Reprinted from MYCOLOGIA, Vol. XXXVIII, No. 2, 120–143, Mar.–Apr., 1946
Printed in U. S. A.

THREE NEW ZOÖPAGACEAE SUBSISTING ON SOIL AMOEBAE

THREE NEW ZOÖPAGACEAE SUBSISTING ON SOIL AMOEBAE

Charles Drechsler [1]

(with 6 figures)

Three additional members of the family Zoöpagaceae have recently come to light in Petri plate cultures in which miscellaneous microörganisms introduced with decaying plant material were given prolonged opportunity to develop on maizemeal-agar medium overgrown with *Pythium* mycelium. All three forms were found subsisting on amoebae, one of them attacking the animals after the manner of an endoparasite, through germination of ingested spores, while the other two attack in a predaceous manner, by capturing the protozoans through adhesion to mycelial hyphae. Apart from descriptions of these new species, supplementary comment is herein supplied relative to an amoeba-capturing form I presented earlier under the binomial *Stylopage rhabdospora* (**3**: 374–377).

A SPECIES OF COCHLONEMA PRODUCING WARTY AZYGOSPORES

The new endoparasitic fungus made its appearance in many maizemeal-agar plate cultures that after being permeated with mycelium of *Pythium arrhenomanes* Drechsl. had been further planted with small quantities of friable vegetable detritus consisting mainly of partly decayed cucumber (*Cucumis sativus* L.) leaves and partly decayed lilac (*Syringa* sp.) leaves. The particular lot of vegetable detritus here in question was gathered near Greeley, Colorado, in October 1944, and has received mention in an earlier paper (**9**) as a source of the nematode-capturing basidiomycetous form I described under the binomial *Nematoctonus haptocladus*. Often, indeed, the amoeba parasite was found developing abundantly in the same cultures with *N. haptocladus*, its presence being betrayed

[1] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

by scattered aerial tufts of ascending conidial chains readily detectable to microscopical examination carried out with a dry objective. As under such examination the sporiferous tufts closely resembled those of both *Cochlonema symplocum* Drechsl. (**6**: 258–266) and *C. euryblastum* Drechsl. (**7**: 283–289), closer scrutiny was necessary to determine whether one of these species or possibly some allied form was active here in destruction of animal life. Accordingly, portions of the cultures were removed and examined under a water-immersion objective of high magnification. Though the conidiiferous tufts disintegrated badly in the moist preparations, they clearly had their origin in parasitized amoebae referable manifestly to a single species which in normal condition, as also in the earlier stages of attack, varied in diameter between 60 and 100 μ when drawn into a rounded shape (FIG. 1, *A–C*; FIG. 2, *A, B*; FIG. 3, *A*). Its firm, thickish pellicle, cast into broadly undulating or more delicately rippled folds, surrounded a colorless, somewhat dispersedly granular sarcode within which could be distinguished a single prolate ellipsoidal nucleus, 20 to 25 μ long and 13 to 20 μ wide, that contained normally a slightly darker, globose or prolate central body, about 8 to 11 μ wide (FIG. 1, *A–D, n*; FIG. 2, *B, n*; FIG. 3, *A, n*; FIG. 4, *A, n*). The animal endured infection with much fortitude, continuing its pseudopodial locomotion and the operation of its contractile vacuole (FIG. 1, *A–D, v*; FIG. 2, *A–B, v*; FIG. 3, *A, v*) until most of its contents were expropriated. Often its cytoplasm revealed a variable number of digestive vacuoles which sometimes were filled with massed bacteria (FIG. 1, *A, w*; *C, w*; *D, w*; FIG. 3, *A, w–z*) and sometimes contained a clump of spores (FIG. 1, *B, w*) belonging evidently to a mucoraceous fungus; such ingested spores being, however, more usually found imbedded individually here and there in the sarcode, without noticeable vacuolar development (FIG. 1, *A–D*; FIG. 3, *B*; FIG. 4, *A*). From its morphology the animal was assigned to *Amoeba verrucosa* Ehrenb.—to the same species, therefore, earlier found preyed upon by *Dactylella tylopaga* Drechsl. (**2**) and parasitized both by *C. megalosomum* Drechsl. (**4**: 128–137) and by *C. symplocum*.

As has been intimated, attack on the animal is always found initiated by germination of ingested conidia; the individual spore,

