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VARIOUS ZOÖPAGACEOUS FUNGI SUBSISTING ON PROTOZOANS AND EELWORMS

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(WITH 6 FIGURES)

Several animal-destroying conidial phycomycetes are reported herein that came under observation in agar plate cultures following the addition of small quantities of decaying vegetable detritus. Two of the fungi, both endoparasitic in *Amoebae,* are being described as new members of the Zoöpagaceae, one of them being readily assigned to the genus *Cochlonema,* while the other differs sufficiently from related forms to merit recognition as the type of a separate genus. Some discussion is given to two other species which subsist endoparasitically on protozoans and embody features that in some degree extend the scope of morphological diversity in the family. The earlier account of my *Acaulopage lophospora* (**10**: 133–137) is amplified by a description of the sexual stage belonging to that fungus. Incomplete observations on a nematode parasite referable to the curious genus *Euryancale* are recorded.

A COCHLONEMA WITH ZYGOSPORANGIA HAVING DISTALLY LOBED COLUMNAR PROTUBERANCES

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted by adding a few pinches of decaying marsh grass (*Spartina* sp.) detritus taken up near Mayo, Maryland, on August 20, 1946, showed three weeks later some scattered *Amoebae* that contained spirally convolved thalli of a zoöpagaceous parasite (FIG. 1, *A–E*). The infected animals usually measured 45 to 70 μ in width when

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they were drawn into a rounded shape. Each was enveloped by a thin pellicle which for the most part appeared cast into broadly undulating folds. Each contained a single contractile vacuole (FIG. 1, *B–D*: *v*). In newly invaded specimens the finely granular protoplasm surrounded a prolate ellipsoidal nucleus (FIG. 1, *B*, *n*), often 18 to 24 μ long and 11 to 14 μ wide, that showed slightly dark elongated bodies arranged peripherally, close under the delimiting membrane. With respect to shape and internal organization, therefore, the nucleus here corresponded fairly well to that of *Amoeba terricola* Greeff—the rhizopod earlier found captured by my *Acaulopage marantica* (**7**: 143–149) and also parasitized both by my *Endocochlus gigas* (**5**: 368–371) and my *Cochlonema megaspirema* (**6**: 235–241). In infected animals where the fungus was found in a more advanced state of vegetative development the peripheral bodies often seemed shorter and more nearly round (FIG. 1, *C*, *n*; *D*, *n*), thereby in some degree approaching the shape and dimensions of the peripheral bodies in the nucleus of the animal attacked by my *Cochlonema euryblastum* (**9**: 283–289). Perhaps in such instances the peripheral bodies may have undergone pathological modifications, especially as the interior of the nucleus was noticeably abnormal in its darkened mottled appearance. Nevertheless, the identity of the animal with Greeff's species cannot be regarded as wholly certain.

The manner in which the fungus gains entrance into its protozoan host has never come directly under observation. However, since the thallus has never been found with an empty conidial envelope attached to it, infection most probably takes place as in *Endocochlus gigas*. At an early stage in its development the thallus is recognizable as a prolate ellipsoidal body (FIG. 1, *A*, *a*) about 10 μ long, either straight or slightly curved along its major axis. On elongating it soon shows rather pronounced curvature (FIG. 1, *A*, *b*). When it attains a length of 20 to 30 μ its growing tip often reaches a position near its proximal end, thereby forming a tight coil of a single turn (FIG. 1, *B*, *a*; *C*, *a*). Although some further elongation often takes place nearly in the plane of the first turn (FIG. 1, *C*, *b*, *c*), the second turn for the most part is applied somewhat laterally to the first (FIG. 1, *C*, *d*).

Usually when a coil of 2 or 2¼ turns has been formed the hypha bifurcates distally (FIG. 1, *A, c–e; C, d*). The two branches, after describing an additional quarter (FIG. 1, *A, c–e*) or half turn (FIG. 1, *D, a*), again bifurcate. A third (FIG. 1, *D, a*) and a fourth dichotomy (FIG. 1, *E*) often ensue, following increases in length not greatly exceeding 15 μ. In each dichotomy the width of the resulting branches is noticeably reduced. The narrowed branches of the second, third, and fourth orders show increasing curvature, thereby adding to the distinctive appearance of the massive hyphal clew (FIG. 1, *D, a; E*). Owing to general similarities in the convolvement and branching of its thalli, the fungus recalls more especially *Endocochlus gigas, Cochlonema euryblastum,* and my *Cochlonema agamum* (**10**: 120–133).

Like many related forms the fungus begins asexual reproduction when the animal host becomes incapable of further locomotion, mainly from loss of protoplasm. While assimilation of the remaining host material continues the vegetative thalli put forth slender reproductive filaments. A small thallus usually gives off a single reproductive filament from a position near its proximal end and on the outer convex side of the coil (FIG. 2, *A, a; B, a*). Larger thalli give rise commonly to several reproductive filaments (FIG. 2, *C, a–d*) from positions mostly 5 to 10 μ apart, along the convex side of the first turn of the coil. Many reproductive filaments, after breaking through the host pellicle, push their way through the overlying or surrounding solid materials, and then elongate in the air. The submerged portions of such filaments show no outward modification, but in the aerial prolongations narrowed constrictions spaced at regular intervals set off a series of expanded portions (FIG. 2, *D*). As the reproductive filaments continue their growth the thallus supplying them becomes more and more vacuolate (FIG. 2, *A–C*). When the thalli have been emptied of all living contents, terminal growth in the filaments ceases and each aerial prolongation is converted into a chain of spores by the laying down of cross-walls in the narrowed isthmuses (FIG. 2, *E, a–d*). On slight disturbance the chains break up, leaving the substratum strewn with conidia (FIG. 2, *F, a–z; G, a–l*) ready to attack any suitable host animal that may come. In their somewhat

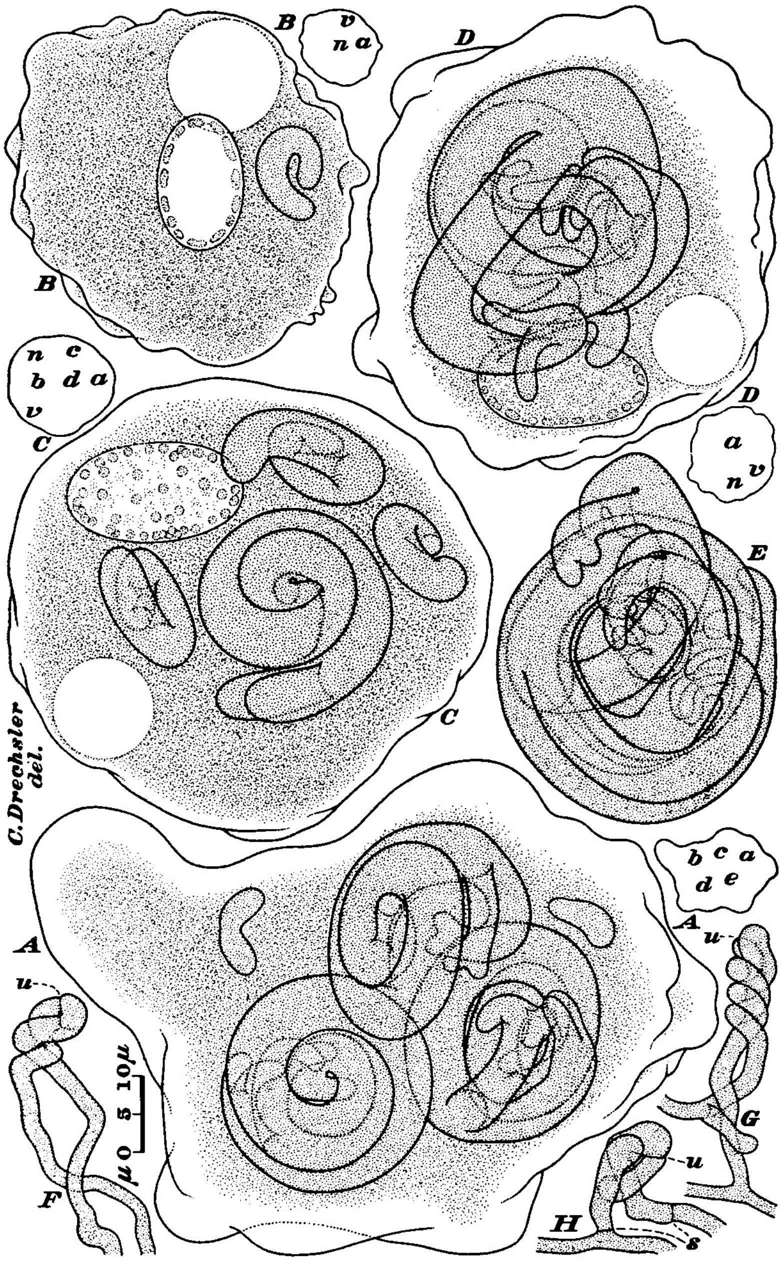
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FIG. 1.

