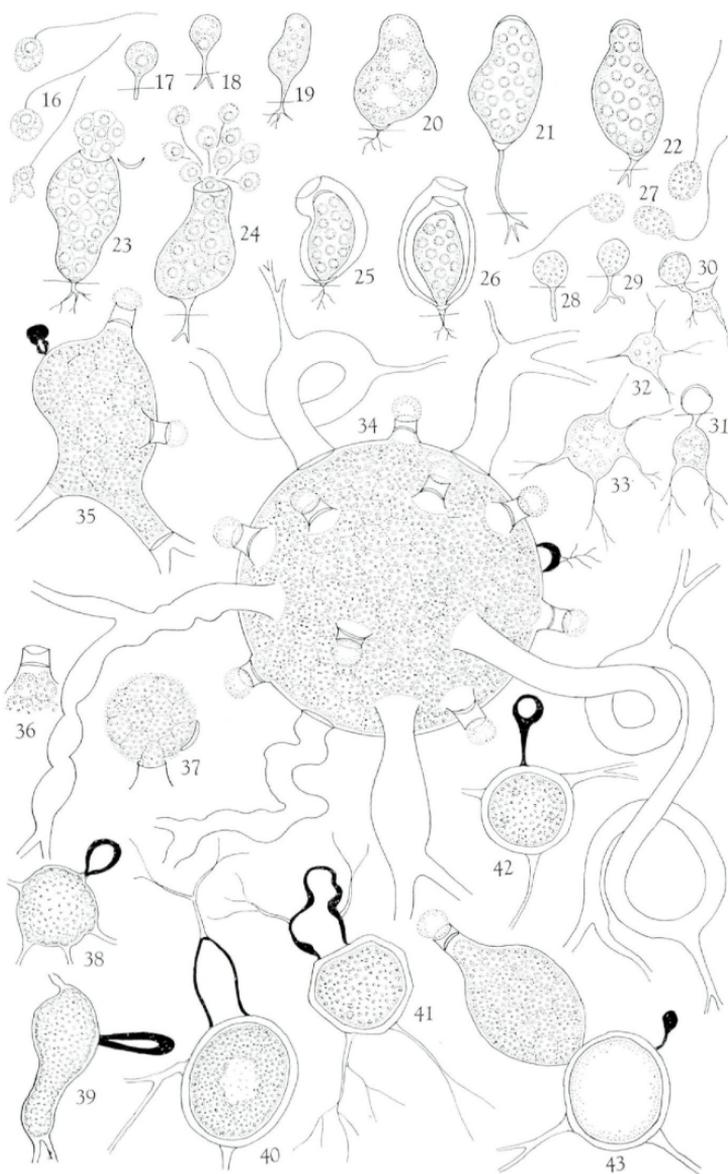
Fig. 1—7. *Chytriomycetes cosmarii*. Fig. 1. *Cosmarium* sp. cell parasitized by four thalli; developing sporangia surrounded by a halo. — Fig. 2. Germination of zoospore and infection; tip of germ tube enlarging. — Fig. 3. Discharge of zoospores. — Fig. 4. Zoospores swarming in and escaping from a vesicle. — Fig. 5. Zoospores. — Fig. 6. Empty sporangium showing persistent wall, operculum, and basal peg. — Fig. 7. Resting spore.

Fig. 8—15. *Chytriomycetes hyalinus* var. *granulatus*. Fig. 8. Sporangium with apical exit papilla. — Fig. 9. Discharge of zoospores. — Fig. 10, 11. Zoospores swarming within and escaping from vesicle, respectively. — Fig. 12. Zoospores with granular content. — Fig. 13, 14. Resting spores. — Fig. 15. Germination of resting spore.

Fig. 16—28. *Chytridium proliferum*. Fig. 16. Zoospores. — Fig. 17, 18. Germination of zoospores and branching of germ tube. — Fig. 19. Young thallus. — Fig. 20. Sessile thallus. — Fig. 21, 22. Stalked sporangia on snake skin; stalked bluntly and sparingly branched in substratum. — Fig. 23, 24. Discharge of zoospores. — Fig. 25, 26. Proliferation of sporangia.

Fig. 27—43. *Karlingia chitinophila*. Fig. 27. Zoospores with granular content. — Fig. 28—31. Germination of zoospore and development of sporangium rudiment as an enlargement in the germ tube. — Fig. 32, 33. Development of sporangium rudiment as an enlargement of the zoospore. — Fig. 34. Large appendiculate spherical sporangium growing free of the substratum with coiled, straight and irregular rhizoids and 9 exit papillae. — Fig. 35. Irregular appendiculate sporangium with two exit papillae. — Fig. 36. Exit papilla shortly before discharge of zoospores. — Fig. 37. Discharge of zoospores, operculum at side of emerging mass of zoospores. — Fig. 38, 39. Stages in the development of appendiculate resting spores. — Figs. 40—42. Variations in sizes and shapes of resting spores and appendages; in fig. 40, 41 appendages vesicular and bearing rhizoids. — Fig. 43. Germination of resting spore.





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