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F. K. Sparrow
University of Michigan

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F. K. SPARROW[1]

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Eight different zoosporic fungi were recovered from soil in the environs of the sanctuary and oracle of Apollo at Delphi, Greece. Two of these, *Rhizophydium sibyllina* and *Phlyctochytrium neuhausii*, are described as new species.
INDEX DESCRIPTORS: Zoosporic Fungi from Delphi; *Rhizophydium sibyllina*, sp. n.; *Phlyctochytrium neuhausii*, sp. n.

The past 50 years of intensive investigation by those interested in zoosporic fungi ("phycomycetes") has resulted in a tremendous increase in our knowledge not only of the diversity of these organisms but of their ubiquity. It is now apparent that they abound in a great variety of types in all land masses of whatever size, origin, or age, not excluding the Arctic, Antarctica, and remote Pacific islands and atolls. It is somewhat surprising, therefore, that apart from phytopathological reports, data on zoosporic fungi from Greece appear to be nonexistent, or at least have escaped this investigator's attention. For this reason, and because our distinguished colleague whom we honor in this volume has his antecedents in this legendary land, and has himself carried on mycological investigations there, it seemed appropriate to present this modest contribution.

The eight zoosporic fungi herein reported were obtained from a six ml soil sample from one of the most famous and hallowed sites of antiquity, the sanctuary and oracle of Apollo at Delphi.[2] Indeed, the ancients would have had little difficulty in identifying the exact spot of collection since it was only a few feet from the Omphalos (as a root seemingly, involved in no less than nine generic names of fungi) marking the exact center of the earth!

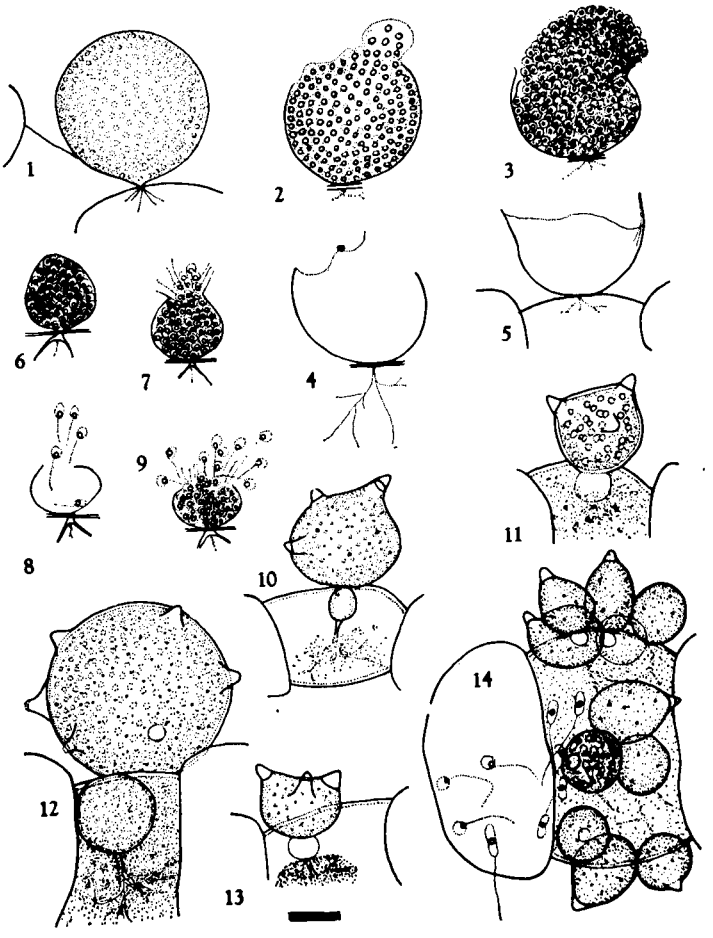
MATERIALS AND METHODS

The soil sample was divided into four equal parts, put in petri dishes, flooded with sterile distilled water and "baited" with a variety of substrates such as pollen, snake skin, shrimp chitin split, hemp seed, etc. Fungi of varying nutritional requirements were obtained by this method.

