# Some Zoosporic Fungi of New Zealand IV.

## Polyphlyctis gen. nov., Phlyctochytrium and Rhizidium

By John S. Karling

(Department of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.)

With Plates XV and XVI

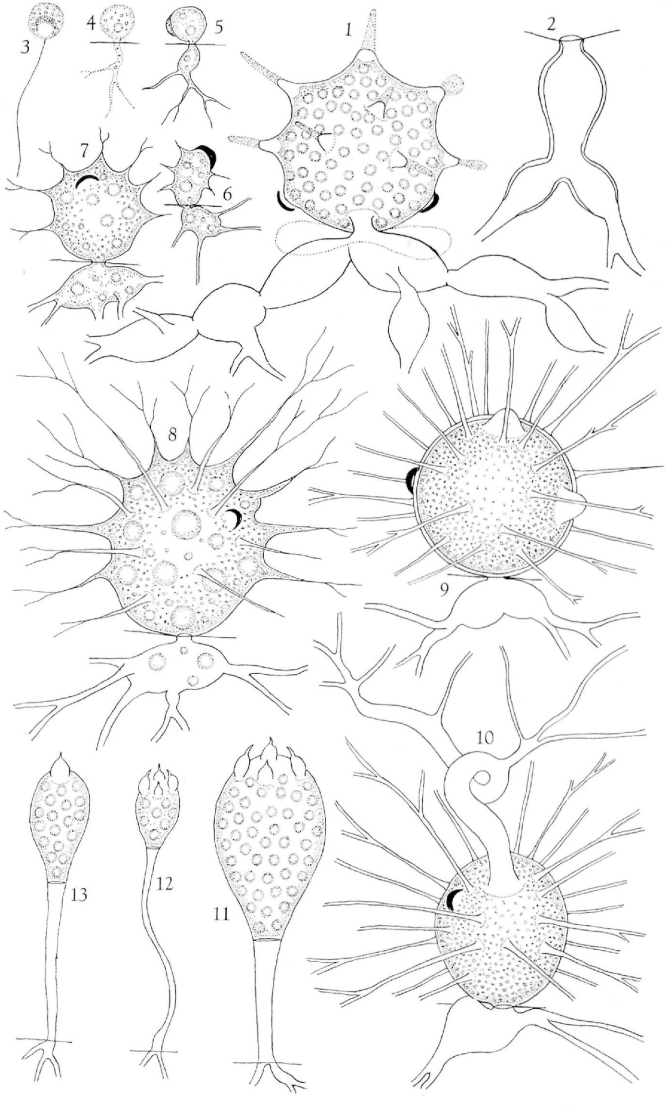
Previous publications by the author (1966, 1967 a, 1967 b) on the zoosporic of New Zealand dealt with members of the Olpidiaceae, Synchytriaceae and several genera of the eucarpic rhizidiaceous chytrids. The present contribution concerns species of *Polyphlyctis* gen. nov. *Phlyctochytrium,* and *Rhizidium* which were observed in various localities on the North and South Islands. Most of the species were obtained from watered soil samples by the methods described previously, and the localities as well as the soil types which they inhabited are indicated by symbols which were listed and described in the first publication of this series.

