Some Zoosporic Fungi of New Zealand. VI.

Entophlyctis, Diplophlyctis, Nephrochytrium and Endochytrium

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With Plates XX—XXII

In previous publications by the author (III to V) in this journal on the chytrids of New Zealand, several extra-intramatrical rhizidiaceous species were described. The present paper relates to intramatrical species of the genera *Entophlyctis, Diplophlyctis, Nephrochytrium,* and *Endochytrium* which were isolated and identified in New Zealand in 1965 and 1966. Among these species *Entophlyctis crenata* and *Diplophlyctis nephrochytrioides* are described as new.

Entophlyctis crenata sp. nov.

Saprophytica. Thallus intramatricalis, monocentricus, eucarpicus. Sporangia hyalina, plerumque oblonga, 10—26 × 28—70 μ, ovoidea vel late fusiformia, 12—25 × 18—37 μ, raro subglobosa, papillula parum proninula praedita, interdum in matricis cellulis duabus evoluta. Rhizoidea parce ramulosa, 2.5—4 μ diam., in sporangiis oblongis plerumque utrinque orta et duas vel complures matricis cellulas occupantia. Zoosporae globosae, 3.8—4.3 μ diam., globulo hyalino splendididissimo, 1.5—2 μ diam. ornatae, flagellum 20—24 μ longum. Sporae perdurantes ovoideae, 12—16 × 20—26 μ, et globulis numerosis, splendidis repletae. Germinatio ignota.

Sporangia hyaline, smooth, usually oblong, 10—26 × 28—70 μ, to narrowly ovoid or broadly fusiform, 12—25 × 18—37 μ, rarely subspherical with a low exit papilla; sometimes occupying 2 host cells. Rhizoids delicate, sparsely branched, main axes 2.5—4 μ diam., usually attached at ends of oblong sporangia and extending into two or more host cells. Zoospores spherical, 3.8—4.3 μ diam., with a conspicuous hyaline refractive globule, 1.5—2 μ diam.; flagellum 20—24 μ long; emerging in a globular mass and soon dispersing. Resting spores usually ovoid, 12—16 × 20—26 μ, with a hyaline crenate wall, and filled with refractive globules; germination unknown.

Saprophytic or weakly parasitic in the epidermal cells of *Vallisneria* sp. growing in a small brook on the Rangimaire Farm, Judgeford Dist., Wellington Province.

The mature thalli of this species do not appear at first sight to belong to *Entophlyctis* because the rhizoids usually arise at the ends of the oblong sporangium (fig. 7) or sometimes from several points on its periphery (fig. 11) as in species of *Rhizophlyctis*. However, a study of the developmental stages (fig. 2—6) shows that this species develops intramatrically and has an *Entophlyctis* type of development, and the author is, accordingly, classifying it as member of this genus. In germination the zoospores usually develop a branched germ tube (fig. 2), and the incipient sporingium begins as an enlargement in one of the branches (fig. 3). The zoospore cyst and upper part of the germ tube degenerate and disappear, and as the sporangial rudiment enlarges the branches occur usually at the opposite ends (fig. 4). The appearance of such young stages may give the impression that germination of the zoospores may have been bipolar as in *Allomyces*. Occasionally, the sporangial rudiment begins at the junction of the branches, and as it enlarges they are moved apart and occupy several points on the periphery (fig. 5, 11). At maturity the rhizoids are sparingly branched, stiff-looking (fig. 7) and usually extend through the walls into several host cells.

The sporangia are predominantly oblong (fig. 7, 8, 10) and occupy the greater part of the elongate epidermal cells. Occasionally, they bud into an adjacent cell, as shown in fig. 10. Nearly spherical (fig. 6), broadly and narrowly ovoid (fig. 9), or irregular (fig. 11) sporangia sometimes occur, and these, also, are intercalary in relation to the rhizoids. The zoospores are discharged through a low papilla or a relatively short tube (fig. 7). The resting spores, also, are intercalary (fig. 12), and have a thick crenate wall, and are filled with fairly large refractive globules.

Entophlyctis confervae-glomeratae (Cienkowski) Sparrow, 1943. Aquatic Phycomycetes, p. 258.

