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A TULASNELLA PARASITIC ON AMOEBA TERRICOLA[1]

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ABSTRACT

A microfungus attacking *Amoeba terricola* through germination of an adhering basidiospore is newly described as *Tulasnella zooctonia*. After intruding a branched haustorium into the animal, the spore extends one to three divergent procumbent hyphae externally, which at intervals give rise to obovoid hypobasidia. Some hypobasidia are oriented ascendingly and produce two to five globose epibasidia on the distal end, but many others lie prostrate on the substratum and form epibasidia on the exposed upper side. In either positional relation the epibasidia readily produce tapering sterigmata, each bearing an obovoid basidiospore provided with a conspicuous basal protrusion. Clamp connections have not been observed, nor any elaboration of waxy or gelatinous material.

THE DESTRUCTION of soil rhizopods by predacious and parasitic fungi is often conveniently revealed in agar plate cultures inoculated with portions of plant materials that have undergone slow decay in moist contact with the ground. Among the fungi recorded as being active in such destruction are many species belonging in the Zoopagales, and a smaller number of moniliaceous forms taxonomically related to the familiar, widely distributed series of nematode-capturing Hyphomycetes. A single species, *Pagidospora amoebophila* Drechsler (1960), which from its production of clamp-connections may be reckoned with the basidiomycetes evidently subsists habitually through the capture of amoebae. Similarities to *P. amoebophila*, with respect to adhesive knobs and endozoic hyphae, suggest some likelihood that kinship to the basidiomycetes is shared also by an unnamed clampless sterile fungus found capturing and consuming amoebae (Drechsler, 1954). Recently an indubitable member of the basidiomycetes was observed subsisting on a rhizopod species, but unlike *P. amoebophila* it attacked after the usual manner of parasites, that is, through the germination of an adhering spore.

MATERIAL—The fungus in question developed in a maize-meal-agar plate culture on which had been planted 35 days earlier a pinch (about 0.03 g) of mealy detritus sifted from forest duff collected in upland woods 0.5 km west of Butternut Lake, near Park Falls, Wis., on 12 October 1968. During the 35 days of incubation the culture remained covered under an inverted jar at a temperature near 18.5 C. The fungus arrested attention through the presence here and there of groups of three to five spores (Fig. 1–3) held aloft above a corresponding number of clustered globose cells. Closer examination revealed each group of aerial spores as sister basidiospores supported, as in the genus *Tulasnella*, on tapering sterigmata arising from plural epibasidia sessile on a hypobasidium. The hypobasidia were attached at intervals to procumbent hyphae (Fig. 4–9) that originated from parasitized amoebae. In instances where the animal's nucleus was discernible in the granular cytoplasm, it appeared as an elongate-ellipsoid body, mostly 19–25 μ wide (Fig. 4n, 7n, 12), which showed close under its peripheral membrane a rather thin, irregularly interrupted, slightly darkish layer. The animal, accordingly, is held referable to *Amoeba terricola* Greef (sensu strictiore), a widespread soil protozoan earlier found subject to destruction by *Endocochlus gigas* Drechsler (1936), *Cochlonema megaspirema* Drechsler (1937), and *Acaulopage marantica* Drechsler (1939).

Owing apparently to the slow multiplication of *Amoeba terricola* among the various microbes present in the agar plate culture, only about 25–30 specimens of the rhizopod, scattered very sparsely over an area of 4–5 square cm, would seem to have been available to the parasite. Probably all these available specimens had already become infected when the fungus was first seen, for efforts to increase the supply of material by transferring occupied slabs of substratum to newly poured agar plates were unsuccessful. Within 7 days the destruction of all individuals of the host rhizopod brought further development of the parasite to an end. Much of the limited supply of reproductive apparatus formed by the fungus incurred visible impairment in being bustled by vigorous eelworms that abundantly infested the culture. These eelworms, apparently being strongly attracted by light, provided additional difficulty by gathering in the areas