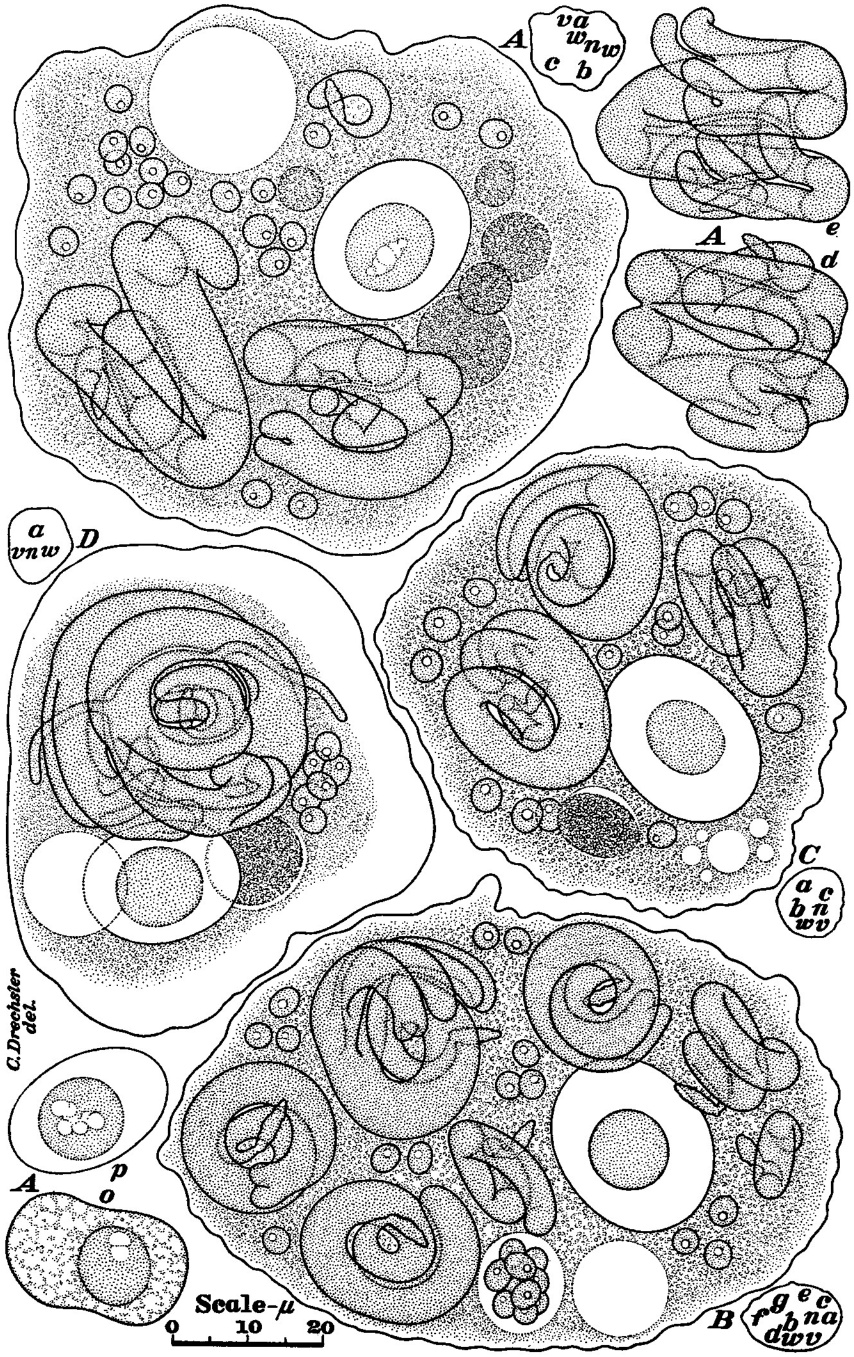

FIG. 1. *Cochlonema agamum*

soon after its ingestion, giving rise somewhat obliquely from one of its ends to a germ tube, which then prolongs itself into a thallus by feeding on the ambient host cytoplasm. In respect to the manner of establishing itself, the fungus thus shows general parallelism with *Cochlonema symplocum* and *C. euryblastum* rather than with *C. megalosomum*. Despite this parallelism and the similarity of host relationship, the young growing thallus is soon distinguishable from correspondingly young thalli of *C. symplocum* not alone by reason of its noticeably greater proximal width but also by reason of its more strongly flaring conformation (FIG. 1, *A*, *a*; FIG. 2, *A*, *a–d*); though on the other hand, it shows generally no close approach to the very stout proximal attachment and abrupt widening so conspicuous in the thallus of *C. euryblastum*. A strong tendency toward curvature becomes manifest early, and soon leads to a somewhat snail-like coiling which because of its geometrical regularity appears much more suggestive of *C. euryblastum* than of *C. symplocum*. The thallus maintains its unbranched condition during a rather extended period of elongation (FIG. 1, *B*, *a–d*; FIG. 2, *A*, *d–m*, *o*; *B*, *a–i*), but after describing 1½ to 2 spiral turns it usually bifurcates a first time (FIG. 1, *B*, *e*, *f*; *C*, *a–c*; FIG. 2, *A*, *p*; *B*, *j–m*, *o*, *p*); a second dichotomy taking place after growth of the two branches has augmented the coil in some instances by a half turn (FIG. 1, *A*, *b*, *c*) and in other instances by only one-tenth of a turn (FIG. 1, *B*, *g*). Where the four branches resulting from a second dichotomy have opportunity for substantial elongation, a third dichotomy often ensues (FIG. 1, *A*, *d*, *e*; *D*, *a*); this being followed, under appropriate conditions, by a fourth dichotomy in some, if not in all, of the terminal elements (FIG. 3, *A*, *a*; *B*). The thalli with three or four successive bifurcations usually have a volume in a general way commensurate with the number (8 to 16) of their terminal branches; for although the tertiary and quaternary branches are for the most part too narrow and too short to contribute much bulk in themselves, their production is accompanied usually by rather marked increase in width (and possibly also in length) of the proximal trunk as well as of the primary and secondary branches.

Since the eventual size of a thallus is determined by the quantity of host protoplasm available for assimilation, maximum dimensions are attained especially in instances where only a single thallus is present in an individual animal (FIG. 1, *D*; FIG. 3, *A*, *B*). Such instances of unitary infection are not infrequent when the fungus first begins its development in a culture. Later, as conidia become strewn about more and more abundantly, they are ingested in increasing numbers, with the result that many animals will then be found harboring more than a dozen thalli (FIG. 2, *A*, *B*); and the eventual size of the thalli will be reduced proportionately.

Regardless of whether one or several conidia have been engulfed, growth of the parasite for a considerable period seems to work no injury on the animal other than progressive reduction of its cytoplasmic contents. Usually the cytoplasm will have been reduced to about a quarter of its original volume before the nucleus begins to look abnormal either from excessive vacuolization of the central body or from mottling of the peripheral layer. There is reason to believe that under suitable circumstances an animal host can recover as long as nuclear degeneration has not gone beyond an incipient stage. After the drawing reproduced in figure 1, *A*, had been prepared, the infected amoeba shown therein was kept under observation 6 hours longer at a temperature close to 23° C. During these 6 hours all three of the thalli (FIG. 1, *A*, *a–c*) within the animal continued growing actively, so that the host cytoplasm, already reduced to about one-half of its original mass when observations were begun, suffered further reduction to about one-fourth or one-fifth of its original mass, while the outer layer of the nucleus became rather conspicuously mottled (FIG. 1, *A*, *o*). The preparation was then stored overnight at a temperature of 15° C. Evidently the lower temperature greatly benefited the amoeba, for 16 hours later its contractile vacuole was again operating briskly, and the outer layer of its nucleus again presented a clear homogeneous appearance (FIG. 1, *A*, *p*). The thalli of the parasite, on the other hand, had not only failed to continue growing, but gave evidence of debility in a noticeably vacuolated condition of their contents.

Where no environmental change intervenes in behalf of the infected amoeba its dwindling sarcode sooner or later becomes incapable of further locomotion. Thereupon the fungus promptly puts forth reproductive filaments even though the host nucleus (FIG. 1, *D*, *n*) may yet present a normal appearance; so that the

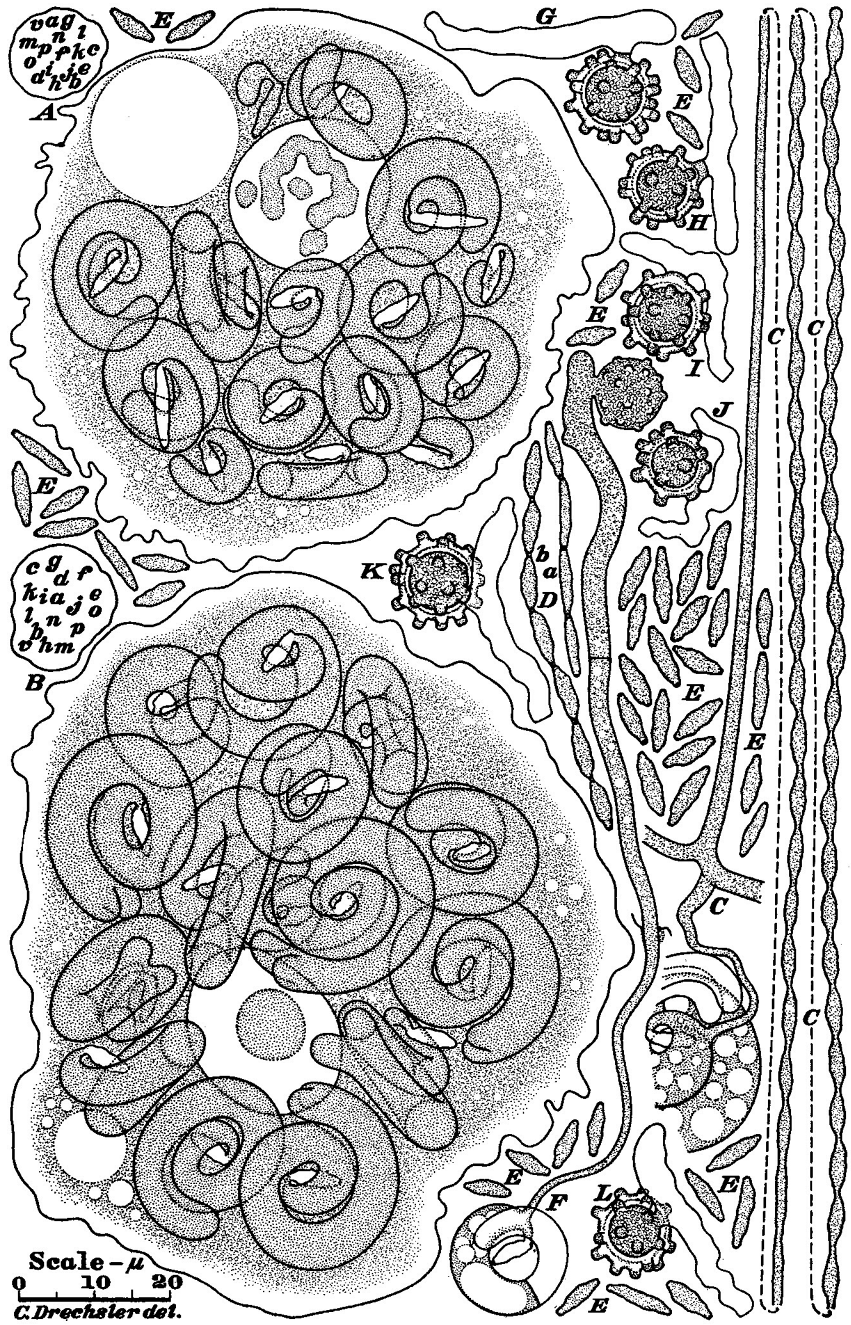

FIG. 2. *Cochlonema agamum*

final stages in the extinction of the animal's life and in the expropriation of its contents are accomplished simultaneously with the earlier stages in the reproduction of the parasite. The filaments growing from the thalli are scarcely half as wide as those of *Cochlonema euryblastum*. Generally the smaller thalli, including unbranched and once-dichotomous specimens together with some twice-dichotomous specimens, will put forth only a single reproductive filament, usually from a position on the convex side 3 to 10 μ from the parent conidium (FIG. 2, *F*; FIG. 3, *C*, *a*, *b*, *c*, *e*, *f*; FIG. 4, *A*, *a–e*). Some thalli of greater size, with 2, 3, or 4 successive bifurcations, will put forth two reproductive filaments (FIG. 1, *D*, *a*; FIG. 3, *C*, *d*, *g*; *D*, *a*, *b*), the second one arising beyond the first and often about a half turn from the origin of the coiled structure. Thalli of still larger size are found provided with three reproductive filaments (FIG. 3, *B*, *a–c*); the third one arising from a position a quarter turn beyond the second. The presence in one observed instance of an additional outgrowth (FIG. 3, *B*, *d*) suggests that the largest thalli may, perhaps, at times give rise to four reproductive hyphae.

