SOME CONIDIAL PHYCOMYCETES DESTRUCTIVE TO TERRICOLOUS AMOEBAE

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(with plates 1–7)

Introduction

In isolating parasites referable to the genera *Pythium, Phytophthora* and *Aphanomyces* from decaying plant tissues, I have been employing for some years a method which as described earlier (**7**) entails no attempt through surface disinfection at avoiding contamination of the isolation plates by the extraneous organisms habitually present on such material. After serving their immediate purpose, the isolation plates, as might be expected, often come to display a varied intermixture of adventitious plant and animal life, the character of the mixture in each case evidently depending somewhat on the nature and in larger measure on the source of the material used. Subterranean parts like roots, tubers, stolons and basal portions of stems, or even aerial parts like fruits and leaves that have been in contact with the ground, generally yield *Amoebae* of different species, the smaller forms apparently feeding mostly on bacteria, the larger ones ingesting cysts of the smaller *Amoebae*, testaceous protozoans, nematode eggs and more especially fungous spores of all descriptions.

While *Amoebae* in agar cultures are thus revealed as very active in the rather indiscriminate destruction of fungous life, they are in turn subject to wholesale destruction here and presumably also in nature by specific parasitic and predacious fungi. In a previous note (**9**) the morphological features of five fungi predacious on *Amoebae* were set forth, four of these being readily recognizable as Phycomycetes in spite of their Hyphomycete-like aerial conidia and of a vegetative mycelium which in three of the species is comparable in delicacy to the mycelium in species of *Actinomyces*. Several other forms similarly producing non-catenulate conidia and similarly displaying moderately extended mycelial or haustorial development within the smaller *Amoebae* captured by them, have since been observed. Although the sexual reproductive bodies of one of these additional forms are somewhat larger than those of any of the three for which such bodies were figured earlier (**9**, *figs. 3–5*), they are yet of dimensions too small to provide in themselves entirely decisive evidence as to their exact structure; or, for that matter, in view besides of the little differentiated and hence undistinctive character of the fusing elements, as to their correct interpretation. In referring earlier to the ambiguous sexual apparatus in the three delicate predacious Phycomycetes as consisting individually of oögonium, antheridium and oöspore, rather than of gametangia and zygospore, consideration was given more particularly to resemblances in general arrangement to the sexual apparatus figured by Arnaudow (**1**, *figs. 4, 5*) for *Zoophagus insidians* Somm., a predacious fungus of larger dimensions, whose position among the Oömycetes there has been little reason to question.

These resemblances and the possible taxonomic significance attaching to them are by no means yet to be dismissed. In any event, however, it is believed that the troublesome obscurity in structural detail like the attendant morphological ambiguity may in some measure be dispelled through an examination of several fungi of generally greater dimensions and more obvious distinctiveness, which have been found destructive to some of the larger species of terricolous *Amoebae*. Moreover, the fungi in question are well deserving of study in themselves, presenting remarkable novelties in vegetative development together with noteworthy peculiarities in sexual reproduction. And though the two variations in asexual reproduction would seem, when considered by themselves, rather commonplace both with reference to the shape of the conidia and to the arrangement in which the conidia are formed, when considered as pertaining to the Phycomycetes, they constitute departures as unexpected as they are difficult to homologize with the different types of asexual reproduction recognized in that class. Associated with the diversity in shape of thallus is a diversity in biological habit embracing endoparasitic, ectoparasitic and predacious relationships. Indeed, there are scarcely any distinctive features that are shared by all five species; yet each of the five shows intimate similarities to one or more of the others, and the several correspondencies interlock in a manner leaving little doubt that one is dealing here with an assortment of naturally related forms.

ENDOCOCHLUS ASTEROIDES

Of the endoparasites, the one most frequently encountered on agar plate cultures attacks an *Amoeba* (PLATE 1, A) having a fairly substantial pellicle and finely granular transparent protoplasmic contents. The larger animals when in a more or less rounded condition measure approximately 60 μ in diameter, and exhibit clearly a single prolate elliptical nucleus (PLATE 1, A, *n*) about 15 μ long and 10 μ wide which contains two darker concavo-convex structures lying close within its periphery, one at each of the poles. A search through the literature has uncovered no description of any *Amoeba* with a nucleus of exactly such structure; though from the frequency of its occurrence on agar plates, the animal would seem to be abundant in soil and in decaying vegetable materials, and therefore could hardly be supposed not to have been encountered previously by protozoölogists. Undoubtedly the animal comes within the scope of Penard's (**15**, *p. 83*) statement: "Il me paraît donc préférable—de considérer comme *A. terricola* toutes les amibes terrestres, revêtues d'une pellicule, et pourvues d'un noyan unique et toujours ellipsoïdal." It will accordingly be designated here by Greeff's binomial, together, when necessary for more specific reference, with a numeral: *Amoeba terricola* I.

Contact between host and parasite is established as the animal moves along on the surface of the substratum on which conidia of the fungus are distributed; the distribution showing usually a noticeably linear arrangement wherein the positions of the aerial sporiferous hyphae, now evacuated and disintegrating, remain recognizable. The conidia thus encountered adhere to the pellicle of the host with sufficient firmness to withstand the jostling incident to continuing locomotion. Germination follows, a somewhat bulbous body being first produced, which apparently functions as an appressorium. From this bulbous part is thrust forth a tube of lesser diameter that perforates the pellicle and extends a distance of about 10 μ into the protoplasmic interior of the animal (PLATE 1, A, *a*). The tip of this tube now swells up into a globular body which increases in size with the gradual transfer to it of all the material in the conidium (PLATE 1, A, *b*). When the transfer is accomplished, the globular body soon becomes disarticulated from the germ tube as the result of slight strains incident to the movements of the animal. The evacuated germ tube is then usually promptly expelled by the animal, but the globular body distinguished by the presence of a noticeable apiculum where it was earlier attached is retained within (PLATE 1, A, *c, c*) to be moved and jostled about passively in the protoplasmic streams together with the nucleus and often also with other inclusions like ingested protozoan cysts, testaceous rhizopods, and miscellaneous fungous spores in various stages of digestion. Sometimes the conidium, instead of remaining external to the *Amoeba,* is ingested either previous to germination or after development has begun, so that structures like that represented in plate 1, A, *a* may be observed being carried about in the protoplasmic streams. Even in these cases the evacuated membrane of conidium and germ tube apparently becomes separated from the globular body and is expelled, since instances of such a membrane remaining attached to thalli of larger sizes have never been seen in material of this species.

The number of infections to an animal depends obviously on the quantity of conidia encountered, which in turn stands in direct relation to the abundance of matured conidiophores. Early in the course of an epizoötic within a petri dish culture, an infected animal usually contains a single parasitic thallus, cases in which two or three thalli are visible occurring only now and then. As the conidia when produced are spaced at intervals approximately equal to the diameter of the *Amoeba,* it is apparent that a single infection would ordinarily result from the passage of an animal over a matured conidiophore at approximately a right angle, while two or three infections might well eventuate from passage at an oblique angle. Later when the epizoötic is well under way and conidiiferous hyphae span restricted areas here and there in an open arachnoid pattern, larger numbers of infections become more usual, so that animals carrying as many as a dozen thalli may be found. When such larger numbers of parasitic thalli are present, they may often be divided according to size into two or three categories, suggesting as in plate 1, A that the infections occurred in several successive installments corresponding to successive passages of the animal over terrain strewn with conidia.

Vegetative growth of the young globular thallus takes place through widening and elongation at the distal end, at first apparently extending the original axis in a straight line (PLATE 1, A, *d, e*). When a length of approximately 10 μ has been attained, a rather abrupt curvature in direction intervenes, resulting first in an obese U-shaped structure (PLATE 1, A, *f, g, h, i*). With continued elongation at a more moderate, rather uniform degree of curvature and at a tolerably uniform diameter, modified sometimes by dichotomous branching at one or two points, a helicoid structure of striking appearance is brought into being (PLATE 1, B, C; PLATE 2, A, B). The ultimate size of this structure is conditioned naturally by the size of the animal as well as by the number of other thalli competing with it for the nourishment which the protoplasm of the host affords.

For a considerable period after infection the *Amoeba* shows virtually no evidence of ill effects from the presence of the fungus. The animal shown in plate 1, A, for example, although burdened with four thalli of more than negligible bulk in addition to five smaller ones, was at the time it remained under observation still moving about with apparently undiminished briskness. As the protoplasmic materials become more and more depleted, locomotion becomes gradually slower, the infected *Amoeba* at the same time evidently contracting in volume; yet even in decidedly advanced stages of debilitation (PLATE 2, A) when the bulk of the host has been reduced by perhaps more than a half and the cumbersome mass of fungous thalli on occasion visibly interferes with the free movement of the pellicle, some locomotion may be observed. Finally, of course, movement ceases, and the remaining protoplasmic host materials are absorbed, leaving virtually nothing of the animal except the collapsing pellicle (PLATE 2, B). With exhaustion of its food supply the fungus necessarily terminates its vegetative growth, and devotes its substance to reproduction, either sexual or asexual, or both.

In asexual reproduction the vegetative thallus gives rise usually to a single delicate filament, which, after perforating the enveloping pellicle, forces its way through the overlying substratum to emerge in the air (PLATE 1, B, C). Aerial growth is continued until the protoplasmic contents of the fungous body have been used up, a length of 1.5 to 2 mm. being usually attained with fairly well developed specimens, while the largest thalli may give rise to aerial filaments twice as long. Sometimes a filament may give off one or more branches, usually near the point of egress from the substratum (PLATE 2, C). Branching however is never abundant, and in any case entails no increase in total length of filament produced.

Once the delicate aerial filament has attained its definitive length septa make their appearance in it at intervals usually of about 50 or 60 μ. Thereupon, mostly in positions approximately median between these septa, protrusions bud forth laterally (PLATE 2, G, *a*) and increase in size as the contents of the parent hyphal segments migrate into them (PLATE 2, H; G, *b*; I). The progressive evacuation of the segments is accompanied by the laying down of successive septa in the same manner as in aging mycelium of many species of *Pythium* and *Phytophthora*. When, therefore, the aerial filament becomes entirely evacuated it reveals cross-walls at intervals of from 5 to 30 μ, and bears at distances of 30 to 80 μ the individual conidia into which the protuberances have in the meantime developed (PLATE 1, C). The completed conidium is set off by a basal septum in a plane parallel with and usually tangent to the parent filament (PLATE 2, J, *b*, *c*), though in many instances the septum may be inserted a short distance from the axial hypha, so that the spore instead of always being accurately sessile is sometimes borne on a perceptible spur (PLATE 2, J, *a*, *d*, *e*, *f*). During maturation the protoplasm is withdrawn from the attenuated tip of the conidium, and the evacuated part, after being set off by a wall, persists as a small apical appendage (PLATE 2, J, *a–f*). Exclusive of this appendage the spore consists of a single fusiform colorless cell, surrounded by a thin smooth wall and filled with finely granular protoplasm. Under low or medium magnification, in undisturbed material, the slightly bristling and markedly linear arrangement of the conidia on the empty parent filaments, now matted down or loosely draped on the substratum, or often suspended like gossamer threads between projecting bits of solid material, provides a distinctive view. Such a view, however, is not frequently afforded when cultures are infested with energetic creatures like mites, springtails, nematodes or small earthworms, for not only do the conidia become very readily detached from the parent filaments, but the parent filaments themselves become readily broken up even while the conidia are still in course of development.

