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NEW SPECIES OF ACAULOPAGE AND COCHLONEMA DESTRUCTIVE TO SOIL AMOEBAE

NEW SPECIES OF ACAULOPAGE AND COCHLONEMA DESTRUCTIVE TO SOIL AMOEBAE

Charles Drechsler

(with 6 figures)

In continuation of observations on biotic relationships of soil microörganisms often revealed in agar plate cultures that after being well permeated with oömycetous mycelium have received some addition of decaying vegetable material, 3 conidial Phycomycetes apparently not hitherto described have been found destroying particular species of terricolous amoebae. Two of the phycomycetous forms are presented herein as new members of the predaceous genus *Acaulopage,* while the third is set forth as a new member of the parasitic genus *Cochlonema.* Further, a rather pronounced morphological variant of *C. bactrosporum* Drechsl. (5) is described as a new variety of that species; and occasion is taken to report supplementary findings pertaining to the vegetative stage of *Acaulopage tetraceros* Drechsl. (2), and to the asexual reproductive stage of *Stylopage cephalote* Drechsl. (4).

A species of Acaulopage producing conidia beset with stubbly appendages

Several maizemeal-agar plate cultures that after being permeated with mycelium of *Pythium splendens* Braun had been planted with small quantities of leaf mold collected near Beltsville, Md., early in January 1941, showed on cursory examination four weeks later scattered conidia bristling with stubbly appendages. In their scant distribution on the surface of the medium, as well as in their unusual ornamentation, the spores bore a suggestive resemblance to the conidia of *Acaulopage acanthospora* Drechsl. (4). It was not surprising, therefore, that on closer scrutiny they were found to arise from a sparse unseptate mycelium to which were attached here and there specimens of a naked rhizopod undergoing expropriation of protoplasmic contents.

The rhizopod which thus served the sparse mycelium as food supply, apparently to the exclusion of other nourishment, varied in width between 10 µ and 40 µ when drawn into a somewhat rounded shape. When moderately extended the larger individuals often measured between 50 µ and 55 µ in length. Some, though not all, of the newly captured animals revealed from 5 to 10 vacuoles, which from their successive enlargement and contraction appeared to operate as contractile vacuoles (FIG. 1, *A*). Occasionally a few rather small subspherical bodies could be distinguished within an animal, but more frequently no structure having any similarity to a protozoan nucleus was recognizable in the peculiarly turbid, almost opaque, very finely and densely granular, yellowish protoplasm. In newly captured prey, whose normal structure had not yet been noticeably affected, a pellicle could hardly be made out, though the presence of a somewhat firm enveloping membrane was indirectly betrayed through the adhesion of the animal to one or more minute deposits of yellow substance secreted by the hypha. The haustorial system which soon came to be extended inward from each place of adhesion likewise was at first either indiscernible or only faintly discernible (FIG. 1, *A*; *B*; *C*, *a*, *b*; *D*). However, as the contents of the prey became more and more attenuated, the haustorial system emerged with increasing clearness, and surrounding it the pellicle became visible as a faint contour (FIG. 1, *E*, *a*, *b*; *F*, *a*, *b*; *G*; *H*; *I*). With respect to branching habit the haustorial system was essentially of the rangy arbuscular type, but owing to unusually prolonged extension of the assimilative branches in the more distant portion of the animal these branches converged and overlapped distally in such manner that in profile they presented a characteristic intertangled appearance alien to the haustoria of any predaceous fungus yet described. Once the animal's protoplasm had been completely absorbed, the protoplasm of the haustorial system was withdrawn backward into the parent filament, and soon all vestiges of the rhizopod and of the ramifying apparatus that encompassed its destruction were lost to view.

The fungus initiated asexual reproduction by giving rise here and there to relatively short hyphal branches, each of which became enlarged terminally to form a subspherical body on the surface of the culture medium. During its growth this body remained smooth (FIG. 1, *J–P*), but after attaining definitive size it put forth numerous digitate protuberances from all portions of its surface exposed to the air. Naturally these protuberances while actively elongating contained protoplasm (FIG. 1, *Q*), which, however, was promptly withdrawn when elongation came to an end (FIG. 1, *R, a, b; S*). A septum was now laid down in the short supporting branch to delimit the spherical body as a conidium. Evacuation of a short stalk-like part above the septum occasionally left the spore with a small empty basal appendage (FIG. 1, *T*), but much more often the proximal end was marked only by a pedicellate protrusion (FIG. 1, *U; V, a–g*).

Despite obvious similarities, the conidia thus formed differ conspicuously from those of *Acaulopage acanthospora*. In the present fungus the empty appendages, instead of tapering perceptibly, maintain a virtually uniform width from base to blunt apex. When the spore is viewed laterally the number of digitations directly visible in upper aspect and in profile varies commonly from about 15 (FIG. 1, *R, a*) to about 75 (FIG. 1, *V, d*); wherefore the total number, including those concealed underneath, probably ranges from 25 to 125, rather than from 7 to 18 as in *A. acanthospora*. Again, in the present fungus the digitations sometimes are distributed only over a distal region of limited extent, and at other times are distributed over the entire surface of the conidium; whereas the tapering appendages of *A. acanthospora* are distributed more constantly over the distal hemisphere of the spore. The fungus predaceous on the yellowish amoeba undoubtedly represents a separate species, which according will be described as new under a name meaning in part "rough" or "shaggy."

Acaulopage lasiospora sp. nov.

Mycelium sparsum, parce ramosum; hyphis incoloratis, aliquantum flexuosis, .9–1.4 μ crassis, ad animalia minuta inhaerentibus, pelliculam cujusque capti perforantibus, haustorium (subinde 2 vel 3 haustoria) intus evolventibus quod protoplasma exhaurit; haustorio ex 2–15 ramulis 10–50 μ longis, 1–1.3 μ crassis, saepius recurvis et inter se intricatis constante. Ramuli fertiles saepius 5–40 μ longi, interdum repentes, conidia singulatim super materiam subjacentem ferentes; conidiis hyalinis, saepe aliquantum pedicellatis,