Rhizidium confervae-glomeratae Cienkowski, 1857. Bot. Zeit 13: 233, pl. 5 A, fig. 1—6.

Rhizidium cienkowskianum Zopf, 1884, Nova Acta Acad. Leop.-Carol. 47: 196, pl. 17, figs. 14—24; pl. 18, figs. 1—4.

Entophlyctis cienkowskiana (Zopf) Fischer, 1892. Rabenhorst Kryptog.-Fl. 1 (4): 118.

Saprophytic in moribund cells of *Cladophora* sp., Lake Ohau, Canterbury Province.

Entophlyctis helioformis (Dang.) Ramsbottom, 1915. Trans. Brit. Mycol. Soc. 5: 318.

Chytridium helioformis Dangeard, 1886. Bull. Soc. Bot. France 33: 356.

Chytridium heliomorphum Dangeard, 1888. J. de Bot. 2: 143, pl. 5, fig. 19—23.

Entophlyctis heliomorpha (Dang.) Fischer, 1892. Rabenhorst Kryptog.-Fl. 1 (4) : 118.

Saprophytic in internodes of *Nitella* sp. and *Chara* sp. in a pond at Soil Bureau, Taita, Wellington Province and in Lake Ohau, Canterbury Province.

This species occurred in great abundance in the two collections noted above, and some of the spherical sporangia were up to 60 μ in diameter. Also, the spherical, 5.2—6.2 μ diam., zoospores were considerably larger than those reported by previous workers (Dangeard, 1886, Karling, 1928) and bore a 26 to 28 μ long posterior flagellum. In other respects the New Zealand specimens were similar to those described by Dangeard in France, the author (1928, 1931), and Sparrow (1943) in the United States.

Entophlyctis texana Karling, 1941. Torreya 41: 106.

Saprophytic in bleached corn leaves in soil samples WK3, HBJF and C102.

This species was reported previously from Texas, U.S.A. and Kerala State, India (Karling, 1964), but so far it has never been illustrated. Accordingly, the author is doing so herewith from the New Zealand specimens which did not differ significantly from those reported previously. As shown in figs 13 to 35, the development of the sporangial and resting-spore thalli as well as the sporangia and resting spores is basically similar to that of other species of *Entophlyctis* and need not be described in detail. Attention is called again, however, to the marked variability in size and shape of the sporangia. They were subspherical (fig. 18, 28), ovoid, elongate, broadly pyriform, (fig. 19, 23, 27), or irregular and deeply lobed (fig. 24—26) and occasionally occupied more than one substratum cell. In one instance, the sporangium had budded out to occupy parts of six adjacent cells. Although the rhizoidal axes usually arose from the base of the sporangium (fig. 18, 19, 24, 26, 27), they sometimes arose from as many as four distant points on the periphery (fig. 22, 25). As shown in the developmental stages in figs. 14—19 and 20—23, the position of the rhizoids relative to the surface of the sporangium depends on the number of times the germ tube branches and the point of origin of the sporangium rudiment in the germ tube, as was pointed out by the author in 1931. If the latter begins considerably above the branches (fig. 16) the rhizoids in the mature thalli usually occupy a basal position (fig. 18, 19, 27, 28). On the other hand, if the rudiment begins at the juncture of several branches, (fig. 20, 21) the rhizoidal axes are carried apart by the expanding incipient sporangium. As a result they occur at divergent points on the periphery (fig. 22, 23, 25).

This species is quite striking in appearance and readily recognized by the large, deep-red globules in its mature sporangia and zoospores (fig. 13), and the large, yellowish to golden-red or rust-colored resting spores. The only other known authentic species of *Entophlyctis* with pigmented globules is *E. Rhizina* (Schenk) Minden (1911), but it differs from *E. texana* by smaller sporangia, and 2 μ diam., zoospores with a reddish-yellow globule. It should be noted here, however, that Haskins (1946) described another saprophytic species, *E. aurea*, in grass leaves with yellow to bright orange sporangia, but it is obvious from his description and figures that it is a species of *Rhizophlyctis* or *Karlingia*.

Diplophlyctis nephrochytrioides sp. nov.