cylindrical shape and slightly warty sculpturing the conidia resemble those of my *Cochlonema odontosperma* (**6**: 229–235) and of my *C. megalosomum* (**7**: 128–137). Their dimensions would seem a little larger than the corresponding dimensions of *C. odontosperma*, and a little smaller than those of *C. megalosomum*.

Sexual reproduction is initiated by the pairing of some reproductive hyphae soon after they have broken through the host pellicle. As far as could be determined from the meager material available, the paired hyphae regularly have their origin in separate thalli. Once they have made apical contact with each other, the two hyphae grow conjointly in length, winding about one another with either right-handed (FIG. 1, *F*) or left-handed rotation (FIG. 1, *G*). Often, again, the two hyphae show no spiral intertwining, but are interlocked in a more haphazard manner by means of irregular lobes or short lateral branches (FIG. 1, *H;* FIG. 2, *H*). Through deposition of a wall (FIG. 1, *H, s;* FIG. 2, *H, s*) in each of the hyphae a pair of gametangia are delimited which become united when the membranes at the contiguous tips (FIG. 1, *F, u; G, u*) are dissolved (FIG. 1, *H, u;* FIG. 2, *H, u*). A globose excrescence thereupon buds out laterally from one of the gametangia in a position close to the union. This excrescence gradually expands to a diameter of 12 to 15 μ. In the later stages of its growth it puts forth many columnar protuberances that terminate individually in three or four well marked lobes (FIG. 2, *H, c*). The

FIG. 1. *Cochlonema calosperma*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing two very young thalli, *a* and *b*, of the fungus, as well as three larger thalli, *c–e*. *B*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing a rather small thallus, *a*, of the parasite; *n*, host nucleus; *v*, contractile vacuole of animal host. *C*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing three coiled unbranched thalli, *a–c*, and a large coiled thallus with one bifurcation, *d; n*, nucleus of animal host; *v*, contractile vacuole of animal host. *D*, Specimen of *Amoeba* sp. (possibly *A. terricola*) containing a large thallus, *a*, bifurcating successively three times; *n*, nucleus of animal host; *v*, contractile vacuole of host. *E*, Large thallus with four successive bifurcations. *F*, Pair of sexual branches intertwined with right-handed rotation; *u*, apical contact between the branches. *G*, Pair of sexual branches intertwined with left-handed rotation; *u*, apical contact between the branches. *H*, Pair of sexual branches not spirally entwined; *s*, cross-walls delimiting the two gametangia basally; *u*, place of union between gametangia.

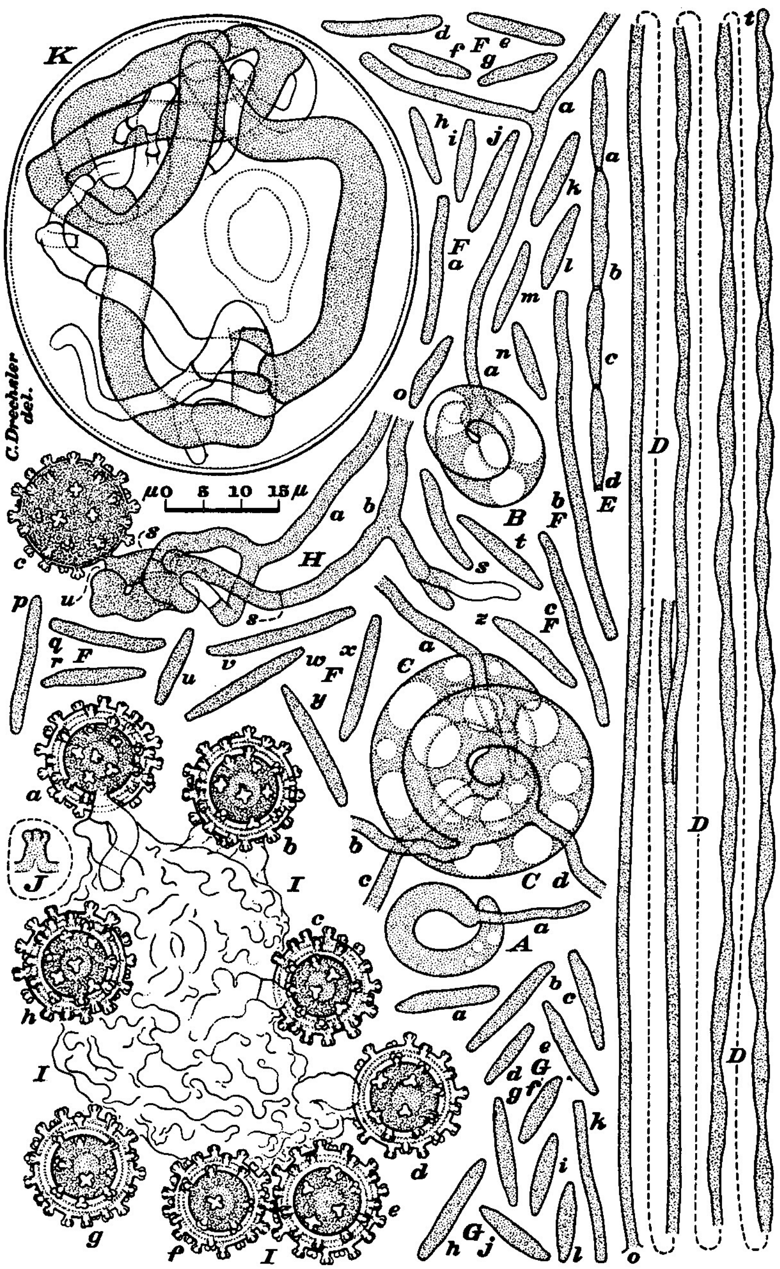

FIG. 2.

handsomely ornamented globose body now undergoes the usual internal changes entailed in the ripening of a zygospore. At maturity it contains a spherical protoplast often 8 to 9 μ in diameter (FIG. 2, *I, a–h*). This protoplast seems composed of a homogeneous central mass and a parietal layer of granular texture. Further details in structure of protoplast and wall are alike obscured through the presence of the many protuberances, which apparently consist of solid wall material (FIG. 2, *J*). It remains uncertain whether a single thick envelope of plural layers lies between the protoplast and the protuberances, or whether a zygospore wall proper is loosely surrounded by a separate zygosporangial envelope.