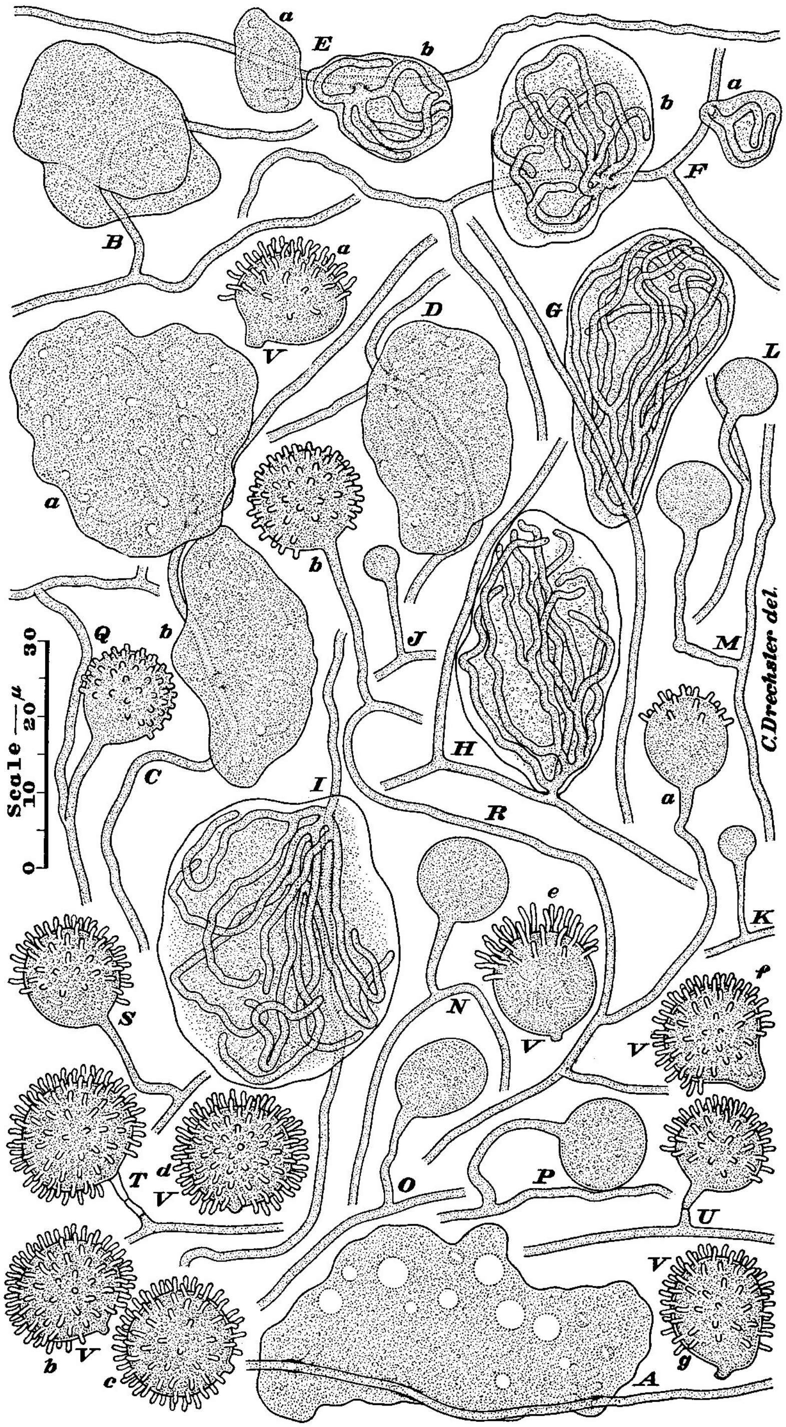

FIG. 1. *Acaulopage lasiospora.*

quoque ex cellula viventi et 25–125 appendicibus vacuis constante; cellula viventi globosa vel aliquid applanata, plerumque 12–16 μ longa, 11–16 μ lata; appendicibus 1.5–4 μ (plerumque circa 2 μ) longis, .6–.7 μ crassis, cylindratis, rectis vel leniter curvatis, apice abtusis vel truncatis, nunc ubique circum cellulam viventem nunc tantummodo in parte supera ejusdem positis.

Amoebas flavidas vulgo 10–40 latas capiens consumensque habitat in humo silvestri prope Beltsville, Maryland.

Mycelium sparse, sparingly branched; vegetative hyphae colorless, somewhat flexuous, .9 to 1.4 μ wide, capturing minute animals through adhesion, perforating the pellicle of each captive, and extending into it a haustorium (or sometimes 2 or 3 haustoria) to appropriate the protoplasmic contents; haustorium bush-like, with 2 to 15 branches, which vary from 10 to 50 μ in length and from 1 to 1.3 μ in width, and which often recurve distally to appear as if intertangled. Fertile branches often 5 to 40 μ long, sometimes prostrate, each bearing terminally a single conidium on the surface of the substratum; conidium hyaline, consisting of a living cell densely filled with protoplasm, subspherical or often oblate ellipsoidal in shape, mostly 12 to 16 μ long and 11 to 16 μ wide, usually somewhat pedicellate at the base, beset everywhere or sometimes only in its distal portion with empty digitate appendages; the latter from 25 to 125 in number, 1.5 to 4 μ (mostly about 2 μ) long, .6 to .7 μ wide, cylindrical or slightly curved, obtuse or tuncate at the tip.

Capturing and consuming amoebae yellowish in color and commonly 10 to 40 μ wide, it occurs in leaf mold near Beltsville, Md.

A SPECIES OF ACAULOPAGE WITH LATERAL CONJUGATION

An agar plate culture which after being permeated with *Pythium* mycelium had received some addition of decaying grass detritus gathered near Beltsville, Md., early in January 1941, showed on microscopic inspection 24 days later numerous slender erect conidia provided individually with a withered distal appendage—the bristling display offering general similarity to a sporulating tract of *Acaulopage rhicnospora* Drechsl. (2). However, the mycelium from which the conidia arose (FIG. 2, *A–F*) was noticeably coarser than that of *A. rhicnospora,* although the hyphae composing it tapered to widths of only .6 μ or .7 μ in their terminal portions (FIG. 2, *G*). These hyphae subsisted, apparently to the exclusion of other nourishment, on amoebae varying from 10 to 40 μ in diameter when drawn into an approximately round shape; the protozoans being captured through adhesion to minute deposits of a yellow substance. Owing to turbidity normal to the animal's sarcode, details of nuclear structure could not be made out in newly captured specimens. After its invasion by a haustorium bearing on a narrow stalk several wider digitate branches, and consequent to the ensuing depletion of its protoplasmic materials, the captive usually came to reveal internally a prolate ellipsoidal structure containing 3 to 6 bodies in peripheral positions (FIG. 2, *A, n; B, n; C, n; D, n; E, an, bn*). This structure probably represented the animal's nucleus, perhaps modified in some degree by incipient pathological changes, though its continued functional capacity was manifested in prolonged operation of the contractile vacuole (FIG. 2, *A, v; B, v; C, v; D, v; E, av, bv; F*). Later the structure disintegrated, and its materials together with remnants of cytoplasm were assimilated by the fungus. Thereupon the contents of the haustorium were withdrawn into the parent hypha; and the empty haustorial membrane, as well as the collapsed pellicle surrounding it, was soon lost to view.

Development of asexual spores was initiated by the production of erect aerial processes from the mycelial hyphae extended on the surface of the culture medium (FIG. 2, *G–I*). On reaching full stature (FIG. 2, *J, a*) the individual process showed noticeable constriction about 1 μ above its origin, and farther upward, approximately midway between base and tip, it tapered into a delicate awl-like prolongation. Through retraction of contents from the attenuated distal part, and deposition of a cross-wall at the basal constriction (FIG. 2, *J, b*), a terminally appendaged conidium came into being at the tip of a short tapering sterigma. On exposure to moderately dry air the empty appendage soon shriveled, much like the similar appendages of various other zoöpagaceous forms (FIG. 2, *K, a–v*). As a general rule the cylindrical or somewhat fusiform living cell of the conidium tapered less markedly toward the base than in *Acaulopage rhicnospora,* and accordingly was somewhat more blunt at the proximal end.

Sexual reproduction took place simultaneously with asexual reproduction. Zygospores were formed in branches (FIG. 2, *C, b; L; M; N; O, a–c; P; Q; R*), which when relatively short—between 15 μ and 25 μ in length—were usually a little wider throughout than the parent filament (FIG. 2, *O, a, c*). When the branches were longer such widening was evident only in a terminal portion, often measuring about 15 to 25 μ in length. At a stage when differentiation with respect to width first became noticeable, a cross-wall was laid down well toward the proximal limit of the swollen part, and a process grew out, sometimes from a position immediately above the septum (FIG. 2, *L*), sometimes from a position a few microns farther toward the tip (FIG. 2, *M*). The process, apparently, would then arch backward somewhat after the manner of clamp-connections in the basidiomycetes, and effect a junction with the parent branch just below the septum (FIG. 2, *N*). Soon after anastomosis was accomplished, if not at an earlier stage, a second cross-wall would be laid down to delimit a proximal gametangium frequently only one-half or one-third as long as the distal gametangium cut off by the first cross-wall (FIG. 2, *O, a–c*). The young zygosporangium thereupon developed as a subspherical enlargement, most often midway between base and apex of the distal gametangium, and less frequently in close proximity to the conjugation-tube, whether at the base of the distal gametangium (FIG. 2, *C, b*) or at the distal end of the proximal gametangium (FIG. 2, *P*). Relatively wide spatial separation of conjugation-tube and zygosporangium resulted occasionally from development of the latter toward the tip of the distal gametangium (FIG. 2, *N, Q, R*).