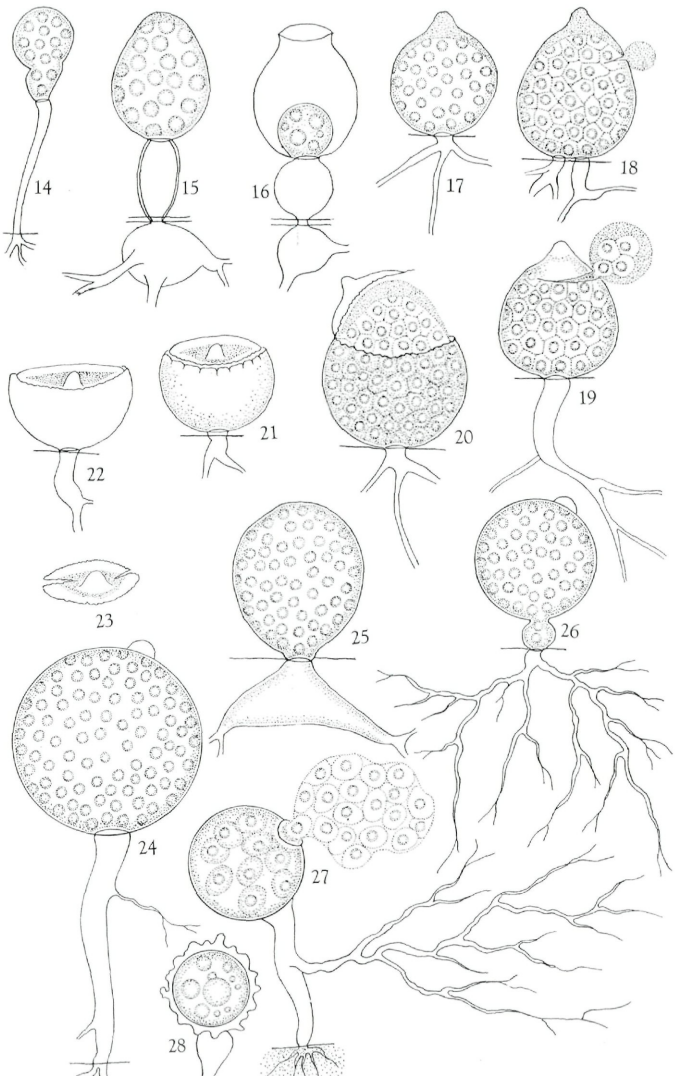
**Polyphlyctis** gen. nov.

Saprophytica. Thallus monocentricus, eucarpicus, extra- et intramatricalis. Sporangia extramatricalia, inoperculata, zoosporarum vesicularum amplificatione formata. Apophysis intramatricalis, raro simplex, plerumque e 2—6 vesiculis composita, rhizoideo ramosissimo aucta. Zoosporae postice uniflagellatae. Sporae perdurantes extramatricales, eodem modo ut sporangia formata, germinatione prosporangii ratione vigentes.

Fungus saprophytic; thallus monocentric, eucarpic, extra-and intramaterical. Sporangia extramatrical, inoperculate, formed by expansion of the zoospore cyst; apophysis occasionally single, usually consisting of 2—6 vesicles. Zoospores posteriorly uniflagellate; encysting on surface of host cell or substrata and usually splitting into 2 parts which are retained as lateral projections on the sporangium wall. Resting spore extramatrical and formed in the same manner (?) as the sporangia; functioning as a prosporangium during germination.

This genus is created for an unusual chytrid which Paterson (1956) described as *Phlyctochytrium unispinum*. Its type of development is unique and differs in several respects from that of *Phlyctochytrium* species, so far as they are known at present. The development of the thallus and sporangia appears to be endo-exogenous (Karling, 1926) as in species





of *Chytridium*. Growth and development are inward until the apophyses and rhizoids are well established, and then it become outward, expanding and splitting the zoospores cyst as the sporangium begins to develop and enlarge. The splitting of the cyst and the retention of the halves as projections on the sporangium wall are unusual, so far as is known. Another unique feature is the development of a series of intramatrical catenulate apophyses as in *Catenochytrium*, and in this respect, also, it differs from the known species of *Phlyctochytrium*. Accordingly, a new genus, *Polyphyctis*, is proposed, which bears the same relation to *Phlyctochytrium* as *Catenochytrium* does to *Chytridium*.

**Polyphlyctis unispina** (Paterson) Karling comb. nov.

*Phlyctochytrium unispinum* Paterson 1956 Mycologia 48: 270. figs. 1 a—1 i.