A term having reference to the handsome and very distinctive ornamentation of the sexual reproductive body is deemed appropriate as specific name for the fungus.

Cochlonema calosperma sp. nov.

Hyphae assumentes incoloratae, primo continuae, in modum cornus dilatatae, 3–11 μ crassae, usque 150 μ longae, in spiram cochleatim semel vel bis vel ter volutae, interdum (plerumque quandocumque tantummodo semel vel bis volutae) simplices interdum (plerumque quandocumque bis vel ter volutae) semel usque quater repetite dichotomae, prope originem saepe 1–4 quandoque plures hyphas genitabiles emittentes; hyphis genitabilibus 1.2–2 μ crassis, animali debilitato vel moribundo pelliculam ejus perforantibus, denique in aërem catenulas conidiorum ascendentes proferentibus aut in materia subjacenti vel ambienti ramos zygosporiferos gignentibus; conidiis incoloratis,

FIG. 2. All parts except *J* drawn to a uniform magnification with the aid of a camera lucida; × 1000 in all parts except *J*. *A–I, Cochlonema calosperma: A, B,* Small unbranched thalli from each of which a reproductive hypha, *a*, is being extended. *C,* Large coiled thallus from which four reproductive hyphae, *a–d,* are being extended. *D,* Reproductive hypha having its origin, *o*, in a thallus; its proximal sterile portion showing little variation in width but its distal portion showing successive constrictions at which conidia will later be delimited; *t,* growing tip; for lack of space the filament is shown in four parts whose proper connection is indicated by broken lines. *E,* Distal portion of conidial chain, showing four separate conidia, *a–d.* *F* (*a–z*), *G* (*a–l*), Random assortment of detached conidia. *H,* Immature unit of sexual apparatus, showing: *a, b,* the two sexual branches; *c,* the nearly fully grown zygosporangium with its distally lobed protuberances; *s,* cross-walls delimiting the gametangia basally; *u,* place of union between gametangia. *I,* Empty *Amoeba* pellicle around which are grouped eight mature zygospores, *a–h*. *J,* Protuberance of zygosporangium, showing lobed distal end; about × 2500. *K,* Testa of *Arcella discoides* containing a mycelium of a zoöpagaceous fungus.

cylindraceis vel elongato-ellipsoideis, plerumque leviter verrucosis, rectis vel leviter curvis, 10–47 μ (saepius 10–27) longis, 1.5–2.7 μ latis; ambobus ramis zygosporiferis vulgo ex aliis hyphis assumentibus oriundis; gametangiis saepe circa 25 μ longis, sursum 2–4 μ rarius usque 5 μ latis, ambobus quandoque sed non semper inter se spiraliter in modum caulis Phaseoli vulgaris vel in modum caulis Humuli lupuli circumvolutis, apice conjugentibus, denique prope junctionem zygosporangium emittentibus; zygosporangio globoso, 25–45 prominentiis speciose ornato, sine prominentiis vulgo 12–15 μ crasso, in maturitate flavidulo, cellulam viventem 8–9 μ crassam circumdante; prominentiis columnaribus, 1.5–2 μ longis, apice in 3–4 ramulos rotundos abeuntibus.

Amoebam saepe 45–70 μ latam (forsitan Amoebam terricolam) enecans habitat in foliis caulibusque Spartinae putrescentibus prope Mayo, Maryland.

Assimilative hyphae colorless, originally continuous, widening out from the narrowly rounded proximal end in the manner of a horn, usually 3 to 11 μ in width, sometimes as much as 150 μ long, coiled in a snail-like spiral of 1 to 3 successive turns, when composed of only 1 or 2 turns often simple, but when comprising 2 to 3 successive turns more usually bifurcate or two, three, or four times successively dichotomous, putting forth from the convex profile near the proximal end 1 to 4 (sometimes more) reproductive filaments 1.2 to 2 μ wide, which, after disablement of the host animal, push through its pellicle either to extend into the air somewhat moniliform prolongations that later are converted into conidial chains or to produce zygophoric branches in the underlying or surrounding material. Conidia colorless, cylindrical or elongated ellipsoidal, usually with a slightly warty lateral outline, straight or slightly curved, 10 to 47 (mostly 10 to 27 μ) long, 1.5 to 2.7 μ wide. Zygophoric branches in a pair commonly originating from separate assimilative hyphae, each by deposition of a cross-wall supplying a terminal gametangium: gametangia about 25 μ long, distally 2 to 4 μ (rarely up to 5 μ) wide, the two of a pair sometimes spirally intertwined with right-handed or left-handed rotation and sometimes more haphazardly interlocked by means of short branches or protuberances, after fusing apically burgeoning forth a zygosporangium near the union; zygosporangium subspherical, handsomely ornamented externally with 25 to 45 columnar protuberances, exclusive of the protuberances measuring usually 12 to 15 μ in diameter, at maturity containing a subspherical protoplast 8 to 9 μ in diameter; protuberances mostly 1.5 to 2 μ long, terminating in 3 or 4 subglobose lobes.

Parasitic on an *Amoeba* often 45 to 75 μ wide (probably *Amoeba terricola*) it occurs in decaying leaves and culms of *Spartina* sp., near Mayo, Maryland.

AN AMOEBA PARASITE WITH A DESMID-LIKE THALLUS

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted by adding a small quantity of decaying willow (*Salix* sp.) leaf detritus gathered in a moist thicket near College Park, Maryland, on February 5, 1950, showed on examination 14 days later, numerous small specimens of *Amoeba* undergoing destruction by an endoparasitic fungus that in scattered positions pushed up into the air small groups of delicate spore chains composed of small conidia. The animals attacked measured 20 to 25 μ in width when drawn into a rounded shape (FIG. 3, *A–E*). They were observed only on the surface of the agar, never being seen moving about under the surface. Their finely granular protoplasm was surrounded by a delicate pellicle with a gently undulating or minutely rippled contour. Apart from their single contractile vacuole (FIG. 3, *A, v; C–E: v*), they showed internally a prolate ellipsoidal nucleus (FIG. 3, *A–E: n*), usually 4 to 5.3 μ long and 3.3 to 4.3 μ wide, within which a slightly darker, globose endosome, 1.8 to 2.5 μ in diameter, was distinguishable. Evidently they all belonged to a single species of *Amoeba* generally resembling *A. sphaeronucleolus* Greeff and *A. verrucosa* Ehrenberg in nuclear make-up, but having dimensions much smaller than either of these familiar rhizopods. Many individuals, whether healthy (FIG. 3, *A*) or infected (FIG. 3, *B–F*), contained a variable number of colorless subspherical bodies, mostly 2.3 to 3.5 μ in diameter, which from their close resemblance to other such bodies strewn about abundantly everywhere on the agar plate seemed best interpretable as ingested fungus spores.