When an infected animal succumbs on the surface of an agar culture held under ordinary conditions of storage—right side up, and with exposure to weak or moderate illumination mainly from above—all the reproductive filaments invariably make their way to the ceiling of the host pellicle. The direction of their growth is evidently not governed by chance, for where the coils of the parent thallus or of some other thallus are in the way, as is frequently the case, the filaments take a circuitous course around the interposed structures (FIG. 3, *D*, *a*, *b*; FIG. 4, *A*, *b*). They soon push out through the ceiling of the pellicle, and after widening rather markedly, extend a few procumbent branches over the upper surface of the amoeba. Where reproduction is exclusively asexual these branches do not attain any considerable length; yet since they are usually present in some number and are concentrated in a rather small area on top of the animal, they become intermingled into a loose overlying meshwork (FIG. 3, *C*). From the meshwork is sent up a tuft of 15 to 25 conidiiferous hyphae (FIG. 3, *C*, *h–z*) similar to the aerial tufts whereby the fungus was first detected; the individual reproductive filament contributing usually two or three conidiiferous hyphae, though if the parent thallus is small (FIG. 3, *C, a*) it may supply only one such hypha (FIG. 3, *C, h*).

In its proximal portion the conidiiferous hypha shows no special modification, but beginning at a height of 25 to 100 μ constrictions appear together with minute warty irregularities (FIG. 2, *C*). Thence upward for a distance of about 50 μ the constrictions become progressively more pronounced and occur at diminishing intervals, while concomitantly the warty irregularities become more numerous and somewhat more prominent. Beyond the transitional region of increasing modification the hypha is prolonged with constrictions at equal intervals and with equal display of warty sculpturing (FIG. 2, *C*; FIG. 3, *C, h, j, o, q, s, v*). After the hypha has attained definitive length, its modified portion is converted into a conidial chain through evacuation of contents from the middle of each constriction, followed by deposition of a wall at both ends of each empty isthmus (FIG. 3, *C, i, m, n, p, r, t, u, z*); the number of spores delimited in a chain varying commonly between 25 and 65. An aerial filament that has given rise to one chain (FIG. 3, *C, k, x*) may grow out below the proximal conidium to produce a second sporogenous hyphal element (FIG. 3, *C, l, y*). Such successive development would seem more frequent where very large thalli are concerned in reproduction than with thalli of moderate size, and, of course, is wholly absent where a thallus is too small to produce more than a single conidial chain. As might be expected, the several lowermost conidia originating from the transitional portion of an aerial hypha are longer, narrower, and smoother (FIG. 2, *D, a*) than the generality of their fellows that come from the more distal, better differentiated hyphal portions (FIG. 2, *D, b*). On slight disturbance the mature conidial chains break up, leaving the disarticulated spores (FIG. 2, *E*) strewn about on the substratum ready to be ingested by any specimen of *Amoeba verrucosa* visiting the seeded area. In size and shape the conidia differ little from those of *Cochlonema symplocum*.

When an infected animal succumbs in a submerged position the reproductive hyphae, after pushing through the host pellicle, find their way to the surface of the substratum by rather widely divergent paths; wherefore the aerial conidial apparatus produced by them is not aggregated in a luxuriant tuft but is dispersed over an extensive area as multiple floccules, each composed of 1 to 3 conidial chains. Such scattered sporulation presumably corresponds no less truly to the normal development of the fungus than tufted sporulation. What would seem, in contrast, to represent thoroughly abnormal behavior is often observable when reproductive filaments are put forth from thalli that are undergoing microscopical examination; for these filaments then consistently make their way to the floor rather than to the ceiling of the host pellicle. Manifestly this curious misdirection of growth results from positive phototropic response to the wholly unnatural upward illumination usual with vertical microscopes. As similar perversion has been noted frequently in congeneric forms, all development of reproductive filaments taking place from thalli directly under microscopical observation should be mistrusted.

Apart from asexual reproduction by conidia the fungus shows an equivalent of sexual reproduction in its frequently abundant formation of azygospores. While, as in related fungi, lower temperatures seem in some degree to favor development of the more durable spores, the two types of reproduction are often closely associated; both conidia and azygospores originating not only from the same animal host, but from the same thallus and even from the same reproductive filament. In instances where an infected amoeba has succumbed on the surface of the substratum azygospore development is initiated, like conidial formation, by extension from the thalli (FIG. 4, *A*, *a–e*) of reproductive filaments that make their way to the upper part of the animal, and then push out through the ceiling of the pellicle, to give rise, after widening rather markedly, to a few (2, 3, or 4) stout prostrate branches (FIG. 4, *A*, *k–m*, *q–z*); or if the thallus is of small size the reproductive filament may continue growth without branching (FIG. 2, *F*). The prostrate branches or prolongations grow out, often somewhat crookedly, to a length of 50 to 125 μ, and thus come to extend some distance into the material underlying or surrounding the animal. Through continued gradual widening they acquire typically an elongated clavate shape. A cross-wall is now laid down usually about 40 μ from the tip. The terminal cell thereby delimited soon burgeons forth a globose body laterally; the new excrescence more often arising from a position near the tip of the cell, or between the