Saprophytica; thallus intramatricalis, monocentricus, eucarpicus, e rhizoideis, ramosissimis et apophyside vacuo et appendiculato nec non e sporangio hyalino compositus. Sporangia hyalina levia, quoad formam variabilia, ovoidea, ad basim applanata vel subreniformia, 12—25 × 18—38 μ, irregularia vel lobota, subglobosa, 14—26 μ diam. vel elongata, curvula et late clavata, papillula laterali vel tubulo 5—9 × 12—22 μ praedita. Apophysis plerumque lateralis, quoad formam variabilis, scrotiformis vel clavata, 5—6 × 10—16 μ, ovoidea vel subglobosa, 10—20 μ diam., lobata, digitata vel elongata. Rhizoidea e apophysidis et sporangii sutura orta, 4—6 μ diam., ramulis usque ad 180 μ extendentibus. Zoosporae globosae, 4—4.8 μ diam., globulo hyalino splendido ornatae, post ejectionem aliquamdiu quietae tunc enantes. Sporae perdurantes quoad formam variabilissimae, globosae, 12—26 μ diam., subglobosae, 14—18 μ diam., ovoideae, 16—20 × 21—25 μ, oblongae vel late clavatae, 15—18 × 22—26 μ, interdum lobatae, pariete obscure brunneo 1.5—2.8 μ crasso, granulis minutissimis globulum unum vel globulos centrales complures majores circumdantibus farctae; germinatio ignota.

Sporangia hyaline, smooth, greatly variable in shape, ovoid with a flattened base or slightly reniform, 12—25 × 18—38 μ, irregular and lobed, subspherical, 14—26 μ diam., elongate, curved and broadly clavate with a basal or lateral exit papilla, or a tubular, 5—9 μ in diameter by 12—22 μ long, tapering exit canal. Apophysis usually lateral, variable in shape and size, bladder-like, clavate, 5—6 × 10—16 μ, ovoid, subspherical, 10—20 μ diam., lobed and digitate or elongate. Rhizoidal axis usually arising at juncture of apophysis and sporangium, main axes 4—6 μ diam., branches extending for distances up to 180 μ. Zoospores spherical, 4—4.8 μ diam., with a conspicuous hyaline refractive globule; flagellum 22—25 μ long; wall of zoospore and germ tube thickening during germination and remaining attached to sporangium and resting spore as an appendage. Resting spores greatly variable in shape, spherical, 12—26 μ diam., subspherical, 14—18 μ diam., ovoid, 16—20 × 21—25 μ, oblong, broadly clavate, 15—18 × 22—26 μ, or lobed, with a dark-brown, 1.5—2.8 μ thick, wall and filled with globules, usually surrounding one or more larger central ones; germination unknown.

Saprophytic or weakly parasitic (?) in moribund internodes of *Nitella* sp., Lake Ohau, Canterbury Province.

This species varies markedly in morphology and development and combines characteristics of both *Diplophlyctis* and *Nephrochytrium*. Many of its thalli develop like those of the latter genus, but the sporangia are inoperculate as in *Diplophlyctis*. So far three variations or types of development of the thalli has been observerd:

First, the germ tube may branch almost at right angles, (fig. 37) and these branches enlarge to form two bladder-like vesicles (figs. 38, 39). At the same time a third branch arises at the juncture of the two vesicles and becomes the rudiment of the rhizoids. The two vesicles may enlarge equally for awhile (fig. 39, 40), but eventually one becomes larger as the content of the other moves into it (fig. 41). Thus, it becomes the incipient sporangium, and the empty vesicle becomes the lateral apophysis.

Secondly, only one vesicle is formed in the germ tube near or at the rhizoidal rudiment (fig. 45), and it enlarges with further development. A bud is formed at its side or apex which increases in size (fig. 46, 47) as the content of the initial vesicle or swelling moves into it and eventually becomes the sporangium (fig. 48). Thus, as in *Nephrochytrium appendiculatum* Karling (1938) the sporangium is formed from the apophysis, and in such cases the apophysis lies at the base (fig. 49) or at the side of the sporangium.

Thirdly, a swelling or vesicle develops in the germ tube, usually above the rudiments of the rhizoids (fig. 50) and it enlarges to become the sporangium (figs. 51—52). In such cases the apophysis is lacking, and many of the thalli observed lacked the apophysis.