Once the globose zygosporangium had attained definitive size it was delimited proximally and distally by septa laid down in approximately tangential planes. Its originally smooth enveloping membrane would ultimately collapse somewhat loosely about the bullate contours of the yellowish zygospore. At maturity the zygospore, like that of other Zoöpagaceae, revealed an internal organization more familiar in oöspores: the thick spore wall surrounding a parietal layer of granular protoplasm, within which a largish reserve globule and a smaller oblate ellipsoidal refringent body were to be distinguished (FIG. 2, *S, a–q*).

Although formation of sexual spores on slightly thickened branches is known also in *Zoöpage cladosperma* Drechsl. (**3**), lateral conjugation has not hitherto been observed in any other member of the Zoöpagaceae. Frequently, indeed, union of the adjacent gametangia is accomplished by the fungus after a manner hardly familiar in any groups of cryptogams. For while the conjugation tube here is sometimes present as a commonplace short direct connection comparable to the lateral connections in species of *Spirogyra,* it more often takes a curiously circuitous course (FIG. 2, *O, c; Q; R*), winding about the parent branch in a complete turn, to give somewhat the appearance of a circular flange or collar. In most instances of such circumvolution the intricated parts are too badly obscured to permit their relationship to be made out with any clearness. This circumstance, together with the small dimensions of the apparatus generally, has made it difficult to determine whether the conjugation-tube may not in some cases originate from the proximal rather than from the distal gametangium, or, again, whether the tube may not result from apical fusion of two processes put forth separately by the two gametangia.

A term suggested in part by the frequent similarity of the conjugation-tube to a circular fastening, and in part by the development of this unusual structure on branches, may serve appropriately as specific name for the fungus.

Acaulopage gomphoclada sp. nov.

Mycelium sparsum, parce ramosum; hyphis continuis, hyalinis, leniter flexuosis, .6–1.3 μ crassis, ad animalcula inhaerentibus, pelliculam cujusque capti perforantibus, haustorium intrudentibus quod protoplasma exhaurit; haustoriis pediculatis, pediculo saepius 1.5–3 μ longo, .6–1 μ crasso, abrupte latescente, apice semel vel ter repetite bifurco, ita 2–8 ramulos assumentes divaricatos 1.5–8 μ longos 1.2–1.8 μ crassos ferente. Conidia hyalina, erecta, ex sterigmatis 1 μ altis oriunda, ex partibus duabus composita: parte supera vacua, 8–20 μ longa, circiter .5 μ crassa, vulgo plus minusve marcida vel collapsa; parte infera protoplasmatis repleta, cylindrata vel elongato-fusiformi, 11–22 μ longa, 1.3–1.8 μ crassa. Ramuli zygosporiferi vulgo 15–50 longi, 1.2–2 μ lati, quoque binas cellulas sexuales (gametangia) ferente, una terminali et saepius 12–20 μ longa, altera huic proxime posita et saepius 2–10 μ longa; tubulo conjugationis a latere excrescente, interdum circum ramulum voluto; zygosporangio plerumque ex cellula sexuali terminali orto, primum levi, sphaeroideo, 7–10 μ crasso, membrana hujus mox circum zygosporam laxe collapsa; zygospora flavida, globosa, verrucosa, saepius 6–9 μ crassa, maturitate membrana ejus .6–1.8 μ crassa, corpus protoplasmatis sphaerale 4.5–6 μ crassum circumdante.

Amoebas 10–40 μ latas capiens consumensque habitat in foliis putrescentibus *Poae pratensis* prope Beltsville, Maryland.

Mycelium sparse, sparingly branched; the vegetative hyphae continuous, hyaline, slightly flexuous, .6 to 1.3 μ wide, capturing minute