Sexual reproduction of the fungus is initiated by the proliferation of hyphal outgrowths about twice as wide as those concerned with asexual reproduction. The outgrowths perforate the loosely enveloping host pellicle, and after growing out into the substratum a variable but usually very short distance, make apical or approximately apical contact with one another in pairs. Each pair of hyphae fuse at the place of contact, after which a spherical body develops, sometimes from the junction (PLATE 2, C, *d*), but much more frequently on a short extension arising from the junction or prolonging one of the hyphae (PLATE 1, B; PLATE 2, C, *a, b, c, e, g*; F). This body continues to increase in size with the movement into it of protoplasm from below until its definitive dimensions are attained, when it becomes delimited usually by a single basal septum (PLATE 2, C). The protoplast within the spherical cell thus formed then undergoes slow reorganization, developing a rather thick wall of its own (PLATE 2, D, *a–d*), which in time becomes contracted so as to present a stellate outer profile with six, seven or eight apices (PLATE 2, E, *a–h*). Between these apices the originally spherical enveloping membrane usually collapses slightly, resulting in relaxed, flat or even depressed facets. The living material occupying the spherical locule within the colorless or slightly yellowish stellate endogenous structure, shows at maturity a parietal layer of rather uniformly coarse granules surrounding a homogeneous central vacuole.

Although the structural relationships of the sexual apparatus are thus tolerably evident, some difficulties of interpretation remain. The very obvious, pronounced separation of the stellate structure from the membrane within which it is formed, is highly suggestive of the separation of oögonial wall and oöspore usual in such monosporous genera as *Pythium* and *Aphanomyces*. In a general way, too, the organization of the protoplasm would seem to conform to the organization usual in oöspores, though the stellate sculpturing injects optical difficulties that make it impossible to determine definitely whether or not a refringent body, the "helle Fleck" of de Bary, is present in the parietal granular layer. On the other hand, the relationships of the sexual hyphae to one another and to their joint product are certainly not those ordinarily obtaining between oögonium, antheridium and oöspore, but correspond essentially to relationships known among some Zygomycetes, such as those figured, for example, by Thaxter for his *Empusa echinospora* (**16**, *pl. 19, fig. 298–302*) and by Van Tieghem for *Syncephalis Cornu* Van Tiegh. & Le Monn. (**18**, *fig. 88–93*). From the account of the latter author, it would appear besides that *S. Cornu* presents similarity to the *Amoeba* parasite also in the development of a spiny spore within the spherical cell arising directly from the fusion of sexual elements, and in the presence of a large "oil drop" in the mature sexual spore. As in accordance with the very plausible "théorie dualiste" referred to by Lendner (**12**) the sexual spore proper of the Zygomycetes, no less than that of the Oömycetes, is essentially endogenous in origin, the development of the sexual apparatus in the present fungus would seem to require interpretation of the spherical cell as a zygosporangium, and of the stellate structure as a zygospore.

Not infrequently while the zygosporangium is still in course of enlargement, a septum appears in one of the sexual hyphae somewhere between its origin and its juncture with the other hypha. It seems at least questionable whether any special significance can be read into this septation, which in its inconstancy invites comparison with the occasional septation occurring in conjugating hyphal bodies of species of *Empusa*. There is little reason for believing that a septum in one of the sexual hyphae is different in function from a septum in the vegetative thallus itself, where a partition would seem to serve a purpose no more abstruse than the separation of a relatively large mass of protoplasm into portions destined for the production of separate reproductive units. In any case cross-walls are more wont to appear in the larger thalli that participate in the production of several zygosporangia besides giving rise, perhaps, to a conidiiferous filament, than in thalli so small that they become exhausted by participation in the development of a single zygosporangium or by proliferation of a single conidiiferous hypha.

To the extent to which the individual thalli can be distinguished from one another in the more or less intricate tangle into which they are usually compacted through the movements of their animal host, two conjugating hyphae seem regularly to originate from different thalli. Conjugation between filaments from thalli in separate *Amoebae* has never been observed, but presumably might well occur if two infected animals should die about at the same time and in close proximity to one another. Apart from the exigencies of a behavior strongly suggestive of heterothallism, the type of reproduction, whether sexual or asexual, is influenced appreciably by proximity to the air. When an infected *Amoeba* containing a number of thalli succumbs on or near the surface of the substratum, a more abundant development of conidia relative to zygospores ordinarily ensues than when the animal dies at a greater depth in the substratum. In the end, whatever the type of reproduction, the evacuated thallus membranes together with the pellicle of the host shrink into an inconspicuous wrinkled mass in which the separate constituents are indistinguishably confused (PLATE 2, D, E).

The parasite is apparently not eligible for inclusion in any genus of the Zygomycetes hitherto described. It would seem to represent the type of a new genus for which a name having reference to its curious spiral endozoic vegetative habit may be appropriate.

Endocochlus gen. nov.

Hyphae nutritae intra corpus amoebarum viventium evolutae, primo continuae, breviusculae, latae, simplices vel parum dichotomae, in spiras convolutae, animali emortuo extus hyphas conidiferas et hyphas zygosporiferas emittentes. Conidia aeria, hyalina, fusoidea vel ellipsoidea, hinc inde ex hyphis aeriis repentibus tenuibus arachnoideis oriunda assurgentia. Zygosporangia globosa intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Vegetative hyphae endozoic, stout, simple or sparingly branched, when well developed often rather regularly and compactly convoluted, at first non-septate, after death of animal giving rise to conidiiferous and zygophoric hyphae. Conidia aerial, hyaline, fusoid or elliptical, borne singly at intervals on long aerial hyphae. Zygosporangia globose, produced in substratum outside of animal host at junction of two similar conjugating branches or on a short hyphal extension from such junction.

Endocochlus asteroides sp. nov.

Hyphae nutritae 4.5–8 μ diam., semel vel bis spiraliter convolutae. Conidia fusoidea, 11–19 × 3.2–4 μ, in apice appendicula 1.5–5 μ longa praedita, ex hyphis arachnoideis 1.2–1.4 μ crassis 1–4 mm. longis ad intervalla 30–80 μ longa enata. Zygosporae hyalinae vel luteolae, echinatae, intra zygosporangium spheroideum 11–14 μ diam. formatae, loculo 5–7.5 μ diam. Hyphae zygosporiferae 2.3–4 μ crassae, 30–65 μ longae.

Hab. in *Amoeba terricola* Greeff (in sensu lato), Washington, D. C.

Vegetative hyphae 4.5 to 8 μ in diameter, simple or often when well developed sparingly branched dichotomously and compactly convoluted in 1 to 2 turns. Conidia spindle-shaped, measuring 11 to 19 μ (mostly 12 to 16 μ) in length by 3.2 to 4 μ (average about 3.6 μ) in diameter, exclusive of an empty apical appendage 1.5 to 5 μ (mostly 1.5 to 3 μ) long and about 1 μ wide at base; produced more or less erect and sessile or nearly sessile at intervals of 30 to 80 μ (mostly 50 to 60 μ) on aerial hyphae 1 to 4 mm. long and 1.2 to 1.4 μ wide. Zygospore colorless or slightly yellowish; at maturity broadly echinulate, the rather thick wall provided with about 20 protuberances of which 6 to 8 are visible in the stellate profile; containing a locule 5 to 7.5 μ in diameter; produced within a spherical zygosporangium 11 to 14 μ in diameter which arises mostly from a short hyphal extension from the junction of zygophoric hyphae that measure usually 30 to 65 μ in length and 2.3 to 4 μ in width.

Destructive to *Amoeba terricola* (in the broad sense more particularly of Penard) in cultures prepared from decaying roots of various herbaceous plants collected near Washington, D. C.

COCHLONEMA VERRUCOSUM

In a single agar plate culture a species of *Amoeba,* somewhat smaller than *A. terricola* I, was found parasitized by a fungus having much the vegetative habit of *Endocochlus asteroides,* but differing from it especially in asexual reproduction. When in a moderately rounded condition the larger individuals of the species of *Amoeba* attacked measured between 50 and 60 μ in diameter. This dimension, considered together with the slightly elliptical or nearly spherical shape of the nucleus (PLATE 3, E), the finely granular transparent character of the protoplasm and the delicate yet firm pellicle, permitted fairly plausible identification of the animal as *A. sphaeronucleolus* Greeff.

At the time the epizoötic was discovered it evidently had already progressed well towards its end, as all the individuals of the susceptible species still alive in the culture bore within themselves thalli of the parasite, varying in number from one to three, at different stages of development (PLATE 3, D, E). Consequently the entrance of the fungus into the animal could not be directly observed, a circumstance of lesser moment, however, as the mode of ingress could here be inferred even from material in advanced stages of development. For very generally to each of the endozoic thalli was still attached a small fusoid body with minutely verrucose membrane, readily recognizable as the conidium from which growth had proceeded (PLATE 3, C, D, E). Manifestly the conidium, after having been ingested by the hapless animal, had germinated laterally, the germ tube immediately widening out and directing its growth spirally, so as to yield a convolute thallus slightly less stout but otherwise resembling that of *Endocochlus asteroides*.

Asexual reproduction of the fungus is initiated when the contents of the animal have been reduced to such an extent that locomotion has virtually ceased (PLATE 3, D), though nucleus, contractile vacuole and remnants of cytoplasm usually continue for some time to present a fairly normal appearance (PLATE 3, E). Mostly from a position near the proximal end of the thallus is put forth a delicate hypha which perforates the pellicle and, if the animal is at all submerged, makes its way to the surface of the substratum. Near or on this surface, if it has not already become branched, the filament undergoes some ramification (PLATE 3, C, E; PLATE 4, A). The resulting branches after spreading in divergent directions for variable distances, grow up into the air, where each gives rise to a more or less erect aerial chain of usually 30 to 40 fusoid verrucose conidia (PLATE 4, A).

The chains of conidia show the general features associated with the genus *Fusidium* in the Hyphomycetes. In spite of the considerable attention devoted to the details of spore formation in this species as well as in the other three catenate forms to be described, it has not been possible, owing to difficulties attending observation, to determine this phase of developmental morphology with as much certainty as might be desired. The structures concerned are too small to be studied successfully in their normal aerial state under a dry objective. On the other hand when developing and hence still immature sporogenous filaments are mounted in water, their appearance suggests so strongly a prompt intervention of degenerative change that the normal course of events remains somewhat a matter of inference. However, judging from the more satisfactory microscopic preparations, it would seem that the spore chains have origin in continuous aerial filaments which from an early stage, if, indeed, not from the very beginning, are characterized by *Leptomitus*-like constrictions spaced at equal intervals (PLATE 3, E; PLATE 4, A, *b, c*). These regularly constricted filaments exhibit minute verrucose sculpturing that does not extend proximally into the sterile parts of the supporting hyphae. Apparently some slight increase in size takes place before the distended parts become separated from one another through withdrawal of protoplasm from the constricted isthmi and formation of a delimiting partition at either end of the individual swellings (PLATE 4, A, *a, d*). In a mature condition the chains are very easily broken up, so that the conidia soon come to lie separately on the surface of the substratum (PLATE 3, F), awaiting the passage of a susceptible animal, and a repetition of the developmental cycle just outlined.

Sexual reproduction of the fungus was observed in connection with but a single animal, and in this was represented by only two unions (PLATE 3, G). Unfortunately, moreover, the membranes of the empty thalli and their hyphal outgrowths were already so badly collapsed that none of the zygophores could with certainty be followed backward any considerable distance from junction to origin. The junction, which in both cases was revealed plainly, appeared similar to that in *Endocochlus asteroides*, and as usually in the latter fungus bore the zygosporangium on a short prolongation. The zygosporangium here, however, was much more distinctly yellowish in coloration, and instead of being smooth, bore numerous prominent bullate protuberances. Within this sculptured primary fusion cell, to extend the contrast, was revealed in the one nearly mature specimen a zygospore with a smooth wall of moderate thickness.