In all of the types of development noted above the wall of the zoospore cyst and part of the germ tube became thickened and remained attached to the thallus as an appendage. The position of the appendage relative to the other portions of the thallus depends on the growth and expansion of the sporangia, resting spores, apophysis and rhizoids in the development stages. In some thalli it was attached directly to the sporangium and resting spore by a short or comparatively long tube (figs 42, 44, 54, 55, 56, 61, 62, 63, 65, 66). In others it was attached to the main rhizoidal axis (figs. 43, 49, 64), and in such instances, particularly if the germ tube was long, it had the appearance of a contributing male (?) thallus such as have been described in *Siphonaria* and *Rhizoclosmatium*. However, a careful study of the development of such thalli has shown that it is nothing more than the zoospore cyst and germ tube It is to be noted in nearly all of the figures illustrating this species that the appendage lies near the juncture of the sporangium or resting spore with the apophysis and rhizoidal axis as in *Nephrochytrium appendicu-*

latum Karling and *N. stellatum* Couch (1938). Occasionally, however, the germ tube becomes involved and expanded as part of the sporangium with the result that the appendage appears as a cyst at the apex of the sporangium (fig. 53).

As noted in the above diagnosis, the apophysis is usually lateral and varies greatly in size and shape. It may be lobed or digitate (fig. 42), broadly or narrowly clavate (figs. 43, 44, 58, 64), elongate (fig. 62, 64), subspherical, or ovoid (fig. 55, 57), small (fig. 56), or almost as large as the sporangium (fig. 55). Frequently, it contains some residual protoplasm and a few granules (fig. 55). The apophysis shown in fig. 62 has a small rhizoid at its end. Sometimes it becomes involved in the development of the resting spore and becomes a part of the latter to form unusually-shaped spores (fig. 63, 65).

The sporangia, also, varied greatly in size and shape. Although a great many of them were ovoid with a flattened base and slightly reniform, others were irregular (figs. 43, 44) to deeply-lobed, subspherical (fig. 52) pyriform, broadly clavate, elongate and curved (fig. 54, 56). Some sporangia developed in the layers of the *Nitella* cell wall and became flattened and markedly irregular in shape. Most of the sporangia, however, were confined to the primordial utricle of the host cell and developed either short papillae (fig. 43, 52, 54) or long tubes which sometimes penetrated the host cell wall and discharged zoospores on the outside (fig. 42, 44, 53, 56). Others discharged the zoospores within the host cell. Prior to discharge the tip of the papilla or tube deliquesced, and the initial zoospores accumulated at the orifice as a globular mass before dispersing (fig. 56).

In the very early stages of development the resting-spore thalli are indistinguishable from the sporangial thalli, but with further development they may be distinguished by a denser and more coarsely granular content. The development of these thalli and resting spores varies in the same manner as described above for the sporangial thalli with the exception that the apophysis, when present, usually remains comparatively small, or becomes a part of the resting spore (fig. 63, 65), as noted previously.

Diplophlyctis intestina (Schenk) Schroeter, 1897. Engler and Prantl. Natürlich. Pflanzenf. 1 (1) : 78.

Rhizidium intestinum (Schenk), 1958, pro parte, Ueber das Vorkommen contractiler Zellen im Pflanzenreich, Würzburg, p. 5, figs. 1—9.

Entophlyctis intestina (Schenk) Fischer, 1892. Rabenhorst, Kryptog.-Fl. 1 (4) : 116.

Saprophytic in moribund internodal cell of *Nitella* sp., Taita, Wellington Province, and Lake Ohau, Canterbury Province.

Diplophlyctis chitinophila Willoughby, 1962. Trans. Brit. Mycol. Soc. 45: 128, fig. 5.

Saprophytic in exuviae of mosquito larvae and bits of shrimp chitin water cultures from the Jury Farm, Hatuma-Waipukurau, Hawkes Bay Province.

The New Zealand specimens conformed very closely in sizes and shapes to those described by Willoughby in England, and no marked differences were observed.