FUNGI COLLECTED

1. *Rhizophydium sibyllina* Sparrow, sp. n.

Sporangium sessile, omnino sphericum, membrana levis ad basim paululo crassiore, 14-32 μm, saepissime 25-32 μm diam., sine papillis dimissionis; systema rhizoidale delicatum, vix



Figures 1-14. Chytrids from Delphi. Figures 1-9. *Rhizophydium sibyllina* Sparrow, sp. n., on pine pollen. Figure 1. Mature sporangium. Figures 2, 3. Early stages in liberation of zoospores. Figures 4, 5. Empty sporangia showing nature of wall tears from discharge. Figure 6. Small sporangium. Figure 7. Same showing discharge with peculiar protrusion of flagella from sporangium. Figure 8. Nearly empty sporangium. Figure 9. Similar to Figure 7 with protruding flagella. Figures 10-14. *Phlyctochytrium neuhausii* Sparrow, sp. n., on pine pollen. Figures 10, 11, 13. Small sporangia. Figure 12. Large sporangium. Figure 14. Cluster of small sporangia, one of which is discharging zoospores through a single pore.

Note variation in number of discharge papillae on sporangia of various sizes. The solid bar is 10 μm.

[1] Department of Botany, University of Michigan, Ann Arbor.

[2] I am greatly indebted to Margaret Neuhauss Everett of the Great Lakes Division, University of Michigan, for this sample, collected Nov. 3, 1970. Inasmuch as it proved so rich in interesting fungi and since the collection data clearly state that the oracle indicated the place of collection, we see a modern instance of the power of the Delphic sibyl.

pertinens, e cauliculo brevi parce ramosum. Zoosporae plurimae, 2.5 μm diam., sphericae, globulum parvum sine colore atque flagellum modice longum (10 μm) habentes; in una massa sine mobilitate singula liberatae, postquam membrana distalis sporangii fissa, contracta, atque delicuit; mox postquam emergentes zoosporae flagella movere incipiunt atque provolant. Spora quiescens non observata.

Habitatio: Planta in solo in polline pini saprophytica, in loco Greece dicto reperta.

Rhizophydium sibyllina Sparrow, sp. n. Sporangium sessile, completely spherical, the smooth wall slightly thicker near the base, 14-32 μm, mostly 25-32 μm in diameter, without discharge papillae; rhizoidal system delicate, very limited in extent and branching from a short stalk. Zoospores very numerous, spherical, 2.5 μm in diameter, with a small colorless globule and moderately long flagellum (10 μm), liberated in a coherent mass without individual motility after splitting, shrinking and some deliquescence of the upper part of the sporangium wall, assuming individual flagellar motion several minutes after discharge, movement a rapid darting; resting spores not observed.

Saprophytic on pine pollen, Greece.

Type: Figures 1-9.

The mature sporangium of this species is sessile, completely spherical, smooth-walled (14-32 μm in diameter), without visible discharge papilla (Figure 1). There is a delicate, branched rhizoidal system often of limited extent arising from a short, usually barely perceptible, endobiotic stalk (Figures 1,6).

Great numbers of zoospores are formed within a single sporangium. The zoospores are spherical, 2.5 μm in diameter, with a small colorless globule and fairly long, somewhat rigid flagellum (Figures 8,9). Zoospore discharge takes place in a rather unusual manner, somewhat like that described for a parasite of the resting spores of *Ceratium* (Canter, 1968).

Zoospore discharge includes the following sequence of events. The wall of the distal end of the sporangium becomes distinctly thinner. Sometimes the entire distal half is involved (Figure 3); on others only the upper third is thus transformed; occasionally only a broad subapical spot appears (Figure 2). In any case, the thin-walled area gradually gives way, and a mass of quiescent spores bulges out (Figures 2,3). For a very short time the wall covering persists around the mass, but it eventually bursts and retracts. A part of the wall may even deliquesce (Figures 4,5). The emerging mass of spores soon separates into individuals which then become motile and swim away. In partially empty sporangia it was not uncommon to see "bundles" of flagella pointing outwards (Figure 7). During spore discharge the differences between the two parts of the sporangium (the split, shriveled, and partially deliquesced distal portion, and the rigid base) become strikingly evident. Often the distal remnants of the wall can be seen only with difficulty.

