Some Zoosporic Fungi of New Zealand. IX

Hyphochytridiales or Anisochytridiales

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With Plates XXVI—XXVIII

Chytrid-like fungi with anteriorly uniflagellate zoospores are fairly common in the soils of New Zealand, and up to the present time several species have been isolated and identified on cellulosic substrata such as bits of bleached corn leaves floated on the surface of watered soil cultures. These fungi, which resemble the chytrids very closely in thallus structure and organization and differ primarily by the presence of an anterior flagellum on the zoospores, have been classified (Karling, 1943) into three families, the Anisolpidiaceae, Rhizidiomycetaceae, and Hyphochytriaceae, on the basis of thallus structure. In the Anisolpidiaceae the monocentric thallus is holocarpic without rhizoids as in the Olpidiaceae of the chytrids, and in the Rhizidiomycetaceae it is eucarpic, monocentric and differentiated into reproductive structures and absorbing rhizoids as in the chytrid family Rhizidiaceae. In the Hyphochytriaceae the thallus is polycentric as in the Chladochytriaceae but distinctly hyphal-like without rhizoids.

So far no members of the Anisolpidiaceae have been found in New Zealand, but in the family Rhizidiomycetaceae five species were observed, including a new one. In the Hyphochytriaceae, *Hyphochytrium oceanum* sp. nov. and *H. catenoides* were found in several localities.

Rhizidiomycetaceae

Rhizidiomycopsis (?) saprophytica sp. nov.

Saprophytica. Thallus monocentricus eucarpicus, in partes duas divisus, parte prima epibiotica maturitate in sporangium transformata parte altera endobiotica ramosa, rhizoidii instar. Sporangia non-apophysata, sessilia, globosa, 16—32 μ diam. et tubis 1—3, 4—45 μ diam., 3—5 μ longis praedita. Pariete laevi, 1.5—2 μ crasso, rubro-brunneo. Zoosporae ovoideae, 4—4.2 × 5—5.2 μ; flagello 14—16 μ longo, in sporangio separatim maturantes et singulatim dispersae. Sporae perdurantes ignotae.

Sporangia non-apophysate, predominantly spherical, 16—36 μ diam.,

with a reddish brown, 1.5—2 μ thick, wall. Rhizoids arising at the base of the sporangium and extending for distances up to 112 μ. Zoospores delimited within the sporangium and emerging fully formed through 1—3 short necks, 3—5 μ long by 4—4.5 μ diam.; ovoid 4—4.2 × 5—5.5 μ, to almost spherical with several small granules; tinsellate flagellum 14—16 μ long. Resting spores unknown.

Saprophytic on bleached corn leaves in soil sample AWRKF along the Waipoua River, Waipoua Kauri Forest, Auckland Province.

This species is included only tentatively in the genus *Rhizidiomycopsis* which S p a r r o w (1960) erected for monocentric eucarpic species whose zoospores are fully formed in the sporangium before emerging. However, in view of the fact that in some well-defined species of *Rhizidiomyces* and *Hyphochytrium* cleavage into zoospores may occur occasionally within the sporangia (K a r l i n g, 1939, 1944), it is questionable that the place of cleavage and maturation of the zoospores is a sharply-defined generic criterium. The thalli of *Rhiziomycopsis* are strikingly similar to those of *Rhizidiomyces,* and on the basis of present knowledge of the anisochytrids in general this genus is highly questionable.

So far, only one other species, *R. japonicus,* is included in this genus. Kobayaski and Ookubo (1954) found it parasitizing the oogonia of *Aplanes* sp. and described it as a species of *Rhizidiomyces.* The new Zealand species differs from it primarily by its non-apophysate sporangia with reddish-brown walls and its saprophytic nature. Also, the zoospores become globular to almost spherical in shape as they become actively motile, and apparently have a shorter flagellum.

The development stages of this species, figures 1 to 10, are similar to those of *R. japonicus* except that no apophysis is formed, and at maturity 1 to 3 exit papillae or short necks develop on the sporangia (fig. 8, 9). As is characteristic of other anisochytrids the protoplasm of young and mature sporangia is coarsely and greyish-granular in appearance (fig. 6—8) with a few interspersed refractive globules. At maturity it cleaves into zoospore initials (fig. 8, 9) which mature and emerge as the tip of the necks deliquesce (fig. 10).

R h i z i d i o m y c e s a p o p h y s a t u s Zopf, 1884. Nova acta Acad. Carol.-Leop. 47: 188, pl. 20, fig. 1—7.