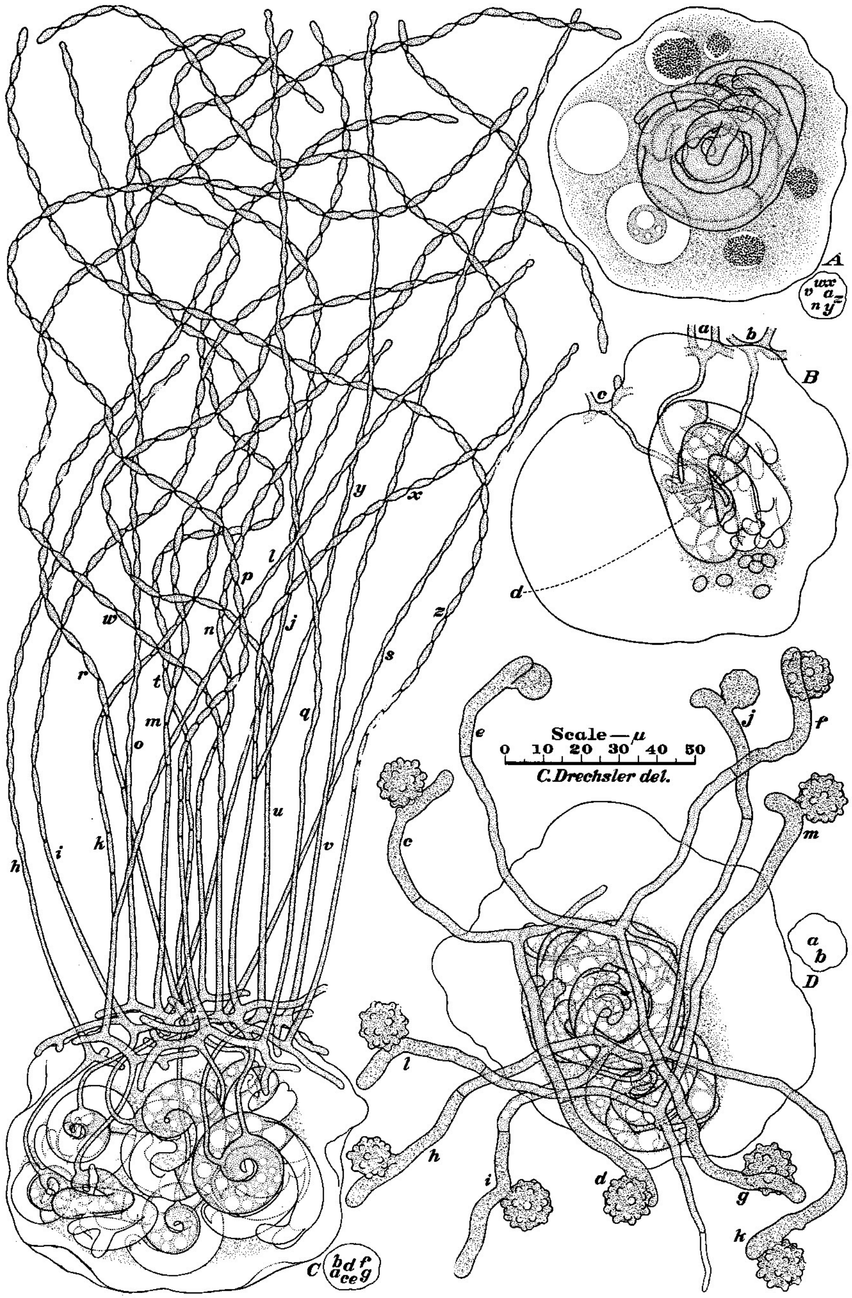

FIG. 3. *Cochlonema agamum*

middle and the tip (FIG. 2, *F*; FIG. 3, *D*, *c–h*, *j–m*; FIG. 4, *B–J*), than from a position at or slightly below the middle (FIG. 3, *D*, *i*; FIG. 4, *K*). Terminal development of the globose body, either on the tip of the cell (FIG. 4, *L*) or on a recognizable stalk of lateral origin (FIG. 4, *H*), would seem, like branching of the cell (FIG. 4, *K*, *L*), to occur as a departure from the usual. Although the distal cell here is larger than the young gametangia of most Zoöpagaceae whose sexual reproduction has been observed, it probably receives a considerable quantity of protoplasm from below after its delimitation; for, as a rule, when the distal cell has contributed all its contents to the globose body, the proximal portion of the branch similarly appears empty (FIG. 4, *C*, *J*, *L*). In the later stages of its growth the globose body becomes beset with prominent verrucose protuberances. At maturity these protuberances seem largely filled with material of a consistency uniform with the thickened peripheral wall from which they arise (FIG. 2, *G–L*). Often the peripheral wall appears spatially separated from an approximately equally thick membrane surrounding the spherical living protoplast within (FIG. 2, *G–L*); though often, again, the separateness of layers is very indistinct, and the appearance offered is more nearly that of a spherical protoplast surrounded by a thick, homogeneous, yellowish, verrucose wall (FIG. 4, *M–T*). The protoplast is evidently composed, in large part, of densely granular material. Further details regarding its internal make-up could not be ascertained because of the serious optical difficulties resulting from the presence of the numerous protuberances.

The boldly sculptured globose bodies, or azygospores, are formed in slightly submerged positions even when the protozoan host has succumbed on the surface of the substratum. In instances where the amoeba has succumbed below the surface, the zygophoric branches, unlike the reproductive filaments concerned in asexual reproduction, show no special tendency to grow upward, but produce their spores rather indiscriminately all around the animal and at no great distance from the pellicle. Once the animal's contents have been nearly exhausted, the migration of fungous protoplasm necessary to sustain continued development of azygospores, or of conidia, entails progressive evacuation of the thalli—a process accompanied here, as in several congeneric forms, by deposition of

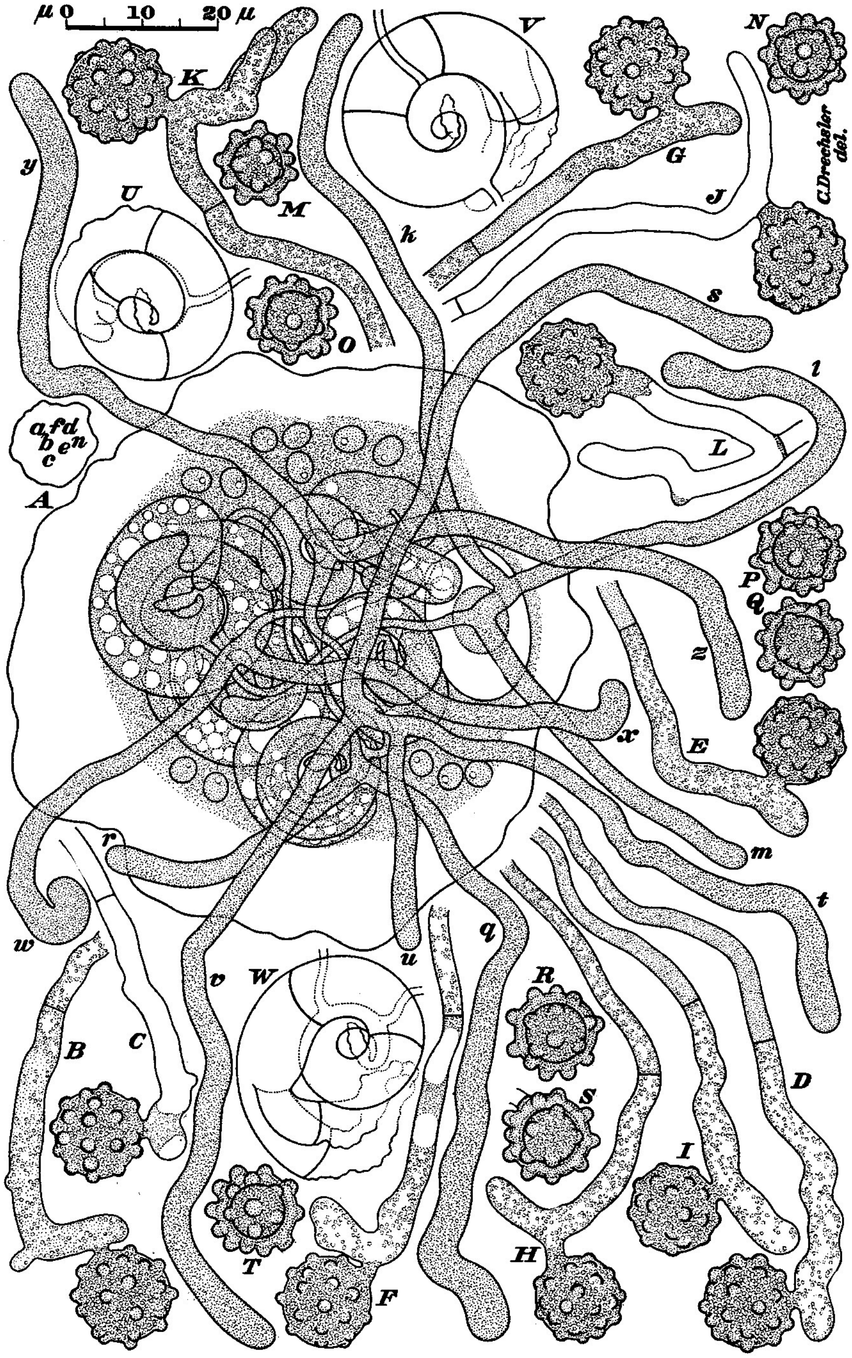

FIG. 4. *Cochlonema agamum*

retaining walls (FIG. 2, *F*; FIG. 3, *B*; *C*, *a–g*). Usually only a single transverse wall will be formed in a small thallus, while eight partitions may be laid down in a large one. Empty thalli of moderate size frequently contain three or four cross-walls (FIG. 4, *U–W*). After some time the empty thallodic envelopes, beginning in the distal portion, progressively collapse and evanesce; and when the host pellicle likewise disappears only a cluster of 10 to 35 azygospores remains as evidence of the animal's destruction.

The absence of conjugation in the development of its more durable reproductive bodies distinguishes the fungus most decisively from *Cochlonema symplocum*. Since similar reproduction is not known to occur elsewhere in the genus, except possibly in the optically difficult species I described earlier as *C. pumilum* (**5**: 398–402; **8**: 9–14), an epithet meaning "unmarried" may be appropriately suggestive.

Cochlonema agamum sp. nov.