No resting spores or resting sporangia have as yet been identified, yet such must be formed for survival of the fungus in the dry soil.

A type of zoospore discharge involving extensive, or all, elements of the sporangium wall, rather than papillae, opercula, etc., is not too uncommon among chytrids. Thus in the genus *Solutoparies* all save a basal part of the wall dissolves. In species parasitic on diatoms (*Rhizophydium cyclotellae, R. melosirae*), green algae (*R. difficilo*), or *Eudorina* (*R. contractophilum*), the wall completely deliquesces. In *Phlyctochytrium megastomum* (Karling, 1968b) and *R. nobile* (Canter, 1968) a large portion of the wall dissolves, although fragments remain. *Chytriomyces willoughbyi* and certain others tear nearly the whole top of the sporangium off at zoospore discharge, and this torn apical part may remain attached as an operculum. A method of discharge closely similar to the present fungus is found in *Rhizophydium collapsum* (Karling, 1964). However, the zoospores of this fungus escape individually through a pore, or a tear in the collapsing wall, and emerge as a large group of active individuals. The Indian species is smaller, its fewer zoospores escape individually, and the rhizoidal system is evidently much more developed in pollen than that of *R. sibyllina.*

2. *Phlyctochytrium neuhausii* Sparrow, sp. n.

Sporangia cum conferta pyriformia, 15 x 7 μm, unam papillam habentia, au aliquantulum depressa, unam ad tres papillas prominentes conicas habentia, cum sparsa, autem, fere omnino spherica, usque ad 47 μ diam., 8-14 vel plures papillas, 4 μm alt. habentia. Systema rhizoidale parum evolutum, ex apophyse spherica, 4-15 μm diam. enascens. Zoosporae sphericae, 4 μm diam., singulum globulum sine colore, atque flagellum 15-20 μm long, habentes. Sporae quiescentes non observatae.

Habitatio: Planta in solo, in polline pini ut esca posita, in loco Greece dicto, reperta.

Phlyctochytrium neuhausii Sparrow, sp. n. Sporangium sessile, spherical and 28-47 μm in diameter, with up to 15 broadly conical discharge papillae, small sporangia 10-15 x 6-7 μm, pyriform with a single conspicuous apical or subapical papilla; rhizoidal system feebly developed, emerging from a generally spherical 4-15 μm in diameter apophysis; zoospores spherical 4 μm in diameter, with a single colorless globule and 15-20 μm posterior flagellum; resting not observed.

On dead pollen of *Pinus resinosa*; Greece.

Type represented by Figures 10-14.

Named for the collector, Margaret Neuhauss Everett.

This fungus belongs to the papillate section of the genus, i.e., those species with sporangia bearing several to many conspicuous discharge papillae on the sporangium. In some, such as *P. papillatum* Sparrow, the contour of the sporangium is little altered by the presence of these structures. In others, such as *P. reinboldtae* Persiel and *P. dichotomum* Umphlett and Olsen, the sporangial shape is greatly modified and becomes "morning-star-like" by reason of the formation of broad-based, coarse, horn-like discharge tubes. The present fungus is allied to the latter group. Under presumably optimal growth conditions a nearly spherical sporangium up to 47 μm in diameter is formed which bears numerous (up to 15 were counted), conspicuous, broadly conical acute papillae of discharge. These are more pointed than those formed by *P. papillatum* but not so horn-like as to greatly modify the general contour of the sporangium (Figure 12). Other sporangia growing singly on pollen grains presumably with less available nutriment are distinctly smaller (15-22 μm in diameter) and are greatly modified in shape by the rather disproportionately large conical papillae (Figures 10, 13). Thus, they might be pyriform with an apical or sub-apical papilla, broader than high with two opposite papillae, or, when densely crowded, pyriform, scarcely 10 x 7 μm, and with a single papilla (Figure 14). All possess a spherical, subsporangial, endobiotic apophysis varying from 4-15 μm in diameter, in proportion to the size of the sporangium. Rhizoids can occasionally be seen but usually are so delicate as almost to escape detection, or are absent.