At the earliest stage of vegetative development in which the parasite can be recognized with certainty, its thallus is often bulb-shaped and consists of a globose part measuring about 4 to 5 μ across, together with a narrower part approximately 1.5 μ in width (FIG. 3, *B, a*). In all likelihood the narrower part here represents the infective body formed at the tip of a germ tube intruded into the protoplasm from an externally adhering conidium. Apparently it soon expands as nourishment is assimilated, for after some increase in size the thallus often has an elongated ellipsoidal outline (FIG. 3, *B, b; C, a, b*) and then offers a little resemblance

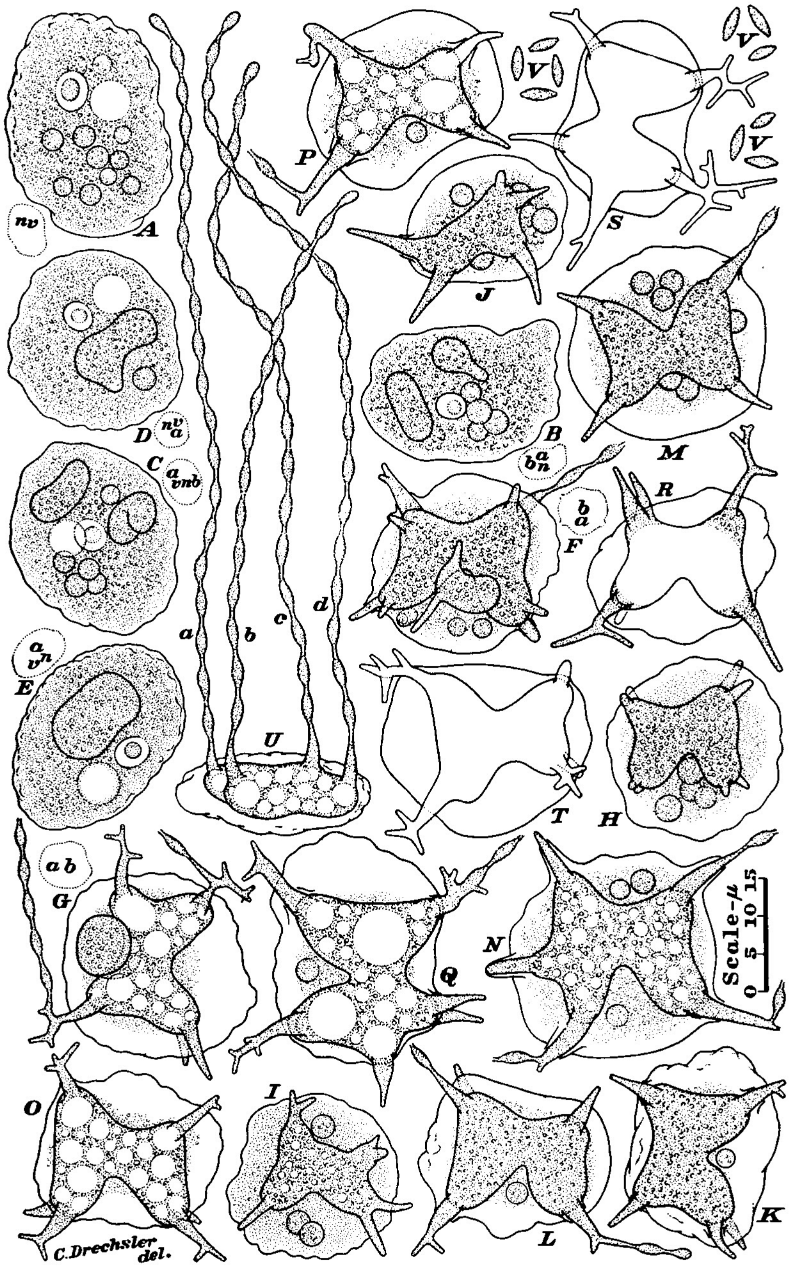
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FIG. 3.

to young thalli of *Cochlonema* or *Endocochlus.* Further growth, however, takes place not through elongation at the distal end as in the two helicoid genera, but through horizontal extension at the margin, so that the thallus acquires a flattened pillowy shape (FIG. 3, *D, a; E, a*). Where two thalli are present in an animal (FIG. 3, *F, a; G, a*) the younger smaller one may find its source of nourishment exhausted early, and may therefore need to become modified for sporulation while still in a juvenile form. More usually only one infection occurs, and the single thallus alters its pillowy shape by expanding unequally in such wise that a major constriction is left midway of its length, and a minor indentation is left at either end (FIG. 3, *F, b; G, b*). From the several arms of the desmid-like body (FIG. 3, *H–T*) tapering hyphal outgrowths are extended that break through the enveloping pellicle to elongate into the air as erect or ascending filaments made up of many minutely verrucose swollen portions connected by narrowed isthmuses (FIG. 3, *U, a–d*). When the swollen portions are separated from one another by the laying down of delimiting walls in the constrictions, each aerial filament is converted into a chain of conidia. The several chains break up on slight disturbance, scattering the individual spores (FIG. 3, *V*) about on the substratum in positions favorable for lodgment on new host animals.

Although microscopical scrutiny with a dry objective most usually shows four conidial chains arising close together from an in-

FIG. 3. *Aplectosoma microsporum,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* Normal specimen of host *Amoeba; n,* nucleus; *v,* contractile vacuole. *B, Amoeba* host containing two small thalli, *a* and *b; n,* nucleus of animal. *C, Amoeba* host containing two small thalli, *a* and *b; n,* host nucleus; *v,* contractile vacuole of animal host. *D, E, Amoebae,* each occupied by a single thallus of larger size, *a; n,* host nucleus; *v,* contractile vacuole of host. *F, G, Amoeba* hosts, each containing a small thallus, *a,* and a large thallus, *b,* from which reproductive hyphae are being extended. *H–R, Amoeba* hosts, each containing a well-developed thallus; the several thalli showing increasing vacuolization as contents are utilized in development of reproductive hyphae and conidia. *S, T,* Pellicles of host *Amoebae,* each empty of protoplasm and occupied only by the empty membranous envelope of a thallus whose contents were spent in producing conidia. (In *F–T* the reproductive outgrowths and sterigmata are shown flattened out considerably, as seen in specimens under a cover-glass when viewed from above.) *U,* Lateral view of host pellicle and of thallus with four ascending reproductive hyphae, *a–d. V,* Detached conidia.

fected animal, groups of five chains occur with some frequency. Such groups generally come from thalli of more than ordinary size (FIG. 3, *N, Q, S*) in which the sequence of development was modified to form five rather than four divergent lobes, and accordingly also five primary hyphal outgrowths. In view of the usual correspondence between the number of thallodic lobes present and the number of conidial chains existent most of the time, it is somewhat surprising to find on closer examination that often a lobe has two primary hyphal outgrowths, and that frequently a hyphal outgrowth bears one to three tips or spurs in addition to the one tip or spur supporting a conidial chain (FIG. 3, *G*). Indeed, the production of supernumerary parts often proceeds freely while material is directly under observation. A well developed thallus that at the time a mount is prepared shows only the usual four hyphal outgrowths (FIG. 3, *M*), each on a separate lobe, will often put forth a second outgrowth from each of the lobes during the ensuing hour. While such behavior may be held to result directly from artificial disturbance of the conditions under which the fungus had made its growth, rather similar disturbance must repeatedly have taken place, without human interference, in my cultures (as also in nature) from the jostling, for example, of robust nematodes. Assuredly in instances where thalli were represented only by empty membranous envelopes when the agar holding them was mounted for microscopical examination, the presence of four or five denuded spurs on one or more of the hyphal outgrowths (FIG. 3, *S, T*) should be considered expressive of normal morphology.

The protoplasm in the flat thallus shows a somewhat more granular structure than is usual in the helicoid thalli of *Cochlonema* and *Endocochlus*. In respect to the texture of its contents the flat thallus invites comparison, among the Zoöpagaceae, more especially, perhaps, with the filamentous mycelium of my *Stylopage hadra* (4). The migration of contents into the elongating aerial hyphae proceeds with increasing vacuolization in the thallus (FIG. 3, *G, b; N–R*) until only the empty desmid-like envelope remains visible within the empty protozoan pellicle (FIG. 3, *S, T*). Retaining walls similar to those laid down in some species of *Cochlonema* to mark successive stages of evacuation, have never been seen in the fungus. Formation of conidia takes place here much as in *Cochlonema* and in the two other catenulate genera—*Bdellospora* and *Zoöpage*—earlier made known in the Zoöpagaceae. The flat pillowy thallus embodies a design for which no provision has hitherto been made in the family. A thallus of such outward shape would, of course, be quite commonplace among some groups within the Chytridiales, but among the Zoöpagaceae its departure from a filamentous condition seems no less unusual than that of the globose thalli developed in my *Bdellospora helicoides* (2). Accordingly the fungus would seem to merit recognition as the type of a separate genus. A generic name compounded of words meaning "unplaited" and "body," respectively, may be helpful in recalling its chief distinctive feature.