In shape and sculpturing of conidium as well as in the persistence of that structure on the endozoic thallus, the fungus shows obvious similarities to the form which Penard (**15**) found parasitizing *Amoeba alba* Greeff and discussed as *Saprolegnia* B. Although the Swiss zoölogist did not succeed in observing the production of the conidia described by him, very probably because he employed mostly cultures of aquatic rather than terrestrial character, their close correspondence to those of the present fungus gives much ground for believing that he may have been dealing with an intimately related catenulate form. It may be mentioned that a cochleate endoparasite with catenulate conidia of approximately the large dimensions given by Penard occurred some years ago on an unidentified *Amoeba* in one of my agar plate cultures, though because of unfamiliarity with the type of fungus in question, which was then mistakenly judged to be referable to *Fusidium*, the scanty material was dissipated before more accurate records had been made of morphological details. However, the method of reproduction by zoöspores that was attributed, even if only with partial assurance, to *Saprolegnia* B, has never been observed either in the inadequately studied species or in any of the catenulate forms dealt with herein; nor would the swollen rather irregularly branching mycelium figured by Penard appear to conform any too well to the regularly involute type of endozoic thallus.

Since the mode of asexual reproduction represented here differs conspicuously from that set forth as characteristic of *Endocochlus*, a separate genus is proposed:

Cochlonema gen. nov.

Hyphae nutritae intra corpus amoebarum viventium evolutae, primo continuae, breviusculae, latae, simplices vel parum ramosae, in spiras convolutae, animali emortuo vel moriente extus hyphas conidiferas et hyphas zygosporiferas emittentes. Conidia aeria, hyalina, fusoidea vel elongata, in catenulas longiusculas simplices plus minusve erectas digesta. Zygosporangia globosa, intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Vegetative hyphae endozoic, stout, simple or sparingly branched, when well developed often rather regularly and compactly convoluted, at first continuous, with decline or on death of host animal giving rise to delicate conidiiferous and usually somewhat stouter zygophoric filaments that pass through the host envelope. Conidia aerial, hyaline, fusoid or elongated, produced in long chains from more or less erect aerial hyphae. Zygosporangium globose, produced on a short prolongation from the apical junction, or directly from the junction of two similar filaments, in the material surrounding or underlying the animal.

Cochlonema verrucosum sp. nov.

Hyphae nutritae 4.5–7 μ diam., semel vel bis spiraliter convolutae. Conidia verruculosa, fusoidea, 6–9 × 1.4–2 μ, ex hyphis saepius 1.2–1.5 μ crassis enata, in quaque catenula tricena usque quadragena. Zygosporangia flavida, globosa, circa 12 μ diam., 20–30 verrucis ornata; verrucae 1.5–2 μ altae, basi 2.5–3 μ diam.; zygosporae globosae. Hyphae zygosporiferae breviusculae, 2–3 μ crassae.

Hab. in *Amoeba sphaeronucleolo*, Washington, D. C.

Vegetative hyphae usually 4.5 to 7 μ in diameter, simple or often when well developed sparingly branched and rather compactly convoluted in 1 to 2 turns. Conidia minutely verrucose, fusoid, measuring 6 to 9 μ in length by 1.4 to 2 μ in diameter, produced in chains usually of 30 or 40, on hyphae mostly 1.2 to 1.5 μ wide. Zygosporangia yellowish, globose, measuring about 12 μ in diameter exclusive of the warty protuberances 20 to 30 in number that measure often 2.5 to 3 μ in basal diameter and 1.5 to 2 μ in height; zygospores smooth, globose; zygophoric hyphae rather short, mostly 2 to 3 μ wide.

Destructive to *Amoeba sphaeronucleolus* in a laboratory culture, Washington, D. C.

COCHLONEMA DOLICHOSPORUM

A fungus very similar to the one just described was found in small quantity in another agar plate culture destroying *Amoebae* having approximately the same dimensions as *Amoeba sphaeronucleolus*. Animals in stages of infection early enough to reveal their nuclear condition were not observed, however, so that the identity of the host remains for the time being uncertain. As in the case of *Cochlonema verrucosum* the conidium here is evidently ingested bodily, since it likewise is regularly to be seen attached to the endozoic thallus, though attached somewhat less closely by a delicate germ tube of appreciable length (PLATE 3, A; PLATE 4, B). Asexual reproduction results here also in chains of aerial conidia (PLATE 4, B, *a, b, c*), the individual conidium being, however, two or three times as long, somewhat more prominently sculptured, and often when fully mature evacuated of protoplasm in the distal narrower part, which then nevertheless persists as an empty appendage (PLATE 3, B). Accordingly, whereas the conidium when newly delimited is ordinarily slightly obclavate in shape, after evacuation of the appendage the cell left filled with protoplasm is more nearly symmetrical, that is, roughly elongated fusoid in shape. Sexual reproduction has not been observed.

Cochlonema dolichosporum sp. nov.

Hyphae nutritae circa 6 μ diam., semel vel bis spiraliter convolutae. Conidia verrucosa, 15–25 × 1.2–2 μ, ex hyphis 1–1.5 crassis enata, in quaque catenula quina usque vicena, primo saepius nonnihil obclavata, maturitate apice saepius vacuo, tum cellula viventi plus minusve fusoidea vel cylindracea. Zygosporae ignotae.

Hab. in *Amoeba* sp., Washington, D. C.

Vegetative hyphae approximately 6 μ in diameter, convolved into a spiral of 1 to 2 turns. Conidia verrucose, measuring 15 to 25 μ in length and 1.2 to 2 μ in width, borne in chains of 5 to 20 each on hyphae 1 to 1.5 μ wide, at first somewhat broader in proximal than in distal portion, later often becoming evacuated in the narrower apical part, which then persists as an appendage up to 7 μ long on the cylindrical or somewhat fusoid living cell. Zygospores unknown.

Destructive to *Amoeba* sp. in a laboratory culture, Washington, D. C.

BDELLOSPORA HELICOIDES

A fungus strongly resembling the two species of *Cochlonema* in its asexual reproduction was observed in a number of agar plate cultures, usually in quantity, habitually parasitizing a species of *Amoeba* the larger individuals of which when fairly well rounded up measured from 60 to 90 μ in diameter. The cytoplasm of the animal was in general finely granular, colorless and decidedly transparent; its pellicle firm, perhaps rather thicker than in most terrestrial forms, and therefore inclined to be cast into fewer but more pronounced folds. The single nucleus (PLATE 5, B, *n*) having the shape of a prolate ellipsoid of revolution showed at the poles accumulations of darkish irregular lumps that thinned out to a single layer in the equatorial periphery. The structural features of the animal thus correspond tolerably well to those that Penard (15) ascribed to his *A. terricola* var. *papyracea*. Yet no close approximation to the considerably larger dimensions cited by Penard has ever been observed in my material, so that a presumption of identity would seem at least doubtful. The animal, of course, readily conforms to Penard's broad concept of *A. terricola,* and may thus be conveniently referred to as *A. terricola* II, the appended numeral being intended to distinguish it from the habitual host of *Endocochlus asteroides,* as well as from the *Amoeba* attacked by the fungus to be described as *Zoopage phanera.*

Infection is initiated in much the same way as was described in the account of *Endocochlus asteroides.* Conidia of the parasite strewn about on the surface of the substratum (PLATE 5, A) make contact with the *Amoeba* as it passes over them and remains adhering to the pellicle in spite of subsequent locomotion of the animal both on and through the substratum. Each adhering spore soon puts forth a delicate germ tube which perforates the pellicle and continues in its course into the host for a distance often about equal to the length of the conidium (PLATE 5, B, *a*). From this stage on, analogy with *E. asteroides* ceases. The tip of the infective germ tube instead of developing a globular body into which the conidial contents are received, becomes dichotomously branched (PLATE 5, B, *b–f*). Each of the divergent limbs soon branches in its turn, and in a plane at a right angle to that of the original dichotomy (PLATE 5, B, *g*). A third bifurcation follows the second (PLATE 5, B, *h*), and in some cases a fourth bifurcation takes place (PLATE 5, B, *i*), so that eventually a compactly branching apparatus with 8 to 16 terminal elements is brought into being. This apparatus has all the appearance of a haustorium and, indeed, manifestly is one; for coincident with the first bifurcation the spore outside of the animal begins to show perceptible swelling (PLATE 5, B, *a–f*). This swelling continues until the conidium has expanded into an obese ellipsoidal body whose mode of origin is later often betrayed only in two minute protruding polar apiculi representing the apparently inelastic spore extremities (PLATE 5, B, *i*).

While in the early stages of an epizoötic individual *Amoebae* are often found with but a single infection, plural infections become the rule subsequently, when with an increasingly abundant and thoroughgoing distribution of conidia the encounters between host and parasite become more and more frequent. Accordingly in the more advanced phases of an epizoötic, animals beset with ten or a dozen separate plants in various stages of development are not of infrequent occurrence (PLATE 5, B). Locomotion although impeded physically by the presence of the larger swollen spore bodies, usually continues for some time after the progressive exhaustion of protoplasmic contents has entailed a readily noticeable contraction of the animal's bulk. Its cessation, in fact, ordinarily is brought about not primarily by exhaustion of the host as in the case of *Amoebae* infected with *Endocochlus asteroides,* but through virtual anchoring of the host to or in the substratum that takes place with the proliferation of hyphal outgrowths from spore bodies preparatory to reproduction. The anchored animal continues to furnish food materials to the parasite during a protracted period of reproductive development, so that conidial chains (PLATE 6) or sexual apparatus (PLATE 5, C) may be displayed in abundance even while the host animal is still alive. The parasitized *Amoeba* in the last stages preceding death is rounded up into an almost spherical shape, its pellicle showing few irregularities except for the depressions where the spore bodies are attached (PLATE 5, C; PLATE 6). Even with the animal reduced to perhaps a third of its original bulk, the granular material now remaining suffices only to provide a thin parietal layer surrounding an immense central vacuole. Often protruding conspicuously from the parietal cytoplasmic layer into the central vacuole is a vesicle-like vacuole, evidently to be interpreted as the contracting vacuole, now greatly enlarged apparently owing to its incapacity to discharge through the thickened pellicle.

As has been intimated asexual reproduction of the ectoparasite follows the same general course as in *Cochlonema verrucosum* and *C. dolichosporum.* Like the convolute endozoic thalli of these fungi, the swollen spore bodies here put forth a single hypha, or less often several hyphae. Each filament, after traversing the substratum for a variable distance depending in some measure at least on the depth at which the host animal was halted, reaches the surface where immediately or after some prostrate growth its further extension becomes aerial and more or less erect. One or several branches may be given off in either the intramatrical (PLATE 6, C), the superficial (PLATE 6, B), or the aerial (PLATE 6, A–D) part. In any case, on each of the resulting aerial hyphae is formed terminally a chain (PLATE 6, A, *a*, *c*; B, *b*, *d*; C, *a*, *b*; D) of irregularly fusiform conidia, between a half-dozen and a score in number. With an adequate supply of nourishment development of a second chain of spores from a branch having origin just below the base of the first chain (PLATE 6, A, *b*, *d*; B, *a*, *c*), and even of a third chain from a second branch, may ensue. On maturity the chains are broken up on relatively slight disturbance, as, for example, on being brushed by passing nematodes; so that the conidia are soon strewn about on the substratum, ready to infect any susceptible *Amoeba* that may happen to come along.