Hyphae assumentes ex tubo germinationis circa 1 μ crasso in modum cornus latescentes, incoloratae, primo continuae, 4–13 μ crassae, usque 175 μ longae, in spiram cochleatim semel vel bis vel subinde paene ter volutae, nunc simplices nunc semel usque quater dichotomae, prope originem ex latere convexo 1–3 fortasse rarius 4 hyphas genitabiles emittentes: hyphis genitabilibus 1–1.5 μ crassis, animali debilitato vel moribundo pelliculam ejus saepe praecipue in parte superiore perforantibus, denique nunc ex aliquot ramis brevibus (1–30 μ longis) procumbentibus 1–4 hyphas conidiiferas in aerem emittentibus nunc 1–4 hyphas azygosporiferas in materiam subjacentem vel ambientem proferentibus. Hyphae conidiiferae incoloratae, erectae vel ascendentes, sursum in catenulas 25–65 conidiorum abeuntes, quandoque ex apice partis inferioris sterilis 25–100 μ longae repullulantes, denique quoque aliam catenulam gignente; conidiis incoloratis, cylindraceis vel elongato-ellipsoideis vel fusiformibus, plerumque minute verrucosis, 6–11 μ longis, 1.5–2.5 μ crassis. Hyphae azygosporiferae incoloratae, vulgo aliquantum pravae, saepius 50–125 μ longae, basi 2–2.5 μ crassae, sursum leniter latescentes, apice 3.8–5.8 μ crassae, primo continuae mox septo in duas cellulas divisae; cellula ulteriore 33–63 μ (saepius circa 40 μ) longa, subinde ramosa, ex latere vulgo prope apicem subinde medio azygosporam gignente; azygospora flavida, globosa, 9–12.5 μ (saepius circa 10 μ) diametro, 20–35 verrucis 2–2.5 μ diametro ornata, globulum protoplasmatis 6–7.5 μ diametro circumdante.

Amoebam verrucosam necans habitat in foliis plantarum (*Cucumeris sativi*, *Syringae* sp.) putrescentibus prope Greeley, Colorado.

Assimilative hyphae colorless, originally continuous, arising from a germ tube about 1 μ thick, widening out in the manner of a horn, mostly 4 to 13 μ in greatest transverse diameter, up to 175 μ long, coiled in a snail-like spiral consisting of one to two and one-half turns, often simple, but when better developed bifurcate or two to four times successively dichotomous, putting forth from the convex profile near the proximal end one to three (sometimes possibly four) reproductive filaments which after disablement of the animal host push through its pellicle often mostly on the upper side, either to give rise from several short prostrate branches to aerial conidiiferous hyphae in numbers usually from one to four, or to extend one to four zygophoric branches into the underlying or surrounding material. Conidiiferous hyphae colorless, erect or ascending, at maturity terminating in a chain of 25 to 65 conidia, sometimes growing out from the tip of a sterile proximal part 25 to 100 μ long and giving rise to a second conidial chain; conidia colorless, cylindrical, elongate-ellipsoidal or spindle-shaped, nearly always minutely verrucose, mostly 6 to 11 μ long and 1.5 to 2.5 μ wide. Zygophoric branches colorless, usually somewhat crooked, commonly 50 to 125 μ long, 2 to 2.5 μ wide at the base, widening out gradually to a diameter of 3.8 to 5.8 μ near the apex, at first continuous, later divided by a cross-wall into two cells; the distal cell 33 to 63 μ (mostly about 40 μ) long, occasionally branched, producing an azygospore laterally sometimes from a median position but much more often from a position closer to the apex; azygospores yellowish, subspherical, studded with 20 to 35 warty protuberances 2 to 2.5 μ wide, exclusive of these protuberances measuring 9 to 12.5 μ (commonly about 10 μ) in diameter, at maturity surrounding a globose protoplast 6 to 7.5 μ in diameter.

Parasitizing *Amoeba verrucosa* it occurs in decaying leaves of *Cucumis sativus* and *Syringa* sp. near Greeley, Colorado.

AN AMOEBA-CAPTURING FUNGUS PRODUCING CONIDIA CRESTED WITH APPENDAGES

Aside from the decaying material that yielded *Cochlonema agamum*, reference was made in my account of *Nematoctonus haptocladus* to a second lot of vegetable detritus likewise collected near Greeley, Colorado, in October 1944. In this second collection, consisting mainly of partly decomposed remnants of tamarisk leaves, cottonwood leaves, and oleaster leaves, *N. haptocladus* was never found accompanied by the endoparasite just described, but instead occurred a few times in association with an equally new predaceous member of the same family. The new predaceous form came to light in 3 maizemeal-agar plate cultures 14 days after pinches of the decaying mixture had been superimposed on an established mycelium of *Pythium undulatum* Petersen *sensu* Dissmann; its presence being detected when microscopical explorations carried out with a dry objective revealed a sparse scattering of conidia whose general similarity to the conidia of my *Acaulopage tetraceros* (**1**: 195–197; **7**: 289–291) was relieved by readily noticeable differences pertaining to the number and size of their empty appendages.

On examining under higher magnification several tracts of substratum over which these conidia were distributed only one kind of predaceous mycelium was found present. The hyphae of this mycelium had affixed to them numerous delicately wrinkled empty membranous envelopes measuring 10 to 20 μ across (FIG. 5, *A*, *a–e*; *B*, *a–d*; *C*), together with some smooth-contoured envelopes usually only about 2.5 μ in diameter (FIG. 5, *B*, *e*, *f*). It is problematical whether the envelopes of the latter sort came from active individuals of some very minute species of *Amoeba*, or whether they belonged to motile spores of some less familiar type of protozoan; they could, in any case, have held only meager nourishment for the hyphae to which they were fastened. The more capacious wrinkled envelopes unquestionably were pellicles of amoebae referable presumably to a single species. In some instances, where a substantial portion of the protoplasmic contents still remained (FIG. 5, *B*, *a*; *C*), a globose or ellipsoidal structure, measuring 2.5 to 3 μ in its greatest dimension and containing a slightly darker central body within its hyaline outer layer, appeared to represent a solitary nucleus. Expropriation of the animal's contents was accomplished by a basally branched haustorium consisting, as in *Acaulopage tetraceros*, of assimilative elements approximately equal in width to the mycelial filaments (FIG. 5, *C*).

Connection between the submerged amoeba-capturing mycelium and the appendaged conidia on the surface of the agar substratum was less clearly observable than might have been desired. Development of the conidia evidently entailed here, just as in congeneric forms, evacuation of long stretches of filament; and since the empty membranes soon became indiscernible, the hyphal attachments (FIG. 5, *D*, *E*) of the spores could not often be followed backward as much as 25 or 50 μ. Nevertheless, it seems fairly certain that two