Parasitic on the oogonia of *Achlya* sp. in soil sample ADSIR.

R h i z i d i o m y c e s b i v e l l a t u s Nabel, 1939. Arch. f. Mikrobiol. 10: 357, fig. 1—7.

Saprophytic on the oogonia *Achlya* sp. in soil sample ADSIR.

Rhizidiomyces hansonii Karling, 1944. Amer. J. Bot. 31: 396, fig. 35—64.

Saprophytic on bleached corn leaves in soil samples AR and AAD.

This species occurred abundantly in the two soil samples noted above, and frequently the surface of leaf strips was almost completely covered with sporangia.

Rhizidiomyces hirsutus Karling, 1945. Bull. Torrey Bot. Club 72: 47, fig. 1—19.

Saprophytic on bleached corn leaves, bits of hemp seeds, and fibrin film in soil samples AAD, ATKF, WT1, CHT, CTG, OD4, and OAL.

As noted above, this is the most widely distributed species of *Rhizidiomyces* observed in New Zealand. Frequently, the surface of leaf strips and bits of hemp seed was almost completely covered with hairy sporangia.

Hyphochytriaceae

Hyphochytrium oceanum sp. nov.

Saprophyticum. Thallus praecipue monocentricus, eucarpicus intramatricalis. Sporangia hyalina laevia, globosa, 15—75 μ diam., ovoidea, 20—45 × 30—62 μ, elongata vel irregularia, 30—40 × 65—98 μ, 1—30 hyphis 2—4 latis et tubulis 1—6 rectis vel contortis, 6—10 × 18—132 μ praedita. Zoosporae ovoideae vel elongatae, 3.3—3.8 × 5—5.5 μ; flagello 12—15 μ longo. Sporae perdurantes ignotae.

Thalli monocentric or polycentric, intramatrical, consisting of sporangia bearing radiating hyphae. Hyphae tubular, relatively narrow, 2—4 diam., or fairly broad, straight, irregular, curved or eneven in diameter, short or extensive, and usually ending bluntly, or inflated at the tip. Sporangia hyaline, highly variable in shapes and sizes which usually depend on the sizes and shapes of substratum cells, ovoid, 20—45 × 30—62 μ, spherical or subspherical, 15—75 μ diam., elongate and irregular, 30—40 × 65—98 μ, sometimes deeply lobed and extending into several substratum cells; bearing a few or up to 30 hyphae or apophysis-like swellings on the periphery; developing 1 to 6 straight or contorted exit tubes, 6—10 μ diam. by 18—132 μ in length, through which the protoplasm emerges as a naked mass and usually undergoes cleavage on the outside. Zoospores ovoid to elongate 3.3—3.8 × 5—5.5 μ, with several small refractive granules; tinsellate flagellum 12—15 μ long. Resting spores unknown.

Saprophytic in grass leaves in soil samples ARSR, ARVAT, and WOG.

This species was first observed in corn leaves added to watered soil cultures from several of the Cooks Islands, Niue, Fiji, Pitcairn and Western Samoa, and later from several localities in New Zealand.

Apparently, it is widely distributed in the South Pacific Islands, and for this reason it is named *oceanum.*

Whether it should be included in the Rhidiomycetaceae or in the Hyphochytriceae is open to question. So far as is known the thallus is predominantly monocentric as in the former family, but it's tenuous absorbing portions are distinctly hyphae-like and may become very extensive. Furthermore, these usually end bluntly like most hyphae, and the terminal branches do not run out to fine threads or points as rhizoids do. For these reasons and the added observation that the thalli frequently become polycentric and very extensive this species is classified for the time being in the Hyphochytriaceae.

Infection of the bleached corn leaves usually occurs at the stomatal pores (fig. 15). The broad germ tube branches once (fig. 19) to several times (fig. 16), and the sporangial rudiments usually begins as an enlargement at the juncture of the branches. The branches continue to elongate and increase in diameter and eventually become hyphae which radiate from the surface of the incipient sporangium. In the meantime, the latter enlarges and additional branches or hyphae may form from it's surface. In this manner a monocentric thallus consisting of a sporangium and radiating hyphae is formed. Occasionally, the sporangial rudiment may develop above the germ tube branches (fig. 20) with the result that a small sporangium with basal hyphae is formed (fig. 21). A few thalli with only one basal hyphae (fig. 22) have been found, and in such cases the sporangium was probably formed in the germ tube above its unbranched tip.