The external position of the spore bodies on the animal, and their globose shape that makes it easy to distinguish them from one another even under crowded conditions, provide in this species circumstances more favorable for determining the relationships involved in sexual reproduction than obtain in any of the related convoluted endozoic parasites. In spite of a frequently somewhat intricate arrangement here of the zygophoric hyphae themselves, two conjugating filaments can always be traced back to separate spore bodies, never to the same spore body (PLATE 5, C). These filaments whether representing direct outgrowths of their respective spore bodies (PLATE 5, C, *d*), or primary (PLATE 5, C, *c*) or even secondary branches of such outgrowths (PLATE 5, C, *a*, *b*), usually engage one another very close to the swollen structures, if not in immediate proximity to them. After establishing apical contact they continue to elongate, twisting about one another in conspicuously regular close helical turns. Each of the hyphae makes from two to four turns, the total number of windings in the intertwined helices thus varying usually between four and eight. No discrimination with respect to direction of rotation, whether dextrorse or sinistrorse, is evident.

The intervolved hyphae now fuse apically or approximately apically. On the end of a short cylindrical part continuing one of the hyphae or arising more symmetrically from the junction, a globose body buds out and increases in size to form a zygosporangium (PLATE 5, C). By this time a septum is present in each of the zygophores usually at a point about median in the intervolved part. The distal halves of the zygophoric filaments delimited proximally by these septa constitute elements that would seem to correspond more plausibly to the gametangia of the more familiar genera in the Zygomycetes than any structural constituents of the sexual apparatus produced by any of the other fungi herein described, or by any of the minute predacious forms figured earlier (**9**, *figs. 3–5*). After the terminal zygophoric parts have contributed their contents to the zygosporangium, the latter is partitioned off from its supporting element by a basal septum. In the meantime the originally smooth zygosporangium has become sculptured through the putting forth of noticeably thick-walled wart-like or bullate protuberances. Internal development now ensues with the result that finally the sculptured wall comes to envelop rather closely a zygospore proper, which at maturity has a smooth wall of moderate thickness, and within this wall a parietal layer of uniformly and rather coarsely granular material surrounding an apparently homogeneous, relative large reserve globule (PLATE 5, D–H, I, *a–h*). A clustered arrangement of these distinctly yellowish sexual structures in the substratum about a wrinkled residual mass in which the shrunken pellicle of the host and the collapsed membranes of the parasite are mostly unrecognizably confused, remains to mark the place where an animal came to its end (PLATE 5, I). Indeed, just as in the case of *Endocochlus asteroides*, the presence of such clustered arrangements often constitutes the only testimony to a former abundance of the susceptible *Amoeba* species in cultures from which it has been exterminated.

The close resemblance of the zygosporangium and zygospore to those of *Cochlonema verrucosum* suggests the possibility that the remarkable intervolution of zygophores in the present fungus, so reminiscent of the relationships of *Syncephalis nodosa* van Tiegh. as illustrated in the beautiful figures of Bainier (**2**, **3**) and of Thaxter (**17**), may constitute a feature having significance only as a character pertaining to the species. A consideration of sexual as of asexual reproduction, therefore, reveals no decisive reason for not assigning the fungus to *Cochlonema*. However, the remarkable epizoic habit with its striking analogy to the habit prevalent among the Rhizidiaceae in the Chytridiales, offers so direct an antithesis to that exemplified in *Cochlonema* and *Endocochlus* that assignment to either of these genera would seem inappropriate. A separate genus is accordingly proposed under a name which is intended to bring into relief the leech-like behavior, so to speak, of the conidium.

Bdellospora gen. nov.

Conidia aeria, hyalina, fusoidea vel elongata, in catenulas longiusculas simplices plus minusve erectas digesta; ad pelliculam animalium adhaerentia, hypha germinationis pelliculam perforantia, haustorium ramosum intus evolventia, tum magnopere tumescentia, mox hyphas conidiferas et hyphas zygosporiferas emittentia. Zygosporangia globosa intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Conidia aerial, hyaline, fusoid or elongate, arising in frequently long chains from more or less erect aerial hyphae; adhering to animals and after individually perforating the pellicle or integument by means of a germ tube that develops into a haustorium inside, swelling into large globose-ellipsoidal bodies from which conidiiferous and zygophoric hyphae grow out. Zygosporangia globose, developed in the material surrounding or underlying the dying animal from the junction of two similar conjugating filaments.

Bdellospora helicoides sp. nov.

Conidia fusoidea, nonnihil angulata, 6–16 × 2–3 μ, ex hyphis 1.3–2 μ crassis enata, in quaque catenula quina usque vicena; post amplificationem globoso-ellipsoidea, usque 15 μ diam., 20 μ longa, haustorio pedicellato usque ter vel quater breviter bifurcato. Hyphae zygosporiferae inter se quater vel octies spiraliter circumplicantes, utraque ex conidio turgido alio enata, basi saepius 2–2.5 μ crassae, sursum 3–4.5 μ crassae, 30–75 μ longae, septo in partes duas fere subaequaliter divisae. Zygosporangia 8–13 μ diam., flavida, 10–30 verrucis 1–1.5 μ altis, 1.5–3 μ diam. ornata. Zygosporae globosae.

Hab. in *Amoeba terricola* (in senso lato), Washington, D. C.

Conidia fusoid, measuring 6 to 16 μ (average 10 μ) in length by 2 to 3 μ (average 2.7 μ) in width, somewhat angular in outline, produced in chains usually of 5 to 20 on aerial hyphae mostly 1.3 to 2 μ wide; after vegetative enlargement globose-ellipsoidal, measuring up to 15 μ or more in transverse diameter, and up to 20 μ or more in length, each conidial body being provided with a haustorium consisting of a germ pedicel 3 to 7 μ long and up to 1.5 μ wide together with short elements up to 2.5 μ wide in a terminal closely dichotomous branching system. Zygophoric hyphae 4 to 8 times intervoluted, each of a conjugating pair arising from a separate swollen conidium, measuring 2 to 2.5 μ in diameter at the base but widening toward the apex to a diameter of 3 to 4.5 μ, mostly 30 to 75 μ long, and regularly divided by a transverse septum into 2 nearly equal parts. Zygosporangia mostly 8 to 13 μ in diameter, distinctly yellowish, ornamented with 10 to 30 (mostly about 25) wartlike protuberances measuring 1 to 1.5 μ in height and 1.5 to 3 μ in basal diameter. Zygospores globose, smooth.

Parasitic on *Amoeba terricola* (in the broad sense especially of Penard) in laboratory cultures made from decaying rootlets collected near Washington, D. C.

ZOOPAGE PHANERA

More readily visible in its results than any of the parasitic relationships herein discussed is a predacious relationship involving as prey a large and relatively opaque species of *Amoeba* that often becomes very abundant in aging plate cultures. Though the species is somewhat larger even than the one attacked by *Bdellospora helicoides,* measuring between 35 and 110 μ in diameter when drawn up into a more or less rounded shape, its pellicle is so delicate as to appear under high magnification as a single-contoured membrane. The cytoplasm of the animal consists of granular material in much larger proportion than in most terricolous forms, and accordingly presents an appearance closely simulating that of the plasmodia of various myxomycetes often present in the same cultures. In its rather dark and almost opaque cytoplasmic matrix the single nucleus is not easily to be found (PLATE 7, A, *d*), yet at opportune moments it can be discerned as a prolate elliptical body composed of a central hyaline part surrounded by a slightly darker external layer (PLATE 7, A, *n*). While the animal thus conforms to Penard's broad concept of *A. terricola* it has an appearance much different from the appearance of the habitual host of either *Endocochlus asteroides* or *B. helicoides*; and would seem, moreover, to be as completely immune from being parasitized by these fungi as their habitual hosts are immune from capture by the fungus preying upon it. In view of these differences, and of what would seem to be a somewhat indiscriminate application of Greeff's binomial, requirements for more specific reference suggest again the use provisionally of an appended numeral in the designation *A. terricola* III.

The fungus destructive to this *Amoeba* is represented in its vegetative stage by a non-septate mycelium branching at moderate intervals both within and on the surface of the substratum, the branches being directed usually at wide angles to their parent filaments in a seemingly ill-defined haphazard manner (PLATE 7, A). Here and there the hyphae show noticeable though usually not pronounced variations in diameter. The commonplace morphology of the diffuse mycelium together with the absence of visible adhesive material is not at all suggestive of a predacious habit. When, however, an animal in the course of its customary wanderings makes contact with a mycelial filament, the contact proves unexpectedly persistent and tends to increase in extent, so that a certain measure of inwrapment often results, especially in cases where additional filaments nearby are also engaged (PLATE 7, A, *a*). From its applied surface the mycelium buds forth at intervals delicate processes that perforate the pellicle, penetrate a short distance into the body of the animal to give rise there individually to several swollen lobules in botryoid arrangement (PLATE 7, A, *a–d*). Though these endozoic processes are of rather small dimensions and ordinarily do not exceed a half-dozen in number, their intrusion is soon followed by obvious degenerative changes in the animal's cytoplasm. The *Amoeba* here concerned, unlike the forms parasitized by *Endocochlus asteroides* and *Bdellospora helicoides,* shows little endurance to internal attack, usually succumbing before the cellular contents have become greatly reduced (PLATE 7, A, *b, c*).

If the general course of the predacious relationship can be followed without any difficulty, the precise manner of capture is a matter of inference rather than of direct observation. The stalked processes with their botryoid terminations present, it is true, much the appearance of grappling organs, but whether they actually function as such seems somewhat unlikely, as the semi-liquid or perhaps softly gelatinous consistency of the protoplasm in which they are immersed can hardly be assumed to furnish anchorage firm enough to resist the ordinary locomotor pull of the animal.

Moreover the very production of the processes must have as an antecedent intimate contact for a more or less protracted period between parent hypha and pellicle. Since this contact is maintained in spite of the animal's restricted but appreciably lively movements in all directions, it may be presumed that adhesion is operative here even though visible deposits of sticky material cannot be distinguished. In mode of application a close similarity to the conidium of *Bdellospora helicoides,* which likewise adheres to its host without an adhesive substance being visible, thus becomes apparent. The similarity is extended in an unmistakable correspondence between the endozoic parts growing from the adhering structures, in accordance with which the delicate stalked processes are interpretable as haustoria rather than as capturing organs.

In whatever the apparatus of capture may consist, as to its efficacy at least there can be no question. When a soft agar medium has been used in making a culture, the *Amoebae* move about freely in or through as well as on the substratum, and are then caught largely here and there on the submerged mycelial hyphae without becoming concentrated anywhere so as to cause much of a display. With the employment of a harder agar medium, however, the animals are constrained to live, feed and move about exclusively on the surface of the culture; and naturally it is then only on the surface that they are captured. As a result scores and even hundreds of *Amoebae* in various stages of decline or disintegration then often accumulate on the restricted areas occupied by separate mycelial tracts of the fungus, becoming readily visible to the naked eye as a superficial deposit of greyish stippled aspect. At later stages the deposit usually is obscured somewhat under a whitish pulverulent efflorescence, which upon microscopic examination is revealed as consisting of a profuse tangle of conidial chains.

In addition to their origin in catenulate arrangement the conidia of the fungus show resemblances to those of *Cochlonema verrucosum* and *Bdellospora helicoides* in their generally fusiform shape as well as in the sculpturing of their enveloping membranes (PLATE 7, A, *f*, *g*; B, *a–c*). The spore chains are borne on usually rather short, erect, distally attenuated branches that arise without much regularity from the superficial hyphae. Because the chains are usually long—lengths of from .5 mm. to 1 mm. being not infrequent—relative to their width especially at the constricted connections, they usually droop until the distal portions come in contact with the substratum. On maturation they are broken up into the separate conidia when disturbed even slightly as by the jostling of a passing nematode.