Nephrochytrium and Endochytrium

These genera are the operculate counterparts of *Diplophlyctis* and *Entophlyctis,* respectively. Some species of *Nephrochytrium* are so similar to members of *Diplophyctis* in morphology and development, as shown by *D. nephrochytrioides,* that it often is difficult to differentiate between them unless an operculum is found. This is further exemplified by Willoughby's (1961) dilemma in classifying two species of *Nephrochytrium* which he found in England. Yet, despite these close similarities, Sparrow (1960) places both *Nephrochytrium* and *Endochytrium* in a different and widely separate family from that of *Diplophlyctis* and *Entophlyctis* — a disposition which places undue taxonomic emphasis on the presence of an operculum above the generic level and relegates close similarities in development, morphology and life cycles to secondary positions.

Willoughby (1961) includes both operculate and inoperculate species in *Nephrochytrium,* but this is contrary to current concepts of the genus. In establishing the genus in 1938 the author failed to mention the presence of an operculum, but subsequent studies by Couch (1938), Whiffen (1941) and the author (1964, 1966) have shown that the sporangia are operculate.

So far only one species each of *Nephrochytrium* and *Endochytrium* have been found in New Zealand.

Nephrochytrium appendiculatum Karling, 1938. Amer. J. Bot. 25: 211, 2 figs.

Saprophytic in moribund *Nitella* sp. internodes in a pond at the Soil Bureau, Taita, Wellington Province.

The sporangia of the New Zealand specimens were less reniform in shape than in the American ones and usually subspherical, 18—52 μ, with the thickened appendage frequently almost adnate to the sporangium wall. This was particularly characteristic of the sporangia which developed in the outer layer of the *Nitella* wall.

Endochytrium operculatum (de Wild.) Karling, 1937. Amer. J. Bot. 24: 353, fig. 1—37.

Rhizophlyctis operculata de Wildeman, 1895. Ann. Soc. Belg. Micro. (Mem.) 10: 108, pl. 4, fig. 1—9.

Saprophytic in dead *Nitella* internodes, Taita, Wellington Province and corn leaves in soil sample CLO 2.

The New Zealand specimens of this species exhibited the same degree of variability as those found by the author on various substrata and synthetic media in America. A detailed description of its development and variability was made by the author in 1937 who regarded Sparrow's (1933) *E. ramosum* as identical with it.

Sumary

Nine species of the genera *Entophlyctis, Diplophlytis, Nephrochytrium* and *Endochytrium* were isolated and identified in New Zealand. Among these *Entophlyctis crenata* and *Diplophlyctis nephrochytrioides* are described as new, and *Entophlyctis texana* is illustrated for the first time.

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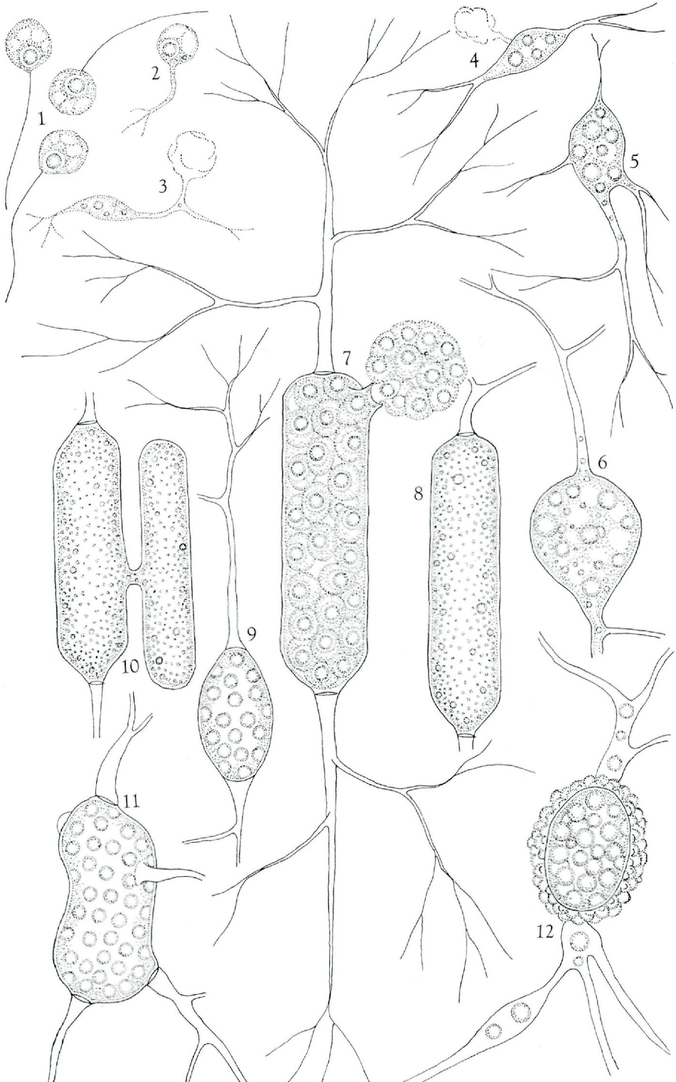