Aplectosoma gen. nov.

Corpora assumentia incolorata, intra animalia viventia praecipue intra Amoebas minores planas crescentia, subdiscoidea vel pulvinata, saepe mox in margine plus minusve lobulata, animali moribundo vel debilitato ex apicibus gibborum hyphas conidiferas emittentia; conidia hyalina, saepe fusoidea vel elongato-ellipsoidea, in catenulas erectas vel ascendentes digesta.

Assimilative thallus colorless, growing endoparasitically in small living animals, especially in rather small *Amoebae* of flattened shape, somewhat disc-shaped or cushion-shaped, at the margin eventually often becoming more or less lobulate, from the apices of the several lobules putting forth conidiiferous hyphae after the disablement or death of the animal; conidiiferous hyphae erect or ascending, at first continuous, later through partitioning becoming transformed into chains of conidia; conidia colorless, often spindle-shaped or elongate-ellipsoidal.

Aplectosoma microsporum sp. nov.

Corporibus assumentibus pulvinatis, in margine saepius quadrifidis vel interdum quinquifidis, plerumque 13–24 μ longis, 10–18 μ latis, ex 4 vel 5 gibbis hyphas fertiles emittentibus; hyphis fertilibus primo continuis, mox in sterigmate infero et catenula conidiorum supera constantibus; sterigmatis simplicibus vel aliquoties breviramosis, plerumque 4–15 μ longis, basi 1.5–3.5 μ crassis, sursum attenuatis, apice circa 0.5 μ crassis; conidiis in catenula erecta vel ascendente denis usque tricenis oriundis, fusoideis vel elongato-ellipsoideis, plerumque 3.5–5.2 μ longis, 1.4–1.9 μ crassis.

Amoebam speciem vulgo 20 usque 25 μ, latam enecans habitat in foliis Salicis putrescentibus prope College Park, Maryland.

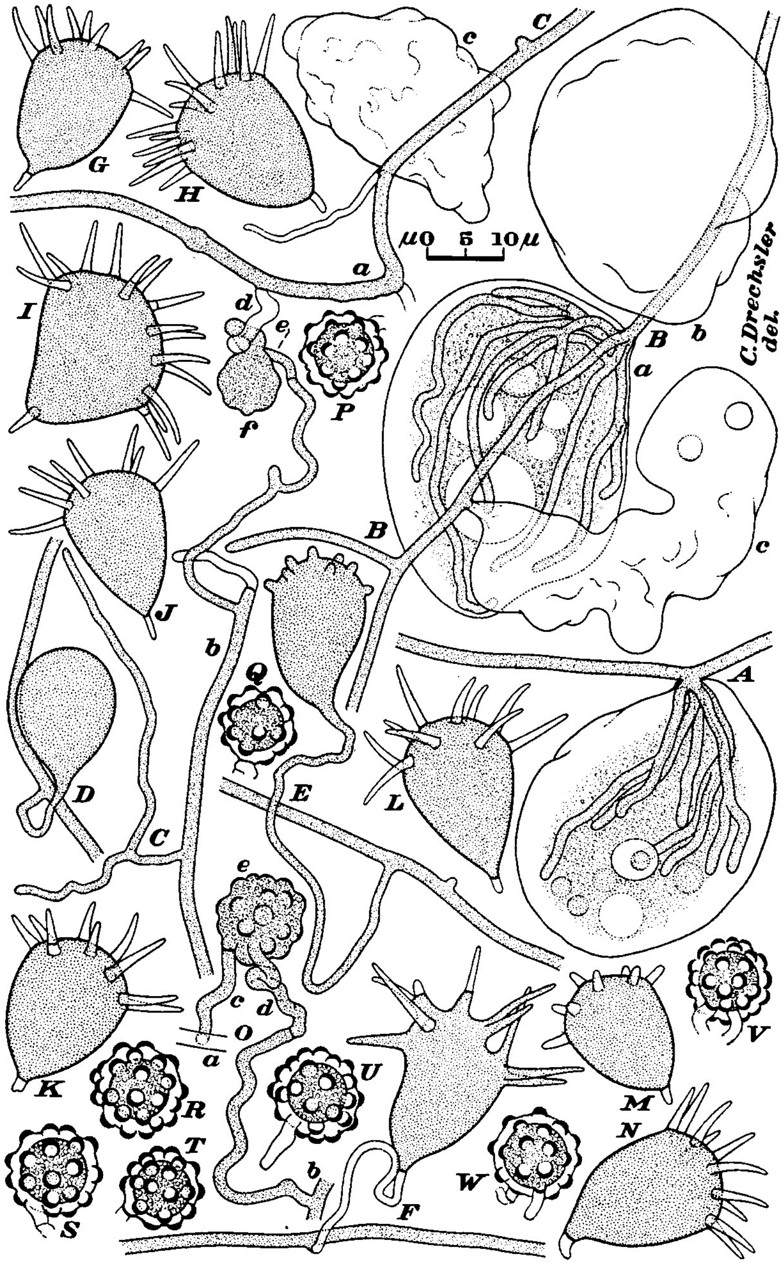
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FIG. 4.

Assimilative thallus cushion-shaped, at the margin usually quadrilobate but sometimes quinquelobate, mostly 13 to 24 μ long and 10 to 18 μ wide, from the several lobes putting forth hyphae which later are converted individually into a basal sterigma and a distal, erect or ascending conidial chain; the sterigma simple or with several short spurs, mostly 4 to 15 μ long, 1.5 to 3.5 μ wide at the base, tapering upward, about 0.5 μ wide at the tip; the conidia spindle-shaped or elongate-ellipsoidal, mostly 3.5 to 5.2 μ long, 1.4 to 1.9 μ wide, formed in numbers from 10 to 30 in a chain.

Endoparasitic in an *Amoeba* mostly 20 to 25 μ wide, it occurs in decaying *Salix* leaves near College Park, Maryland.

THE SEXUAL REPRODUCTIVE STAGE OF ACAULOPAGE LOPHOSPORA

Acaulopage lophospora was originally described from an agar plate culture that had been planted with decaying vegetable material gathered in Colorado. The species at the time had never been observed in cultures prepared with vegetable detritus from other sources, though a closely related form, my *Acaulopage tetraceros* (**3**: 195–197; **9**: 289–291), would seem widely and abundantly distributed in Maryland and Virginia. More recently mycelium and conidia evidently referable to *A. lophospora* developed in a maizemeal-agar plate culture which after being overgrown by *Pythium ultimum* had been further planted with a small quantity of partly decayed honeysuckle (*Lonicera* sp.) leaves collected near Aberdeen, Maryland, on February 14, 1950. The mycelium (FIG.