Sexual reproduction of the fungus takes place abundantly in the substratum underlying the conidial chains. Two branches, one often more or less contorted (PLATE 7, B, *d*, *e*; C–G), the other more nearly straightforward in course, arising from separate elements, whether mycelial filaments (PLATE 7, B, *d*, *e*; C–H) or germinating conidia (PLATE 7, N, O), encounter one another, fusion takes place, and at the junction a spherical body makes its appearance. This body increases steadily in size, being supplied with protoplasm from both branches, with each of which it communicates directly, never indirectly through a single common prolongation as in the sexual apparatus of the parasites previously described herein. During the earlier stages the branches remain continuous, but later, usually when the globular body is well along in development, a cross-wall appears in one or both of them at some distance from the body. On attaining definitive size the spherical body is delimited from each of the branches by an approximately tangential septum (PLATE 7, G, H). Within the smooth zygosporangium thus formed is developed the zygospore proper, a distinctly yellowish structure which at complete maturity reveals a handsomely sculptured, bullate wall, a parietal layer of uniformly coarsely granular material and a central reserve globule (PLATE 7, I–M). The zygosporangial membrane generally collapses somewhat between the bullate protuberances, though remaining recognizable usually as a separate envelope.

The predacious habit of the fungus entails such a marked difference in morphology of vegetative thallus from the genera *Cochlonema* and *Bdellospora* that the proposal of an additional genus, under a name constructed from two words meaning " animal " and " anything that fixes or fastens " respectively, appears justified.

Zoopage gen. nov.

Mycelium effusum; hyphis ad pelliculam animalium adhaerantibus, ramulis hanc perforantibus, haustoria intus evolventibus. Conidia aeria, hyalina, fusoidea vel elongata, in catenulas saepe longas plus minusve erectas digesta. Zygosporangia globosa, intra materiam ambientem vel subjacentem ex copulatione hypharum similium orta.

Mycelium effuse, the hyphae adhering to the pellicle or integument of an animal, perforating it and producing a haustorium inside. Conidia aerial, hyaline, fusoid to elongated, arising in more or less erect chains from aerial branches. Zygosporangia globose, produced in the substratum at the junction of two conjugating hyphae.

Zoopage phanera sp. nov.

Mycelium ramosum; hyphis hyalinis, 1.2–2.7 μ crassis; haustoriis pedicellatis, pedicello .5–1 μ crasso, 3–6 μ longo, 3–7 lobulos turgidos saepe 2–3 μ longos et crassos ferente. Conidia minute verrucosa, elongato-fusoidea, 25–60 μ, saepius 35–45 μ longa, 2.2–2.8 μ crassa, ex apice hypharum saepius brevium oriunda, in quaque catenula quina usque vicena quina. Zygosporangia 9–12 μ diam., primum levia, maturitate nonnihil collabentia. Zygosporae flavidae, 6.5–10 μ diam., loculo 5–7 μ diam., membrana .6–1.5 μ crassa 15–30 verrucis ornata.

Hab. in terra, *Amoebam terricolam* (in senso lato) capiens et consumens, Washington, D. C.

Mycelium branched; hyphae 1.2 to 2.7 μ wide; haustoria pedicellate, the pedicels, mostly .5 to 1 μ in width and 3 to 6 μ in length, bearing apically in botryoid arrangement from 3 to 7 lobulations measuring 1.5 to 6 μ, mostly 2 to 3 μ, in length and in thickness. Conidia elongated fusiform, minutely verrucose, measuring 25 to 60 μ, mostly 35 to 45 μ (average 40 μ) in length and 2.2 to 2.8 μ (average 2.4 μ) in width, produced in chains of 5 to 25 on distally attenuated, mostly short branches. Zygosporangium 9 to 12 μ in diameter, at first smooth, at maturity collapsing somewhat about the sculptured zygospore. Zygospore yellowish, 6.5 to 10 μ in diameter, with a locule 5 to 7 μ in diameter and a wall .6 to 1.5 μ thick; the wall provided with 15 to 30 verrucose protuberances, of which 6 to 8 are visible in profile on its outer sigillate contour.

Capturing and destroying *Amoeba terricola* (in the broad sense more particularly of Penard), Washington, D. C.

TAXONOMIC CONSIDERATIONS

The similarity of the intervolved zygospores of *Bdellospora helicoides,* even in the absence of bladder-like appendages, to the spirally intertwined sexual branches of *Syncephalis nodosa* directs attention to the possibility of a homological relationship between the conidial chains produced in three of the newly erected genera with the rows of asexual spores characteristic of *Syncephalis, Piptocephalis* and *Syncephalastrum*. An important difference is, of course, at once apparent in the absence here of anything at all corresponding to the simple or branching, usually rather stout, erect hyphae whose remarkably varied capitate development must have facilitated the plausible interpretation of the ultimate cylindrical elements borne on them as constituting either linear sporangia or sporangial ramuscules, wherein the spores though formed in a row are nevertheless formed endogenously much as in species of *Mucor* or *Rhizopus*. It might be argued with some cogency, however, that endogenous formation of asexual spores could conceivably occur in the Zygomycetes unassociated with the easily recognizable correspondencies of external morphology through which homologies with the very familiar spherical type of sporangium have been brought into relief; that, in fine, an element approximately homologous to a linear sporangium or sporangial ramuscule might be formed, without the intervention of a conspicuously differentiated sporangiferous hypha, directly on a commonplace filament. The proof of endogenous spore production here would necessarily depend more nearly exclusively on optical evidence, like that adduced by Thaxter (**17**) in the case of *Syncephalastrum racemosum* Cohn, showing physical separateness of the spore wall from a sporangial wall or ramuscule wall enveloping it.

No such conclusive evidence bearing on sporulation in the four catenulate fungi described herein has come to light, though a few morphological details permit of possible alternative interpretations favorable to endogeny. Thus the development of spore chains from originally continuous filaments with regularly spaced constrictions might be taken to imply parallelism with the type of spore formation illustrated by Thaxter in several figures (**17**, *pl. 1, figs. 9–11*) of his *Syncephalis Wynneae*; or, on the other hand, it might be construed as representing merely a modification of the type of spore formation familiar in *Oospora lactis* Fres. Again the presence of sculpturing on the conidia of all four catenulate species, when the conidia of comparable non-catenulate *Amoeba-*

destroying fungi are uniformly smooth, might or might not be taken to indicate parallelism with *Syncephalis intermedia* Van Tiegh. and *Syncephalis nodosa*, where according to Van Tieghem (**18**) the wrinkled exterior is attributable to the persistence of the segment of the sporangial envelope surrounding the conidium proper.

In any case, concerning the intimate relationship of these non-catenulate *Amoeba*-destroying forms with the catenulate forms there assuredly can be no serious doubt. The strong similarity in vegetative habit between *Endocochlus asteroides* and *Cochlonema verrucosum*, supported by a general parallelism in make-up of sexual apparatus, provides adequate testimony to a close affinity between these two species. Nor are the two types of conidial production exemplified in these species entirely irreconcilable with one another. The segments resulting from the insertion of the first order of septa in the conidiiferous hyphae of *Endocochlus* can without much straining be homologized with the seriate swellings in the young spore chains of *Cochlonema*; the lateral conidia proliferated from them then becoming interpretable as the immediate exogenous products of structures equivalent to catenated conidia. The sessile conidia of three of the delicate Phycomycetes capturing rather small *Amoebae*, which were figured in my earlier note (**9**, *figs. 2, 4, 5*), appear certainly morphologically equivalent to the conidia of *Endocochlus*, even though they are produced from prostrate, predacious and hence primarily vegetative hyphae rather than from primarily reproductive aerial filaments. In the two (**9**, *figs. 4, E*; *5, E, F*) of the three forms for which sexual reproduction is known, this type of reproduction is closely similar to that found in *Zoopage*, the small originally smooth zygosporangium communicating separately with each of the outwardly almost undifferentiated zygophoric branches; and its wall later collapsing slightly over a sculptured zygospore. Precisely the same make-up of sexual apparatus is represented also in a fourth delicate predacious form whose small conidia are produced successively on a delicate erect sporophore (**9**, *fig. 3*). A simplification of this multiple proliferation of conidia appears in an *Amoeba*-capturing fungus yet to be described, which gives rise to an elongated obovoid conidium, measuring on an average about 15 μ in length by 6.4 μ in diameter, at the tip of an erect conidiophore usually about 200 μ long and .8 μ wide. This form is of especial importance as it reveals such thoroughgoing parallelism with the much larger Phycomycete capturing nematodes by adhesion, which was figured in a brief summary (**8**, *fig. 8*), that a close natural relationship is sufficiently obvious.

Through the ramification of overlapping resemblances, therefore, the five fungi newly described are shown to be related rather intimately with forms differing greatly from them and from one another in the arrangement and dimensions of their conidial apparatus. It may be presumed that fungi of similar morphology have been encountered by investigators from time to time; yet, aside from some descriptions of filamentous growths associated especially with protozoans, hardly any mycological writings can be referred at all plausibly to the group under consideration. The *Saprolegnia* B of Penard seems almost certainly to belong here, though the production of zoöspores attributed to the fungus is little consonant with development in the Zygomycetes generally. The mycelium which Dangeard (**4**) described as attacking an *Amoeba* by means of a bifurcating haustorium, and to which in the absence of asexual and sexual reproductive structures he applied the binomial *Rhizoblepharis amoebina*, might be referred to the group under discussion with more plausibility if dichotomous branching of the haustorium did not also occur, and, indeed, in much greater measure, in the *Amoeba*-capturing fungus figured earlier (**9**, *fig. 1*), whose freely septate mycelium and septate, appendaged conidium rather clearly indicate mucedinaceous affinities. Possibly the filamentous outgrowths that on being found attached rather consistently to two species of *Amoeba* by Leidy (**11**) were mistaken by him for normal appendages and thus made the basis for a separate genus *Ouramoeba*, may belong here, the chains of fusoid segments shown in the figures of *O. botulicauda* Leidy (**11**, *pl. 9, figs. 13–17*) being especially suggestive of relationship. While the filamentous attachments (*Amoebophilus Korotneffi* Dang.) that led Korotneff (**10**) likewise to propose a new genus of *Amoebae*, *Longicauda*, show somewhat less resemblance to the known members of the group in question, the possibility of relationship is yet not to be excluded; and the same situation obtains apparently in regard to the different outgrowths (*Amoebophilus caudatus* Dang.) which Penard (**14**) figured and discussed as being fungi attached to *Amoeba nobilis* Pen. and *Amoeba vespertilio* Pen. Dangeard's figures of the fungus outgrowths on his *Pelomyxa vorax* to which he (**5**) applied the binomial *Amoebophilus Penardi* reveal *Leptomitus*-like constrictions through which a condition not greatly unlike that evident in immature spore chains of *Cochlonema verrucosum* and *Bdellospora helicoides* is brought about.

In view of the extensive study devoted to *Amoebae* for many decades the paucity of literary references to fungi possibly assignable to the series under consideration might seem remarkable, especially as much more definite descriptions and records of parasites belonging to such relatively difficult chytridiaceous genera as *Sphaerita* and *Nucleophaga* are available in some number. The explanation for this paucity very probably lies in the fact that protozoölogists have very largely kept or cultivated in water the animals studied by them. All of the five newly described fungi as well as the various allied predacious forms are pronouncedly terrestrial in their asexual reproduction, if not also in their sexual reproduction and biological adaptations. It appears very doubtful whether the adhesion of conidium or filament in *Endocochlus asteroides, Bdellospora helicoides* and *Zoopage phanera* necessary for penetration can occur when the host animal is bathed in free liquid water. Even if vegetative development were provided for under aquatic conditions, the production of conidia, so important in the multiplication of these fungi, would generally be meager as evidently it can proceed only in the air. In cultures consisting of irrigated decaying plant materials, one of the predacious forms figured earlier (**9**, *fig. 2*) has often been seen to extend its mycelium, partly submerged, partly floating, some distance from the solid substratum, but its conidia even then were always formed in the air on floating filaments. Indeed, appearances suggest that in nature the special function of the curious empty appendages, whose presence on the conidia of this species as on those of several of its predacious allies constitutes a feature anomalous among the Phycomycetes, might be to give the buoyancy necessary to prevent submergence in local accumulations of water following rains or heavy dews.