The hyphae vary greatly in length and diameter and may be continuous or branched once to several times (fig. 24, 32). They may be straight, curved or quite irregular in diameter (fig. 33), or inflated at various places. Sometimes, they may become greatly inflated near the periphery of the sporangium and develop into apophysis-like swellings. The latter may develop further into secondary sporangia so that the thallus becomes polycentric and consists of a central primary sporangium surrounded by secondary sporangia (fig. 32). In other instances the hyphae may extend into several substratum cells and become inflated intercalarly or at the tips. Such inflations or swellings may develop into secondary and tertiary sporangia with hyphal branches on their peripheries. This results in extensive thalli which are distinctly polycentric and composed of several sporangia with connecting isthmus of various lengths, diameters and shapes. The most extensive polycentric thallus observed so far consisted of a interconnected sporangia.

A smaller but extensive polycentric thallus is shown in figure 33. It occupied 8 adjacent corn leaf cells and consisted of 8 sporangia of various sizes and shapes. The unusually large, irregular, and lobed sporangium at the top of the figure developed 6 exit canals within

5 hours after mounting in water, and the sporeplasm began to emerge from 5 of them. In preparation for discharge the sporeplasm began to separate into 7 masses which are shown in fig. 33 to be connected by protoplasmic strands. Emission of the sporeplasm occurred from the sporangium in the center of the figure, also, and two other sporangia developed exit canals. Figure 33, also, illustrates quite well the variations in the sizes and shapes of the connecting isthmuses as well as the hyphae which are centered on the sporangia.

Discharge of the sporeplasm and its cleavage into zoospores on the outside of the sporangium are similar to those of other species of *Rhizidiomyces* and *Hyphochytrium*. A few hours after mounting the sporangium in fresh water one or more broad papillae develop on it's periphery, and these elongate to become the exit tubes, which vary from 6—10 μ in diameter by 18—132 μ in length and may be straight, curved or contorted. As the tip of the tube expands (fig. 25, 33), its wall becomes so thin that it is no longer perceptible as such. The sporeplasm moves outward very slowly at first, but its movements increases in rapidity and becomes readily perceptible. At it pours out, a large globular mass is formed at the tip of the tube. This mass is naked and devoid of a wall or membrane. Within a short time it usually invaginates on the periphery nearest the exit canal (fig. 26), and cleavage furrows become visible at the periphery. These eventueally delimit zoospores which remain in a group for a while with their flagella beating (fig. 27) before dispersing. Fairly often a portion of the sporeplasm may remain in the sporangium (fig. 29) and undergoes cleavage into zoospores (fig. 31) which swim around and finally emerge through the exit canal. In other instances such protoplasmic masses round up in the sporangium without cleaving into zoospores (fig. 30).

In very large sporangia where discharge may take a long time or the rate of discharge is unusually slow cleavage of the sporeplasm may begin at the periphery of the discharged mass while the remainder of protoplasm is still flowing out (fig. 28). In such cases zoospores may become fully formed at the periphery and move away while cleavage is still occurring in the central mass.

The zoospore (fig. 11) are ovoid to elongate 3.3—3.8 × 5—5.5 μ, and considerably larger than those of *H. catenoides*. Their behavior and movements are quite characteristic and similar to those of other anisochytrids. Sometimes the zoospores do not become actively motile but creep around as amoebae with the anterior flagellum beating back and forth slowly (fig. 12). Eventually they come to rest like the actively motile ones and become cystospores (fig. 13). Some of these may grow and become abnormal in size. So far, they have not been found to form secondary zoospores.

Hyphochytrium catenoides Karling, 1939, Amer. J. Bot. 26: 512, fig. 1—18.

Saprophytic in bleached corn leaves in soil samples ATRC and OD4.

Summary

Species of anisochytrids occur commonly in the soils of New Zealand, and so far five species of the family Rhizidiomycetaceae and two of the Hyphochytriaceae have been isolated and identified. Among these *Rhizidiomycopsis saprophytica* and *Hyphochytrium oceanum* are described as new species. No members of the monocentric, holocarpic family Anisolpidiaceae, have been observed.