FIG. 4. *Acaulopage lophospora* (Maryland strain), drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Portion of hypha with a captured *Amoeba* that has been largely expropriated of its protoplasmic contents by means of a rangy bush-like haustorium. *B*, Portion of hypha showing one captured *Amoeba*, *a*, extensively invaded by a rangy bush-like haustorium, and the empty pellicles of two other captured *Amoebae*, *b* and *c*. *C*, Two hyphae, *a* and *b*, without close mycelial connection; one of them, *a*, has attached to it the empty pellicle of a captured *Amoeba*, *c*; the two hyphae have produced two gametangia, *d* and *e*, respectively, which have united to put forth the young zygosporangium, *f*. *D*, Portion of hypha with a branch bearing a nearly full-grown conidium. *E*, Portion of hypha with a branch bearing a conidium that is putting forth distal protuberances. *F*, Portion of hypha with a branch bearing a full grown conidium whose protuberances are being evacuated of protoplasmic contents. *G–N*, Mature conidia. *O*, Immature unit of sexual reproductive apparatus: *a*, *b*, mycelial hyphae without close mycelial connection; *c*, *d*, gametangia; *e*, immature zygosporangium. *P–W*, Mature zygospores.

4, *A–F;* FIG. 5, *A*) here appeared, in general, a little coarser than in the earlier culture, some of the filaments attaining a width of 2 μ (FIG. 4, *C, a, b*). The *Amoebae* (FIG. 4, *A; B, a–c; C, c*; FIG. 5, *A*) captured in large numbers through adhesion to the mycelial filaments included individuals measuring as much as 35 μ in average diameter. In some of the captured animals a nearly spherical nucleus with a slightly darker central body could be recognized (FIG. 4, *A*). Expropriation of the animal's contents was accomplished by means of longish assimilative branches that arose in bush-like arrangement (FIG. 4, *A; B, a;* FIG. 5, *A*) from the lateral spur intruded by the external mycelial hypha through the adhering pellicle.

Conidia were produced freely by the Maryland fungus. During the earlier stages of development the lateral branch of somewhat variable length connecting the mycelial filament with the growing spore could often be made out clearly (FIG. 4, *D–F*). As a rule the young growing conidium showed a smooth obovoid contour (FIG. 4, *D*). In the final stages of enlargement it would burgeon forth most usually about ten tapering digitate protuberances from its upper side (FIG. 4, *E*). Once the protuberances were fully extended their protoplasmic contents were withdrawn backward. A cross-wall having in the meantime been laid down to delimit the conidium basally, the supporting branch was likewise evacuated of contents (FIG. 4, *F*). The completed conidium with its short empty stipe, and its crest of six to fifteen empty membranous appendages (FIG. 4, *G–N;* FIG. 5, *B–F*) corresponded well to the conidia produced by the Colorado strain of the fungus (FIG. 5, *G–J*).

In addition to forming conidia the Maryland strain gave rise to zygospores. Units of sexual reproductive apparatus regularly had their origin in two mycelial filaments (FIG. 4, *C, a, b; O, a, b*) without any close hyphal connection. From these filaments would arise two sexual branches which after becoming in some degree interlocked would each lay down a septum to delimit a terminal gametangium (FIG. 4, *C, d, e; O, c, d*). After apical fusion of the two gametangia a globose zygosporangium grew out close to the union (FIG. 4, *C, f; O, e*). During the later stages of their growth the zygosporangia became covered with twenty to thirty verrucose protuberances. At maturity the protuberances appeared as handsome yellowish hemispherical warts, often about 1 μ high and 2 μ wide at the base. Owing to their presence the structure of the underlying wall was badly obscured, making it difficult to determine whether only a single thick layer surrounded the living protoplast, or whether a zygosporangial membrane enveloped a separate zygospore wall. The mature globose body (FIG. 4, *P–W*), exclusive of the warts, measured usually 9 to 11.5 μ in diameter; the spherical protoplast, mostly of granular texture, varied from 6.5 to 8.7 μ in that dimension.

A ZOÖPAGACEOUS MYCELIUM FOUND IN TESTACEOUS RHIZOPODS

Aplectosoma microsporum provides an example wherein the shape of the assimilative thallus is adapted in a distinctive manner to the spacial limitations of the host animal. Adaptation to somewhat different spacial limitations is shown in the curious disposition of a mycelium, evidently referable to the Zoöpagaceae, that was observed most often in a circular shield-shaped *Arcella* (FIG. 2, *K;* FIG. 5, *K;* FIG. 6, *A, B*), which from its close resemblance to two small specimens of *Arcella discoides* Ehrenb. figured by Leidy (**13**: *pl. 28, figs. 24–27*) I am referring to Ehrenberg's species. The infected animals were found among a much larger number of healthy individuals of the same species in maizemeal-agar plate cultures which after being overgrown with mycelium of *Pythium ultimum* had been further planted in some instances with small quantities of decaying crab grass (*Digitaria sanguinalis* (L.) Scop.) taken from an old weed pile on moist ground in College Park, Maryland, on March 26, 1950, and in other instances with small quantities of overwintered sedge detritus gathered nearby on the same day. Infection must almost certainly have taken place before the decaying material was added to the cultures, for the endoparasitic mycelium was always seen in a declining rather than in a growing condition. Within the shield-like testa the mycelium was disposed circularly, after the manner of a garland, between the central aperture and the peripheral margin. In specimens where the proximal hypha was clearly visible, even if in part evacuated

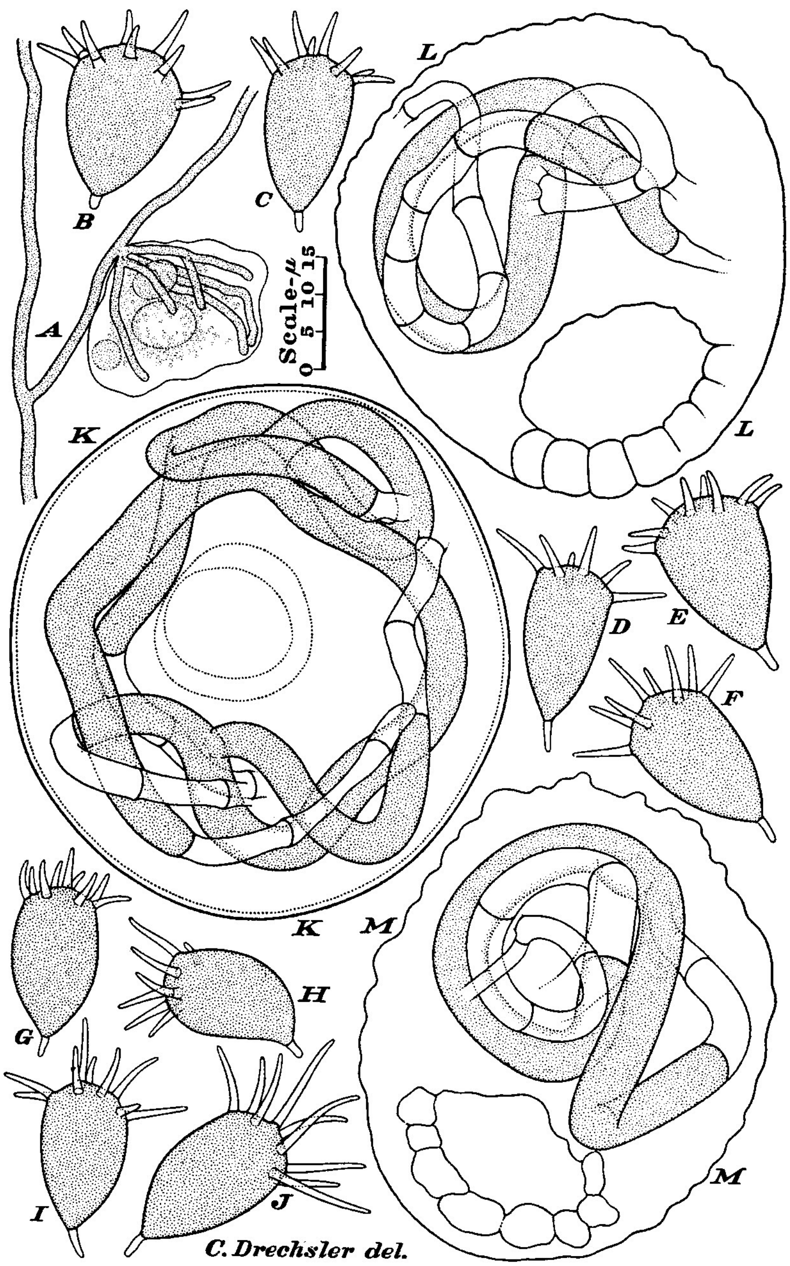

FIG. 5.