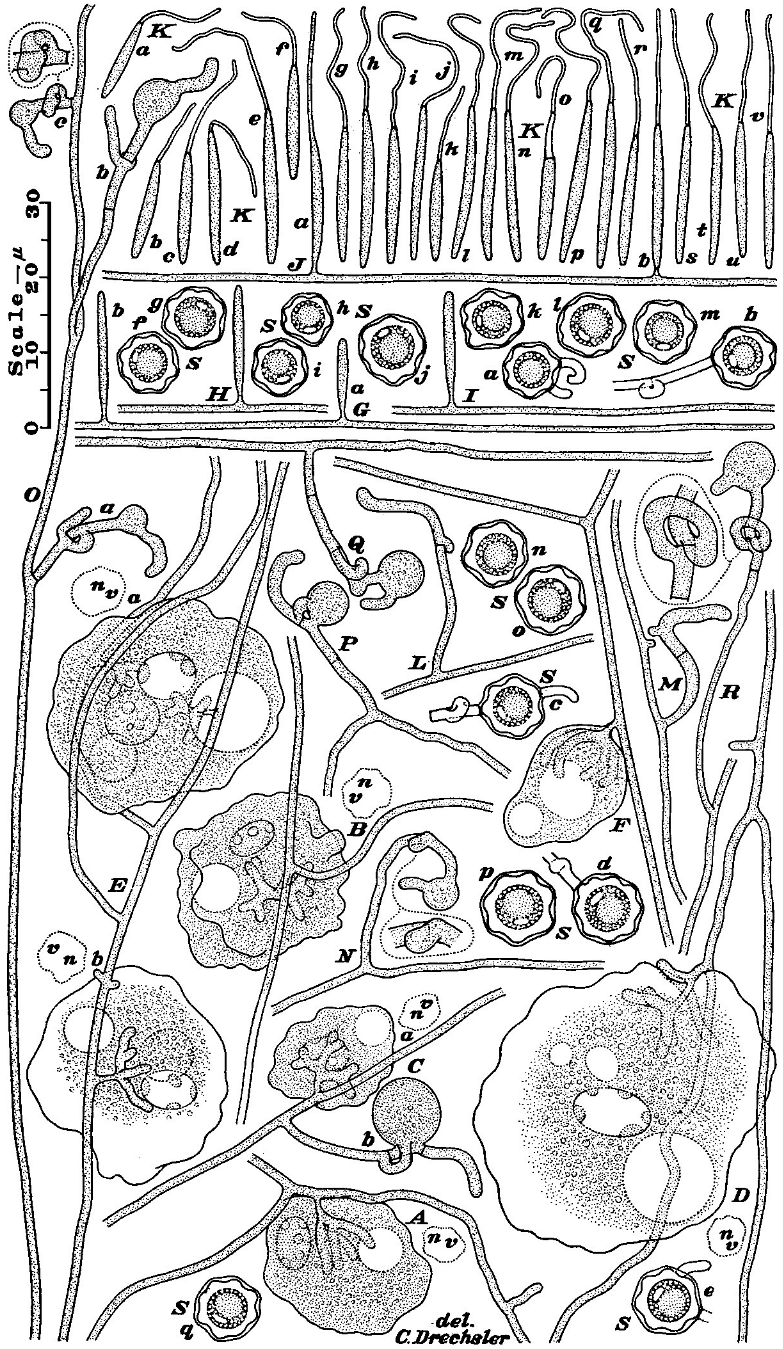

FIG. 2. *Acaulopage gomphoclada.*

animals through adhesion, then penetrating the pellicle of each captive and intruding into it a haustorium to appropriate the protoplasmic contents; haustoria pedicellate, the pedicel usually 1.5 to 3 μ wide and .6 to 1 μ thick, widening abruptly and bifurcating 1 to 3 times to terminate in 2 to 8 divergent assimilative branches 1.5 to 8 μ long and 1.2 to 1.8 μ wide. Conidia hyaline, erect, arising singly from sterigmata 1 μ long, each spore consisting of 2 parts: a distal empty part, mostly 8 to 20 μ long and about .5 μ wide, often present as a withered appendage; and a proximal part filled with protoplasm, cylindrical with somewhat tapering ends or elongate fusiform, measuring 11 to 22 μ in length and 1.3 to 1.8 μ in width. Paired sexual cells (gametangia) formed adjacent to each other by deposition of 2 septa in slightly widened lateral branches which often measure 15 to 50 μ in length and 1.2 to 2 μ in thickness—one of the cells, usually 12 to 20 μ long, constituting the terminal segment of the branch; the other, in penultimate position, varying usually from 2 to 10 μ in length. Conjugation always of lateral type, the tube sometimes short and direct, but more often somewhat circuitous in course and often rather closely enwrapping the lower portion of the distal cell and the upper portion of the proximal cell; zygosporangium most frequently formed about midway between base and tip of the distal cell, subspherical, commonly 7 to 10 μ in diameter, at first smooth, its envelope later collapsing somewhat loosely about the zygospore; zygospore yellowish, subspherical, commonly 6 to 9 μ in diameter, rather prominently verrucose, its wall .6 to 1.8 μ in thickness, surrounding a spherical protoplast usually 4.5 to 6 μ in diameter.

Capturing and consuming amoebae 10 to 40 μ wide it occurs in decaying leaves of *Poa pratensis* near Beltsville, Md.

A ROBUST COCHLONEMA WITH SMALL VERRUCOSE CONIDIA

Several maizemeal-agar plate cultures which after being permeated with mycelium of *Pythium myriotylum* Drechsl. had received some addition of partly decayed bluegrass leaves removed on May 10, 1941, from a heap of old lawn clippings in Arlington, Va., showed 11 days later many scattered white tufts just visible to the naked eye under strong lateral illumination. Examined microscopically under low magnification the tufts were found to consist of conidial chains and of moniliform filaments destined for conversion into conidial chains. The chains and filaments varied in number mostly from 10 to 25, and arose, erect or ascending, in bush-like arrangement, from a common origin. In general appearance the tufts resembled more particularly the conidiiferous tufts of *Cochlonema symplocum* Drechsl. (**6**), and the constituent spores, as in that species, were markedly verrucose. Despite these similarities it was evident, even with low magnification, that the catenated spores here were shorter than the homologous bodies of either *C. symplocum* or *C. verrucosum* Drechsl. (**1**).

Consonant with expectations suggested by the resemblances, the tufts on being examined under high magnification were found to originate from spiral thalli lying within collapsed pellicles of amoebae whose protoplasm had either wholly or in large part disappeared. Many animals (FIG. 3, *A–F*) showing earlier stages of infection moved slowly about on the substratum, the smaller individuals measuring approximately 35 μ across when drawn into a somewhat rounded form (FIG. 3, *A*), the larger ones of similar conformation (FIG. 3, *F*) attaining widths sometimes in excess of 60 μ. Each infected specimen was enveloped in a very thin pellicle, delicately rippled all around except where a broad pseudopodium was actively being pushed forward. During the earlier stages of parasitic attack, before pathological changes became noticeable, the host protoplasm remained of a finely granular consistency, permitting easy recognition of the single nucleus (FIG. 3, *A, n–F, n*) and of the contractile vacuole (FIG. 3, *A, v–F, v*). Prolate ellipsoidal in shape and measuring 10 to 14.5 μ and 8 to 11 μ along its major and its minor axis respectively, the nucleus was distinguished especially by circulatory movement, close under its peripheral membrane, of about 30 to 35 slightly darker subspherical or oblate ellipsoidal bodies ranging between 1 μ and 2 μ in greatest dimension. The number of intranuclear bodies, as also their curious cyclosis, appeared to indicate close kinship of the host rhizopod with the *Amoeba* previously observed being utilized as prey by *Stylopage cephalote* (**4**). While the animals attacked by the catenulate fungus were generally larger than those earlier found being captured by the capitate form, the difference in size was hardly sufficient to exclude the likelihood that the same protozoan species might have been concerned in both instances.

Infection is initiated through germination of a conidium (FIG. 3, *A*) or of several conidia (FIG. 3, *B*) unhappily ingested by the animal. The germ-tube put forth laterally or somewhat obliquely