Sporangia sessile, irregularly ellipsoidal and angular in appearance, 12—50 μ in greatest diameter, with a hyaline smooth persistent wall and 1—10 low exit papillae which are surmounted usually by opaque, gelatinous tapering projections or spines, 2.5—8 μ broad at base to 1.5—9 μ tall; apical portion of spines usually pushed aside or sometimes deliquescing completely at dehiscense of sporangium. Rhizoids extending sometimes for a distance of 180 μ from the sporangium. Primary apophysis up to 30 μ in greatest diameter, frequently becoming thick-walled. Zoospores spherical 6—7.5 μ (?) or 3.5—4.5 μ (?) diam., with hyaline refractive globule, 1.0—1.5 μ diam.; flagellum 26—30 μ long; encysting on host cell wall or substratum and usually splitting; halves of cyst usually becoming part of sporangium wall, occasionally one or both halves being shed. Resting spore smaller than sporangia with a thick smooth, hyaline wall; functioning as a prosporangium during germination.

Saprophytic on *Oedogonium* sp., *Zygnema* sp., and *Stigonema* sp. in Michigan U.S.A., (Paterson, 1956) and grass leaves in England (Willoughby and Townley, 1961) and New Zealand (soil samples ATK and OWL).

According to the observations of Willoughby and Townley and the author, this type species is inappropriately named *unispinum*. Paterson reported only one spine on the sporangium, and his observations probably relate to small, exceptional thalli. On the other hand, his species may possibly be different from the one studied by Willoughby and Townley and the author because of the marked difference in the size of the zoospores. Willoughby and Townley reported the presence of up to 10 spines on some exceptional sporangia, and among the numerous thalli observed by the author in New Zealand only 2 sporangia had a single spine. Five to 8 spines were commonly present (fig. 1). As figured and described by Willoughby and

Townley, the author observed that the so-called spines were frequently subspherical, hemispherical, and ovoid in shape instead of tapering and spine-like. Also, the zoospores in the New Zealand specimes were 3.5 to 4.5 μ in diameter instead of 6—7.5 μ as reported by Paterson. This difference in size seems significant, and it is possible, as noted above, that Paterson's fungus may prove to be a different species.

*Polyphlyctis unispina* occurred abundantly on bleached corn leaves in yellow-brown pumice soil derived from rhyolitic volcanic ash (ATK) at Kaingaroa, Auckland Province, and acid, pH 4.3, peaty soil (OWL) above Wilke Lake, Otago Province. Its structure and development were similar to those described by previous workers, particularly Willoughby and Townley, and no significant differences were observed. Although the cultures were maintained for several months no resting spores developed in them.

## Phlyctochytrium

This genus is well represented in New Zealand and includes parasitic as well as saprophytic species. Up to the present time 11 species have been identified, including a new one. Several other specimes were found, but it was impossible to identify them with certainity from the meager material available.

**Phlyctochytrium hirsutum** sp. nov.

Saprophyticum. Sporangia sessilia, hyalina, appendiculata, globosa, 30—60 μ diam., papillis 1—3 pro ratione humilibus et rhizoideis vel setis 20—60 ramosissimis in sporangii superficie zonam pilosam formantibus praedita, setis ad basim 2.5—4 μ latis, usque ad 120—200 μ extendentibus. Apophysis intramatricalis, minuta 7—12 μ diam., ovoidea vel subirregularis, rhizoideo uno vel rhizoideis nonnullis crassiusculis praedita. Zoosporae globosae 3—4.5 μ diam., granulis nonnullis et pileo conspicuo praeditae, glomeratae emergentes et mox vagantes. Sporae perdurantes ignotae.

Sporangia sessile, hyaline, appendiculate, spherical, 30—60 μ diam., with 1—3 relatively low exit papillae and bearing up to 20—60 frequently branched rhizoids or hairs on its surface which form a hairy zone around the sporangium; hairs 2.5—4 μ diam. at base and extending for distances of 120—200 μ. Intramatrical apophysis small, 7—12 μ diam., ovoid or slightly irregular, bearing one to several fairly coarse rhizoids on its surface. Zoospores spherical, 3—4.5 μ diam. with a few granules and a conspicuous nuclear cap; emerging in a globular mass and dispersing after a few seconds. Resting spores unknown.

Saprophytic on purified shrimp chitin in soil sample WT6.