If aquatic conditions while facilitating observation are unfavorable for normal development of the fungi under discussion, terrestrial conditions attaching to natural substrata like moist soil, leaf mold, decaying plant remains and excrement of animals, favor abundant normal development but impose serious impediments to observation. The rather meager and scattered conidial apparatus is usually neither very conspicuous in the much more luxuriant growth of saprophytic forms often surrounding it, nor disposed in a manner to make easy the manipulations entailed in transfer to a microscopic preparation; and when successfully mounted shows little or nothing to distinguish it from that of commonplace Hyphomycetes. With sexual apparatus, vegetative thallus and all evidence of its curious parasitism concealed in an opaque substratum, *Endocochlus asteroides* might with good fortune be referred perhaps to the genus *Acladium*; while *Cochlonema verrucosum, C. dolichosporum, Bdellospora helicoides* and *Zoopage phanera* might find places among the unsifted species compiled in *Fusidium*. It is therefore in these Hyphomycetous genera and in *Oospora,* closely similar to *Fusidium,* that the fungi herein described and forms strictly congeneric with them most likely need to be looked for in the literature; though my own searches here have so far yielded no immediately useful information.

In this connection it may not be amiss to direct attention to the general similarity of conidial development in *Cochlonema, Bdellospora* and *Zoopage* to that described earlier (6) as occurring in *Actinomyces,* a genus formerly often included in *Oospora.* This similarity gains in suggestiveness from the circumstance that the vegetative mycelium characteristic of *Actinomyces* with its sparse septation largely if not exclusively consequent to protoplasmic degeneration in aging portions, or to withdrawal of protoplasm into younger ramifications, conforms rather well in fundamental design with that of the Phycomycetes. The extraordinary delicacy of the mycelium in *Actinomyces,* while very little reminiscent of the more familiar types of fungi among the Oömycetes and Zygomycetes, is, as has been mentioned, matched in the extremely slender hyphae of the minute *Amoeba*-capturing forms concerning whose close relationship in the Phycomycetes to the three catenulate genera herein described, there can be no reasonable doubt. To be sure the conidia of none of the more minute *Amoeba*-capturing forms now known are either catenulate or of dimensions at all comparable in smallness to the filaments from which they are produced; and in the known members of the catenulate genera neither the filamentous parts nor the conidia can be regarded as especially small. Yet when, as in the present instance, an obviously natural group embodies extreme delicacy of mycelium in some members, and in others formation of catenulate conidia not greatly exceeding in diameter the hyphae on which they are borne, the possibility of its relationship to forms wherein both these features are combined deserves consideration. Until the affinities of *Actinomyces* are definitely revealed through the discovery of a convincing sexual stage—the "Vierhyphensporen" described by Lieske (**13**) are far from impressive when viewed in such character—the morphological resemblances just noted would seem to compare more than favorably with the similarities that have long been cited as justifying the relegation of the genus to the higher bacteria.

For the time being the disposition of the group embracing the four newly described genera and the forms obviously kindred to them presents a more immediate problem. The similarities and possible homologies suggested more particularly in a comparison between *Bdellospora helicoides* and *Syncephalis nodosa* would seem to betoken articulation with the Mucorales through the Piptocephalidaceae. On the other hand the sturdy nematode-capturing form (**8**, *fig. 8*), in the moderate and often even meager development of its vegetative or predacious mycelium, in the habitual migration of its protoplasm from old hyphae to newly proliferated branches, in the large dimensions of its conidia, and in the repetitional production of secondary conidia from primary ones, reveals features suggestive of some sort of interdigitation with the Entomophthorales. What would seem to make for a provoking analogy with the latter order is apparent also in the semi-predacious behavior, as it were, of many species of *Empusa* in fixing their enfeebled insect hosts to the substratum through the production of adhesive rhizoids. A taxonomic position somewhere between the

Mucorales and the Entomophthorales is thus indicated for the group, which it is believed deserves recognition as a separate family, to be designated with perhaps tolerable appropriateness as the Zoopagaceae. Indeed, there is some reason to believe that further study of fungi destructive to terricolous microscopic animal life under approximately natural conditions on suitable transparent solid subtrata, will, without impairing the distinctiveness of the group, bring to light a range in morphological diversity and a plenitude of species commensurate in the Phycomycetes with the taxonomic scope of a suborder or of an order rather than with that of a family.

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EXPLANATION OF PLATES

PLATE 1

A, A well developed and active specimen of *Amoeba terricola* I, showing: *a,* penetration by an infective germ tube from an adhering conidium of *Endocochlus asteroides*; *b,* development of a globular body at tip of infective germ tube; *c,* separation of the globular body or young vegetative thallus from the evacuated germ tube, the former remaining within the animal, the latter being expelled; *d–i,* young thalli within the animal in increasingly advanced stages of development; *n,* nucleus of host animal; *v,* contractile vacuole of host; × 1000. *B,* An animal with two vegetative thalli which have given rise to three pairs of zygophores and a conidiiferous hypha shown in three sections,—*a* and *b* representing corresponding points on these sections, and the dotted line indicating the point of emergence from the substratum; × 500. *C,* An animal with a single well developed thallus that has given rise to a branching conidiiferous hypha with conidia in various stages of development, the branches being shown in sections,—*a–g* indicating corresponding points on these sections, and the dotted line the point of emergence from the substratum; × 500.

PLATE 2

A, A specimen of *Amoeba terricola* I, shortly before succumbing to infection from two well developed thalli of *Endocochlus asteroides*. *B,* An animal soon after its death from the three or four thalli of various sizes massed together in its interior. *C,* Remains of an animal containing eight thalli of *E. asteroides* of small and moderate sizes which have become evacuated in giving rise to seven zygosporangia, *a–g*. *D,* Wrinkled pellicle of an animal enveloping and concealing the membranes of the thalli which have produced four zygospores, *a–d,* approaching maturity. *E,* Remains of an animal showing eight mature stellate zygospores, each loosely enveloped by the collapsing zygosporangial membrane, *a–g*. *F,* A pair of zygophoric hyphae with a nearly fully grown zygosporangium produced on a short prolongation from the junction. *G,* A portion of conidiiferous hypha showing *a,* an early stage, and *b,* a later stage in the lateral proliferation of a conidium. *H,* An intermediate stage in conidial development. *I,* An advanced stage in conidial development. *J,* Mature conidia, *a–f,* showing variation in shape and size. Magnification × 1000 throughout.

PLATE 3

A, Pellicle of an unidentified *Amoeba* containing an empty conidium of *Cochlonema dolichosporum* and attached to it, the thallus, also empty, produced from it. *B,* Conidia of *Cochlonema dolichosporum,* showing variation in size and shape, arrangement in chain, and appendaged condition at late maturity. *C,* Pellicle of a specimen of *Amoeba sphaeronucleolus* enveloping a single thallus of *Cochlonema verrucosum* with the conidium from which it had origin still attached, and showing the basal portions of the conidiiferous hyphae to which it gave rise. *D, Amoeba sphaeronucleolus* about at point of death from the parasitism of three internal thalli of *Cochlonema verrucosum,* two of which have given rise to normal hyphae, while the third, through somewhat abnormal development, has developed externally a thallus-like swollen hypha. *E,* A dying specimen of *Amoeba sphaeronucleolus,* showing in addition to its two contractile vacuoles and its nucleus, three thalli of *Cochlonema verrucosum,* two of which have each given rise to an immature conidial chain shown in sections, whereof *a, b* and *c* represent corresponding points. *F,* Mature conidia of *Cochlonema verrucosum. G,* Remains of a specimen of *Amoeba sphaeronucleolus* with two zygosporangia of *Cochlonema verrucosum,* one still growing, the other containing a nearly mature zygospore. Magnification × 1000 throughout.

PLATE 4

A, Same as Plate 3, *C,* but showing the four conidial chains, *a–d,* in their entirety,—*b* and *c* being still in course of development, *a* and *d* being approximately mature; and the points of emergence of the conidiiferous hyphae from substratum being indicated by dotted lines; × 500. *B,* Same as Plate 3, *A,* but showing the asexual reproductive apparatus of *Cochlonema dolichosporum,* consisting of three conidial chains, *a–c,* in its entirety; points of emergence of conidiiferous hyphae into the air being indicated by dotted lines; the considerable lengths of the portions of conidiiferous filaments submerged in the substratum being due to the depth at which the host animal succumbed; × 500.

PLATE 5

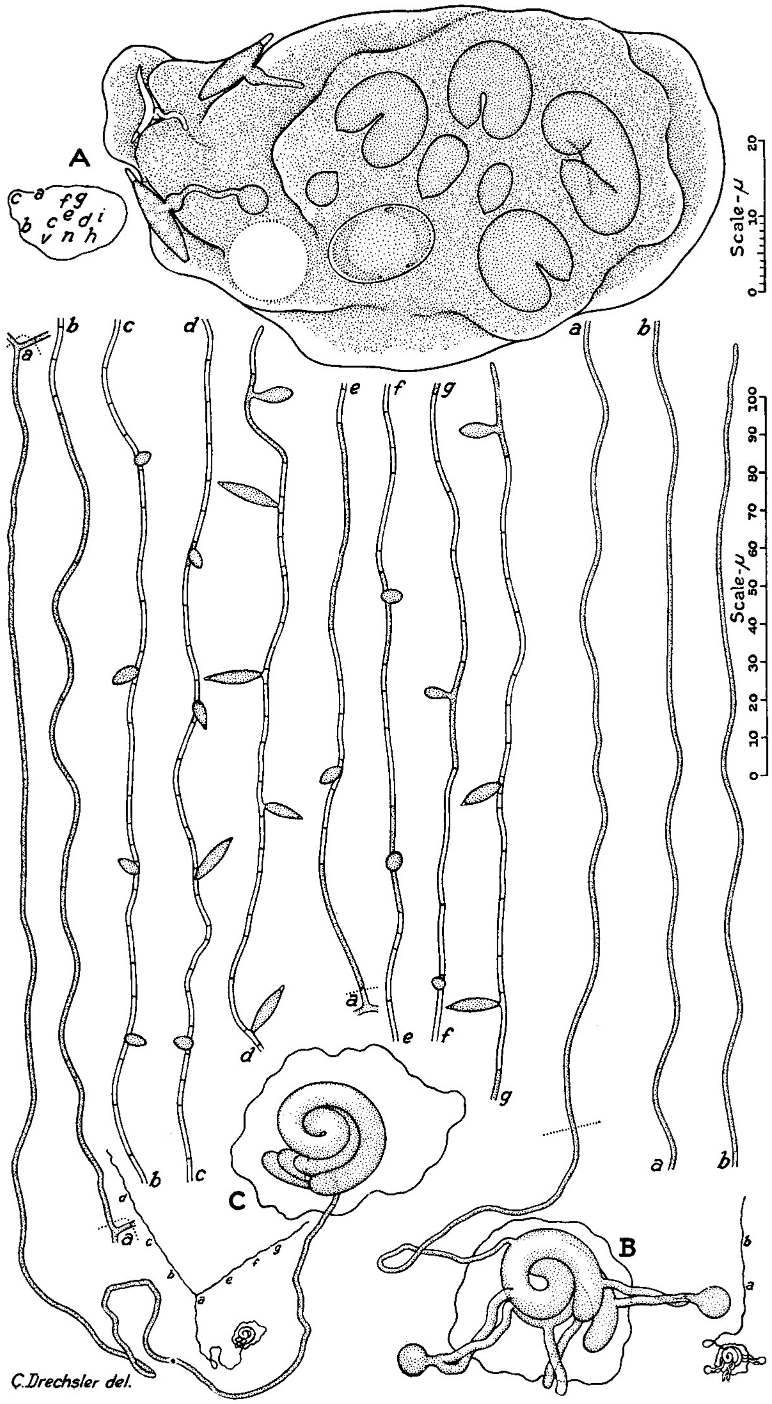
A, Conidia of *Bdellospora helicoides,* showing variation in size and shape. *B,* A specimen of *Amoeba terricola* II with nine infections from separate conidia of *Bdellospora helicoides,* the separate conidia being designated alphabetically *a–i* approximately in the order of their respective stages of development into swollen vegetative bodies; *n,* nucleus of animal; *v,* contracting vacuole. *C,* Another specimen of *Amoeba terricola* II, still alive, on which have developed six swollen spore bodies, which have given rise to four zygosporangia, *a–d*; one pair of the bodies, through branching of their respective sexual hyphae having given rise to two pairs of zygophores. *D–H,* Approximately mature zygosporangia and zygospores of *Bdellospora helicoides,* together with portions of the zygophores. *I,* Remains of a specimen of *Amoeba terricola* II and of vegetative bodies of *Bdellospora helicoides* from which have been produced eleven zygosporangia of which eight, *a–h,* contain each a normal mature zygospore, while the remaining three, *i–k,* are empty as a result of internal degeneration. Magnification × 1000 throughout.