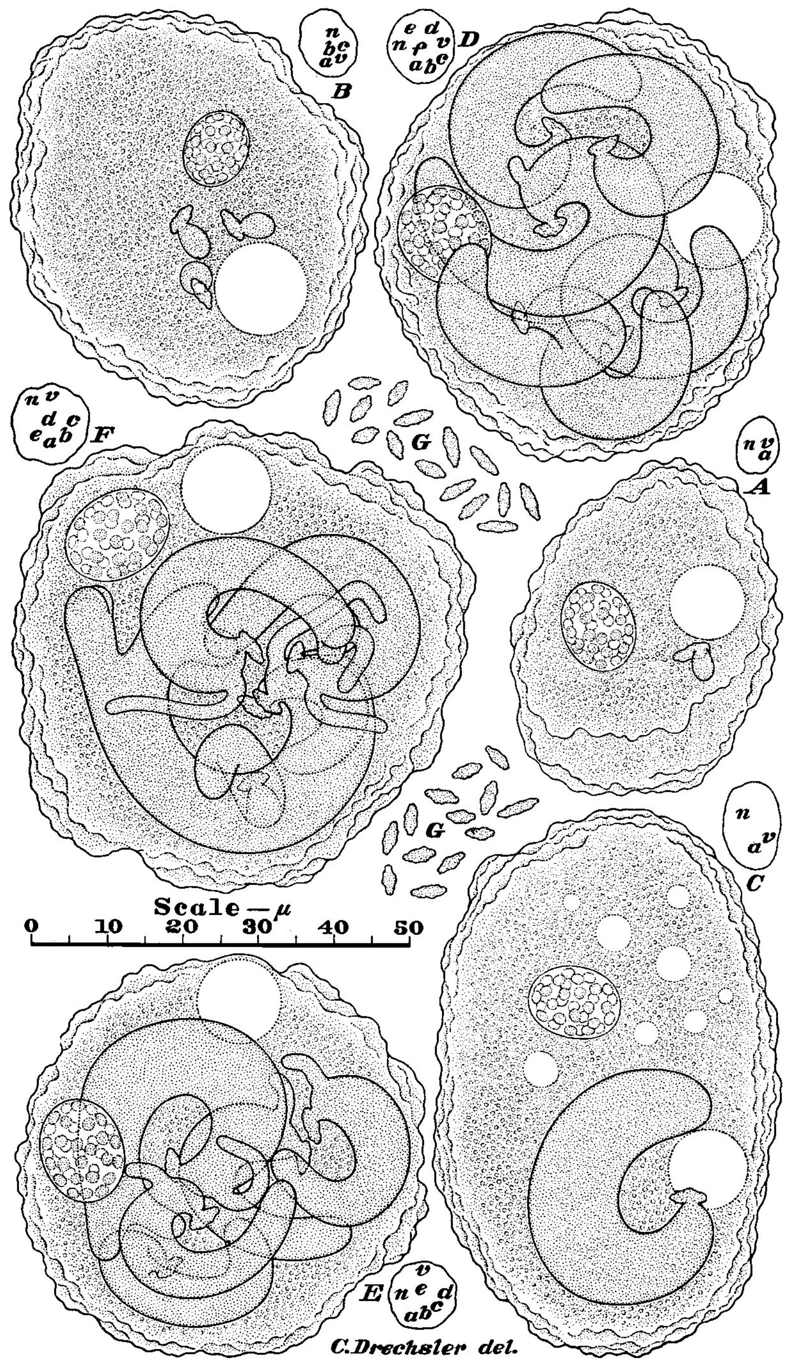

FIG. 3. *Cochlonema euryblastum.*

from the spore is much stouter than the proximal portions of corresponding outgrowths in *Cochlonema symplocum* and *C. verrucosum*. During early stages of growth it widens rather markedly, though soon further elongation proceeds at a nearly uniform or gradually diminishing diameter (FIG. 3, *C, a; D, a–e; E, a, b*). As the young thallus lengthens it curves into a flat spiral. Branching for the most part takes place only after a complete turn has been described, and consequently often remains absent in thalli that have failed to attain the necessary proportions before their food supply has been exhausted. Whether a thallus concludes its development as a simple hypha, or as a branched hypha, depends, therefore, not only on the size of the animal host, but also on the measure in which other thalli participate in the expropriation of available protoplasmic materials. In instances where a host animal, even of relatively large size, is infected simultaneously by 5 or 6 conidia, so that its substance is rather equally divided between a corresponding number of thalli, all of the thalli may remain simple (FIG. 3, *D, a–f*), though in instances of multiple infection at separate times, where, for example, 1 or 2 of the thalli have begun development earlier than their fellows, the older individuals may become branched (FIG. 3, *E, e; F, d, e*). When an animal has been infected simultaneously by only 3 conidia, all of the resulting thalli may show branching (FIG. 4, *A*), though naturally more abundant ramification is afforded when only a single thallus is present (FIG. 4, *B–D*), and especially when a single thallus has developed in an animal of unusually large size (FIG. 4, *C, D*). The first bifurcation, as in *C. megalasomum* Drechsl. (**5**), usually takes place in the plane of the first spiral coil (FIG. 3, *E, d; F, e*. FIG. 4, *A, a, b; B; C; D*), though occasionally a primary dichotomy may be somewhat oblique to that plane (FIG. 4, *A, c*). Some dichotomies of the second order (FIG. 3, *F, e*) as well as some of the third (FIG. 4, *D*) and fourth (FIG. 4, *C*) orders, when such higher ramifications are present, also lie in the plane of the first spiral coil. The generally flat spiral conformation maintained up to the second dichotomies is rather little disturbed by irregularity of angular relationships in the second, third, and fourth bifurcations, since the branches resulting from these later ramifications are so markedly reduced in length and thickness that they constitute only a small portion of the whole thallus.

When the animal host has been disabled for further locomotion, owing to continuing loss of protoplasm, the thallus initiates asexual reproduction by putting forth a reproductive hypha from a position on its convex profile usually 3 to 10 μ from its origin (FIG. 3, *D, f; E, b–e; F, c, d.* FIG. 4, *A, a–c; B*). If the thallus is large a second reproductive hypha is put forth simultaneously from a position on the convex profile usually 3 to 10 μ beyond the first (FIG. 3, *F, e.* FIG. 4, *C, D*). After growing through the enveloping host pellicle each reproductive hypha branches several times (FIG. 4, *B*) to extend into the air eventually from 3 to 15 filaments beset with warty protuberances and noticeably constricted at close intervals. Once the individual filament has reached definitive length, it is converted into a chain of verrucose conidia through deposition of cross-walls at the constrictions (FIG. 3, *G.* FIG. 4, *C, E*). Development of the several spore chains that originate from the same reproductive hypha is in considerable measure successive, additional sporiferous branches being extended until the thallus has yielded up all its contents. Departure of protoplasm from a thallus is accompanied by progressive, conspicuous vacuolization (FIG. 4, *C; D; E*), but apparently does not entail deposition of retaining septa within the thallodic envelope.

The parasite is obviously referable to *Cochlonema,* and in that genus appears most closely akin to *C. verrucosum* and *C. symplocum.* From these species it differs markedly with respect to vegetative habit, especially when its thallus attains a size large enough to permit repeated branching. Since, however, the distinctly broad attachment between germinating conidium and thallus is observable much more often than abundant distal ramification, the fungus is described under an epithet compounded of two words meaning "wide" and "sprout," respectively.

Cochlonema euryblastum sp. nov.

Hyphae assumentes protinus ex tubo germinationis saepius 1.5–2 μ crasso latescentes, hyalinae, continuae, 6–15 μ crassae, usque 125 μ longae, in spiram planam semel subinde paene bis volutae, nunc simplices nunc semel bifurcae nunc etiam bis vel ter vel quater crebro dichotomae, prope originem ex latere convexo unam hypham genitabilem vel quandoque duas hyphas genitabiles emittentes; hyphis genitabilibus 2–3.2 μ crassis, quoque sursum 3–15 ramos erectos vel ascendentes in aerem proferente qui in catenulas 30–80 conidiorum abeunt; conidiis hyalinis, verrucosis, plerumque 3–6 μ longis, 1.5–2 μ crassis.

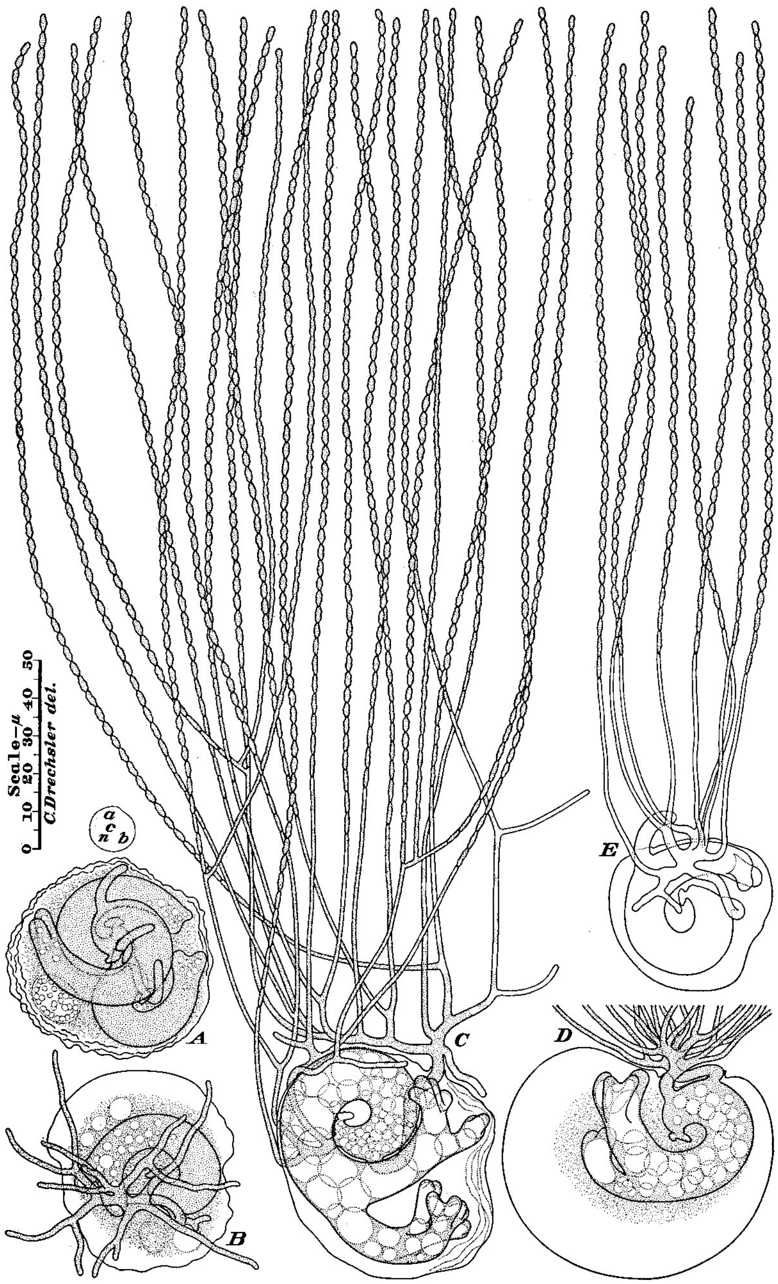

FIG. 4. *Cochlonema euryblastum.*

Amoebas vulgo 35–60 μ latas enecans habitat in foliis *Poae pratensis* putrescentibus in Arlington, Virginia.