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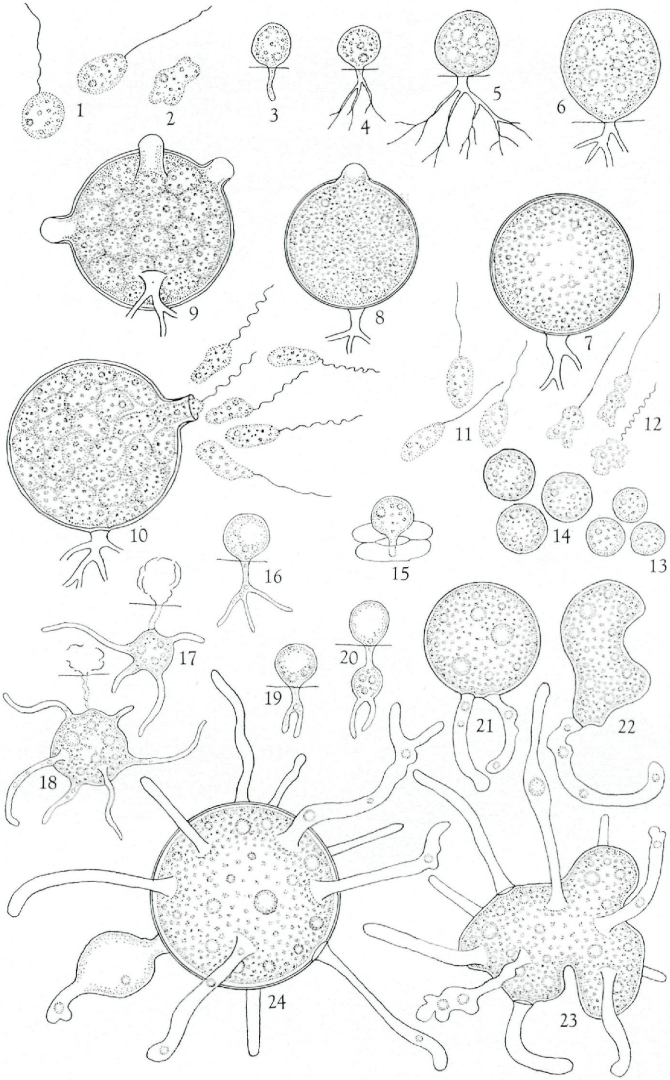
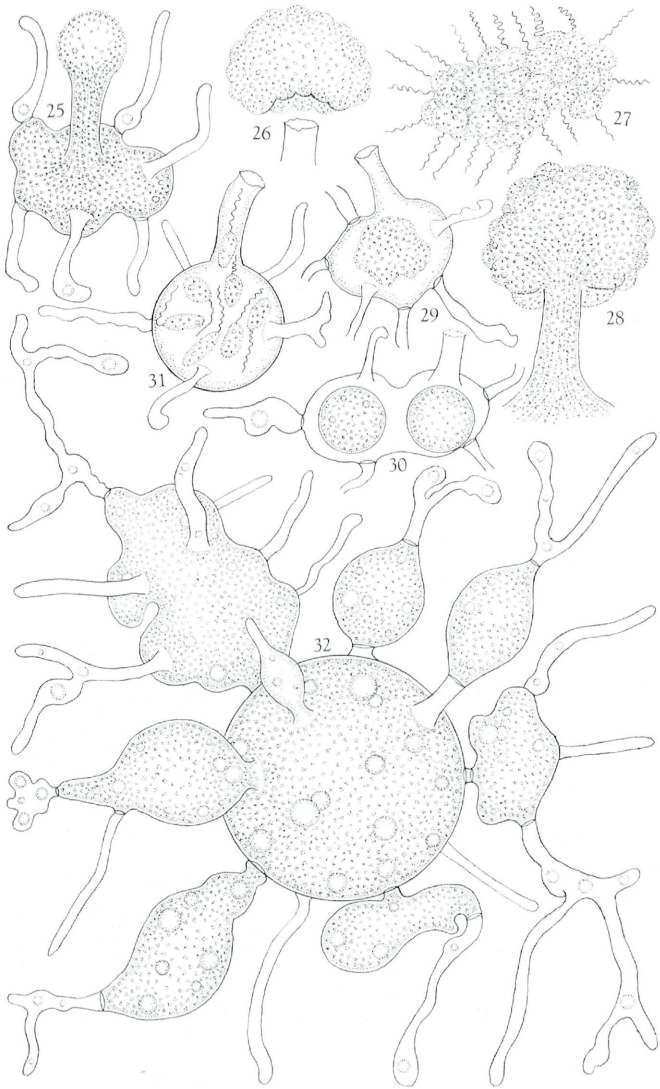
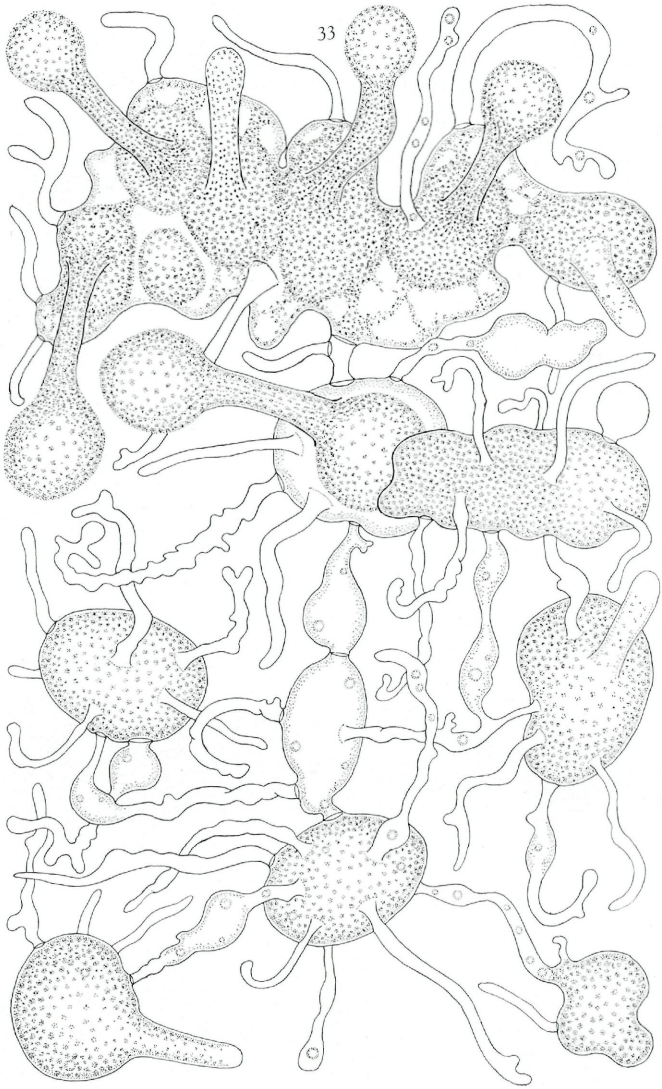
Explanation of Figures.