of protoplasmic contents (FIG. 2, *K;* FIG. 5, *K*), the fungus manifestly had begun its vegetative development as a filament between 2 and 3 μ thick. As it elongated it widened gradually, and thus usually attained a diameter of 6 to 7.5 μ on reaching a length of 75 to 100 μ, when it branched dichotomously. The two branches evidently grew for distances of 50 to 100 μ (FIG. 2, *K;* FIG. 5, *K*), or even for a distance of 150 μ (FIG. 6, *B*), before a second bifurcation occurred. Between the first and second dichotomies the branches maintained a rather uniform diameter, but beyond the second dichotomy the width of all hyphal branches diminished perceptibly. A third dichotomy was sometimes observed (FIG. 2, *K*), with the resulting ramifications measuring only about 2 μ in thickness. At the time the fungus came under observation the proximal hypha as well as the distal branches in all the mycelia was already emptied rather extensively of protoplasmic contents. Evacuation had evidently proceeded step by step, for the empty portions of hyphal membrane revealed a succession of cross-walls similar to the retaining walls bounding the filamentous living cell at its several ends. In one testa (FIG. 6, *A*) two separate living hyphal cells were found present—a condition that may have come about through plural infection. The protoplasm in the living portions of mycelium had the dense, somewhat homogeneous appearance familiar in the helicoid thalli of *Cochlonema* and *Endocochlus.*

Many of the cultures that contained infected specimens of *Arcella discoides* showed mycelia of the same fungus present also in the shells of another testaceous rhizopod (FIG. 5, *L, M*), which would seem referable to *Difflugia constricta* Ehrenb., judging from resemblances in shape and size to some individuals of that species figured by Leidy (**13**: *pl. 18, figs. 17–19*). In the more nearly globose chamber of this animal the individual mycelium formed a loose irregular clew without noticeable helicoid convolvement. Ex-

FIG. 5. Drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A–J, Acaulopage lophospora: A,* Portion of mycelium of Maryland strain, to which is attached a captured *Amoeba* that has been nearly depleted of its protoplasmic contents by means of a bush-like haustorium. *B–F,* Mature conidia of Maryland strain. *G–J,* Mature conidia of Colorado strain. *K–M,* Mycelial stage of endoparasitic zoöpagaceous fungus: *K,* Testa of *Arcella discoides* containing the fungus. *L, M,* Fungus present in testae of *Difflugia constricta.*

tensive evacuation of hyphal portions here likewise had resulted in deposition of successive partitions similar to the retaining walls bounding the living filamentous cell at the ends (FIGS. 5, *L, M*).

As progressive evacuation of mycelium or thallus in the Zoöpagaceae is commonly associated with formation of conidia or zygospores, the fungus in the two species of rhizopods was examined closely for reproductive development. No reproductive structures of any sort could be recognized. In instances where the proximal portion of the mycelium was visible, no empty spore envelope could be seen from which vegetative growth could have proceeded. Since empty shells of *Arcella discoides* sometimes contained a coiled eelworm, the possibility was considered that the fungus might be parasitic on the nematode visitors rather than on the protozoans themselves. The possibility seems a very unlikely one, inasmuch as development within imprisoned eelworms would not have permitted the rather broad garland-like hyphal arrangement often observed (FIG. 2, *K*; FIG. 6, *A*); and besides, no eelworms were ever found occupying empty shells of *Difflugia constricta*. Material showing asexual reproduction will be necessary before the fungus can be assigned to an appropriate genus in the family.

AN AMOEBA PARASITE WITH A SMALL U-SHAPED THALLUS

A Petri plate culture of rather soft maizemeal agar, which, after being permeated with mycelium of my *Pythium arrhenomanes*, had been further planted with a small quantity of barley (*Hordeum vulgare* L.) straw gathered near Greeley, Colorado, in October, 1945, showed on examination 18 days later scattered individuals of a species of *Amoeba* that harbored the smallest endoparasitic thallus hitherto seen in any member of the Zoöpagaceae. The *Amoeba* in question (FIG. 6, *C–G*) may have been of approximately the same size as the species parasitized by *Aplectosoma microsporum*, but was usually of more elongate shape. When in active locomotion it stretched out (FIG. 6, *E, F*) after the manner of *A. limax* Dujardin, though it lacked the curious tuft of posterior processes familiar in that species. In a fully extended condition the animal often measured 50 to 60 μ in length, while its width varied from 5 to 15 μ at different places along its body. It contained a globose or somewhat prolate ellipsoidal nucleus 5.5 to 7 μ in diameter, with a slightly darker subspherical endosome 1.9 to 2.7 μ wide (FIG. 6, *C–G: n*). A single contractile vacuole (FIG. 6, *E, v; F, v*) was usually found operating in a peripheral position, and sometimes two (FIG. 6, *D, v*) or even three (FIG. 6, *G, v*) such vacuoles could be distinguished.

Infected specimens of the *Amoeba* showed fungous bodies of two types immersed in their protoplasm, a straight cylindrical type (FIG. 6, *C, a*) and a strongly curved, semi-circular or U-shaped type (FIG. 6, *C, b*). The straight bodies were often about 8 or 9 μ long and measured approximately 2.3 μ in greatest width; whereas the curved bodies varied commonly from 12 to 18 μ in length, and from 2.5 to 3.5 μ in greatest width. Many of the curved bodies were found connected at one of their two ends by a slender isthmus to an external body which in an early stage of development was of ovoid shape (FIG. 6, *D, a*), but later showed approximately the same dimensions and the same cylindrical or strongly elongated ellipsoidal shape (FIG. 6, *E, a, b; F, a, b*) as the straight bodies immersed in the protoplasm (FIG. 6, *C, a*). Although the curved bodies are small in comparison with the spirally convolved thalli usual in species of *Cochlonema* and *Endocochlus,* they manifestly are of the same character with respect to both function and form. The external bodies would seem best interpretable as conidia that are supplied with protoplasm by the curved thalli within the animal host. As not more than one presumptive conidium was ever seen attached to a thallus, there is reason to believe that each conidium on reaching definitive size becomes disjointed before its successor is burgeoned forth. It seems rather unlikely that only one single conidium is produced, for the thalli that are found connected with full grown conidia (FIG. 6, *F, a, b*) ordinarily show no sign of vacuolization as long as the animal host affords ample nourishment.