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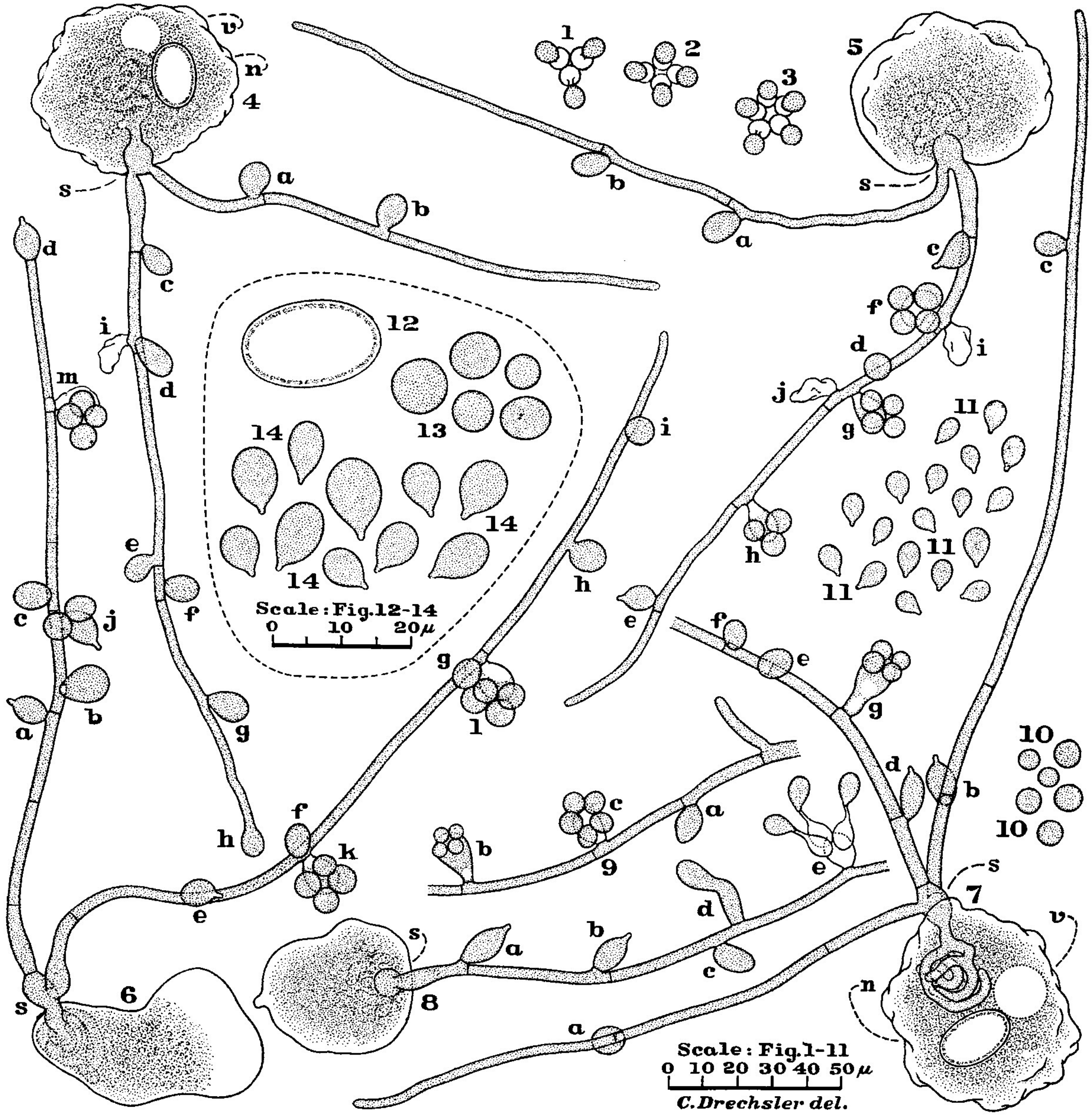