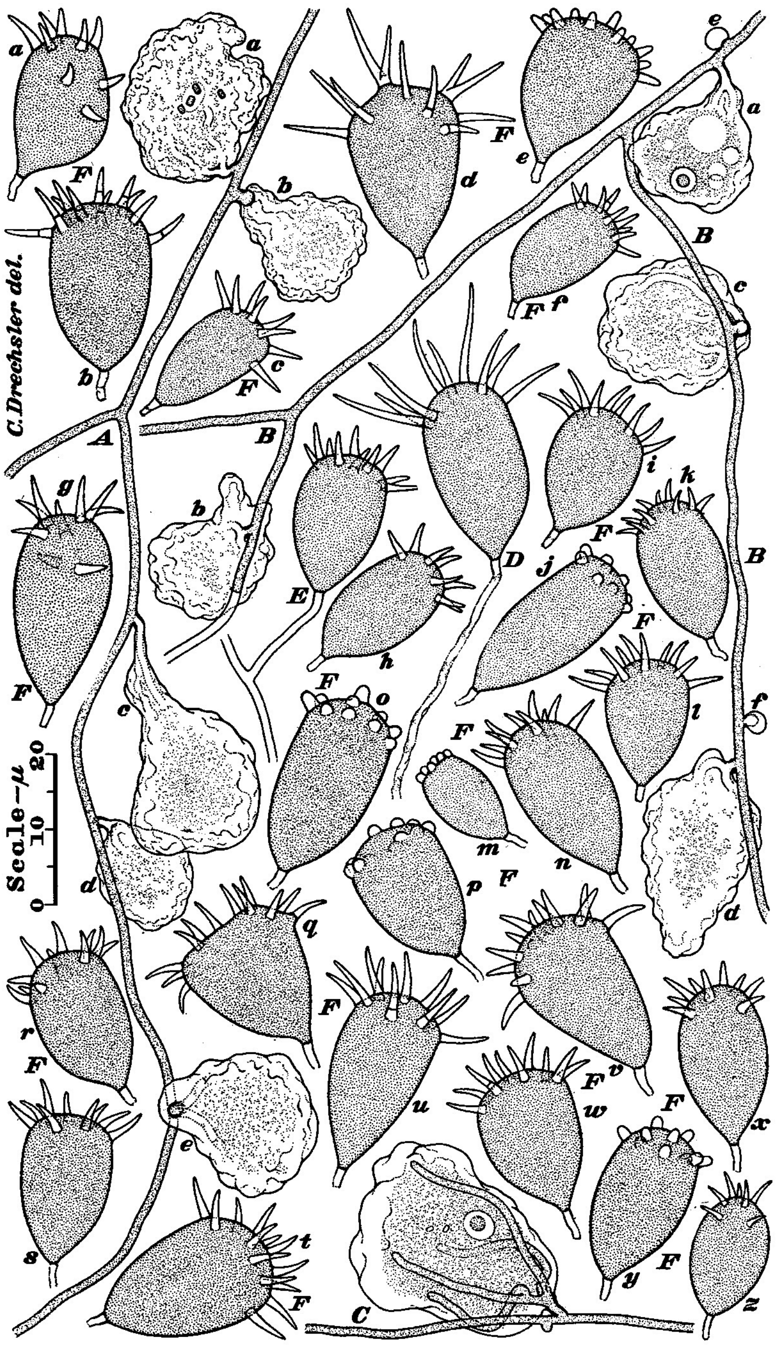

FIG. 5. *Acaulopage lophospora*

developmental states must have belonged together, because no other predaceous mycelium, and no other spores at all suggestive of the Zoöpagaceae, were to be found then or later in the same tracts of substratum.

As has been intimated, the conidia (FIG. 5, *F*, *a–z*) in a general way resembled those of *Acaulopage tetraceros* with respect to size and shape; and, moreover, were similarly provided at the base with a short stipe-like empty hyphal part. At the apical end, however, they bore commonly from 8 to 15 empty appendages, while in *A. tetraceros* the number of such parts ranges usually from 3 to 6. Their appendages, though more numerous, were, on the whole, scarcely half as long as those of *A. tetraceros*, sometimes, indeed, being reduced to wart-like protuberances (FIG. 5, *F*, *c*, *j*, *m*, *o*, *p*, *y*). In *A. tetraceros* the appendages are regularly borne in a circle around the crown of the spore, whereas in the conidia under consideration they were more often found distributed all over the distal end in a bristling crest. An epithet compounded partly of a word meaning "crest" may therefore perhaps be helpful in distinguishing the fungus producing these conidia from the congeneric form most similar to it.

Acaulopage lophospora sp. nov.

Mycelium sparsum; hyphis filiformibus, incoloratis, primum continuis, parce ramosis, plerumque 1–1.5 μ crassis, ad animalia minuta inhaerentibus, pelliculam cujusque capti perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio plerumque ad instar arbusculae ex 3–4 ramulis assumentibus 5–25 μ longis et circa 1.2 μ crassis constante. Conidia in superficie materiae animalia ambientis singulatim oriunda, hyalina, basi stipitata, apice 8–15 appendicibus praedita: cellula viventi subinde inversum oviformi vel ellipsoidea sed saepius inversum lageniformi, plerumque 13–25 μ longa, 8–15 μ crassa; stipite vacuo, 2–4 μ longo, .9–1.4 μ crasso; appendicibus vacuis, vulgo acute subulatis, 3–13 μ longis, basi .8–1.5 μ crassis, subinde obtuse conicis vel verruciformibus, 1.5–3 μ longis, .8–1.5 μ crassis.

Amoebas plerumque 10–20 μ latas capiens consumensque habitat in foliis plantarum (specierum *Syringae, Populi, Tamaricis*) putrescentibus prope Greeley, Colorado.

Mycelium sparse; vegetative hyphae filiform, colorless, sparingly branched, originally continuous, mostly 1 to 1.5 μ wide, adhering to minute animals, perforating the pellicle of each captive and intruding a haustorium to appropriate the protoplasmic contents; haustorium bushlike, often consisting of 3 or 4 assimilative branches

5 to 25 μ long and about 1.2 μ wide. Conidia formed singly on the surface of the material surrounding the animals, colorless, stipitate at the base and provided at the apex with 8 to 15 appendages: the living cell sometimes obovoid or ellipsoid but more often inversely flask-shaped, usually 13 to 25 μ long and 8 to 15 μ wide; the stipe empty, mostly 2 to 4 μ long and .9 to 1.4 μ wide; the appendages empty at maturity, usually acutely awl-shaped, 3 to 13 μ long and .8 to 1.5 μ wide at the base, but sometimes bluntly conical or wart-like, then mostly 1.5 to 3 μ long and .8 to 1.5 μ wide.

Capturing and consuming amoebae, mostly 10 to 20 μ wide, it occurs in decaying leaves of *Syringa* sp., *Populus* sp., and *Tamarix* sp., near Greeley, Colorado.

AN AMOEBA-CAPTURING FUNGUS PRODUCING CONIDIA BESET WITH LONGISH FINGER-LIKE APPENDAGES

A maizemeal-agar plate culture that on May 5, 1945, was planted with softened discolored roots from a wilting pansy (*Viola tricolor* L.) plant newly dug up in Mt. Rainier, Md., showed, when explored microscopically 21 days later, sparsely scattered conidia beset with bristling appendages in a manner reminiscent of the amoeba-capturing fungus I described earlier (**7**: 274–278) as *Acaulopage lasiospora*. On closer examination it was found that the conidia in question were, on the whole, appreciably smaller than those of *A. lasiospora,* and that they more frequently tapered noticeably toward the proximal end (FIG. 6, *A*, *a–x*), thereby acquiring more often a somewhat turbinate shape. Their appendages showed marked distinctiveness in that they were longer than the appendages of *A. lasiospora,* and only about half as numerous.

At the time the conidia first came under observation the mycelium from which they originated was for the most part already empty of contents, making it very difficult to trace the slender hyphae for any distance. Unfortunately, besides, *Acaulopage tetraceros* was present in considerable abundance throughout the culture, so that when membranous remains of amoebae were found affixed to mycelial elements it was usually not possible to ascertain which fungus had been concerned in particular instances of capture. Details relating to haustorial development must consequently await an accession of less ambiguous material. In the meantime the curious conidial ornamentation would seem to justify amply the recognition in the Zoöpagaceae of an additional member to be known by a specific epithet compounded of two words meaning "porcupine" and "seed," respectively.

Acaulopage hystricospora sp. nov.

Mycelium sparsum, parce ramosum; hyphis incoloratis, primo continuis, circa 1 μ crassis, ad animalia minuta inhaerentibus, pelliculam cujusque capti perforantibus, haustorium intrudentibus quod protoplasma exhaurit. Conidia in superficie materiae animalia ambientis singulatim oriunda, incolorata vel subinde languide flavida, globosa vel elongato-ellipsoidea vel applanato-ellipsoidea vel turbinata, plerumque 7.5–12.5 μ longa, 7–14 μ crassa, basi interdum minute stipitata, prope basin semper glabra, medio interdum glabra interdum appendicibus vestita, apice semper appendicibus vestita; appendicibus in summa 10–50, in maturitate vacuis, digitiformibus, plerumque 2–6.5 μ longis, .7–.9 μ crassis.

Amoebas capiens consumensque habitat in radicibus *Violae tricoloris* putrescentibus in Mt. Rainier, Maryland.

Mycelium sparse, sparingly branched; vegetative hyphae colorless, originally continuous, about 1 μ wide, capturing minute animals through adhesion, perforating the pellicle of each captive, and extending into it a haustorium to appropriate the protoplasmic contents. Conidia produced singly on the surface of the material underlying or surrounding the animals, colorless or sometimes faintly yellowish, globose or prolate ellipsoidal or oblate ellipsoidal or turbinate, mostly 7.5 to 12.5 μ long and 7 to 14 μ wide, sometimes minutely stipitate, always glabrous in the region surrounding the hilum, glabrous or beset with appendages in the equatorial zone, regularly beset with appendages at the distal end; the appendages commonly numbering 10 to 50 in all, empty at maturity, finger-shaped, mostly 2 to 6.5 μ long and .7 to .9 μ wide.

Capturing and consuming amoebae, it occurs in decaying roots of *Viola tricolor* in Mt. Rainier, Maryland.