The manner in which the fungus gains entrance into the animal was not observed. Nor was conidial germination—at least germination of any easily recognizable sort—ever seen either inside or outside of host animals. From the similarity of fully grown conidia to the straight cylindrical bodies seen immersed in the host proto-

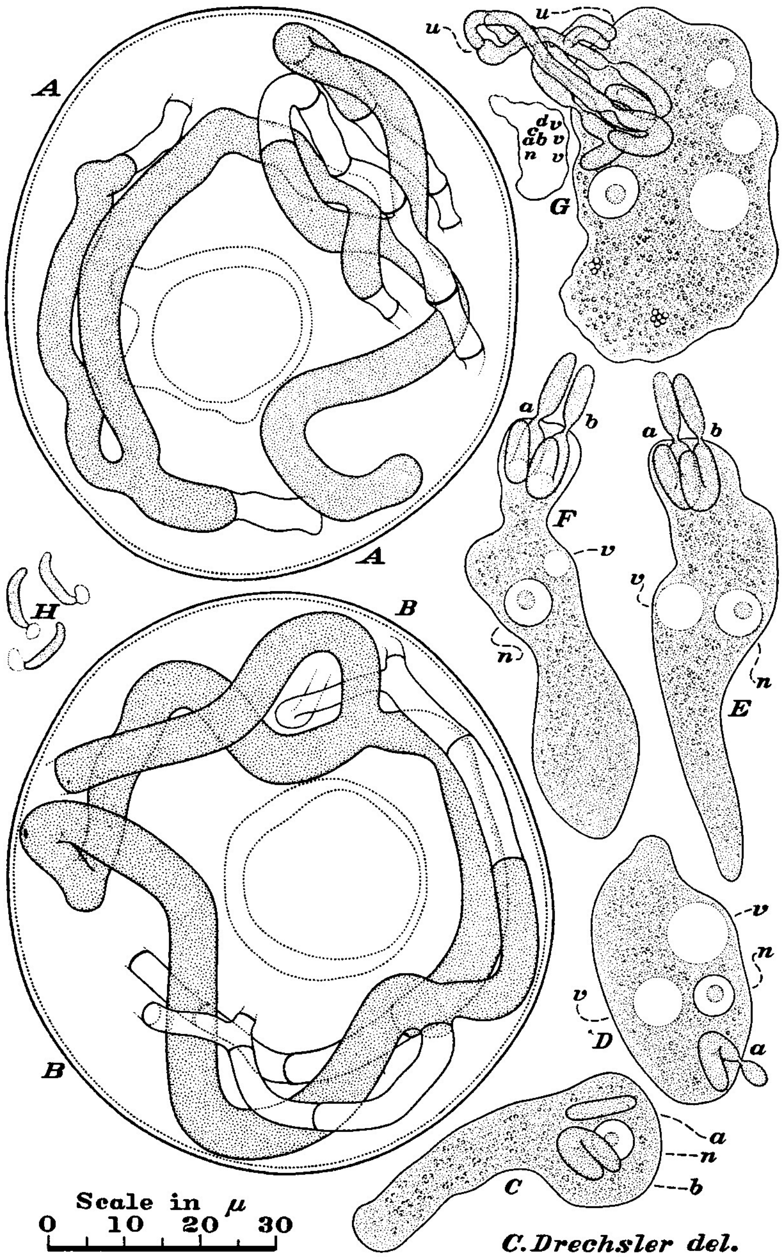
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FIG. 6.

plasm, it seems not improbable that the conidia are ingested by the *Amoeba,* and that they then develop directly into thalli through slight widening, strong curvature, and mere two-fold increase in length. Such immediate enlargement would offer some parallelism to the development of *Bdellospora helicoides,* where the externally adhering conidium expands rather directly into an obese thallus, though emission of the haustorium in that fungus provides a phase of growth somewhat akin to ordinary germination in species of *Cochlonema* and *Endocochlus.*

In some instances where an animal contained several curved thalli (FIG. 6, *G, a–d*) huddled close together, zygospores were produced. Each thallus would extend from one of its ends a narrow process which after perforating the host pellicle widened out externally into hyphae about 2.2 μ in diameter. Some of the hyphae made direct apical contact with one another in pairs (FIG. 6, *G, b, d*), while others became paired through tip-to-tip contact of branches put forth by them (FIG. 6, *G, c, d*). At the places of contact (FIG. 6, *G, u*) fusion occurred after cross-walls had been laid down to delimit gametangia. A verrucose zygospore about 6 μ in diameter resulted from each union. The several zygospores produced from plural thalli huddled in an animal formed handsome clusters outside, close to the pellicle. In mature clusters the continuity of the individual zygospores with the intertangled empty membranes of gametangia and supporting hyphae was found exceedingly difficult to follow.

FIG. 6. Drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A, B,* Mycelial stage of endoparasitic zoöpagaceous fungus: *A,* Two separate portions of living mycelium present in testa of *Arcella discoides. B,* Portion of living mycelium in testa of *Arcella discoides. C–G,* Zoöpagaceous fungus with a small curved thallus parasitic on a small *Amoeba* apparently of the *limax* type: *C. Amoeba* host containing a straight fungus body, *a,* perhaps representing a newly ingested conidium, as well as a curved thallus, *b; n,* host nucleus. *D, Amoeba* host containing a curved thallus, *a,* that is producing a conidium externally; *n,* nucleus of host; *v,* contractile vacuole of host. *E, F, Amoeba* host shown in two views drawn 15 minutes apart: *a, b,* two curved thalli, each of which has nearly completed production of a conidium externally; *n,* host nucleus; *v,* contractile vacuole of *Amoeba* host. *G, Amoeba* host containing four curved thalli, *a–d,* that have extended sexual hyphae which have made close contact in two positions, *u; n,* host nucleus; *v,* contractile vacuole of animal host. *H,* Three conidia of unnamed species of *Euryancale.*

Among the Zoöpagaceae that have hitherto become known the fungus is remarkable for the small size of its curved thallus, and the relatively large size of its conidia. The development of these conidia singly, and, in all likelihood, successively, makes its asexual reproduction different from any hitherto recognized in the family. There is reason to believe that the perseverance of conidial development through a long period when the animal host keeps on moving about actively, is a feature more consistent with an aquatic than with a terrestrial mode of life. In respect to habitat, therefore, the fungus could perhaps be placed in a category with the filamentous appendages observed on some aquatic rhizopods by different authors, including Korotneff (**12**), Leidy (**13**: 67–72), Penard (**14**: 65–70), Dangeard (**1**), and Geitler (**11**). While Leidy's figures of the appendages on his *Ouramoeba botulicauda* (**13**: *pl. 9, figs. 13–17*), and Geitler's figures of filaments attached to *Amoeba proteus* are suggestive of the Zoöpagaceae, catenulate sporulation would seem indicated in the illustrations of both authors rather than the solitary sporulation shown in my fungus.

A SECOND SPECIES OF EURYANCALE SUBSISTING ON NEMATODES

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium ultimum* had been further planted with a small quantity of leaf mold taken from deciduous woods near Butternut, Wisconsin, on November 15, 1945, showed 25 days later a small area occupied by conidial apparatus of a species of *Euryancale* different from my *E. sacciospora* (**8**: 406–411). Its conidia (FIG. 6, *H*) consisted individually of a slightly curved cylindrical living cell usually from 7.5 to 9 μ long and about 1.5 μ wide, together with an empty basal appendage of prolate ellipsoidal shape commonly measuring 2 to 3 μ in length and approximately 1.8 μ in width. The appendage was oriented most often with its longer axis at a right angle to the proximal portion of the living cell. Unlike the empty appendage of *E. sacciospora* its saccate shape was not modified by conspicuous narrowing near the place of attachment. The upcurved lateral branches bearing the spores seemed markedly sturdier than the corresponding branches of *E.*

sacciospora. Further, the main reproductive hyphae giving off the upcurved branches appeared about twice as wide as the homologous hyphae of *E. sacciospora.*

Although several of the reproductive filaments could readily be traced backward to their origin in an assimilative mycelium within the integument of an eelworm, the remains of the eelworm were too largely concealed from view by opaque overlying material to permit identification of the host animal. No further development of the fungus could be noted during ensuing weeks, either because the host animal failed to multiply in the culture or because conditions were unsuitable for infection.

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