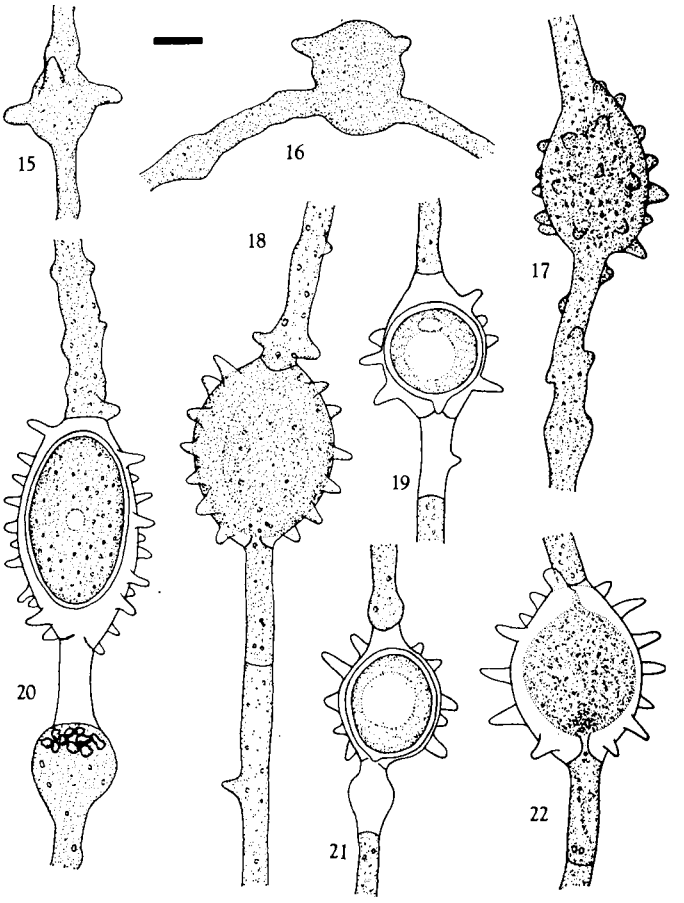
At zoospore discharge all of the papillae deliquesce and form pores through which the zoospores escape rapidly and successively. A very short tubular protrusion remains on the rigid wall after discharge. At first, the zoospores are elongate, 6 x 2 μm, with a minute glistening body. As they continue to swim, they become spherical, about 4 μm in diameter, with a long posterior flagellum (Figure 14, left). No resting spores are known. However, germinated thick-walled resting sporangia with 3-4 prominent discharge tubes are common in old cultures.

The fungus is distinct from *Phlyctochytrium papillatum* Sparrow, its nearest ally, because of its larger, rigid-walled sporangia, more conical and more numerous discharge papillae, and less coarse rhizoidal system. Smaller plants are not unlike those of *P. nematodae* Karling, a virulent parasite of nematodes. The sporangium of *P. californicum* Barr (1969) resembles large specimens of the present fungus but the apophysis (size not given) is seemingly insignificant in size. *Phlyctochytrium palustre* Gaertner from Gaertner's figures (1954) lacks the conspicuous conical discharge tubes and has a coarser rhizoidal system on pollen. The single papilla on small plants of these fungi, in contrast to the Greek fungus (Figure 14), do not have such broad bases as to destroy the spherical contour of the sporangium. The various interpretations of *P. reinboldtae* Persiel (1959) should be considered here. In fully developed sporangia of *P. reinboldtae* a characteristically "morning-star" configuration prevails due to the unusually broad bases to the conical discharge papillae, a feature not seen in the Greek fungus (Figure 12). Furthermore, when crowded on pollen grains, sporangia of stunted plants of *P. dichotomum* Umphlett and Olsen (1967) also have a star-like configuration as well as a coarse, dichotomously branched, blunt-tipped rhizoidal system, not delicate and tapering like the other two fungi. Similarly, *P. semiglobiferum* Uebelmesser (1956) is star-like, but with a strong tap root endobiotic system and multigranulated zoospores.