A STRAIN OF STYLOPAGE RHABDOSPORA PRODUCING LARGISH CONIDIA

Many of the cultures that, after being planted with the cucumber-lilac detritus from Colorado, yielded *Cochlonema agamum*, often in association with *Nematoctonus haptocladus*, permitted abundant development also of *Stylopage rhabdospora*. On the whole the Colorado strain showed satisfactory agreement with the original description of the species, but its conidia, ranging in length from

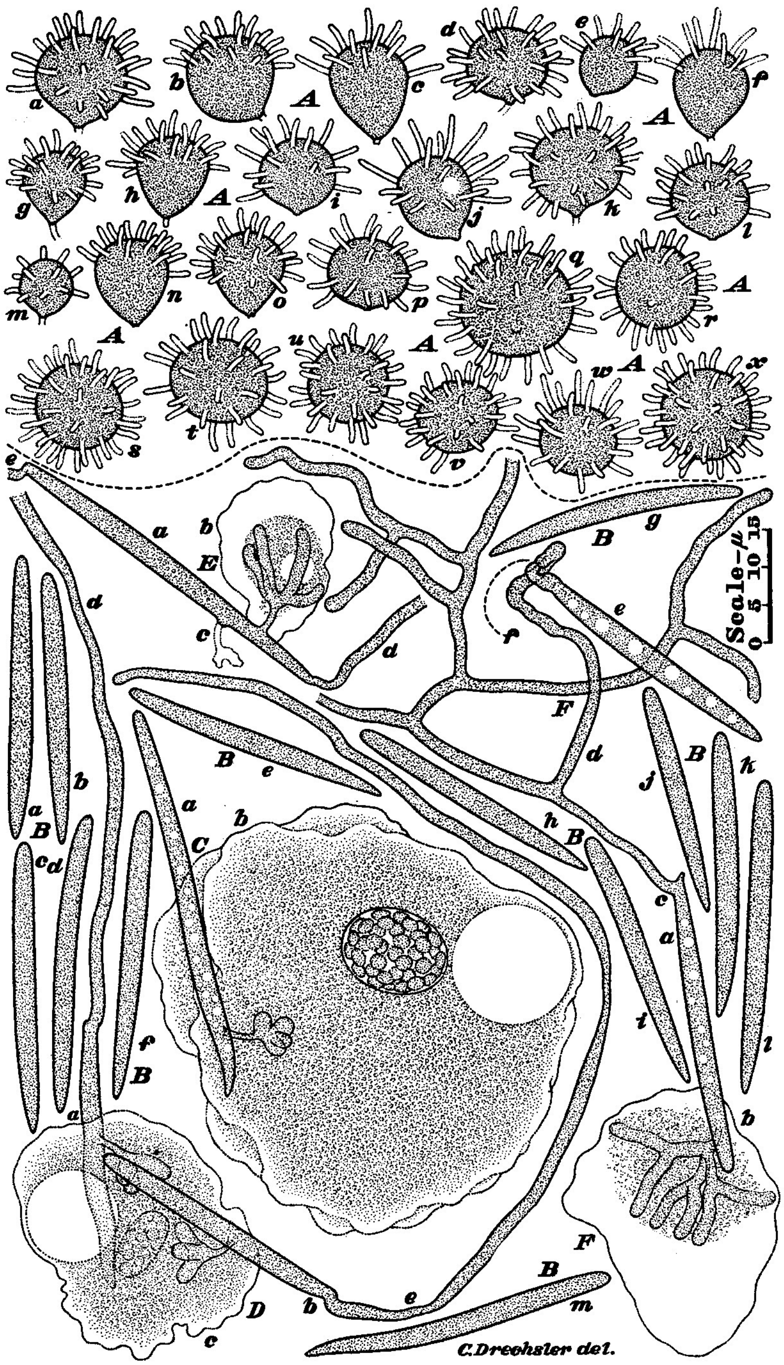

FIG. 6. A. *Acaulopage hystricospora*
B–F. *Stylopage rhabdospora*

30 to 52 μ, and in width from 2.7 to 3.2 μ (FIG. 6, *B*, *a–m*; *C*, *a*; *D*, *a*, *b*; *E*, *a*; *F*, *a*, *e*), exceeded the dimensions previously given. The *Amoeba* attacked by it was manifestly of the same species as the one earlier found serving as prey, though some individuals measured no less than 50 μ when drawn into a rounded shape (FIG. 6, *C*, *b*). While the single prolate-ellipsoidal nucleus present in these larger animals measured as much as 14 μ in length and 11 μ in width (FIG. 6, *C*, *b*) it revealed, like the smaller nuclei figured earlier (**3**: p. 375, FIG. 4, *A*, *C*), about 30 to 35 oblate ellipsoidal bodies distributed peripherally; about 10 to 12 of the bodies being usually visible in profile view. The larger animals as well as the smaller ones (FIG. 6, *D*, *c*; *E*, *b*; *F*, *b*) were often attacked directly by adhering conidia, which in such instances would intrude a haustorium while at the same time putting forth one (FIG. 6, *D*, *d*, *e*; *F*, *c*) or two (FIG. 6, *E*, *d*, *e*) germ hyphae. Again, much as in the earlier material, a hyphal branch (FIG. 6, *F*, *d*) on encountering a germ hypha from a conidium nearby (FIG. 6, *F*, *e*) would often conjugate with it (FIG. 6, *F*, *f*) to initiate development of a zygospore.

BUREAU OF PLANT INDUSTRY,
U. S. HORTICULTURAL FIELD STATION,
BELTSVILLE, MARYLAND

LITERATURE CITED

1. **Drechsler, C.** Some non-catenulate conidial phycomycetes preying on terricolous *Amoebae*. Mycologia **27**: 176–205. 1935.
2. ——. A new mucedinaceous fungus capturing and consuming *Amoeba verrucosa*. Mycologia **27**: 216–223. 1935.
3. ——. New conidial phycomycetes destructive to terricolous *Amoebae*. Mycologia **28**: 363–389. 1936.
4. ——. A few new Zoöpagaceae destructive to large soil rhizopods. Mycologia **31**: 128–153. 1939.
5. ——. Five new Zoöpagaceae destructive to rhizopods and nematodes. Mycologia **31**: 388–415. 1939.
6. ——. Four phycomycetes destructive to nematodes and rhizopods. Mycologia **33**: 248–269. 1941.
7. ——. New species of *Acaulopage* and *Cochlonema* destructive to soil *Amoebae*. Mycologia **34**: 274–297. 1942.
8. ——. Several additional phycomycetes subsisting on nematodes and *Amoebae*. Mycologia **37**: 1–31. 1945.
9. ——. A clamp-bearing fungus parasitic and predaceous on nematodes. Mycologia **38**: 1–23. 1946.

30 to 52 μ, and in width from 2.7 to 3.2 μ (FIG. 6, *B*, *a–m*; *C*, *a*; *D*, *a*, *b*; *E*, *a*; *F*, *a*, *e*), exceeded the dimensions previously given. The *Amoeba* attacked by it was manifestly of the same species as the one earlier found serving as prey, though some individuals measured no less than 50 μ when drawn into a rounded shape (FIG. 6, *C*, *b*). While the single prolate-ellipsoidal nucleus present in these larger animals measured as much as 14 μ in length and 11 μ in width (FIG. 6, *C*, *b*) it revealed, like the smaller nuclei figured earlier (**3**: p. 375, FIG. 4, *A*, *C*), about 30 to 35 oblate ellipsoidal bodies distributed peripherally; about 10 to 12 of the bodies being usually visible in profile view. The larger animals as well as the smaller ones (FIG. 6, *D*, *c*; *E*, *b*; *F*, *b*) were often attacked directly by adhering conidia, which in such instances would intrude a haustorium while at the same time putting forth one (FIG. 6, *D*, *d*, *e*; *F*, *c*) or two (FIG. 6, *E*, *d*, *e*) germ hyphae. Again, much as in the earlier material, a hyphal branch (FIG. 6, *F*, *d*) on encountering a germ hypha from a conidium nearby (FIG. 6, *F*, *e*) would often conjugate with it (FIG. 6, *F*, *f*) to initiate development of a zygospore.