So far as it is known, this species is characterized principally by

the presence of numerous fairly stout and richly branched rhizoids or hairs on the surface of the sporangium, and its occurrence on chitinic substrata. In the latter respect it is the only species of *Phlyctochytrium* known to occur on such substrata.

As shown in fig. 4 and 5 the zoospore forms a germ tube which penetrates the substratum, branches and establishes the rudiments of the rhizoids. Shortly thereafter a swelling develops in the tube, usually at the juncture of or slightly above the rhizoid rudiments, and with further development it becomes the apophysis. As these intramatrical structures become established, part of the extramatrical zoospore wall becomes slightly thickened (figs. 5, 6) and remains as an appendage. The zoospore body begins to expand (fig. 6) and becomes the rudiment of the extramatrical sporangium. As this process continues fine rhizoids or relative coarse hairs begin to develop from its surface (fig. 6, 7, 8), and at this stage the incipient sporangium is angular and irregular in outline (fig. 7, 8). As it matures it becomes spherical (fig. 9), subspherical, or ovoid (fig. 10) in shape. The peripheral rhizoids or coarse hairs increase in diameter, elongate up to distance of 200 μ and branch, whereby the sporangium becomes surrounded by a hairy zone. Sometimes one or more of the extramatrical rhizoids may develop more rapidly and vigorously than the others, attain up to 10 μ in diameter at the base, and become quite extensive (fig. 10). Such thalli may become markedly asymmetrical. Although cultures of this species were maintained for several months on chitin, no resting spores developed.

*Phlyctochytrium lagenaria* (Schenk) Domjan, 1936. Folia cryptogam. 2: 18, pl. 1, figs. 45—46, 48, 52—55, 62—63, 67, 69.
*Chytridium lagenaria* Schenk, 1958, pro parte, Verhandl. Phys.-Med. Gesell. Würzburg A. F. 8: 241, pl. 5, figs. 12, 14—15.
On moribund *Nitella* internodes from Lake Ohau (CLO), Canterbury Province.

*Phlyctochytrium zygnematis* (Rosen) Schroeter, 1897. Engler u. Prantl., Natürl. Pflanzenf. 1 (1): 79.
*Chytrium zygnematis* Rosen, 1887. In Cohn, Beitr. Biol. Pflanz. 4: 266, pl. 13, figs. 1—14, pl. 14, figs. 14—27.
*Rhizidium zygnematis* (Rosen) Dangeard, 1889. Le Bot. 1: 64.
On *Zygnema* sp. from a pond in the Eglinton Valley, Otago Province.

*Phlyctochytrium quadricorne* (deBarry) Schroeter, 1897. Engler u. Prantl., Natürl. Pflanzenf. 1 (1) : 79.
*Chytridium quadricorne* deBary. See Rosen, 1887. In Cohn Beitr. Biol. Pflanz. 4: 266, pl. 14, fig. 28.

*Rhizidium quadricorne* (deBary) Dangeard, 1889. Le Bot. 1: 64.

On *Vaucheria* sp. in a pond at the Soil Bureau, Taita, Wellington Province, and the Eglinton Valley, Otago Province.

In connection with this species note is made of another parasite which occurred on *Vaucheria* sp. in the same culture from Eglinton Valley. As shown in figs. 11—14, its ovoid 8—15 × 12—18 μ, citriform, or elongate non-apophysate sporangia were borne on stalks, 10—28 μ long, which branched as rhizoids after entering the host cell. The apex of the sporangia usually bore four, non-bipartite, converging teeth, but rarely only one (fig. 13), or none at all (fig. 14). Altough its zoospores were similar in size and shape to those of *P. quadricorne*, it differs from this species by the lack of bipartite teeth and an apophysis.

*Phlyctochytrium planicorne* Atkinson, 1909. Bot. Gaz. 48: 337, fig. 7.

Saprophytic on dead bits of sclerenchyma in soil sample WGB.

*Phlyctochytrium chaetiferum* Karling, 1937. Mycologia 29: 179, figs. 1—3.