3. Another *Phlyctochytrium* sp. with perfectly spherical sporangia 30-40 μm in diameter and up to 10 relatively low papillae 3 μm in diameter was found on pollen. The apophysis was spherical and 8-10 μm in diameter. No rhizoids and no discharge of zoospores were seen. This species is possibly allied to *P. spectabile* (Uebelmesser, 1956).
4. *Rhizophlyctis rosea* (de Bary and Waronin) Fischer. Moderately abundant on lens paper bait.
5. *Catenaria anguillulae* Sorokin. On snake skin bait.
6. *Catenophlyctis variabilis* Karling. On snake skin bait.
7. *Anisolpidium saprobium* Karling. On pine pollen bait.

This *Olpidium*-like form occurred in great abundance in the pollen bait. Karling (1968a) and Sparrow (1973) have recently given details of this fungus and only a brief account need be given here to characterize the material from Delphi.

The spherical thalli, 13-22 μm in diameter, lie loosely in the body of the pollen grain. Transference to fresh water results, within 10-15 minutes, in initiation of a discharge tube. After several hours such tubes may attain a length of 100 μm or more and occasionally branch, in which case they fail to function further. Unbranched tubes became hyaline and refractive apically (Figures 28, 29), and eventually such tips suddenly swell up (Figures 30-33), coincident with a forward movement of the "mealy" membranated contents of the thallus. For the most part the coarsely granulated contents emerge in a continuous mass and either remain coherent at the discharge tube apex (Figure 34), or dissociate into several large lumps. In any case, zoospore formation proceeds fairly rapidly, the spore origins becoming visible in approximately 1½ minutes and final maturation achieved within five (Figures 35-38).



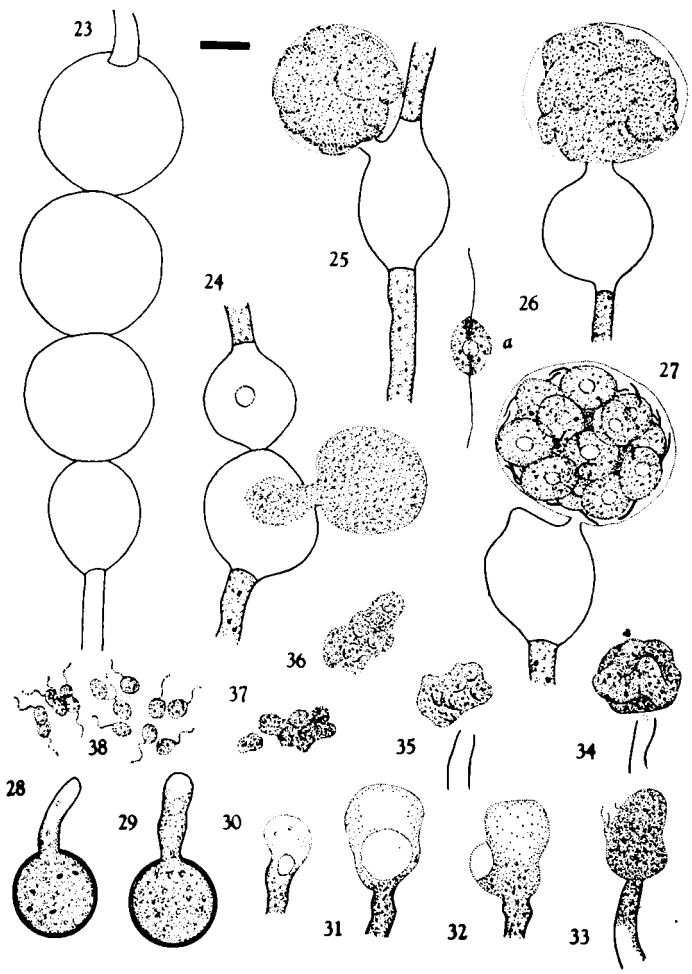
Figures 15-22. *Pythium echinulatum* Matthews, on hemp seed bait. Figures 15-17. Developmental stages of papillate oogonium. Figure 18. Later stage of sex organ development showing an intercalary oogonium with periplasm and ooplasm differentiating. The lower concomitant smooth hyphal segment has been converted into a hypogynous antheridium with fertilization tube; the upper, papillate hypha remains sterile. Figures 19-21. Mature aplerotic oospores within papillate oogonia. Figure 22. An oogonium with egg in surface view during fertilization by lower hypogynous antheridium. Solid bar is 10 μm.