BUREAU OF PLANT INDUSTRY,
U. S. HORTICULTURAL FIELD STATION,
BELTSVILLE, MARYLAND

LITERATURE CITED

1. **Drechsler, C.** Some non-catenulate conidial phycomycetes preying on terricolous *Amoebae*. Mycologia **27**: 176–205. 1935.
2. ——. A new mucedinaceous fungus capturing and consuming *Amoeba verrucosa*. Mycologia **27**: 216–223. 1935.
3. ——. New conidial phycomycetes destructive to terricolous *Amoebae*. Mycologia **28**: 363–389. 1936.
4. ——. A few new Zoöpagaceae destructive to large soil rhizopods. Mycologia **31**: 128–153. 1939.
5. ——. Five new Zoöpagaceae destructive to rhizopods and nematodes. Mycologia **31**: 388–415. 1939.
6. ——. Four phycomycetes destructive to nematodes and rhizopods. Mycologia **33**: 248–269. 1941.
7. ——. New species of *Acaulopage* and *Cochlonema* destructive to soil *Amoebae*. Mycologia **34**: 274–297. 1942.
8. ——. Several additional phycomycetes subsisting on nematodes and *Amoebae*. Mycologia **37**: 1–31. 1945.
9. ——. A clamp-bearing fungus parasitic and predaceous on nematodes. Mycologia **38**: 1–23. 1946.

EXPLANATION OF FIGURES

FIG. 1. *Cochlonema agamum;* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba verrucosa* still active though infected with 3 thalli, *a–c*; *d, e*, same thallus as *c*, but drawn 30 minutes later and 4 hours later, respectively; *n*, host nucleus in virtually healthy condition; *o*, same host nucleus 6 hours later, showing abnormal mottling in outer layer; *p*, same host nucleus, restored to virtually normal condition through incubation for 16 hours at 15° C.; *v*, contractile vacuole of host; *w*, digestive vacuoles crowded with ingested bacteria; 20 ingested spores of a mucoraceous fungus are shown scattered through the protoplasm. *B*, Specimen of *Amoeba verrucosa* still actively motile though infected with seven thalli, *a–g*, of various sizes; *n*, host nucleus; *v*, contractile vacuole containing a group of spores from a mucoraceous fungus; 14 other similar spores are shown distributed through the host cytoplasm. *C*, Small specimen of *Amoeba verrucosa* still motile though infected with three thalli, *a–c*; *n*, host nucleus; *v*, contractile vacuole, *w*, digestive vacuole containing ingested bacteria; 14 ingested spores of a mucoraceous fungus are shown scattered through the cytoplasm. *D*, Small disabled specimen of *Amoeba verrucosa* infected with a large thallus, *a*, that is putting forth two reproductive filaments; *n*, host nucleus; *v*, contractile vacuole; *w*, digestive vacuole crowded with bacteria; six ingested spores of a mucoraceous fungus are shown imbedded in the host cytoplasm.

FIG. 2. *Cochlonema agamum;* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba verrucosa* still capable of slight movement though infected with 15 thalli, *a–m, o, p*, mostly rather small; *n*, host nucleus abnormal in the organization of its darker material; *v*, contractile vacuole. *B*, Large specimen of *Amoeba verrucosa* still capable of slight movement though infected with 15 thalli of moderate size, *a–m, o, p*; *n*, host nucleus of virtually normal appearance; *v*, contractile vacuole. *C*, Portion of a thallus showing a reproductive filament and its connection with a young growing conidiiferous hypha; the latter from lack of space being shown in parts connected in proper sequence by broken lines. *D*, Catenated conidia as found in the proximal portion, *a*, and in a more distal portion, *b*, of a chain. *E*, Random assortment of disarticulated conidia, showing ordinary variations in size and shape. *F*, Small thallus that has yielded most of its contents in prolonging its single reproductive filament into a zygophoric hypha from whose distal cell a young azygospore has burgeoned forth laterally. *G–L*, Mature azygospores to each of which is attached the empty membrane of the distal cell.

FIG. 3. *Cochlonema agamum;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Specimen of *Amoeba verrucosa* still capable of some movement though infected with a large thallus, *a*; *n*, host nucleus of nearly normal appearance; *v*, contractile vacuole; *w–z*, digestive vacuoles to a large extent crowded with ingested bacteria. *B*, Disabled specimen of *Amoeba verrucosa* whose contents have been almost completely assimilated by the large thallus, which in turn has lost most of its protoplasm in putting forth conidial apparatus from three reproductive filaments, *a–c*; a short diverticulum, *d*, evidently representing a fourth repro-

ductive filament that aborted at an early stage. *C*, Specimen of *Amoeba verrucosa* whose contents have been almost completely assimilated by seven thalli, *a–g*, which collectively have given rise to the 19 conidiiferous hyphae and conidial chains *h–z*: *a* having given rise to *h*; *b* to *i*, *j*; *c* to *k*, *l*; *d* to *m*, *n*, *o*, and to *p*, *q*; *e* to *r*, *s*; *f* to *t*; *g* to *u*, *v*, and to *w*, *x*, *y*, *z*. *D*, Specimen of *Amoeba verrucosa* killed by 2 large thalli, *a* and *b*, each of which has given rise to two reproductive filaments; the more proximal filament from thallus *a* having then produced the zygophoric branches *c* and *d*; the more distal filament from thallus *a* in like manner having produced the zygophoric branches *e*, *f*, *g*; the proximal filament from thallus *b* having produced the zygophoric branches *h*, *i*, *j*, *k*; the more distal filament from thallus *b* having produced the zygophoric branches *l* and *m*.

FIG. 4. *Cochlonema agamum;* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba verrucosa* disabled from infection by six thalli, *a–f*, five of which, *a–e*, have each put forth a reproductive filament that in every instance is extending zygophoric branches into the surrounding material—thallus *a* in this manner giving rise to branches *k*, *l*, *m*; thallus *b* to branches *q*, *r*; thallus *c* to branches *s*, *t*, *u*, *v*; thallus *d* to branches *w*, *x*; and thallus *e* to branches *y*, *z*; *n*, host nucleus. *B–L*, Distal portions of zygophoric branches, showing development of azygospore in various positional relationships to the terminal cell, and in two instances (*K*, *L*) showing ramification of the terminal cell. *M–T*, Detached mature azygospores, showing variations in size and sculpturing. *U–W*, Empty thalli of moderate size, with three (*U*) or four (*V*, *W*) transverse partitions, and with one (*U*, *V*) or two (*W*) reproductive hyphae; the distal portion of each thallus collapsing and evanescing.

FIG. 5. *Acaulopage lophospora;* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Portion of mycelium to which are affixed the virtually empty pellicles of five amoebae, *a–e*. *B*, Portion of mycelium to which are affixed: *a*, one moribund amoeba that still reveals a nucleus, a contractile vacuole, and a remnant of cytoplasm; *b–d*, three virtually empty pellicles of conspecific amoebae; *e*, *f*, two empty envelopes of very minute microörganisms. *C*, Portion of hypha to which is attached a moribund amoeba largely expropriated of its cytoplasm. *D*, Conidium shown attached to an empty collapsing evanescent hypha. *E*, Conidium shown attached to a short branch arising from a mycelial filament. *F*, *a–z*, Detached conidia showing variations in size and shape of the living cell, as well as in size, shape, number and arrangement of the appendages.

FIG. 6. Drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, *Acaulopage hystricospora:* Detached conidia, *a–x*, illustrating usual variations in size and shape of the living cell, as well as in number, size, and arrangement of the appendages; 16 of them (*a–p*) being shown as viewed at a right angle to the longitudinal axis; two (*q*, *r*) as viewed from below, with the hilum surrounded by a glabrous area; six (*s–x*) as viewed from above, with appendages arising everywhere from the apical surface. *B–F*, *Stylopage rhabdospora* (strain from Greeley, Colorado): *B*, Detached conidia, *a–m*, showing variations in size and shape. *C*, Conidium, *a*, infecting a large individual amoeba, *b*; nucleus of animal being drawn to show all the oblate ellipsoidal bodies distributed throughout its periphery.

D, Two conidia, *a* and *b*, infecting a small amoeba, *c*, while putting forth the germ tubes *d* and *e*, respectively. *E*, Conidium, *a*, that has intruded one haustorium into a small captured amoeba, *b*, besides producing and later evacuating another haustorium, *c*, while at the same putting forth two germ hyphae, *d* and *e*. *F*, Conidium, *a*, which has largely depleted a captured amoeba, *b*, by means of a haustorium, besides putting forth a germ hypha, *c*, one of whose branches, *d*, has encountered the germ tube from a neighboring conidium, *e*, and is uniting apically (*f*) with it.