On dead oogonium of *Vaucheria* sp. and *Oedogonium* sp. in a pond at the Soil Bureau, Taita, Wellington Province.

*Phlyctochytrium bullatum* Sparrow, 1937. Occ. Papers Boston Soc. Nat. Hist. 8: 296.

Saprophytic on dead oogonia of *Vaucheria* sp. in a pond at the Soil Bureau, Taita, Wellington Province.

*Phlyctochytrium mucronatum* Canter, 1949. Trans. Brit. Mycol. Soc. 32: 240, figs. 1, 2.

Saprophytic on a dead desmid in a pond at the Soil Bureau, Taita, Wellington Province.

*Phlyctochytrium bryopsidis* Kobayasi and Ookubo, 1954. Bull. Nat. Sci. Mus. Tokyo 1: 66, fig. 5.

Parasite on *Bryopsis* sp., Portobello, Otago Province.

This species occurred on *Bryopsis* sp. which was attached to rocks at the University of Otago Marine Biological Laboratory at Portobello, and the structure and dimensions of the thalli, sporangia and zoospore were similar to those reported by Kobayasi and Ookubo. Resting spores were not observed in New Zealand specimens.

*Phlyctochytrium reinboldtae* Persiel, 1959. Arch. f. Mikrobiol. 32: 414, fig. 1.

Saprophytic on deal pollen of *Pinus sylvestris* in soil samples ADB, AKT, OWL, and OMS.

*Phlyctochytrium indicum* Karling, 1964. Sydowia 17: 287, figs. 16—29.

Saprophytic on dead pollen of *Pinus sylvestris* in soil sample HBHW.

In relation to this species attention is called to another member of *Phlyctochytrium* which occurred in abundance on *Pinus sylvestris* pollen in soil sample ASB. The sporangia (fig. 15, 16) were ovoid to broadly pyriform, 11—21 μ in broadest diameter by 14—27 μ tall, citriform and egg-shaped and were borne on a short fusiform to ovoid, apophysis-like, extra-matrical, thick-walled stalk (fig. 15) which connected it with a subspherical, 8—14 μ diam., intramatrical apophysis and the rhizoids. The zoospores were spherical, 2.8—3 μ diam., with a conspicuous hyaline refractive globule, and were discharged through a broad, 6—8 μ diam., apical papilla. Secondary and tertiary sporangia were occasionally formed (fig. 16) within the initial one. This species resembles *P. indicum* fairly closely except for the extramatrical stalk, but this difference may not prove to be taxonomically significant. Similar variations were described by Rosen (1887) in *P. zygnematis*.

## Rhizidium

This genus has been variously interpreted by investigators since the time of Braun (1856), but at the present time it is usually regarded as including all monocentric eucarpic species whose thalli develop free of the substratum or host, form sporangia by the enlargement of the zoospore body, and have a definite central, main rhizoidal axis from which most of the branches of the rhizoids arise. However, these criteria are not sharply defined, and several species have been added to the genus whose thalli do not develop free of the substratum or host and resemble somewhat species of *Rhizophydium* and *Phlyctochytrium*. Furthermore, the thalli of other similar inoperculate chytrid genera may occasionally develop free at the edge of the substratum. In New Zealand the following species have ueen identified, but it is not certain that all of them belong in *Rhizidium*.

*Rhizidium verrucosum* Karling, 1944. Amer. J. Bot. 31: 255, figs 34—58.

Saprophytic on snake skin in soil sample OGBD.

*Rhizidium varians* Karling, 1949. Amer. J. Bot. 36: 681, figs. 1—26.

Saprophytic on bleached corn leaves in soil sample WGB.

The New Zealand specimens exhibited the same degree of variation as the thalli described by the author from Maryland, U.S.A., and the relationships and taxonomic position of this species are uncertain.

*Rhizidium elongatum* Karling, 1949. Amer. J. Bot. 36: 682, figs. 27—48.

Saprophytic on purified shrimp chitin in soil sample WWl.

*Rhizidium richmondense* Willoughby, 1956. Trans. Brit. Mycol. Soc. 39: 128, figs. 1—3.