Assimilative hyphae widening out immediately from a germ-tube often 1.5 to 2 μ in thickness, hyaline, continuous, 6 to 15 μ in diameter, up to 125 μ in length, convolved in a flat spiral of one turn or occasionally of nearly two turns, often simple but sometimes once bifurcate and occasionally even further dichotomizing, though at shorter intervals, a second, third, or fourth time; the smaller specimens putting forth, from a position on the convex profile close to the proximal end, a single reproductive filament, the larger specimens putting forth 2 such filaments. Reproductive filaments 2 to 3.2 μ wide, each extending into the air 3 to 15 branches, which soon are converted in large part into chains of 30 to 80 conidia; conidia hyaline, warty, mostly 3 to 6 μ long and 1.5 to 2 μ wide.

Destroying amoebae commonly 35 to 60 μ wide it occurs in decaying leaves of *Poa pratensis* in Arlington, Va.

UTILIZATION BY ACAULOPAGE TETRACEROS OF THE AMOEBA CAPTURED BY ZOÖPAGE THAMNOSPIRA

In the original description of *Acaulopage tetraceros* little information was supplied relative to the morphology and specific identity of the animals found captured by the fungus. Cultures abundantly bestrewn with inversely lageniform and plurally appendaged conidia have come under observation from time to time in subsequent years, without, however, providing much additional knowledge of the prey; for usually when asexual reproduction had advanced far enough to invite attention, predaceous activity had virtually come to an end. Better success attended observations on an old *Pythium* culture to which had been added a few pinches of leaf mold collected in deciduous woods near Beltsville, Md., on January 7, 1941. Ten days after the decaying refuse was added predaceous activity appeared in two separate areas, and accompanying it, early development of conidia in sufficient quantity to permit identification of the two distinct zoöpagaceous forms concerned. In one of the tracts *Zoöpage thamnospira* Drechsl. (4) was readily recognized both from the morphology of its catenulate conidia, and from the gracefully coiled, thallus-like haustoria it extended into the captured amoebae. As in the cultures whereon the description of *Z. thamnospira* was based, the prey often measured about 40 μ across when

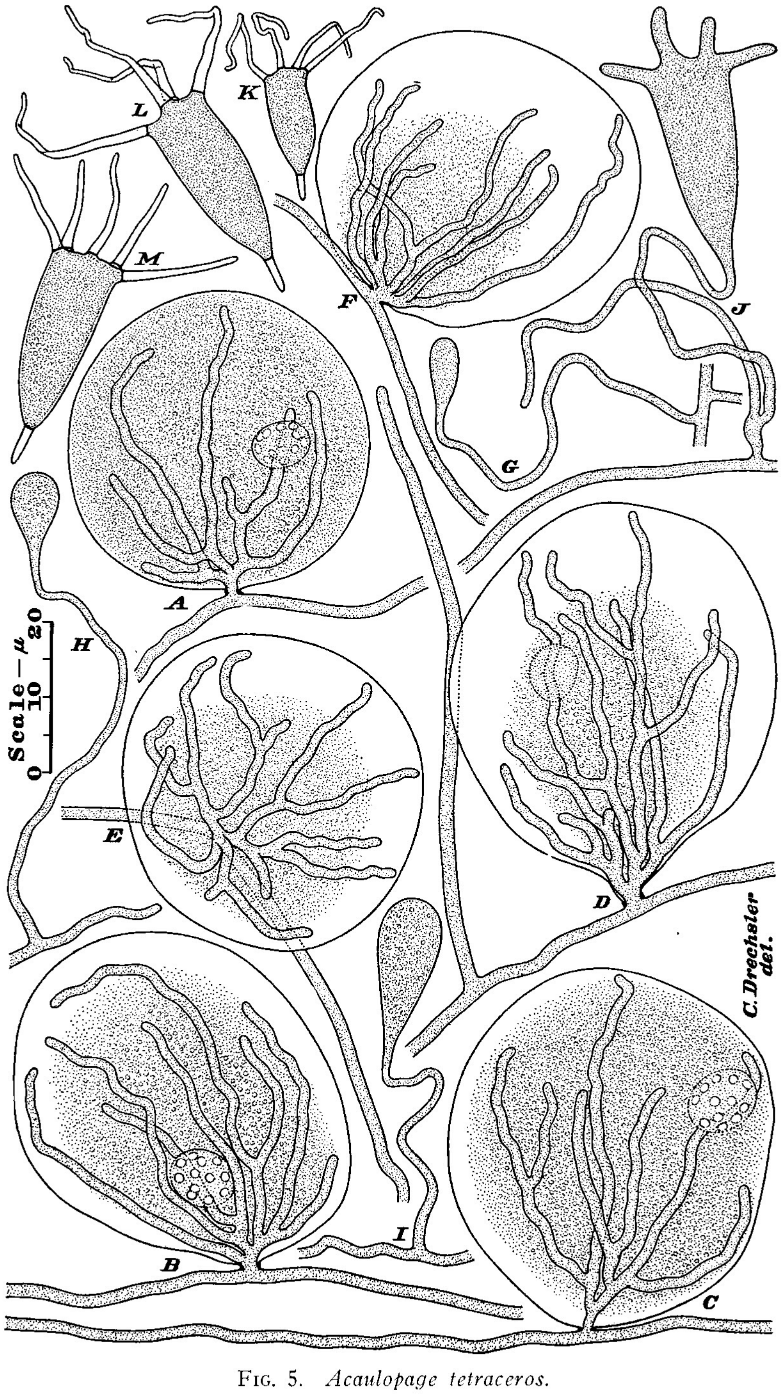

FIG. 5. *Acaulopage tetraceros.*

drawn into an approximately round shape, and contained a prolate ellipsoidal nucleus within which a dozen somewhat flattened orbicular bodies were distributed in positions close under the peripheral membrane. Amoebae entirely similar with respect to dimensions and nuclear organization (FIG. 5, *A–C*) were preyed upon also in the other tract of substratum, where, however, the protoplasmic materials were assimilated by means of more commonplace bush-like haustoria whose rangy branches showed no coiling and did not exceed the parent filaments in width (FIG. 5, *D–F*). Here and there the mycelial hyphae bore prostrate branches on whose erect tips were borne swollen bodies in various stages of development (FIG. 5, *G–J*) into conidia typical of *A. tetraceros* (FIG. 5, *K–M*). Accordingly the species of *Amoeba* habitually captured by *Z. thamnospira* is to be recognized also as prey of *A. tetraceros*. The animal further seems to be an intimate relative of the *Amoeba* parasitized by *Cochlonema euryblastum*, since its prolate elliptical nucleus, like that of the latter protozoan, shows orbicular bodies in gentle rotational movement close under the peripheral membrane. Yet as the rotating intranuclear bodies present here are conspicuously less numerous than those present in the host of *C. euryblastum*, the rhizopods are perhaps better considered to be merely congeneric rather than conspecific.