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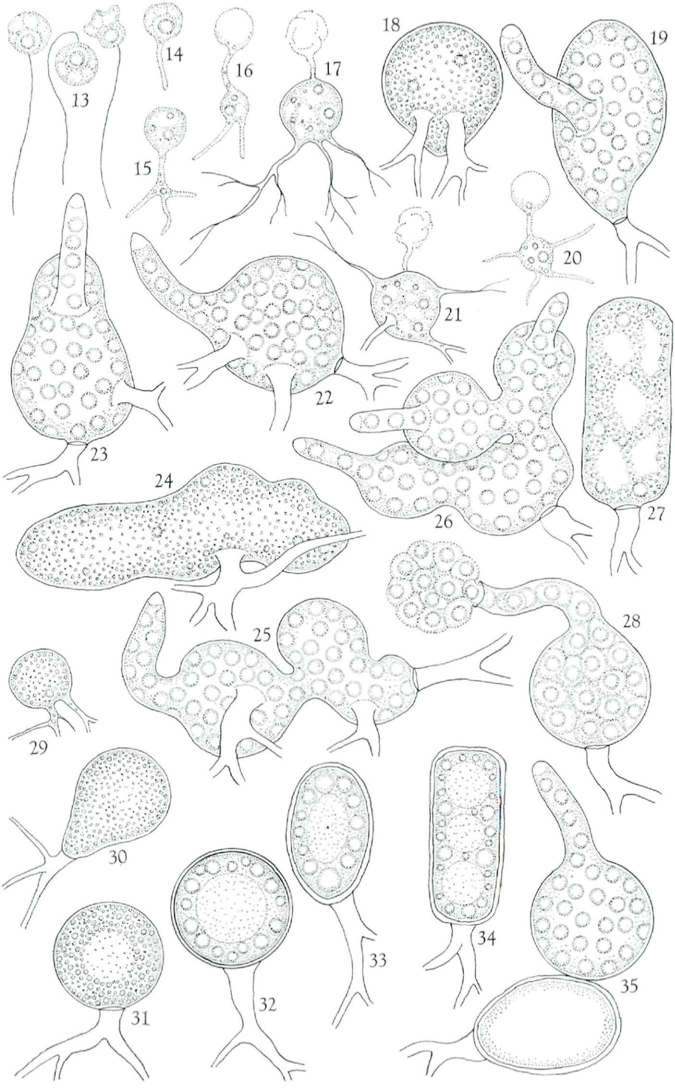
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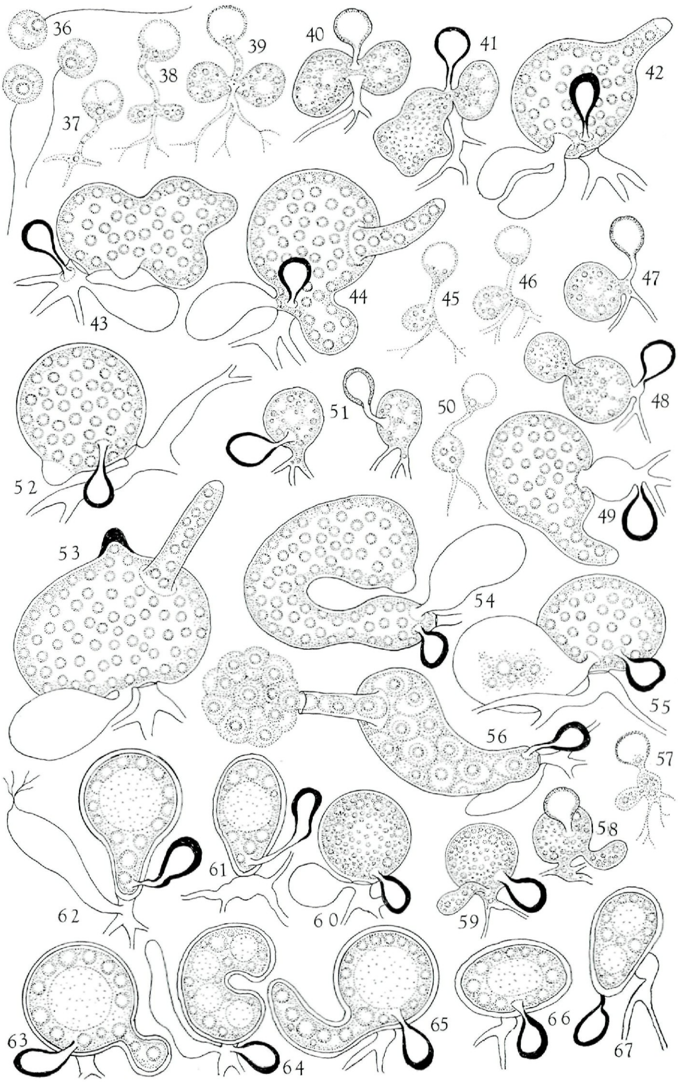
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Explanation of Figures.