PLATE 6

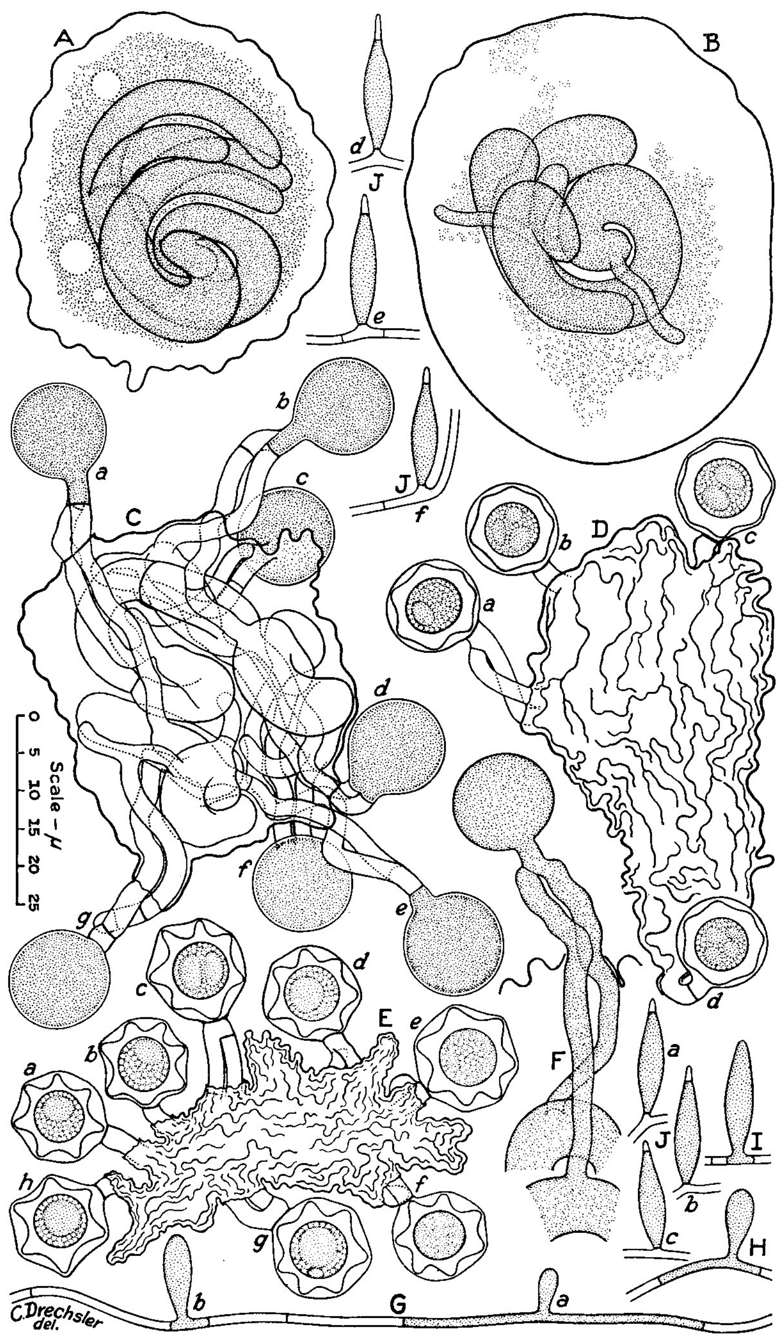
A large specimen of *Amoeba terricola* II about to succumb to four infections from *Bdellospora helicoides,* the four swollen spore bodies having given rise to hyphae *A, B, C* and *D,* on which have been or are being produced chains of conidia *a–d, a–d, a–b* and *a* respectively; of these chains *A, b* and *D, a* are continuous and therefore immature. The dotted lines at *A* and *C* and the lower dotted lines at *B* and *D* indicate the points of emergence of the hyphae from the substratum, the upper dotted lines at *B* and *D* the divisions between prostrate and erect portions. × 500.

PLATE 7

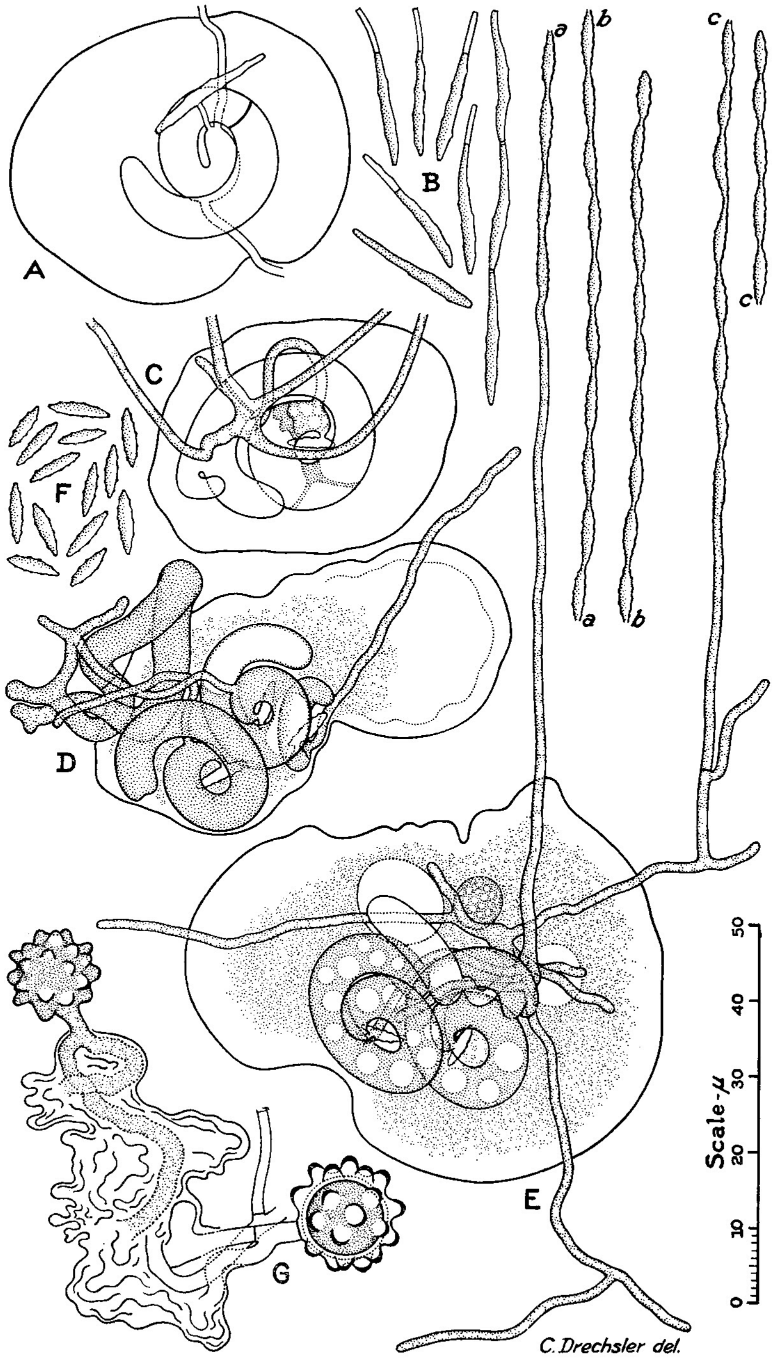
A, Four specimens, *a, b, c* and *d,* of *Amoeba terricola* III captured by the branched hyphae, *e* and *h,* of *Zoopage phanera,* showing the stalked botryoid haustoria of the fungus; hyphae *f* and *g,* perhaps also adhering to animal but without having produced haustoria, bear respectively one and two erect conidiiferous branches with long chains of conidia of which from lack of space only the lowermost individuals are shown; in the animal *d* is shown its nucleus, and the same structure from a healthy specimen is shown in *n*; × 500. *B,* Two hyphae, bearing three conidial chains *a–c* (shown only in part from lack of space) and producing on diclinous sexual branches two zygosporangia, *d* and *e,* shown at early stages of development; × 500. *C–H,* Sexual apparatus of *Zoopage phanera* showing diclinous origin of zygophores, inconstancy of septation during earlier stages in development of fusion product, and the frequently contorted condition of one of the zygophores; × 1000. *I–M,* Approximately mature zygospores, each within its collapsing zygosporangial membrane; × 1000. *N, O,* Sexual apparatus, with a germ tube from a conidium functioning directly as a zygophore; × 1000.