Saprophytic on bleached corn leaves in soil samples AKT, ASB, and OWL.

The identity of this fungus as a member of *Rhizidium* is open to question on the grounds that the thalli vary in several aspects from the generic concepts noted above. In the first place the apiculus and upper part of the sporangium wall may be pushed up like an operculum during dehiscence. In the New Zealand material, at least, this structure appeared to be as well-defined as the operculum in *Chytridium parasiticus* Willoughby (1956, fig. 5 m—p) and *Chytriomyces verrucosus* Karling (1960). Secondly, a large number of the sporangia were sessile on the substratum without a main taproot-like rhizoidal axis (fig. 17, 20, 21). Instead the rhizoids branched almost equally immediately beneath the sporangia in much the same manner described and figured by Willoughby. In other thalli, however, a central rhizoidal axis was present (fig. 19, 22), and these conformed to the general concept of the genus, except that the axes were intramatrical.

Willoughby gave an excellent and fully illustrated account of the development of this species. This has been confirmed in general from the New Zealand specimens, and the present observations will be confined to the dehiscence of the sporangia and the fate of the so-called apiculus and sporangium wall. At maturity and just before dehiscence the apex of the sporangium under the apiculus is filled with an opaque to hyaline optically homogenous zone or matrix (fig. 17) which may extend down along the sides to a distance of $^1/_4$ to $^1/_3$ the height of the sporangium. In exceptional cases it occupied almost half of the sporangium as in *Chytriomyces spinosus* Fay (1947). At dehiscence in most of the sporangia observed in New Zealand, a visible bulging occurs at one side of the sporangium wall beneath the apiculus, and a tear occurs in the wall. Instantaneously, part of the hyaline matrix emerges, (fig. 18) and at the same time the tear progresses in an almost circular path around the apex of the sporangium (fig. 19). This tear is usually irregular so that its edges appear jagged (figs. 19—23). In rare cases dehiscence was fairly slow so that the tearing of the sporangial wall and emergence of the hyaline matrix and initial zoospores could be observed more closely (fig. 19). In most sporangia, however, it was very rapid and almost explosive in appearance so that the apiculus and upper part of the sporangium wall was pushed up and aside as shown in fig. 20. In such cases it looks strikingly similar to

an irregularly-edged, thin-walled operculum. Sometimes, it may be torn loose and carried away by the enlarging mass of emerging matrix and zoospores.

As more and more of the zoospores emerge, the surrounding matrix thins out, and, apparently, becomes the vesicle in which the zoospores swarm as in other species of *Rhizidium* and *Chytriomyces*. After the zoospores have emerged and escaped from the expanded vesicle, the apiculate operculum-like structure usually flips back and down into the sporangium as shown so well by W i l l o u g h b y and is illustrated in figs. 21 and 23. This structure as well as the sporangium wall is quite thin, and during the rapid dehiscence it may become torn (fig. 23).

Whether one classifies this fungus as a species of *Rhizidium*, or *Chytridium*, or *Chytriomyces* depends on one's interpretation of the operculum-like structure noted above. Inasmuch as there is no gradual deliquescence of an exit papilla at dehiscence as in most inoperculate species, the author is inclined from his study of the New Zealand specimens to regard this species as being operculate and a member of the genus *Chytriomyces*. However, this remains to be proven by the discovery of the resting spores and their manner of germination.

In this connection it may be noted that some of the sporangia were heavily parasitized by *Chytridium parasiticum* as Willoughby found in England.

*R h i z i d i u m c h i t i n o p h i l u m* (?) Sparrow, 1960. Aquatic Phycomycetes. p. 412.

Saprophytic on strip of purified shrimp chitin and snake skin in soil samples OWL and ORC.