A VARIETY OF COCHLONEMA BACTROSPORUM WITH LONG CONIDIA

Seven weeks after some pinches of leaf mold collected near Haugen, Wis., in September 1939, had been added to an old *Pythium* culture on maizemeal agar, the medium adjacent to one of the deposits showed a colony of *Heleopera sylvatica* Penard (**7**), numbering nearly a hundred individuals, being exterminated by a *Cochlonema* corresponding in nearly all respects to the description of *Cochlonema bactrosporum* (**5**). On close scrutiny it was found that the animals undergoing destruction were noticeably larger than those previously found parasitized in the cultures planted with decaying material from Beltsville, Md.; for they measured about 80 μ in average length, and about 50 μ in average width, as compared with corresponding values of 65 μ and 42 μ, respectively, derived from measurements of the earlier specimens. As far as

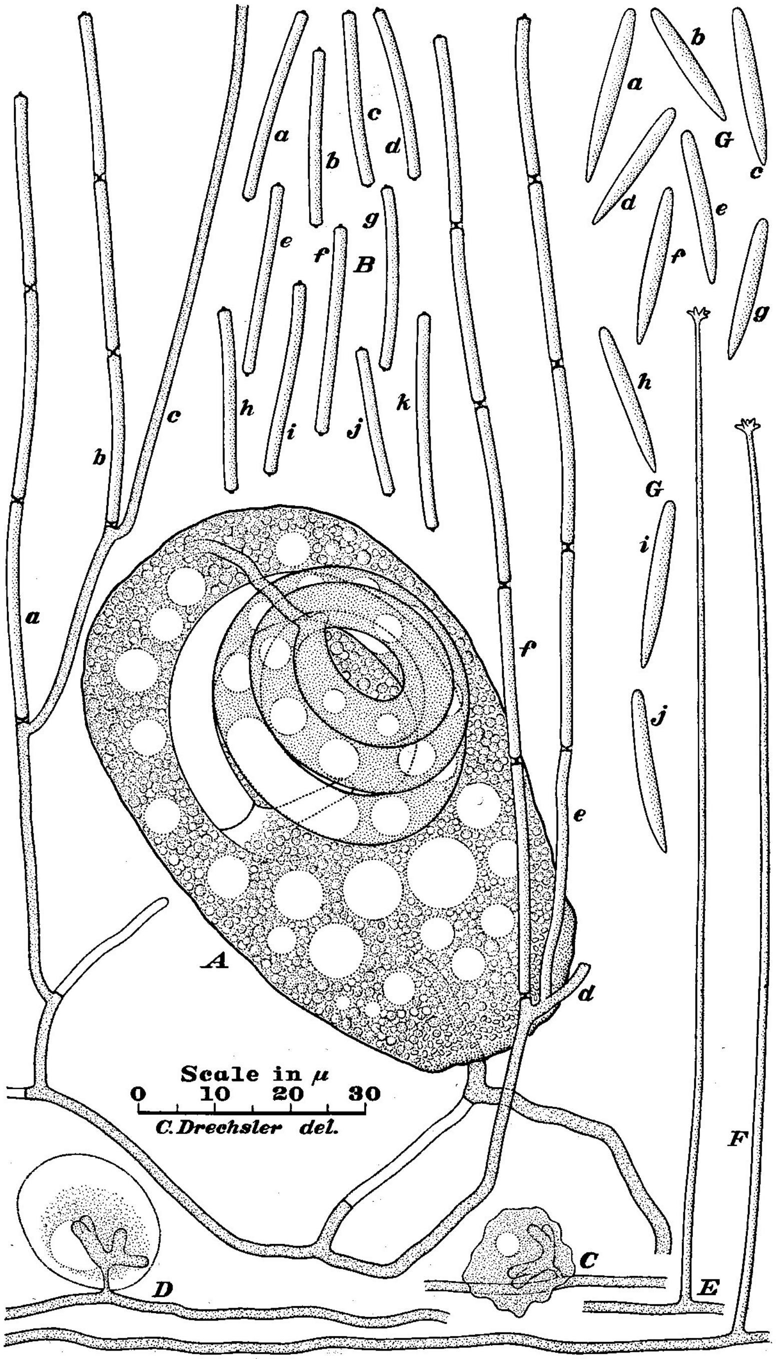

FIG. 6. *A, B, Cochlonema bactrosporum* var. *longius; C–G, Stylopage cephalote.*

could be determined under very troublesome optical difficulties arising from the globulose texture of the degenerating host protoplasm, the grandiose helicoid thalli of the parasite (FIG. 6, *A*) resembled those previously encountered; and the resemblance extended evidently both to the reproductive filaments and to the aerial sporiferous branches while in immature condition. However segmentation of the aerial branches (FIG. 6, *A, a–f*) here resulted in conidia (FIG. 6, *B, a–k*) fully half again as long as those of the Maryland fungus. Since the material from either of the two sources showed only moderate variability in conidial length, the fungus from Wisconsin would seem to merit recognition as a distinct variety.

Cochlonema bactrosporum var. **longius** var. nov.

Speciei typicae simile ad hypham alitam et hyphas fertiles; conidiis catenulatis, hyalinis, levibus, cylindratis, vulgo 20–31 μ longis, 1.6–1.9 μ crassis, utrimque in verruculam minutam abeuntibus.

Heleoperam sylvaticam formae grandis enecans habitat in humo silvestri prope Haugen, Wisconsin.

Similar to the type of the species with respect to vegetative hypha and conidiiferous filaments; conidia catenulate, hyaline, smooth, cylindrical, commonly 20 to 31 μ long, 1.6 to 1.9 μ wide, at each end terminating in a minute warty protuberance.

Destroying *Heleopera sylvatica* of a large type, it occurs in leaf mold near Haugen, Wis.

SUPPLEMENTARY OBSERVATIONS ON STYLOPAGE CEPHALOTE

The same set of cultures that after being planted with partly decayed blue-grass leaves gave rise to *Cochlonema euryblastum* afforded development also of *Stylopage cephalote*. The latter fungus here subsisted through capture of an *Amoeba,* within whose prolate ellipsoidal nucleus about a dozen orbicular bodies appeared in gentle movement close under the peripheral membrane. With respect to number of intranuclear bodies, therefore, the animal agreed rather accurately with the *Amoeba* found subject to capture by both *Acaulopage tetraceros* and *Zoöpage thamnospira.*

Stylopage cephalote also developed rather abundantly in several maizemeal-agar plate cultures that had been planted with a few pinches of leaf mold from a collection of this material made near Charleston, S. C., in February, 1941. At the time observations were begun the fungus had nearly concluded its vegetative growth. Only a few small amoebae were found adhering to the hyphae in newly captured condition (FIG. 6, *D, C*); the captives measuring about 15 μ across when drawn into an approximately round shape, and revealing no nucleus in their turbid protoplasm. Remnants of pellicles more capacious than any that could have been left by such small animals were found attached here and there, indicating that larger prey may previously have been exterminated in furnishing a richer supply of nourishment. Many of the conidiophores arising from the predaceous filaments showed dimensions in tolerable agreement with the original description of the species (**4**); though others, again, gave measurements for height in excess of 120 μ or 130 μ (FIG. 6, *E, F*), and measurements for subterminal width as small as .6 μ or .8 μ. The conidia (FIG. 6, *G, a–j*) produced on these taller and more slender supporting hyphae showed no concomitant departure in morphology.