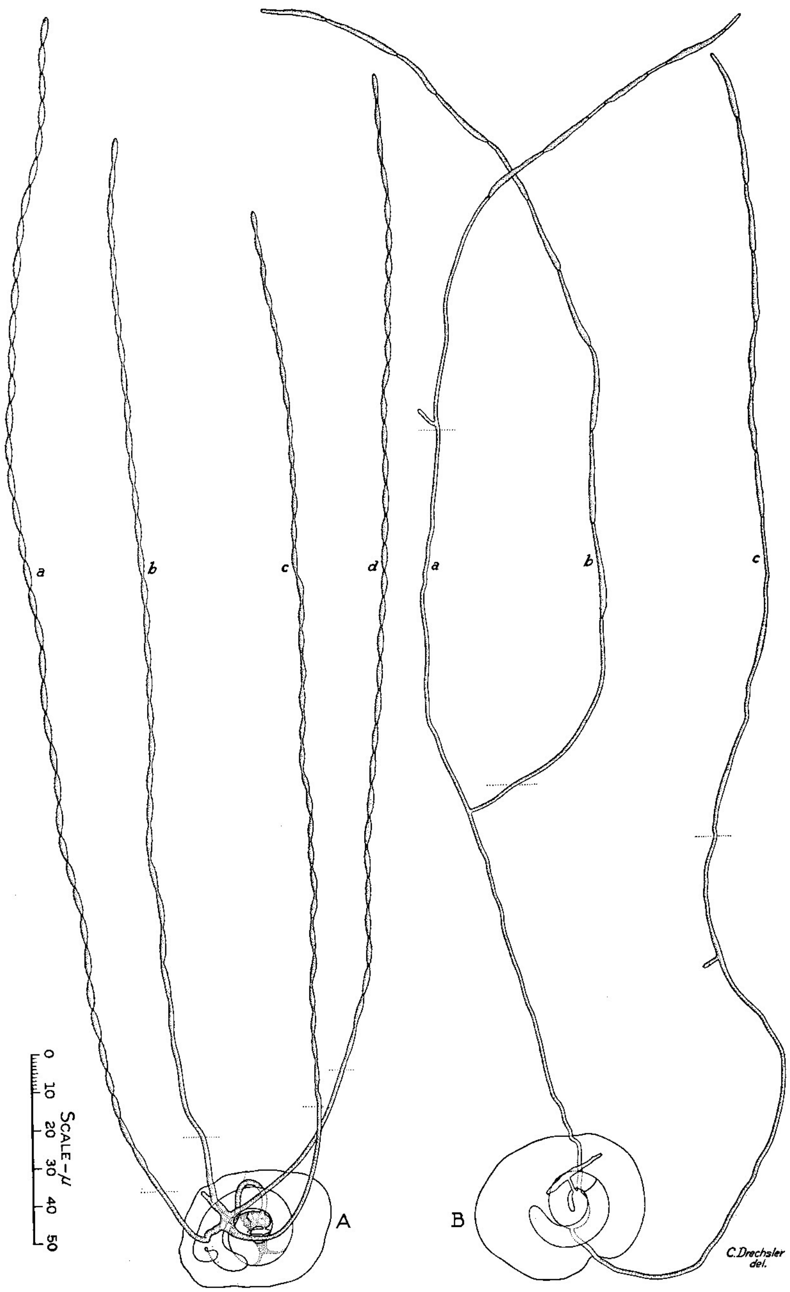
ENDOCOCHLUS ASTEROIDES



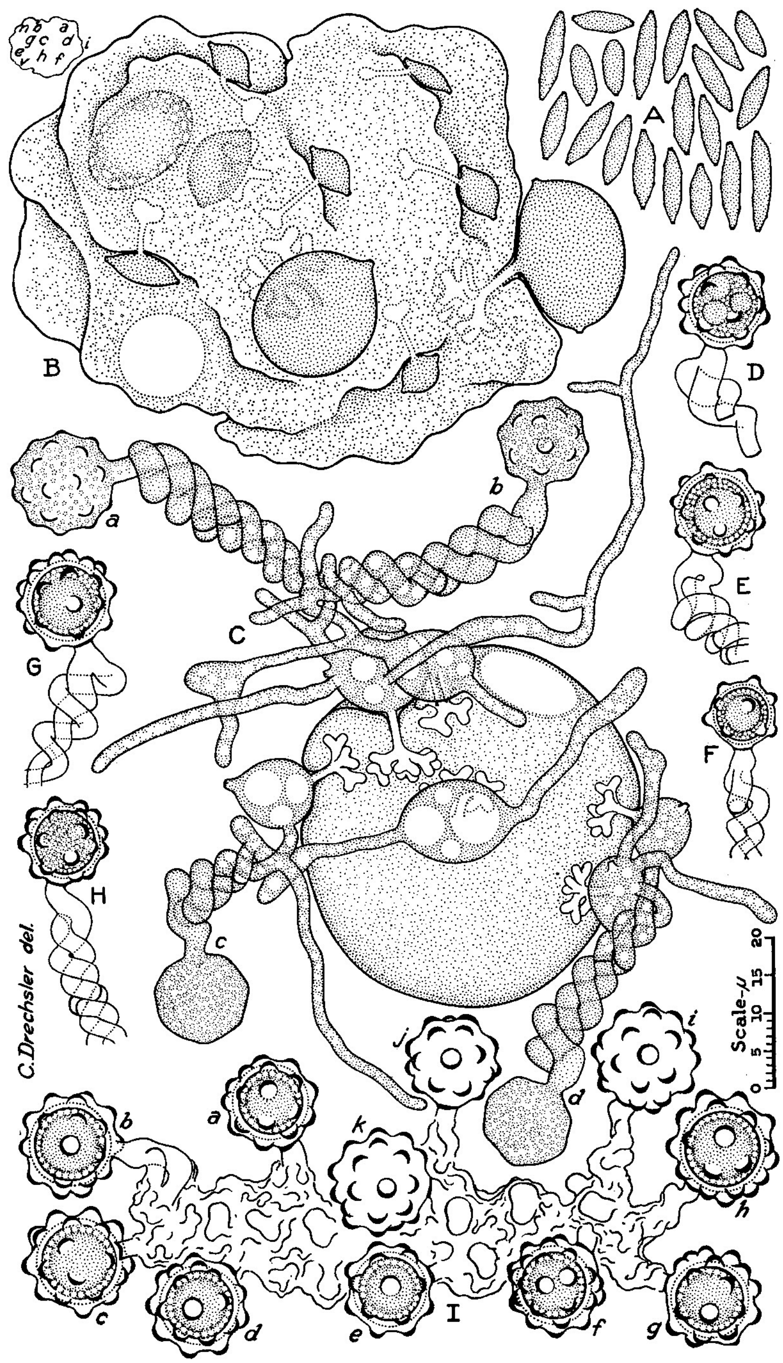
ENDOCOCHLUS ASTEROIDES



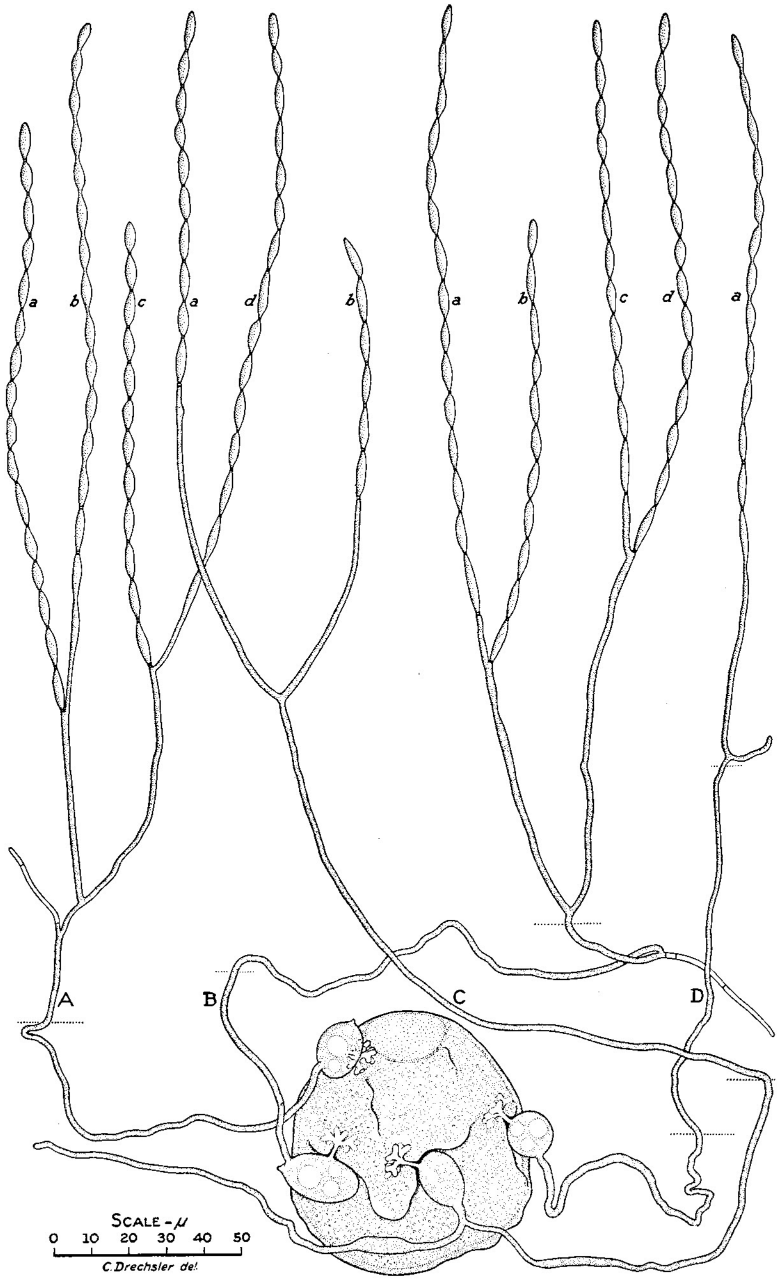
A–B. COCHLONEMA DOLICHOSPORUM
C–G. COCHLONEMA VERRUCOSUM



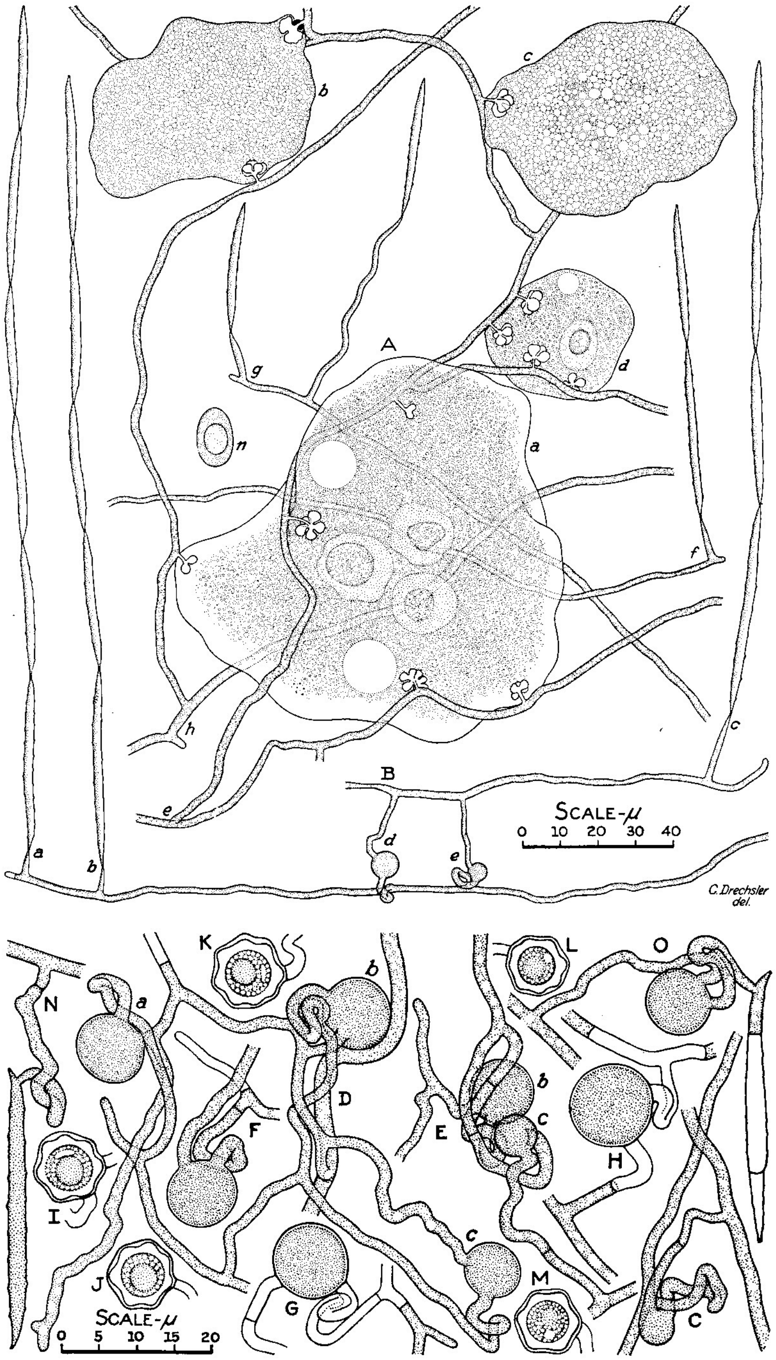
A. COCHLONEMA VERRUCOSUM
B. COCHLONEMA DOLICHOSPORUM



BDELLOSPORA HELICOIDES



Bdellospora helicoides



ZOOPAGE PHANERA