Fig. 1–14. *Tulasnella zooctonia* parasitic on *Amoeba terricola* in a maize-meal-agar plate culture 35–40 days old, except for Fig. 1–3, drawn from material covered with a cover glass.—Fig. 1–3. Upper view of basidiospores in uncovered material, showing groups of three, four, and five, respectively, arising from clustered epibasidia. × 500.—Fig. 4. Infected amoeba showing two fertile hyphae with eight hypobasidia (a-h) at various stages of growth, an empty hypobasidium (i), host nucleus (n), infective spore or cell (s), contractile vacuole (v). × 500.—Fig. 5. Infected amoeba showing two fertile hyphae with five full-grown hypobasidia (a-e), three hypobasidia (f-h) surmounted by epibasidia, two empty hypobasidia (i-j) and the infective spore (s). × 500.—Fig. 6. Infected amoeba showing two fertile hyphae with nine globose hypobasidia (a-i), three hypobasidia (j-l) with developing epibasidia, an empty hypobasidium (m), and the infective basidiospore (s). × 500.—Fig. 7. Infected amoeba showing three fertile hyphae with six globose or ellipsoidal hypobasidia (a-f), a hypobasidium (g) producing four epibasidia, host nucleus (n), infective basidiospore (s), and contractile vacuole (v). × 500.—Fig. 8. Infected amoeba showing one fertile hypha with four delimited hypobasidia (a-d), a fully developed basidium (e) bearing three basidiospores, and the infective spore or cell (s). × 500.—Fig. 9. Portion of fertile hypha with a delimited ellipsoidal hypobasidium (a), a hypobasidium (b) surmounted by four young epibasidia, and an empty hypobasidium (c) bearing five delimited globose epibasidia. × 500.—Fig. 10. Random assortment of detached globose epibasidia. × 500.—Fig. 11. Random assortment of detached basidiospores showing usual variations in size and shape. × 500.—Fig. 12. Host nucleus. × 1000.—Fig. 13. Detached globose epibasidia. × 1000.—Fig. 14. Detached basidiospores. × 1000.

lit up by the beam from the microscope condenser, and thereby often jostled reproductive parts directly under examination. Enough material, however, was found in an undisturbed condition to permit trustworthy observations on normal development of the fungus.

RESULTS—Like other fungi given to a similar mode of subsistence, the basidiomycetous parasite cannot reasonably be expected to develop in such quantity as to form on opaque natural substrata any layer visible to the naked eye. In spite of its microscopic proportions and of its failure to elaborate any waxy or gelatinous coating, the distinctive development of its basidia establishes the fungus as a congener of the many saprophytic species of *Tulasnella* familiar from their extensive resupinate fructifications on decaying plant remains.

Tulasnella **zooctonia** sp. nov.[2] (Fig. 1-14)—Fungus minute, not visible to the naked eye. Growth initiated through germination of basidiospores in contact with live amoebae; each spore first pushing a haustorium into the host animal, and later giving rise externally sometimes to one but much more often to two or three divaricate fertile hyphae. Haustorium consisting of a proximal stalk, mostly 2.5–5 μ long and 2.5–3.2 μ wide, together with four to eight assimilative branches often irregularly recurved and loosely intertangled, mostly 10–30 μ long and about 3 μ wide. Fertile hyphae procumbent, without clamp-connections, 150–300 μ long, proximally 3–6 μ wide, tapering to a width of 2.5–3 μ at the tip, giving rise sometimes dorsally to erect or inclined hypobasidia, but more often giving rise laterally or terminally to prostrate hypobasidia. Hypobasidia subglobose or obovoid or elongate-ellipsoidal, commonly 11–16 μ long and 7–10.5 μ wide, inclusive in many instances of a digitate apical protrusion about 1.7 μ long and 1 μ wide, each producing distally or dorsally two to five (usually four) sessile epibasidia; epibasidia globose or ellipsoid, mostly 7–9 μ long, 6–8.5 μ wide; basidiospores elongate-obovoid, narrowed proximally into a somewhat protruding base, mostly 8–11.5 μ long and 5–7.8 μ wide.

The jostling of nematodes was evidently sufficient to detach full-grown epibasidia from their empty parent hypobasidia, for many of the globose spore-like bodies (Fig. 10, 13) were found on the surface of the agar, often being intermixed with detached basidiospores (Fig. 11, 14). It seems possible, even rather probable, that epibasidia can infect host animals much like basidiospores. Assuredly the nearly spherical bodies (Fig. 4s, 8s) from which infection of some individuals of *Amoeba terricola* proceeded, showed, in their outward form, close resemblance to epibasidia; whereas the more elongated bodies (Fig. 5s, 6s, 7s) on other animals revealed convincing similarity to basidiospores.