This identification is made tentatively of a species which occurred on chitin in soil sample OWL and later in abundance on snake skin in soil sample ORC. Its sporangia and zoospores are similar in sizes and shapes to those reported by Sparrow, but it differs in some respects from his species. Many of the sporangia stood off from the chitinic substratum on a long stalk (fig. 24) and were frequently broken off in mounting them for microscopic study. Other sporangia were almost sessile with a somewhat triangular, intramatrical apophysis underneath (fig. 25). In none of the sporangia were the apophyses or a portion of the stalk separated from the rhizoidal branches by a septum. On snake skin the sporangia were frequently sessile with a sparingly branched, wavy system of intramatrical rhizoids (fig. 26). Occasionally, the sporangia were borne on a long stalk and anchored to the snake skin by a tuft of rhizoids. Also, the stalk branched outside of the substratum and developed an extramatrical system of wavy rhizoids as shown in fig. 27. A few extramatrical, brown verrucose, subspherical to spherical, 10—13 $\mu$ diam., resting spores were formed on snake skin

which contained one or two large refractive globules and several smaller ones (fig. 28). Germination of such resting spores was not observed. Whether or not this fungus is identical with Sparrow's species is uncertain, but the author is tentatively identifying it as such instead of describing it as a new species.

Another *Rhizidium* species which might be related to *R. chitinophilum* was found in soil sample OWL on a small larva of an insect. More than 200 hundred thalli were attached to it in such a way that its surface was almost completely covered by sporangia. The broadly pyriform, 37—45 × 50—60 μ, subspherical, 25—55 μ diam., and slightly flattened sporangia were borne on and stood off on stalks, 20—79 μ long, by 3—11 μ wide which occasionally bore a few fine lateral rhizoids or were slightly inflated below the sporangia. The stalks branched within the larval body and produced, thus, a system of rhizoids. The zoospores were ovoid, 3—4 × 5—6 μ, or spherical, 5—5.6 μ and contained a conspicuous hyaline refractive globule. Swarming of the zoospores in a vesicle was not observed, nor were resting spores found.

## Summary

A new genus *Polyphlyctis*, is created to include *Phlyctochytrium unispinum* Paterson on the basis of studies on this species in New Zealand. *Phlyctochytrium hirsutum* is described as a new species. In addition 10 known species of this genus and 5 of *Rhizidium* are identified as saprophytes in the soil and as parasities of algae.

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## Explanation of Figures.

Fig. 1, 2. *Polyphlyctis unispina*. Fig. 1. Portion of a thallus dissected out of grass leaf. Fig. 2. Thickened wall of primary apophysis.

Fig. 3—10. *Phlyctochytrium hirsutum*. Fig. 3. Zoospore. Figs. 4—7. Germination of zoospore and development of young thallus. Fig. 8. Young irregular thallus. Fig. 9. Mature sporangium with numerous radiating rhizoids or coarse hairs. Fig. 10. Ovoid sporangium with one coarse extramatrical rhizoid and numerous smaller radiating ones.

Figs. 11—14. *Phlyctochytrium*? sp. Fig. 11, 12. Stalked, non-apophysate sporangia with 4 teeth. Fig. 13, 14. Sporangia with 1 and no teeth, respectively.

Fig. 15, 16. *Phlyctochytrium* sp. Fig. 15. Mature sporangium with fusiform extramatrical stalk. Fig. 16. Proliferating sporangium.

Figs. 17—23. *Rhizidium richmondense*. Fig. 17. Sporangium shortly before dehiscence. Fig. 18. Asymmetrical swelling of sporangium and rupture of wall at one side. Fig. 19. Later stage of dehiscence; hyaline matrix and zoospores oozing out and lifting up apiculus and sporangium cap. Fig. 20. Apiculus and sporangium cap pushed off by emerging zoospores and hyaline matrix. Fig. 21—23. Empty sporangia with collapsed sporangium cap and apiculus.

Fig. 24—28. *Rhizidium chitinophilum* (?). Fig. 24. Stalked sporangium on chitin. Fig. 25. Apophysate sporangium on chitin. Fig. 26. Thallus on snake skin with wavy, sparsely-branched rhizoids. Fig. 27. Thallus attached to snake skin by a holdfast-like tuft of rhizoids with a branch of extramatrical wavy rhizoids. Fig. 28. Resting spore.