Figs. 1—12. *Entophlyctis crenata.* Fig. 1. Zoospores. Figs. 2, 3. Germination of zoospore and early development stage. Figs. 4, 5, 6. Later stages of development. Fig. 7. Mature thallus with sparsely branched rhizoids arising from both ends of dehiscing sporangium. Fig. 8. Elongate cylindrical sporangium. Fig. 9. Ovoid sporangium. Fig. 10. Sporangium which has budded into an adjacent epidermal cell. Fig. 11. Irregular sporangium with rhizoids arising from 4 points on its periphery. Fig. 12. Resting spore with crenate wall and filled with refractive globules.

Figs. 13—35. *Entophlyctis texana.* Fig. 13. Zoospores with dark-red refractive globules. Figs. 14—17. Germination of zoospores and formation of sporangium rudiment in the germ tube above the rhizoid branches. Fig. 18. Subspherical sporangium with 2 rhizoid axes near the base. Fig. 19. Mature ovoid sporangium with 1 basal rhizoid axis. Fig. 20, 21. Rudiment of sporangium forming at the juncture of 4 branches of the germ tube. Figs. 22, 23, 25. Mature sporangia with rhizoid axes at 2 and 3 points on the periphery. Fig. 24. Elongate, slightly irregualr sporangium which filled an elongate host cell. Fig. 25, 26. Deeply-lobed sporangia. Fig. 27. Cylindrical sporangium Fig. 28. Discharge of zoospores. Figs. 29—31. Stages in the development of resting spores. Figs. 32—34. Some variations in sizes and shapes of resting spores. Fig. 35. Germination of resting spore.

Figs. 36—67. *Diplophlyctis nephrochytrioides.* Fig. 36. Zoospores. Fig. 37. Germinated zoospore with 2 oppositely-directed branches of germ tube. Fig. 38—39. Stages in the inflation of the branches to form 2 vesicles. Fig. 40. Later stage; one vesicle becoming larger. Fig. 41. Later stage; protoplasm flowing into larger vesicle; zoospore cyst and germ tube persistent. Fig. 42.

Mature sporangium with digitate apophysis. Figs. 43, 44. Variations in shapes of sporangia and positions of apophysis and appendage. Figs. 45—47. Development of a single vesicle from a branch of the germ tube. Fig. 48. Later stage showing incipient sporangium budding out from vesicle or apophysis. Fig. 49. Sporangium thus formed with a basal apophysis. Figs. 50—52. Stages in development of sporangium without an apophysis. Fig. 53. Sporangium with a basal apophysis and apical appendage. Fig. 54. Curved sporangium. Fig. 55. Apophysis almost equal in size to sporangium. Fig. 56. Discharge of zoospores. Figs. 57—60. Stages in development of resting spores. Figs. 61—67. Some variations in sizes and shapes of resting spores.