While the procumbent fertile hyphae of *Tulasnella zooctonia* give rise dorsally to some erect or inclined hypobasidia (Fig. 4d; 5c, d; 6b, c, e, f, g, i; 7e, f) in a rather commonplace manner, they show noteworthy distinctiveness in putting forth also many prostrate hypobasidia either terminally (Fig. 4h, 6d) or laterally (Fig. 4a, b, c, e, f, g; 5a, b, e; 6a, h; 7c, d; 8a-d; 9a). The downward orientation of some hypobasidia (Fig. 7a, b) in proximal positions near the infected amoeba is very probably not normal, having almost certainly resulted from slow wrenching earlier exerted by the animal, when the fertile hyphae concerned were still too short to provide firm anchorage. It seems not unlikely that the prostrate posture of hypobasidia here represents an adaptation for achieving better anchorage, since infected amoebae remain alive for several days, revealing through changes in shape, movement of protoplasm, and continued operation of the contractile vacuole (Fig. 4v, 7v), a prolonged capability for action, which, if uncontrolled, might cause difficulty for the parasite.

As prostrate hypobasidia in an agar culture are embedded lengthwise to some little depth in the substratum, they put forth their epibasidia not from the partly submerged broad distal end but from the exposed dorsal surface (Fig. 5f-h; 6j-m; 7g; 9b, c). Nevertheless, if an agar slab showing groups of epibasidia at an incipient stage (Fig. 9b) is mounted on a slide and covered with a coverglass, their further growth proceeds unhindered and can be observed under a microscope of high magnification. However, development of sterigmata and basidiospores (Fig. 8e) has not ensued in covered mounts, thus this final phase of reproduction would seem to require actual exposure to air. The empty membrane of a spent hypobasidium usually remains visible for some time, though often only in a somewhat

[2] Fungus minutus, oculo nudo vulgo non visibilis. Basidiospora germinans primo haustorium in amoebam vivam intrudens, postea extra animal interdum 1 hypham fertilem sed saepissime 2–3 hyphas fertiles divaricatas emittens. Haustorium in stipula (plerumque 2.5–5 μ longa, 2.5–3.2 μ lata) et 4–8 ramulis assumentibus consistens; his ramulis assumentibus vulgo irregulariter recurvis, laxe tortuosis, plerumque 10–30 μ longis, circa 3 μ latis. Fertiles hyphae procumbentes, non septatonodosae, 150–300 μ longae, basi vulgo 3–6 μ latae, sursum attenuatae, apice 2.5–3 μ latae, in superficie hypobasidia erecta vel acclivia ferentes, in apice vel a latere hypobasidia procumbentia emittentes. Hypobasidia globosa vel obovoidea vel elongato-ellipsoidea, apice saepius eminentia digitiformi circa 1.7 μ longa et 1 μ lata praedita, plerumque 11–16 μ longa, 7–10.5 μ lata, 2–5 (vulgo 4) epibasidia ferentia; epibasidiis globosis vel ellipsoideis, plerumque 7–9 μ longis, 6–8.5 μ latis. Basidiosporae elongato-obvoideae, basi attenuatae, plerumque 8–11.5 μ longae, 5–7.8 μ latae.

Amoebam terricolam interficiens habitat in humo silvestri prope Park Falls, Wisconsin. Typus: Figurae 1–14.

collapsed condition (Fig. 4i; 5i, j).

Apparently the apical protuberance found on many hypobasidia (Fig. 5c, e; 6a, d, e, j; 7b, d; 8a, b) of *Tulasnella zooctonia* has no significant relation either to the parasitic character of the fungus or to its development in an agar culture. Although, obviously, such a protuberance might occur equally well in a saprophytic species growing on a natural substratum, no mention of any similar distal prolongation is made by Rogers (1933) in his discussion of 19 congeneric fungi. The apical digitation thus would seem of moment chiefly as a supplementary diagnostic feature, less constant and correspondingly less determinative than the papillate protuberance on the hypobasidia of *T. papillata* (Olive) Olive (1954, 1957).

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