Fig. 1—10. *Rhizidiomycosis saprophytica.* Fig. 1. Zoospores. — Fig. 2. Amoeboid zoospore. — Fig. 3—6. Germination of zoospore and development of thallus. — Fig. 7. Mature sporangium with coarse greyish-granular content. — Fig. 8. Development of exit canal; faint cleavage lines in protoplasm. — Fig. 9. Sporangium with three exit canals and zoospore initials. — Fig. 10. Discharge of fully-developed zoospores.

Fig. 11—33. *Hyphochytrium oceanum.* Fig. 11, 12. Elongate and amoeboid zoospores. — Fig. 13. Cytospores. — Fig. 14. Cystospores after enlarging. — Fig. 15. Stomatal infection by zoospore. — Fig. 16—18. Stages in the development of a multi-hyphal thallus. — Fig. 19—21. Stages in the development of a thallus with two hyphae at the base of the sporangium. — Fig. 22. Small irregular sporangium with one hyphae. — Fig. 23. Lobed sporangium bearing 10 hyphae. — Fig. 24. Large spherical sporangium with 11 hyphae. — Fig. 25. Beginning of discharge of sporeplasm from an irregular sporangium. — Fig. 26. Invagination of sporeplasm and beginning of cleavage. — Fig. 27. Mass of zoospores with beating flagella shortly before dispersing. — Fig. 28. Cleavage beginning at periphery while sporeplasm is still flowing







out of exit canal. — Fig. 29. Small sporangium containing undischarged sporeplasm. — Fig. 30. Undischarged encysted sporeplasm. — Fig. 31. Undischarged sporeplasm which has undergone cleavage into zoospores. — Fig. 32. Large polycentric thallus with a central spherical primary sporangium and 7 secondary attached sporangia. — Fig. 33. Unusually extensive polycentric thallus consisting of 8 sporangia bearing hyphae of various lengths and shapes and connected by long or short, and inflated isthmuses; upper irregular and lobed sporangium with 6 exit canals; sporeplasm separating into masses and emerging from 5 of the canals.