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EXPLANATION OF FIGURES

FIG. 1. *Acaulopage lasiospora;* drawn with the aid of a camera lucida to a uniform magnification; × 1000 throughout. *A,* Portion of hypha on which a relatively large amoeba has been captured by adhesion; at each of the three places of adhesion a haustorium is shown growing into the protoplasm; within the animal's body are visible also ten small contractile vacuoles

and three round bodies of uncertain function. *B*, Portion of mycelium with a captured amoeba; the latter being shown at an early stage of invasion by the haustorium. *C*, Portion of hypha on which two amoebae, *a* and *b*, have been captured by adhesion; within the sarcode of each animal portions of haustorial branches are faintly visible here and there. *D*, Portion of hypha with a captured amoeba within whose dense protoplasm portions of haustorial branches are faintly visible. *E*, Portion of hypha on which two small amoebae, *a* and *b*, have been captured; the smaller captive, *a*, has been depleted of protoplasm in sufficient measure to make the haustorium faintly visible throughout; in the slightly larger captive, *b*, depletion of protoplasm is further advanced, so that the haustorium has become clearly visible throughout. *F*, Portion of mycelium with two captured amoebae, *a* and *b*; each captive having been expropriated of its contents in such large measure that the haustorium has become clearly visible. *G, H, I*, Portions of mycelium, each with a captured amoeba; each captive has been almost wholly depleted of its protoplasm, so that the haustorium is clearly visible. *J–P*, Fertile branches, each bearing a growing conidium at its tip. *Q*, Portion of mycelium with a young conidium from which protuberances are being extended. *R*, Portion of mycelium with two conidia, *a* and *b*, whose protuberances are fully extended. *S*, Portion of hypha with a conidium whose fully extended and evacuated protuberances are arranged asymmetrically relative to the conidial axis. *T*, Mature conidium shown attached to a supporting branch from which the protoplasmic contents have been mostly withdrawn. *U*, Mature or nearly mature conidium attached to a branch that is still filled with protoplasm. *V*, Mature conidia, *a–g*, showing variations in size and shape of living cell, as well as in number, dimensions, and distribution of the empty appendages.

FIG. 2. *Acaulopage gomphoclada;* drawn with the aid of a camera lucida to a uniform magnification; × 1000, except for supplementary drawings (each surrounded by a dotted line) showing details of conjugation in *N, O, R*, which are reproduced at a magnification of about 2000 diameters. *A, B*, Portions of mycelium, each with a captured amoeba into which a haustorium has been intruded; *n*, nucleus of animal; *v*, contractile vacuole. *C*, Portion of hypha which besides intruding a haustorium into the captured amoeba *a*, has given rise to a sexual branch, *b*, showing development of a nearly full-grown zygosporangium; *n*, nucleus of captured amoeba; *v*, contractile vacuole. *D*, Portion of mycelium with a captured amoeba whose contents have been largely assimilated by means of a single haustorium; *n*, nucleus of amoeba; *v*, contractile vacuole. *E*, Portion of mycelium from which two haustoria have been intruded into a captured amoeba, *a*, while a single haustorium has been intruded into a second amoeba, *b*; *n*, nucleus of each amoeba; *v*, contractile vacuole of each amoeba. *F*, Portion of mycelium from which a haustorium has been intruded into a captured amoeba. *G*, Terminal portion of a mycelial filament, showing two conidia, *a* and *b*, in early stages of development. *H, I*, Portions of mycelial hypha, each showing an early stage in development of a conidium. *J*, Portion of mycelium showing one conidium, *a*, in an advanced stage of development, and another, *b*, in mature condition. *K*, Mature conidia, *a–v*, showing variations in the dimensions both of the living cell and of the empty appendage. *L, M*, Portions of mycelial hyphae,

each bearing a sexual reproductive branch in an early stage of development. *N*, Portion of hypha bearing a sexual branch with a conjugation-tube and a young zygosporangium. *O*, Portion of hypha bearing three sexual branches, *a*, *b*, *c*, each showing a conjugation-tube and a young zygosporangium. *P*, *Q*, *R*, Portions of mycelial hyphae, each bearing a sexual branch with a half-grown zygosporangium. *S*, Mature zygospores—some of them, *a–e*, shown with empty attachments; the others, *f–q*, shown without empty parts.

FIG. 3. *Cochlonema euryblastum;* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Small specimen of the susceptible *Amoeba*, within which a single conidium, *a*, has begun to germinate; *n*, nucleus of host animal; *v*, contractile vacuole. *B*, Medium-sized specimen of the susceptible *Amoeba*, within which three conidia, *a*, *b*, *c*, have begun to germinate; *n*, nucleus of host animal; *v*, contractile vacuole. *C*, Rather large specimen of host *Amoeba* containing a single growing thallus, *a;* *n*, nucleus of host animal; *v*, contractile vacuole. *D*, Large specimen of host *Amoeba* containing six thalli, *a–f*, one of which, *f*, has begun putting forth a reproductive hypha; *n*, host nucleus; *v*, contractile vacuole. *E*, Fairly large specimen of host *Amoeba* containing five thalli, *a–e*, of which four, *b–e*, have each begun to put forth a reproductive hypha; *n*, host nucleus; *v*, contractile vacuole. *F*, Large specimen of host *Amoeba* containing five thalli, *a–e*, two of which, *c*, *d*, are each putting forth a single reproductive hypha, while another, *e*, of greater size, is putting forth two reproductive hyphae; *n*, host nucleus; *v*, contractile vacuole. *G*, Random assortment of conidia, showing variations in size, shape and sculpturing.

FIG. 4. *Cochlonema euryblastum;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Specimen of host *Amoeba*, within which three thalli, *a*, *b*, *c*, have developed; each thallus shows a single dichotomy, and each has begun to put forth a single reproductive hypha; *n*, host nucleus. *B*, Specimen of host *Amoeba* whose protoplasmic contents have been assimilated almost entirely in the development of the distally bifurcate thallus, which near its proximal end has put forth a reproductive hypha that has produced several branches destined to grow into sporiferous aerial filaments. *C*, Collapsed pellicle of a parasitized *Amoeba*, within which a large thallus with four successive bifurcations has developed; the thallus, though not yet wholly depleted of contents, has put forth two reproductive hyphae, which together have given rise to three conidiiferous hyphae and twenty chains of conidia. *D*, Specimen of host *Amoeba* containing a thallus with three successive bifurcations; at its proximal end the thallus has put forth two reproductive hyphae that have branched copiously in giving rise to aerial conidiiferous hyphae whereof only the basal portions are shown. *E*, Empty pellicle surrounding membranous envelope of twice bifurcate thallus, which at its proximal end has put forth a single reproductive filament that has branched in giving rise to eight chains of conidia.

FIG. 5. *Acaulopage tetraceros;* drawn with the aid of a camera lucida to a uniform magnification; × 1000 throughout. *A*, *B*, *C*, Portions of hyphae with captured specimens of *Amoeba* sp., into which rangy arbuscular systems have been extended; each captive reveals a nucleus of approximately normal structure. *D*, *E*, *F*, Portions of hyphae with captured specimens of *Amoeba* sp.; the captives have lost nearly all their protoplasmic contents, and their

nuclei are no longer clearly recognizable. *G, H, I, J,* Creeping mycelial branches on each of which a conidium is being formed terminally. *K, L, M,* Mature conidia.

FIG. 6. Drawn with the aid of a camera lucida to a uniform magnification; × 1000 throughout.

A, B, Cochlonema bactrosporum var. *longius: A,* Specimen of *Heleopera sylvatica* containing a helicoid thallus of the parasite; from its proximal end the thallus has put forth a reproductive hypha, which on emerging from the mouth of the animal host has sent a few short branches into the substratum and given rise to two main branches; from one of these main branches two conidial chains *a, b,* and a young sporiferous hypha, *c,* have been extended, while the other main branch has given rise to three chains of conidia, of which two, *e* and *f,* are still intact, whereas the third is represented only by a sterile basal support *d.* (Owing to lack of space only proximal portions of the sporiferous hypha and of the four conidial chains are shown.) *B,* Random assortment of conidia, *a–h,* showing variations in length.

C–G, Stylopage cephalote from a culture planted with leaf mold collected in South Carolina: *C,* Portion of hypha from which a pedicellate haustorium has been intruded into a small amoeba captured through adhesion; though the captive is still alive, as is evident from the normal functioning of its contractile vacuole, no nucleus is visible in the turbid protoplasm. *D,* Portion of hypha from which a haustorium has been intruded into a captured amoeba; as the protoplasm of the captive has been very largely assimilated, the delicate pellicle has become flattened out so as to show a smooth outer contour. *E, F,* Portions of prostrate hyphae from which unusually tall slender conidiophores have arisen. *G,* Random assortment of conidia, *a–j,* showing usual variations in size and shape.