Although this fungus meets the definition of the genus *Canteriomyces* (Sparrow, 1960), it is placed in *Anisolpidium* and identified with Karling's species until an evaluation based on comparative studies of strains determines more precisely the taxonomic significance of zoospore formation (i.e., inside or outside the sporangium).

8. *Pythium echinulatum* Matthews. On hemp seed bait.

This delicate species of *Pythium* was the only filamentous zoosporic fungus found in the sample. It is a rare species. For purposes of comparison material for examination has been obtained from England through the kindness of Dr. Michael Dick, the University of Reading.

Since the fungus has been so infrequently isolated, reproductive details in particular are included here.



Figures 23-27. *Pythium echinulatum* Matthews. Non-sexual reproduction. Figure 23. Rare catenulate series of sporangia. Contents not shown. Figure 24. Discharge of lower sporangium of a pair. Figure 25. Discharge of a single intercalary sporangium. Figure 26. Mid-stage in maturation of zoospores in vesicle of a terminal sporangium. Figure 27. Later stage of zoospore development in terminal sporangium showing nearly mature zoospores with vacuoles and flagella. Figure 27a. Mature liberated zoospore. Figures 28-38. *Anisolpidium saprobium* Karling, in pine pollen. Figures 28-33. Stages in emergence of contents of a thallus. Figures 34-37. Emerged contents dividing into zoospores. Figure 38. Mature anteriorly uniflagellate zoospores. Solid bar is 10 μm.

The mycelium is composed of delicate, branched hyphae, usually 2-5 μm in diameter.

The sporangia are spherical, mostly 18-28 μm in diameter, occasionally ellipsoidal, 30 x 17 μm. They are predominantly singly borne, either terminally (Figures 26, 27) or intercalary (Figures 24, 25), on the hyphae. Rarely, 2-4 sporangia may occur in tandem (Figures 23, 24). The discharge papilla is usually slightly elevated, about 5 μm in diameter, and variously placed on the sporangium. Zoospore discharge is typically pythioid, with production of a vesicle within which the swarmers mature (Figures 24-27). The zoospores are plump, 7-8 x 10-12 μm, with granular contents and the usual vacuole, and with two laterally attached flagellae of about equal length (Figure 27a) and oppositely directed.

Sexual reproduction is by oogonia and by antheridia which in our material are solely hypogynous. Oogonia develop as intercalary irregular swellings accompanied by stout, broadly conical, protrusions (Figures 15, 16). As development proceeds, one (only rarely both) concomitant hypha attending an oogonium also develops protrusions (Figure 17). The smooth element usually becomes the walled-off hypogynous antheridial cell (Figures 18, 20). Meanwhile, protrusions continue to grow and multiply on the oogonium proper. At full maturity, 25 or more of these papillae, each up to 10 μm in height, flare broadly toward the base with sides scarcely opposed convexly to one another in optical section. "Broadly conical" best describes the rounded apices of the papillae. This feature of the Delphic fungus is unlike the papillae with sharp-tipped spines usually with convexly opposed walls described by Matthews (1931).

The mature sexual apparatus consists of a narrowly to broadly ellipsoidal intercalary, or rarely, a terminal oogonium (21-45 x 15-23 μm), set with 25 or more broadly conical protuberances; a cylindrical, usually smooth, hypogynous antheridium (15-22 x 4-9 μm); and an aplerotic, nearly spherical to ellipsoidal oospore, 20-35 x 11-19 μm, with a 2-4 μm thick wall. Occasionally the oospore is papillate at the point of contact with the fertilization tube (Figures 19, 21). Germination of the oospore occurs by hyphal formation.

The complete lack of stalked antheridia, the presence more often of ellipsoidal rather than spherical oogonia, the nature of the oogonial protuberances, and the great rarity of sporangia in tandem are features unlike those in the American fungus, and thus may prove to be sufficient to separate the two fungi at the species level.

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